TECHNICAL MEMORANDUM ◦ AUGUST 2018

Soulajule Reservoir and Arroyo Sausal Methylmercury Control Project: Synthesis of Phase 2 Pilot Studies and Prioritization of Reservoir Management Measures

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EXECUTIVE SUMMARY

Background

Located in western Marin County, California, Soulajule Reservoir is a small surface water impoundment owned and operated by Marin Municipal Water District (District) as an auxiliary water supply. The Soulajule Reservoir watershed includes geologic deposits of mercury in cinnabar (a mercury ore) such that total mercury concentrations in soils and water may be naturally enriched. In 2008, a Total Maximum Daily Load (TMDL) for mercury in the Walker Creek and Soulajule Reservoir watersheds was adopted by the San Francisco Bay Regional Water Quality Control Board (Regional Board) to protect aquatic life, wildlife, and human health in the Walker Creek watershed, including Soulajule Reservoir. Since 2010, the District has undertaken a series of studies to determine mercury occurrence and bioaccumulation patterns in Soulajule Reservoir and its downstream waterbody, Arroyo Sausal. The most recent, ongoing effort focuses on control strategies to reduce mercury methylation and bioaccumulation potential in the reservoir. This report summarizes and synthesizes key findings from Phases 1−2 of the *Soulajule Reservoir and Arroyo Sausal Methylmercury Control Project* (Project) and provides recommendations for Phase 3. The project phases are briefly summarized below.

Phase 1 Develop Study Plan

Phase 1 of the Project included review of existing data, an evaluation of 17 potential in-lake management methods and five watershed management methods for their applicability in addressing overall reservoir management objectives and methylmercury control, and development of a study plan to address information gaps related to potential control strategies. The Project team undertook an initial screening of the broad list of 22 potential management methods and narrowed the list to seven methods judged to have general or potential applicability for controlling methylmercury production and bioaccumulation in Soulajule Reservoir. The Project team subsequently ranked the seven management methods using a set of weighted criteria (e.g., compatibility with water supply objectives, effectiveness for decreasing methylmercury in water and/or fish, risk of failure, cost, potential for multiple benefits, landowner acceptability, engineering requirements, operation and maintenance requirements, permitting requirements, recreation effects). The seven ranked management methods are listed below, with the final weighted rankings shown in parentheses:

- Biomanipulation—sport fish stocking (81)
- Hypolimnetic oxygenation system (HOS) (79)
- Upland erosion control in Eastern Arm (74)
- Vigorous epilimnetic mixing (VEM) in Eastern Arm (73)
- Biomanipulation—prey fish stocking (71)
- Dredging of reservoir sediments in Eastern Arm (65)
- Capping of reservoir sediments in Eastern Arm (59)

Based on results of the ranking effort, the Project team identified six pilot studies to fill data gaps related to the eventual implementation of the top ranked management methods. The pilot studies were described in the *Soulajule Reservoir and Arroyo Sausal Methylmercury Control Study Plan* (Study Plan), which outlined pilot study objectives, hypotheses (where appropriate), sampling design, data analysis and reporting approach, implementation schedule, and estimated costs.

Phase 2 Finalize and Implement Study Plan

Phase 2 of the Project included finalization of the Study Plan and implementation of the six pilot studies. The pilot studies were undertaken in 2016, including a full year of investigation of physical, chemical, and biological conditions in Soulajule Reservoir, as well as characterization of upland mercury source loading. Results of the Phase 2 pilot studies and information from prior investigations indicate that the below factors influence methylmercury production and bioaccumulation in Soulajule Reservoir, forming the basis of an existing conditions conceptual model (Figure ES-1).

- Upland soils surrounding Soulajule Reservoir exhibit total mercury concentrations ranging from the background TMDL allocation (200 ng/g dry weight) to relatively high levels (> 1,000 ng/g dry weight), where the higher levels occur in both undisturbed areas and soils proximal to the historical Franciscan and Cycle mercury mines. There is currently little to no evidence of erosion and transport of mercury-laden soils into the reservoir as surrounding hillslopes are stabilized by heavy vegetation cover.
- Although currently understood to be stable, thick graded fill located at the historical Cycle Mine site exhibits relatively high total mercury concentrations and is in contact with reservoir water.
- Reservoir sediments in the Eastern Arm directly adjacent to the historical Cycle Mine exhibit the highest total mercury concentrations in the reservoir; these sediments also appear to be stable.
- Methylmercury concentrations in reservoir sediments are 1,000-100,000 times lower than total mercury concentrations. Methylmercury release rates from reservoir sediments are approximately 10 times higher from sediments near the dam as compared with sediments in the Eastern Arm, where total mercury concentrations are the highest, and the Western Arm, where total mercury concentrations are at background levels.
- Soulajule Reservoir supports high levels of pelagic (open water) primary productivity and low littoral zone productivity. Primary productivity is dominated by blue-green algae (cyanobacteria). Reservoir-wide blooms (or patchy concentrations of algae) can occur regardless of season.
- Thermal stratification throughout the reservoir, including the shallow Eastern Arm, limits resupply of dissolved oxygen to bottom waters and reservoir

sediments on a seasonal basis. Low to no oxygen for extended periods facilitates methylmercury production in anoxic sediments and/or bottom waters, as well as release of other redox-sensitive compounds like ammonium and orthophosphate that can contribute to overall internal nutrient loading and stimulate algal productivity in subsequent seasons.

- High planktonic algal productivity provides an ongoing source of organic carbon to fuel the microbial community in the reservoir sediments and the water column, which in turn deplete dissolved oxygen from the hypolimnion and reservoir sediments during stratification, and ultimately support mercury methylation.
- The littoral and profundal portions of the aquatic food web are interconnected, whereby trophic level (TL) three (TL3) and TL4 fish move between habitats and their prey include both pelagic (i.e., zooplankton, predatory insects) and benthic (i.e., chironomids, crayfish) forms.
- Methylmercury bioaccumulates in the Soulajule Reservoir food web, whereby tissue concentrations increase with trophic level, although patterns may be more complex than a simple integer conceptual model (i.e., TL1 → TL2 → TL3 → TL4) would predict because many reservoir biota are feeding at intermediate and/or multiple trophic levels.
- Spring-time water column mixing in the Eastern Arm may allow dispersal of methylmercury released from deeper bottom sediments to enter reservoir waters, resulting in water, phytoplankton, and zooplankton methylmercury concentrations that are 2−3 times higher than concentrations exhibited in other seasons and/or locations.
- Zooplankton (TL2) and small fish (TL3) methylmercury concentrations peak in the fall following reservoir overturn, due to a build-up of methylmercury in reservoir bottom waters and subsequent incorporation into the food web.

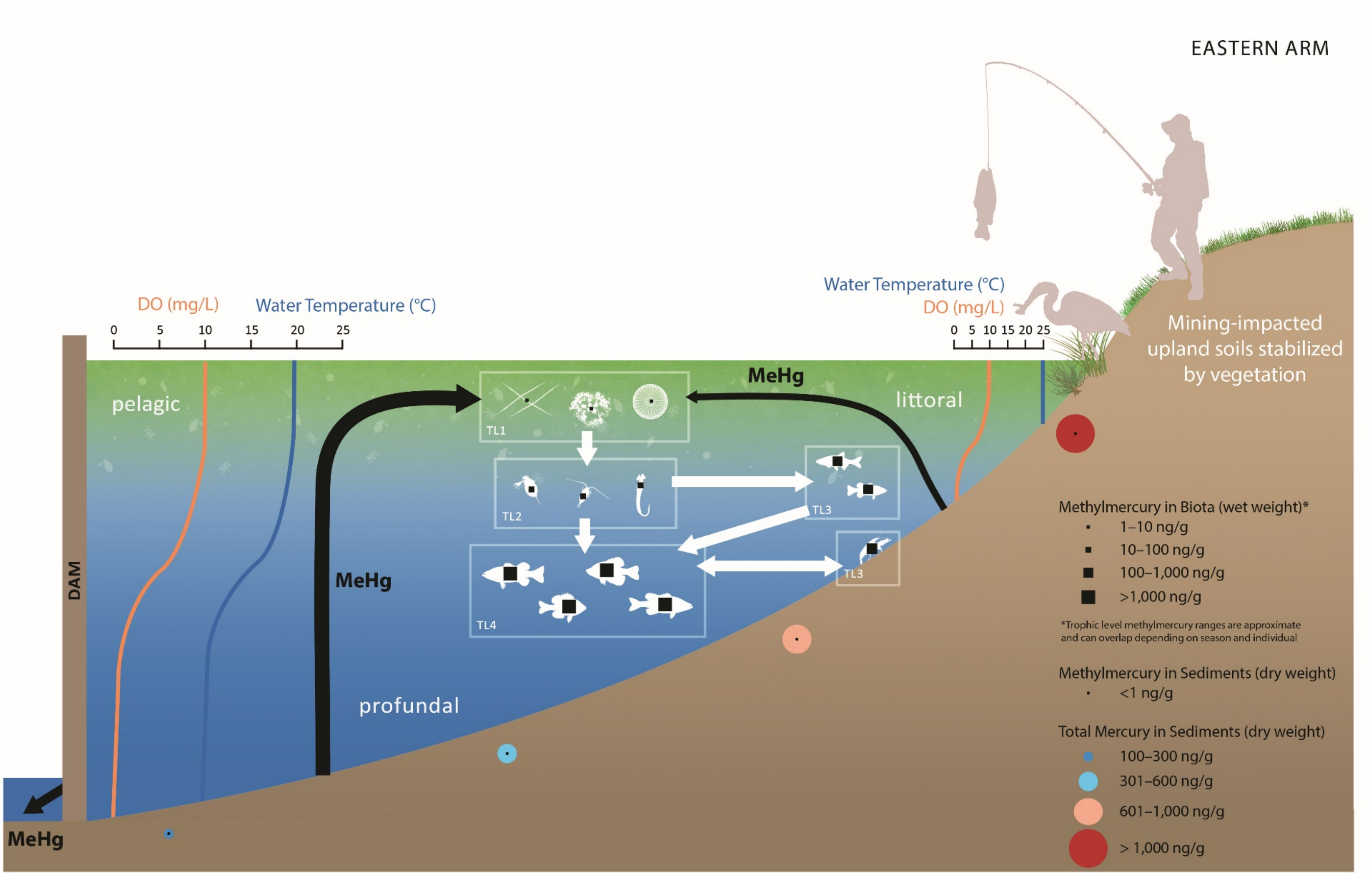


Figure ES-1. Existing conditions conceptual model of key factors controlling methylmercury production and bioaccumulation in Soulajule Reservoir during periods of stratification.

Phase 3 Develop Reservoir Management Plan

As an initial activity under Phase 3 of the Project, the Project team reassessed potential methylmercury control actions for Soulajule Reservoir based on the results of the Phase 2 pilot studies and prioritized the following actions for implementation under the Soulajule Reservoir Management Plan, as follows:

- Priority 1 − Low water survey of Cycle Mine thick fill
- Priority 2 – Biomanipulation - sport fish stocking permitting and implementation
- Priority 3 – Hypolimnetic oxygenation system (HOS) design and implementation
- Priority 4 – Eastern Arm upland erosion control and/or localized in-reservoir sediment capping

In order to best achieve effective and fiscally responsible implementation of the priority methylmercury control actions, the District would implement the actions in additional Project phases (Table ES-1).

The next step of the Project involves development of the Reservoir Management Plan, which will include: management objectives, prioritized control actions, implementation approach, monitoring requirements, success criteria, and responsibilities/roles for carrying out each of the control actions. For high priority control actions, the Reservoir Management Plan will include refined cost considerations compared with those provided in the original Study Plan.

Table ES-1. Reservoir Management Plan (RMP) priorities and phased implementation schedule.

RMP implementation[1]	2018				2019				2020				2021				2021			
Phase 4a – Low Water Survey of Cycle Mine Thick Fill																				
Phase 4b – Sport Fish Stocking																				
Phase 5a – Additional Studies to Inform HOS Design																				
Phase 5b – 30% HOS Design																				
Phase 5c – 100% HOS Design																				
Phase 5d – Construct and Operate HOS																				
Phase 6a – Eastern Arm Upland Erosion Control and Capping[2]																				

1 Phase schedules are shown in dark grey shading. Pink indicates the planning phase prior to implementation. Light grey shading indicates possible implementation of management action based on the success of previous phases.

2 Consideration of these management practices if stocking and Hypolimnetic Oxygenation System (HOS) implementation not significantly improving methylmercury bioaccumulation.

1 INTRODUCTION

Owned and operated by Marin Municipal Water District (District), Soulajule Reservoir is a small (10,200 acre-feet [ac-ft]) surface water impoundment located in western Marin County, California. The District releases water from Soulajule Reservoir downstream to Arroyo Sausal, which flows to the confluence with Salmon Creek, forming Walker Creek, which then flows to Tomales Bay, and eventually to the Pacific Ocean (Figure 1). The District also can transfer Soulajule Reservoir water to other District locations, as needed. The Soulajule Reservoir watershed includes geologic deposits of mercury (Hg) in cinnabar (a mercury ore) such that total mercury concentrations in soils and water may be naturally enriched (SFBRWQCB 2008).The San Francisco Bay Regional Water Quality Control Board (Regional Board) issued a 13267 letter on March 24, 2009, which requested a monitoring plan and time schedule for assessing methylmercury (MeHg) production and bioaccumulation in Soulajule Reservoir and its downstream waterbody Arroyo Sausal. The District subsequently undertook the development and implementation of the Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study (Brown and Caldwell and Stillwater Sciences 2010, 2013). In its May 7, 2014, letter to the District, the Regional Board requested a series of next actions to decrease methylation and bioaccumulation of mercury in Soulajule Reservoir. “Next Action A,” an addendum to the 2013 Study Report, provided Regional Board clarifications and additional data analysis requests with respect to sampling locations, monthly reservoir storage patterns, and methylmercury mass accumulation estimates (Stillwater Sciences 2014). The Regional Board requested that “Next Action B” involve development and implementation of a study plan to identify and pilot test management methods for controlling methylmercury production in Soulajule Reservoir.

Under Next Action B, the District undertook a multi-phased effort entitled the Soulajule Reservoir and Arroyo Sausal Methylmercury Control Project (Project), where the primary Project goal is to identify a set of effective and fiscally responsible management methods for controlling methylmercury in Soulajule Reservoir that the District would include and implement in its long-term reservoir management plan. The Project phases are as follows:

- Phase 1 – Develop Study Plan (2015)
- Phase 2 – Finalize and Implement Study Plan (2016−2017)
- Phase 3 – Develop Reservoir Management Plan (2018)

Each of the Project phases is summarized below. Data collection efforts in support of the Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study (2012) and the Soulajule Reservoir and Arroyo Sausal Methylmercury Control Project (2016) are summarized in Appendix A.

Figure 1. Location of Soulajule Reservoir and Arroyo Sausal, Marin County, California.

1.1 Phase 1 - Develop Study Plan

Phase 1 of the Project focused on development of the Soulajule Reservoir and Arroyo Sausal Methylmercury Control Study Plan (Study Plan), which the District submitted to the Regional Board in August 2015; the Regional Board subsequently approved the Study Plan. The District Board of Directors approved the Final Study Plan in September 2015 (Stillwater Sciences and Brown and Caldwell 2015).

While the Regional Board already had identified several potential methylmercury management approaches in its May 7, 2014 letter, development of the Study Plan commenced with a screening workshop to evaluate 17 potential in-lake management methods, as well as five broader watershed management methods, for their applicability in addressing overall reservoir management objectives (see Section 4.1) as well as methylmercury control (Stillwater Sciences and Brown and Caldwell 2015). District staff and the Stillwater Team undertook an initial screening of a broad list of potential management methods, including those suggested by the Regional Board, to ensure that the Project did not inadvertently overlook possible viable approaches.

Of the 22 total management methods considered during the screening effort, the District and the Stillwater Team judged seven methods to have general applicability or potential applicability for controlling methylmercury production and bioaccumulation in Soulajule Reservoir, given its broader water quality challenges, physical characteristics, and consideration of the Regional Board's four identified mercury management objectives. The Stillwater Team subsequently ranked the seven management methods using a set of 15 weighted criteria (e.g., compatibility with water supply objectives, effectiveness for decreasing methylmercury in water and/or fish, risk of failure, cost, potential for multiple benefits, landowner acceptability, engineering requirements, operation and maintenance requirements, permitting requirements, recreation effects). Results of the ranking exercise (Table 1) suggested three general rating ranges for potential methylmercury control actions:

- 80–100—no negative ratings, most ratings positive
- 70–79—most ratings neutral or positive
- <69—several negative ratings

Table 1. Phase 1 ranking summary for Soulajule Reservoir in-lake and watershed methylmercury control actions[1].

Methylmercury (MeHg) control action	Weighted rating (Scale 1–100)
Biomanipulation—sport fish stocking	81
Hypolimnetic oxygenation system (HOS)	79
Upland erosion control in Eastern Arm	74
Vigorous epilimnetic mixing (VEM) in Eastern Arm	73
Biomanipulation—prey fish stocking	71
Dredging of reservoir sediments in Eastern Arm	65
Capping of reservoir sediments in Eastern Arm	59

[1] Stillwater Sciences and Brown and Caldwell 2015.

Water level fluctuation, while occasionally used to control fisheries, aquatic vegetation, and algal growth, is not currently understood to be a method for controlling mercury methylation and bioaccumulation in lakes and reservoirs. However, at the time Phase 1 was being developed, the District was exploring the possibility of using Soulajule Reservoir for drinking water supply more frequently, which could alter the future magnitude, frequency, and duration of volume and water level fluctuations. Thus, the District and the Stillwater Team recommended that the Study Plan consider the potential effects of increased water level fluctuation on methylmercury production and bioaccumulation. Since water level fluctuation would be a potential change in operating conditions for Soulajule Reservoir rather than a methylmercury-specific control action, it was not ranked alongside the other potential management methods (Stillwater Sciences and Brown and Caldwell 2015).

Ratings for the two top-ranked control actions (biomanipulation – sport fish stocking, HOS) were similar (i.e., 81 versus 79, respectively), and the ratings for the next three control actions (upland erosion control in the Eastern Arm, VEM, biomanipulation – prey fish stocking) were similar (74 versus 73 and 71, respectively). Given the closeness of ratings within these two groups, and the number of generally positive ratings that the control actions within these two groups received, the Project team identified six pilot studies to fill data gaps related to the eventual implementation of the top five ranked management methods. Section 1.2 summarizes the six pilot studies.

1.2 Phase 2 – Finalize and Implement Study Plan

The District undertook Phase 2 of the Project in 2016, which included finalization of the Study Plan via refinement of pilot study elements including study objectives, hypotheses (where appropriate), sampling design, data analysis and reporting approach, implementation schedule, and estimated cost. The Stillwater Team implemented the six pilot studies in 2016, including a full year of investigation of physical, chemical, and biological conditions in Soulajule Reservoir, as well as characterization of upland mercury source loading. Five technical memorandums (Table 2) present the results. Synthesis of the pilot study results is the primary subject of this technical memorandum; Section 3 presents results by topic area.

Table 2. Priority pilot studies for Soulajule Reservoir mercury control actions.

<table>
<tr><th>Pilot study</th><th>Objective</th><th>Technical memorandum</th></tr>
<tr><td>Pilot Study 1—Additional Characterization of Methylmercury in Water and Biota</td><td>Build on existing data describing methylmercury concentrations in water, zooplankton, and small fish to better understand the role of conditions in the Eastern Arm and patterns of mercury bioaccumulation throughout the reservoir.</td><td>Characterization of Seasonal Mercury Cycling and Bioaccumulation in Soulajule Reservoir (Stillwater Sciences 2017a)</td></tr>
<tr><td>Pilot Study 2—Water Level Fluctuation</td><td>Explore the potential for increased mercury methylation and bioaccumulation and biomanipulation management given the potential for more frequent water level fluctuation in future years.</td><td>Assessment of Littoral Zone Extent and Productivity as Related to the Potential for Increased Water Level Fluctuation in Soulajule Reservoir (Stillwater Sciences 2017b)</td></tr>
<tr><td>Pilot Study 3—Soulajule Reservoir Fish Community Composition</td><td>Determine the relative abundance, distribution, and age-size class distribution of fishes as a means of informing potential mercury bioaccumulation management approaches involving biomanipulation.</td><td rowspan="2">Assessment of Soulajule Reservoir Fish Community Composition and Food Web Structure (Stillwater Sciences 2017c)</td></tr>
<tr><td>Pilot Study 4—Soulajule Reservoir Food Web Structure</td><td>Characterize general aspects of the food web as a means of informing potential mercury bioaccumulation management approaches involving biomanipulation and (potentially) water level fluctuation.</td></tr>
<tr><td rowspan="3">Pilot Study 5— Evaluation of Reservoir Seasonal Oxygen Demand and Sediment Response to Hypolimnetic Oxygenation</td><td rowspan="3">Fill data gaps related to the design and implementation of a hypolimnetic oxygenation system (HOS).</td><td>Updated Dissolved Oxygen Demand Calculations for Soulajule Reservoir for Conceptual Sizing of a Hypolimnetic Oxygenation System (Brown and Caldwell 2016a)</td></tr>
<tr><td>Sediment Oxygen Demand Report (Beutel 2016a)[1]</td></tr>
<tr><td>Soulajule Reservoir Methylmercury Control Study-Sediment Flux Study (Beutel 2016b)[2]</td></tr>
<tr><td>Pilot Study 6—Upland Mercury Source Loading Characterization</td><td>Fill data gaps associated with potential upland mercury sources to the Eastern Arm.</td><td>Upland Soil Mercury Contribution to Soulajule Reservoir (Brown and Caldwell 2016b).</td></tr>
</table>

1 Report included as an appendix in Brown and Caldwell 2016a.

2 Report included as an appendix in the technical memorandum Stillwater Sciences 2017a.

1.3 Phase 3 - Develop Reservoir Management Plan

Phase 3 of the Project is development of the Soulajule Reservoir Management Plan, which is currently underway and will incorporate the findings of the six pilot studies conducted during Phase 2 (Table 2), as well as previous relevant work, in a comprehensive plan to implement a set of effective and fiscally responsible management methods for controlling methylmercury in the reservoir. This technical memorandum also includes outcomes from a coordination meeting at the beginning of Phase 3, where the meeting was designed to refine the prioritization of future reservoir management measures based on an overview of Phase 2 pilot study results and further discussion among the District, the Regional Board, and primary Stillwater Team technical members.

2 SUMMARY OF PRIOR STUDY RESULTS

Historical mercury mining activities occurred in the Walker Creek watershed during the 1960s and early 1970s. The Gambonini Mine, the largest mercury mine in the watershed, was active from 1964 to 1970. By 1972, all mining had ceased in the watershed, with remediation and clean-up of the Gambonini Mine site beginning in 1999 (SFBRWQCB 2008a). Although there were anecdotal references to submerged mine works at Soulajule Reservoir, a report by the District located the historic mines along the Eastern Arm of the reservoir (MMWD 2010), well above the water line.

Soulajule Reservoir was initially constructed as an earthen dam in 1969 on privately owned lands to serve a recreational second-home development, Soulajule Ranch. In 1979, after the District acquired lands to encompass its reservoir area, a larger impoundment was created by the construction of a larger dam that was located just downstream of the original dam (Figure 2).

Currently, Soulajule Reservoir has a (maximum) surface area of 320 acre (ac), a storage capacity of 10,200 ac-ft, and a maximum depth of approximately 90 feet (ft). Regulated flow releases occur in the summer and winter to maintain downstream flows to support native anadromous fish (coho salmon and steelhead). Releases to Arroyo Sausal are through a multi-valve discharge pipe located between the original dam and the current dam. Releases occur from valves 3 and 4, which are located approximately 35 vertical feet from the water surface and 45 vertical feet from the bottom sediments. Releases from valves 3 and 4 support designated cold fresh water habitat in Arroyo Sausal (SFBRWQCB 2010). Valve 5 is located too close to the bottom sediments and is not usable. Valves 1 and 2 are located in the reservoir epilimnion and would not consistently support cold water releases. Soulajule Reservoir experiences typical seasonal volume fluctuations between approximately 8,000 and 10,200 ac-ft (roughly 22%), and water level fluctuations of approximately 7.5 ft (elevation between 335 ft and 315 ft) (Stillwater Sciences 2017b).

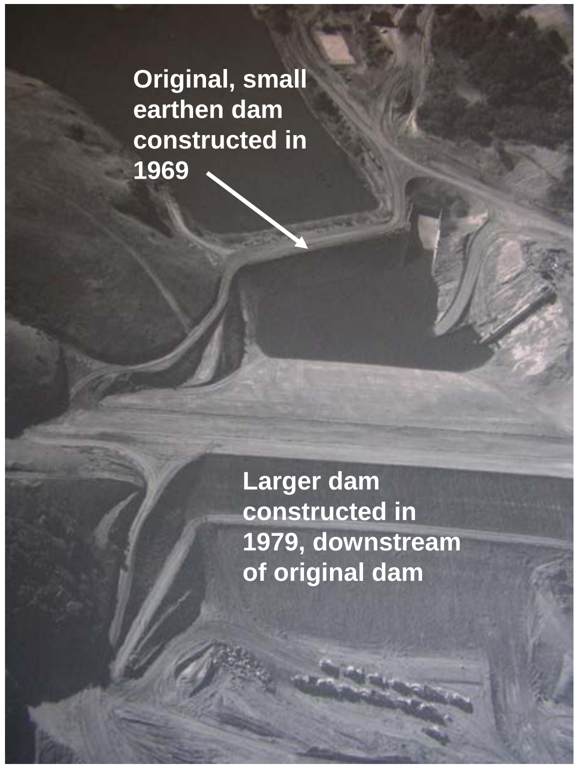


Figure 2. Original dam and construction of larger dam at Soulajule Reservoir. Source: MMWD.

Soulajule Reservoir shorelines tend to be steep and not well vegetated (Figure 3). The eastern and western arms are shallow and exhibit relatively broad shorelines; however, these shorelines tend not to be vegetated other than seasonal grasses which cattle graze when reservoir levels are low (Figure 4).

Figure 3. Soulajule Reservoir high water mark along the main portion of the reservoir east of the dam during November 2009.

Figure 4. Western Arm of Soulajule Reservoir exhibiting low water levels, November 2009.

Soulajule Reservoir is monomictic, typically exhibiting complete water column mixing throughout the winter months. Although thermal stratification tends to develop during late spring/early summer (May to June), stratification onset has occurred as early as the end of February. The shallow reservoir side arms become fully mixed earlier in the season than the deeper portions of the reservoir, which remain fully stratified July through October. Full turnover usually occurs by mid-November (Brown and Caldwell and Stillwater Sciences 2010).

Soulajule Reservoir is also eutrophic, supporting large seasonal algal blooms that typically occur between March and May, but have been observed in the fall during some years (Brown and Caldwell and Stillwater Sciences 2013). The seasonal blooms provide readily degradable organic carbon from decomposing algal cells, which depress dissolved oxygen levels in the hypolimnion and can result in low oxygen to anoxic conditions near the dam during late summer/early fall. Conversely, daytime conditions in reservoir surface waters can become supersaturated with dissolved oxygen during bloom periods, particularly in the shallow Eastern Arm (Brown and Caldwell and Stillwater Sciences 2013). Seasonally warm water temperatures, abundant phosphorus, and limited bioavailable nitrogen in Soulajule Reservoir create conditions where nitrogen fixing blue-green algae (cyanobacteria) thrive. Despite the abundance of blue-green algae (cyanobacteria), levels of cyanotoxins measured in the reservoir have not historically been of concern with respect to drinking water standards (Brown and Caldwell and Stillwater Sciences 2013).

The seasonally low dissolved oxygen and algal organic carbon in Soulajule Reservoir provide ideal conditions for methylating bacteria, including sulfate-reducing and iron-reducing bacteria associated with mercury methylation. Results from a 2012 study of mercury occurrence and bioaccumulation in Soulajule Reservoir (Brown and Caldwell and Stillwater Sciences 2013) indicated that relatively elevated water column total methylmercury (2–3 nanogram per liter [ng/L]) concentrations occurred in the hypolimnion, at the reservoir outlet, and in Arroyo Sausal during the late summer, when the reservoir was stratified and bottom waters were anoxic. Sampling showed the opposite pattern in the reservoir's shallow Eastern Arm during spring and summer months, when surface water total methylmercury concentrations were relatively higher (0.4–1.2 ng/L) and were associated with supersaturated dissolved oxygen and high algal cell counts (Brown and Caldwell and Stillwater Sciences 2013).

Concurrent sediment sampling indicated relatively higher concentrations of total mercury (390–5,940 nanogram per gram [ng/g] dry weight) in surface sediments proximal to the historical Cycle and Franciscan mine sites on the Eastern Arm of the reservoir, with concentrations at or near the reported watershed background concentration of 200 ng/g (SFBRWQCB 2008) moving towards the dam and in the reservoir's Western Arm (Figure 5). The localized nature of the elevated total mercury concentrations in surface sediments, particularly directly adjacent to Cycle Mine, indicates that reservoir sediments are generally stable and there has been little to no migration of the higher concentrations in the 50+ years since the mines were active and/or the almost 40 years since the reservoir was flooded by the larger dam. Sediment sampling results indicated generally low methylation efficiency, as evidenced by ratios of methylmercury to total mercury of much less than 0.01, both in the shallow Eastern Arm near the mine sites and in deeper waters near the dam. The reason for the low methylation efficiency of Soulajule Reservoir surface sediments may be because of a predominance of insoluble mercury sulfides, low organic carbon availability, low sulfate availability, and high pH, or a combination of these factors (Brown and Caldwell and Stillwater Sciences 2013).

While all 2012 fish tissue methylmercury results exceeded the Walker Creek Mercury TMDL numeric targets of 50 ng/g (0.05 milligram per kilogram [mg/kg]) (wet weight) for prey fish (5–15 centimeter [cm] fork length [FL], TL3[1]) and 100 ng/g (0.1 mg/kg) (wet weight) for piscivorous fish (15–35 cm FL, TL4[1]) (SFBRWQCB 2008), Soulajule Reservoir generally exhibits lower overall methylmercury bioaccumulation factors (e.g., $10^{6.0}$–$10^{6.3}$ liter per kilogram [L/kg] for piscivorous fish) and subsequently lower fish tissue total mercury concentrations (e.g., 546–1,080 ng/g wet weight for piscivorous fish) compared with other mercury-mining impacted reservoirs in the San Francisco Bay Area (Brown and Caldwell and Stillwater Sciences 2013).

[1] Aquatic biota trophic levels (TLs) are commonly defined as the following:

- TL1 – primary producers (e.g., phytoplankton)
- TL2 – primary consumers (e.g., zooplankton)
- TL3 – secondary consumers (e.g., small/juvenile fish)
- TL4 – piscivorous fish (e.g., large/adult fish)

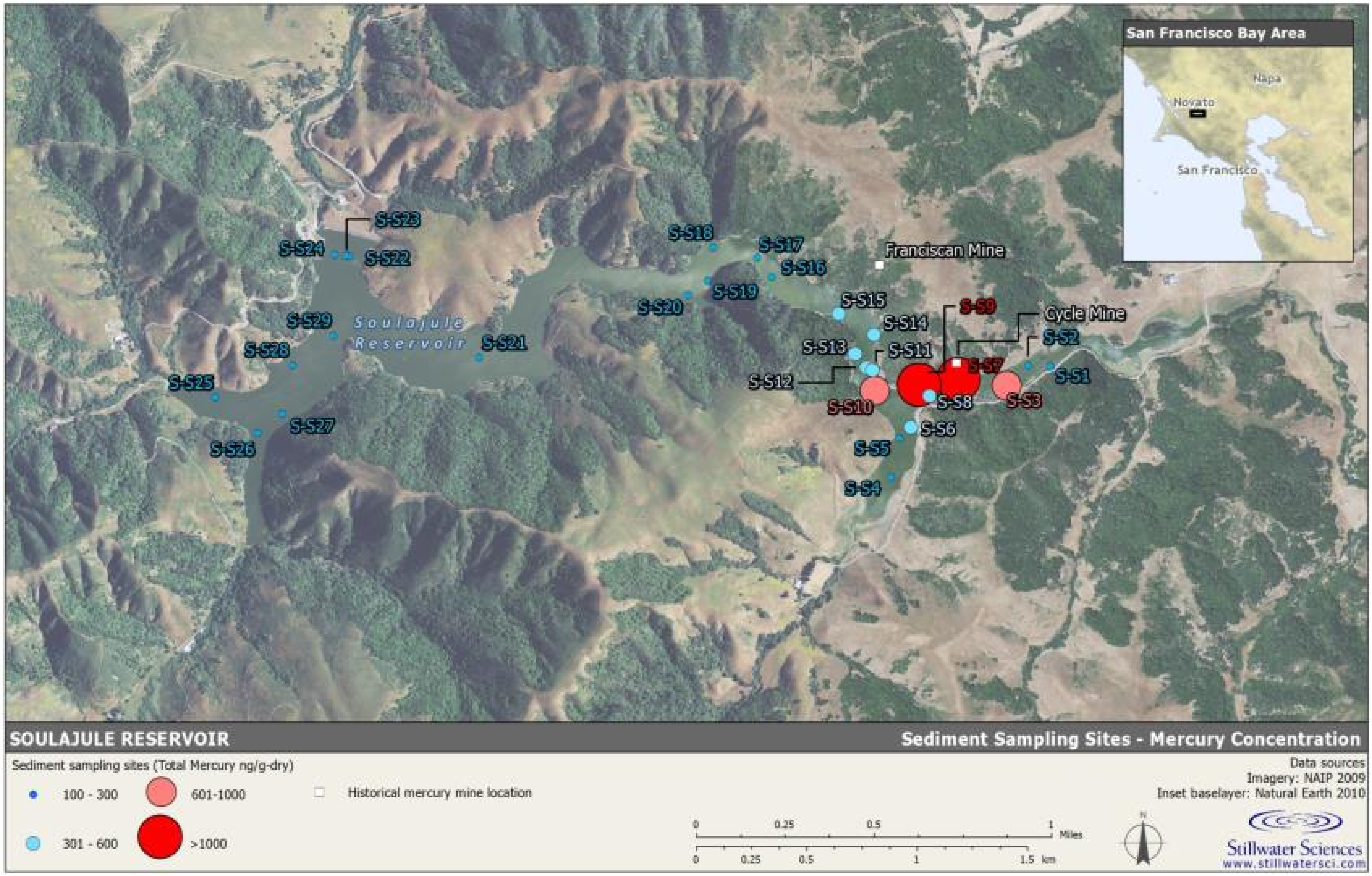


Figure 5. Total mercury in sediments in Soulajule Reservoir, August 29–30, 2012.

3 SYNTHESIS OF PHASE 2 PILOT STUDY RESULTS

The below synthesis of Phase 2 pilot study results is presented by topic area, moving from the predominant reservoir mercury source, which is currently understood to be legacy mining activities, through a discussion of the physical, chemical, and biological factors that contribute to methylmercury production and bioaccumulation in Soulajule Reservoir, and ending with a conceptual model of existing conditions. The topic areas are presented in the following order:

- Potential upland sources of mercury to Soulajule Reservoir
- Reservoir conditions and methylmercury production
- Reservoir primary productivity
- Reservoir food web and mercury bioaccumulation
- Reservoir water level fluctuation
- Existing conditions conceptual model

3.1 Potential Upland Sources of Mercury to Soulajule Reservoir

The *Upland Mercury Source Loading Characterization Study* (Pilot Study 6) evaluated the potential for mercury loading from upland soils near historical mine sites in the Eastern Arm of Soulajule Reservoir and determined whether the District needs to implement uplands erosion control around the Cycle and Franciscan mines (Brown and Caldwell 2016b). Brown and Caldwell staff collected supplemental data to assess whether sediment transport from streams is a major source of mercury to the reservoir. Pilot Study 6 tested the following hypotheses:

> PS6-H1. Upland soils are visibly erodible and are likely to represent a significant source of mercury loading to Soulajule Reservoir.
>
> PS6-H2. Upland soils in the vicinity of the Franciscan and Cycle Mines have a higher total mercury concentration than background upland soils unaffected by mining or other anthropogenic activity.

To address these hypotheses, Brown and Caldwell staff conducted field reconnaissance and collected surface soil samples at the two historical mercury mine sites (the Franciscan and Cycle mines) and two disturbed areas identified by the Regional Board as potential mine sites (Unnamed Mine 1 [UNM-1] and Unnamed Mine 2 [UNM-2]) (Figure 6). Brown and Caldwell staff collected soil samples from disturbed portions of the mine (and potential mine) sites, undisturbed locations adjacent to the mine (and potential mine) sites, and from five creek inlets (Sites EAD-1, EAD-2, EAI-1[2], EAI-2, EAI-3) that drain into Soulajule Reservoir's Eastern Arm (Figure 6). (See Brown and Caldwell (2016b) for additional details regarding soil and sediment sampling sites, frequency, and methodology).

[2] Sediment sample collected from an area where the cows had trampled the stream bank. Sample is probably more representative of bank material than sediment. Site EAI-1 is located near the east end of the reservoir and flows into the reservoir from the south. Location not included on Figure 6.

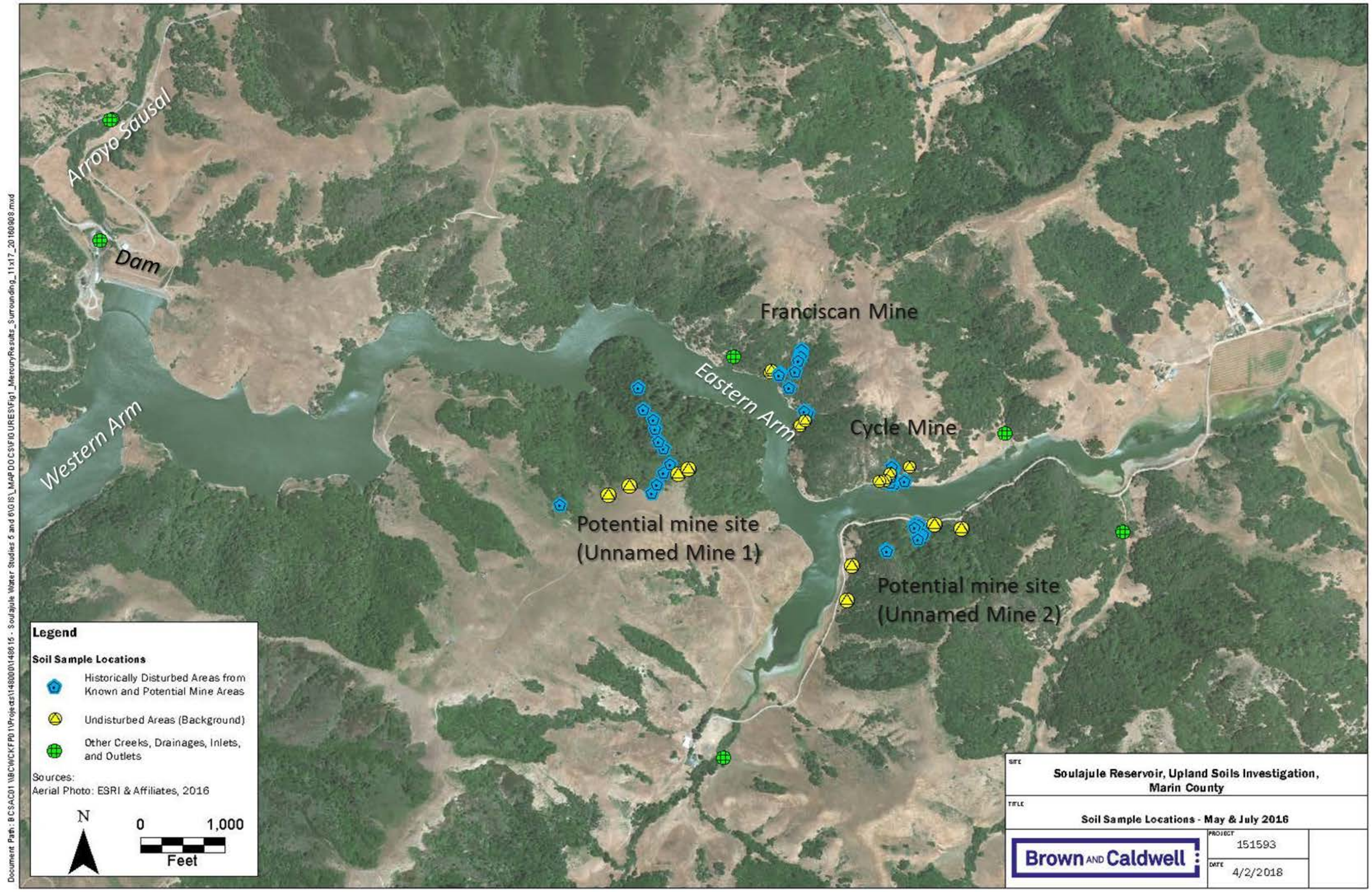


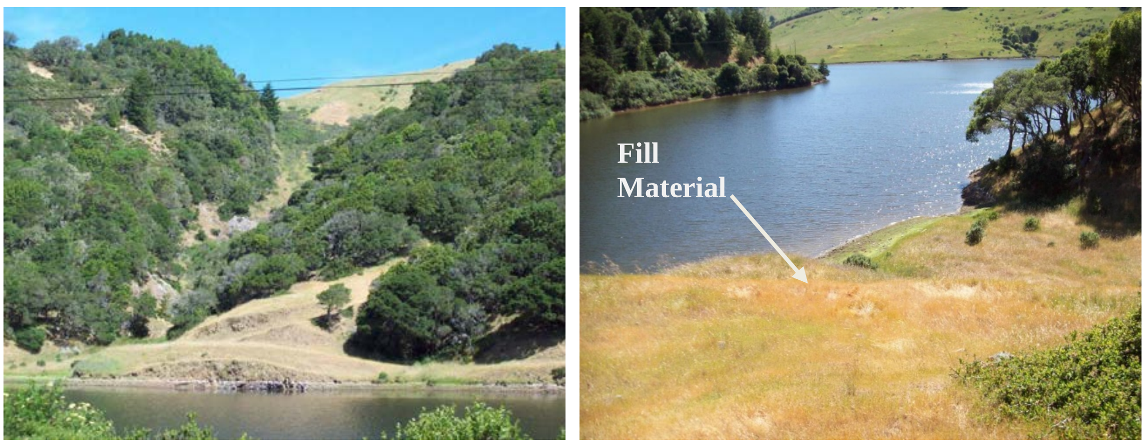
Figure 6. Soulajule Reservoir total mercury in upland soil and creek sediment sampling locations, 2016.

With respect to Hypothesis PS6-H1, information collected during 2016 indicated little to no visible erosion of upland soils at most of the surveyed sites. Visual assessment (i.e., observed slope, vegetation, soil type and other factors) found very little evidence of non-vegetated actively eroding soils at the two former mercury mine sites (the Franciscan and Cycle mines), with the exception of soils with possible erosion potential in the west drainage below Franciscan Mine (Table 3) and in the portion of thick overburden fill at Cycle Mine that is in contact with the reservoir water. There was very little evidence of non-vegetated actively eroding soils at the two disturbed areas identified by the Regional Board as potential mine sites (Unnamed Mine 1 [Site UNM-1] and Unnamed Mine 2 [Site UNM-2]) (Figure 6). Past and current field work show clear evidence of historical mining at the Franciscan Mine site, including abandoned equipment, access roadways, and disturbed but apparently closed work areas. During the 2016 survey at the Cycle Mine, Brown and Caldwell found disturbed road cut material, mine waste, or overburden fill, however, this site had no abandoned mining equipment. Overall, the field assessment indicates that the thick graded fill in contact with the reservoir water at the Cycle Mine site is now stable. Brown and Caldwell found no evidence (i.e., equipment) that mining had ever occurred at Unnamed Mine 1 or Unnamed Mine 2, although at Unnamed Mine 1, there was evidence of slight soil disturbance. All sites surveyed in 2016 exhibited well established vegetation, which stabilizes these soils and reduces erosion and transport into the reservoir. Figure 7 includes representative photographs of the 2016 sampling sites.

Regarding Hypothesis PS6-H2, Brown and Caldwell staff sampled and analyzed upland surface soil samples for total mercury from disturbed areas and undisturbed areas near the Franciscan Mine, Cycle Mine, Unnamed Mine 1, and Unnamed Mine 2. Background soils (110–23,400 ng/g) from undisturbed areas and soils proximal to Franciscan Mine (12,900–158,000 ng/g), Cycle Mine (329–23,700 ng/g), and Unnamed Mine 2 (<79–9,100 ng/g) exhibited relatively high total mercury, with concentrations greater than the background TMDL allocation for the Walker Creek watershed (200 ng/g) (SFBRWQCB 2008) (Figure 8). In contrast, total mercury in soils at Unnamed Mine 1 (<84–398 ng/g) and from five creeks entering the reservoir's Eastern Arm (Sites EAD-1, EAD-2, EAI-13, EAI-2, EAI-3) (<86–1,030 ng/g) (Figure 8), were generally less than the background TMDL allocation (SFBRWQCB 2008).

[3] BC collected sediment sample collected from an area where the cows had trampled the stream bank. Sample is probably more representative of bank material than sediment.

Franciscan Mine area (left) and abandoned mining equipment (right).



Cycle Mine area (left) and fill material (right).

Grassy area in the south of Unnamed Mine 1 (left) and drainage below Unnamed Mine 2 (right)

Figure 7. Representative photographs of Franciscan Mine, Cycle Mine, Unnamed Mine 1, and Unnamed Mine 2, 2016.

More specifically, total mercury concentrations in disturbed upland soils around the Franciscan and Cycle Mines were generally similar to upland soils unaffected by mining or other anthropogenic activity, with the exception of areas near the Franciscan Mine. Brown and Caldwell staff found the highest total mercury concentrations in the northern portion of the Franciscan mine adjacent to the mining equipment (12,900–158,000 ng/g), one to two orders of magnitude greater than background upland soil samples collected from undisturbed areas (153–3,500 ng/g) and the background TMDL allocation for the Walker Creek watershed (200 ng/g) (Table 3). However, in the west drainage below the mine and within 50 ft of the reservoir water level soil mercury concentrations were relatively low (1,280–1,600 ng/g) (Table 3), and Brown and Caldwell staff observed no visible signs of erosion, indicating that mercury loading is not likely to be occurring from the Franciscan Mine. Additionally, low mercury concentrations measured at the Eastern Arm creek inlet sites (Figure 8) suggest that sediment transport from local sub-watersheds are not currently a major Eastern Arm mercury source.

At the Cycle Mine, both disturbed overburden fill and background samples exhibited a range of concentrations (329 – 5,420 ng/g) (Table 3), including some relatively higher total mercury in fill soils (2,370 ng/g and 5,420 ng/g) that extend to the reservoir shoreline, providing a potential mercury loading path to the reservoir. Although Brown and Caldwell staff observed little to no visual evidence of actively eroding upland soils at the Cycle Mine thick fill location due to well-vegetated slopes, wave-action induced erosion of the thick graded fill located along the reservoir shoreline is possible. Since highly variable total mercury is evident in the thick fill (Figure 8, Table 3), the Stillwater Team recommends further investigation of the Cycle Mine thick fill at the reservoir water line during low water conditions to provide visual evidence (or lack thereof) of active erosion as well as additional total mercury measurements (see Section 4.2).

In summary, except for the Cycle Mine thick fill at the reservoir water line, Brown and Caldwell staff found little to no evidence that upland soils represent a significant mercury loading source to Soulajule Reservoir. Further, the localized nature of the elevated total mercury concentrations in the reservoir sediments, particularly directly adjacent to Cycle Mine (Figure 5), indicates that little to no migration of higher concentrations of total mercury has occurred in the 50+ years since the mines were active and/or the almost 40 years since the reservoir was flooded by the larger dam.

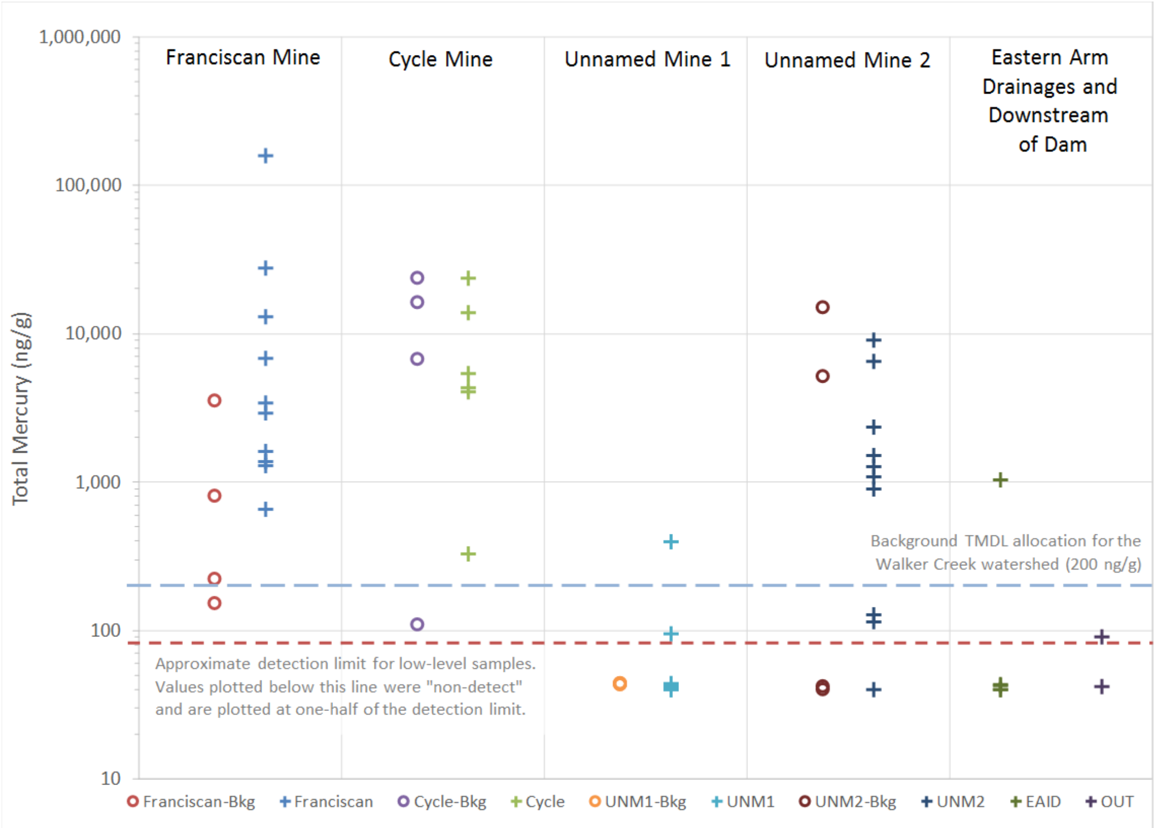


Figure 8. Total mercury concentrations in upland soil and creek inlet sediment samples for the Eastern Arm of Soulajule Reservoir (and downstream of the dam).

Table 3. Summary of total mercury in Soulajule Reservoir upland soils and reservoir sediments for mining and non-mining impacted areas.

Location	Total mercury (ng/g)	Ratio of total mercury (ng/g) to background TMDL allocation for Walker Creek Watershed (200 ng/g)	Upland soils erosion or reservoir sediment displacement potential	Reference
Non-mining impacted areas				
Reservoir sediments in Soulajule Reservoir Western Arm (S-S25 through S-S29) and Nicasio Reservoir (N-S1 through N-S3 and N-S5)	114–183 mean = 153	0.6–0.9	N/A	Brown and Caldwell and Stillwater Sciences 2013
Historical mercury mines				
Franciscan Mercury Mine upland soils in the vicinity of mining equipment (FM-2 through FM-4)	12,900–158,000 mean = 66,233	60–800	Low	Brown and Caldwell 2016b
Franciscan Mercury Mine upland soils in west drainage below the mine (FM-13 and FM-14)	1,280–1,600 mean = 1,440	5–8	Possible at toe of west drainage	
Reservoir sediments in Soulajule Reservoir Eastern Arm downstream of Franciscan Mine (S-S-3, S-S-6 through S-S-15)	390–5,940 mean = 1,176	2–30	Low – no apparent displacement over 40+ years	
Cycle Mercury Mine upland soils at thick graded fill (CM-15, CM-16, and CM-18)	23,700; 5,420; and 329, respectively mean = 9,816	1.6–100	Possible along reservoir shoreline contacting thick graded fill due to wave action	
Reservoir sediments in Soulajule Reservoir Eastern Arm (S-S-3, S-S-6 through S-S-15)	390–5,940; mean = 1,180	2–30	Low – no apparent displacement over 40+ years	Brown and Caldwell and Stillwater Sciences 2013

Location	Total mercury (ng/g)	Ratio of total mercury (ng/g) to background TMDL allocation for Walker Creek Watershed (200 ng/g)	Upland soils erosion or reservoir sediment displacement potential	Reference
Potential historical mercury mine sites				
Unnamed Mine 1 upland soils (UNM1-01 through UNM1-10)	< 88–396; with eight of ten samples < MDL mean[1] = 113	0.4–2	Low	Brown and Caldwell 2016b
Unnamed Mine 2 upland soils (UNM2-01 through UNM2-10)	114–6,530; mean = 3,090	0.4–45	Low	

TMDL= Total Maximum Daily Load, N/A = not applicable, ng/g=nanogram per gram, MDL = method detection limit.

[1] Mean calculated using MDL values.

3.2 Reservoir Conditions and Methylmercury Production

Stillwater Sciences evaluated Soulajule Reservoir conditions in the *Additional Characterization of Methylmercury in Water and Biota Study* (Pilot Study 1), designed to build on prior study results through more focused investigation of spatial and seasonal patterns of mercury production and bioaccumulation in three general areas of the reservoir. Seasonal sampling sites for water and biota established in the following locations (biota results are discussed in Section 3.4) (Figure 9) provided new information:

- Eastern Arm (EA-1) given its proximity to the historical Franciscan and Cycle mines;
- Western Arm (WA-1) where existing information indicates there were no historical mining activities; and,
- Near the dam (D-1) in the deepest portion of the reservoir, between the original earthen dam and the current larger dam.

Additionally, one site was established at the dam outlet to Arroyo Sausal (A-1), to characterize water leaving the reservoir (Figure 9).

Seasonal *in situ* (DO, pH, turbidity, conductivity) vertical profiles were collected at sites EA-1, WA-1, and D-1, and seasonal 48-hour continuous *in situ* data were collected in the bottom waters (approximately 0.15 meter [m] [0.5 ft] from sediments) of Site EA-1 (Stillwater Sciences 2017a). Methylmercury in water samples were collected seasonally at sites EA-1, WA-1, and D-1. At Site A-1, methylmercury in water samples were collected monthly from January through December 2016, plus additional samples approximately every two weeks surrounding the onset of stratification in spring and reservoir destratification in the late fall. Additional details regarding Pilot Study 1 sampling sites, frequency, and methodology are provided in Stillwater Sciences (2017a).

The scientific community has found that anaerobic sediments of mercury-contaminated lakes and/or reservoirs are particularly active zones of methylmercury production and an important internal source of methylmercury, especially in lakes experiencing low to no oxygen in the hypolimnion during the summer (Regnell et al. 1996). Methylmercury can also be produced directly in the hypolimnion of the water column if other electron acceptors such as oxygen and nitrate are in short supply (Eckley and Hintelman 2005, Watras et al. 1995). To help structure the investigation of potential spatial and seasonal patterns of methylmercury production and bioaccumulation in Soulajule Reservoir, the District and the Stillwater Team developed the hypothesis below regarding conditions in the Eastern Arm, where mercury sediment concentrations are relatively high (Figure 5):

> PS1-H2. During algal blooms and periods of stratification, dissolved oxygen in the shallow Eastern Arm varies with water depth and time of day such that anaerobic conditions occur at or near the sediment-water interface.

Lastly, to determine how Soulajule Reservoir water column and/or sediment conditions might affect methylmercury release from sediments in the three general areas of the reservoir, release rates of redox sensitive compounds, including ammonia, phosphate, manganese, iron, ionic mercury and methylmercury, were assessed using sediment cores collected from the "East Arm", West Arm", and "Dam" sediment sites (Figure 9), as part of the *Evaluation of Reservoir Seasonal Oxygen Demand and Sediment Response to Hypolimnetic Oxygenation Laboratory Study* (Pilot Study 5). Field work included collection of an additional sediment core from the area just upstream of the original dam ("Mid-dam") (Figure 9). Once collected, the replicated sediment cores were subjected to alternating oxic and anoxic conditions in bench-scale laboratory chambers designed to mimic reservoir conditions (Beutel 2016b).

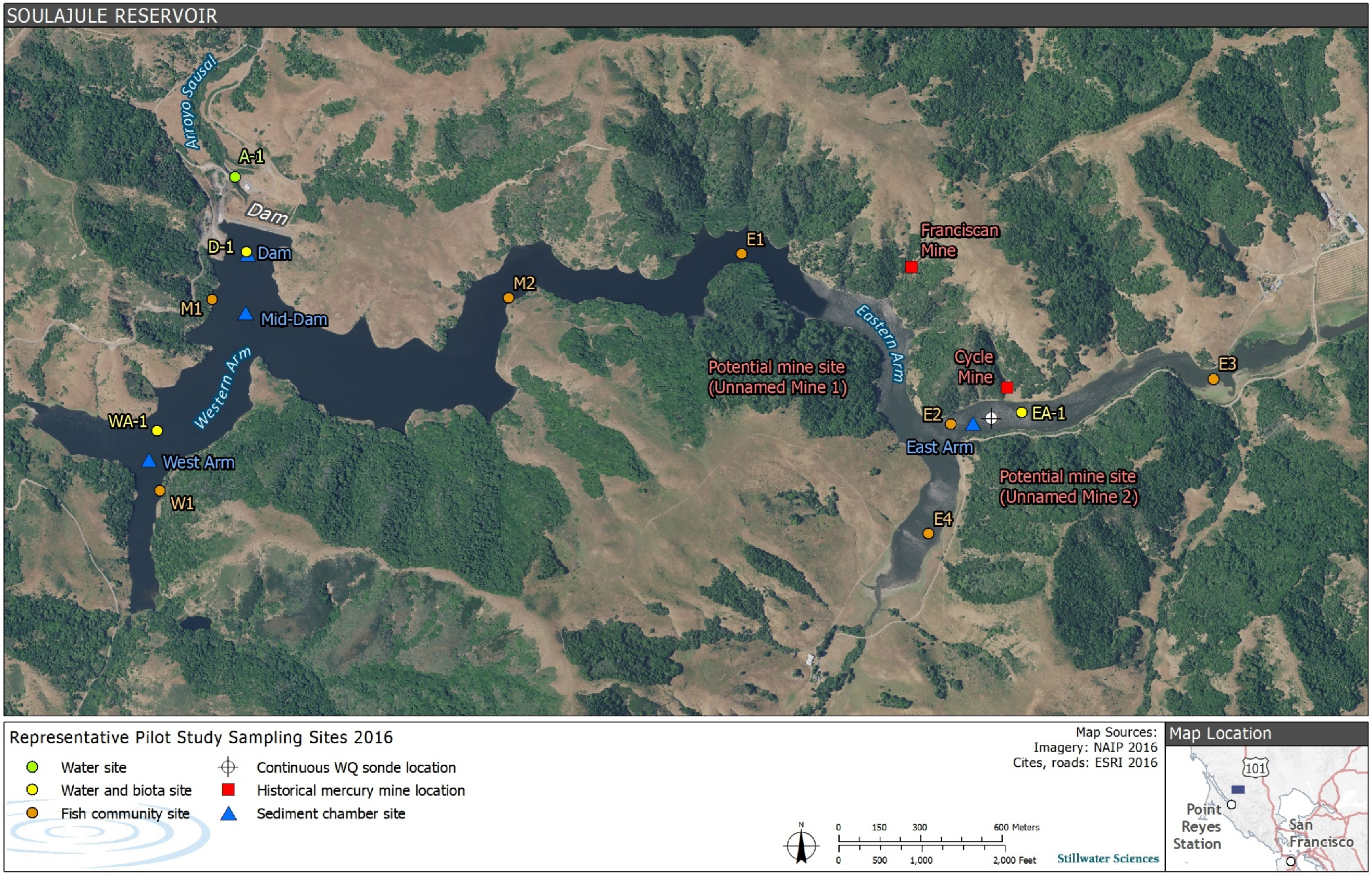


Figure 9. Soulajule Reservoir representative sampling sites for the methylmercury in water and biota and fish population pilot studies, 2016.

3.2.1 Reservoir stratification patterns

Consistent with prior studies, 2016 reservoir stratification developed during late spring/early summer (May to June), and fall turnover occurred by mid-November. Water temperature, dissolved oxygen, pH, and turbidity exhibited seasonal variation with depth at each of the reservoir sites. Except for the near-dam site (D-1), the reservoir thermocline tended to occur as a gradual transition in water temperatures from surface to bottom waters (Figure 10, Figure 11). In contrast, strong dissolved oxygen stratification was apparent during the summer at the Eastern Arm (EA-1) (Figure 10), Western Arm (WA-1) (figure not shown), and near-dam (D-1) (Figure 11) sites. Hypoxia (DO <3 milligram per liter [mg/L] and <30% saturation) was observed in bottom waters of the deeper sites (WA-1, D-1) during the spring and summer surveys, while surface waters at all sites were supersaturated (DO > 9 mg/L and > 100% saturation).

In general, elevated dissolved oxygen, pH, and turbidity measured in surface waters during the spring and summer 2016 surveys are consistent with high levels of phytoplankton productivity in Soulajule Reservoir. Overall, the Eastern Arm site (EA-1) exhibited the warmest water temperatures and lowest water clarity, and the near-dam site (D-1) exhibited the lowest dissolved oxygen concentrations at depth. The shallow Eastern Arm (EA-1) and the deep, near-dam site (D-1) behaved somewhat differently, particularly in the spring, when stratification occurred slightly earlier in the Eastern Arm (EA-1) and there was greater variability in water column *in situ* parameters with depth (Figures 10−12).

As hypothesized in PS1-H2, during spring and summer, when algal blooms were present and the water column was thermally stratified, dissolved oxygen in the shallow Eastern Arm varied with water depth and time of day such that anaerobic conditions occurred at or near the sediment-water interface. In the spring, the highest dissolved oxygen concentrations (11 mg/L; 138% saturation) occurred in surface waters (Figure 10) and lower concentrations occurred in bottom waters (4–9 mg/L; 38−114% saturation), depending on the time of day (Figure 12). Although springtime dissolved oxygen near the sediment-water interface appears to have remained at moderate levels (≥4 mg/L), it is possible that dissolved oxygen concentrations within deeper sediment pore waters decreased to anaerobic or anoxic levels, allowing slow diffusion of redox-sensitive compounds into bottom waters. Water column mixing in the spring, as evidenced by the diurnal patterns in bottom water temperature and dissolved oxygen (Figure 12), could have allowed dispersal of redox-sensitive compounds released from the bottom sediments to enter reservoir waters. The springtime broad diurnal fluctuations in Eastern Arm bottom water pH and turbidity (Figure 12), coupled with indications of elevated algal concentrations (i.e., supersaturated dissolved oxygen concentrations,) in the surface waters (Figure 10), also suggest that algal biomass can influence water chemistry and redox conditions in the water column and at the sediment-water interface in the shallow Eastern Arm. Summertime field measurements showed hypoxia in the Eastern Arm water column, with dissolved oxygen rapidly declining from 5.4 mg/L to 1.2 mg/L between 9 ft and 12 ft depth, such that roughly 50 percent of the water column was anaerobic (hypoxic) (Figure 10). Summertime dissolved oxygen in Eastern Arm bottom waters remained very low (<1 mg/L; 10% saturation) throughout the continuous monitoring period (Figure 12), such that anaerobic conditions were maintained at the sediment-water interface, potentially facilitating the release of redox-sensitive compounds, including methylmercury, into the overlying water column.

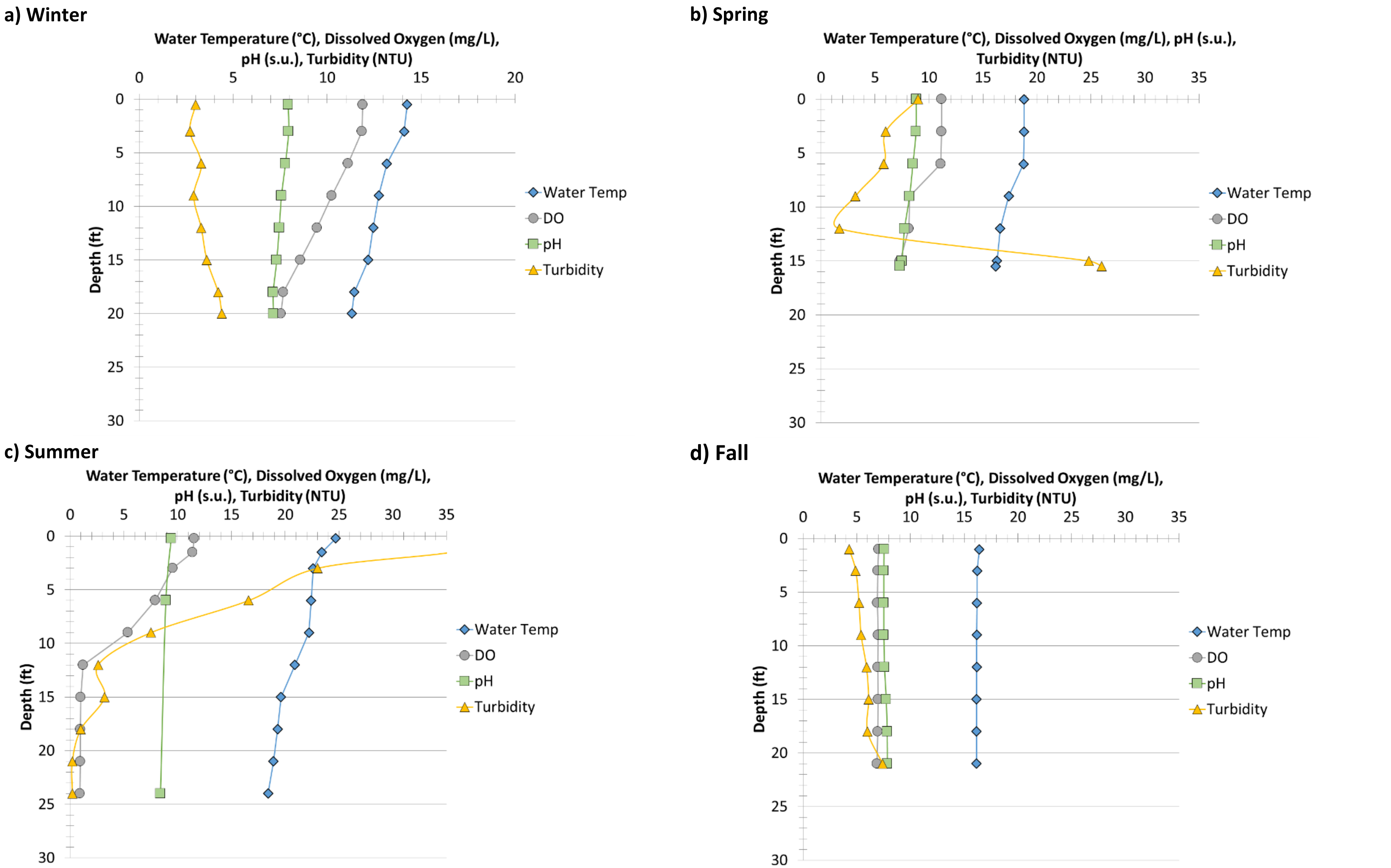


Figure 10. Eastern Arm (EA-1) water temperature, dissolved oxygen (DO), pH and turbidity profiles, 2016.

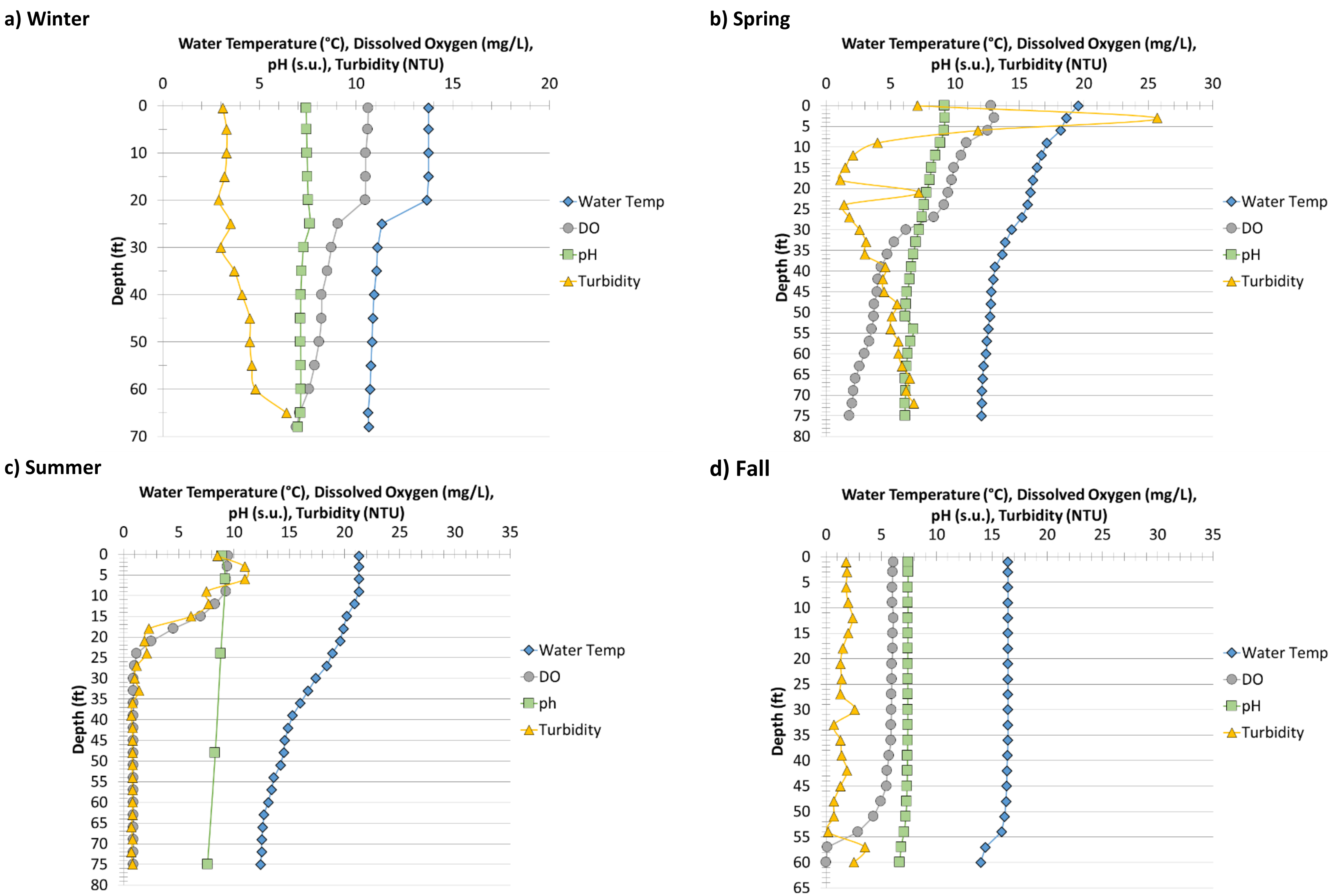


Figure 11. Dam (D-1) water temperature, dissolved oxygen (DO), pH and turbidity profiles, 2016

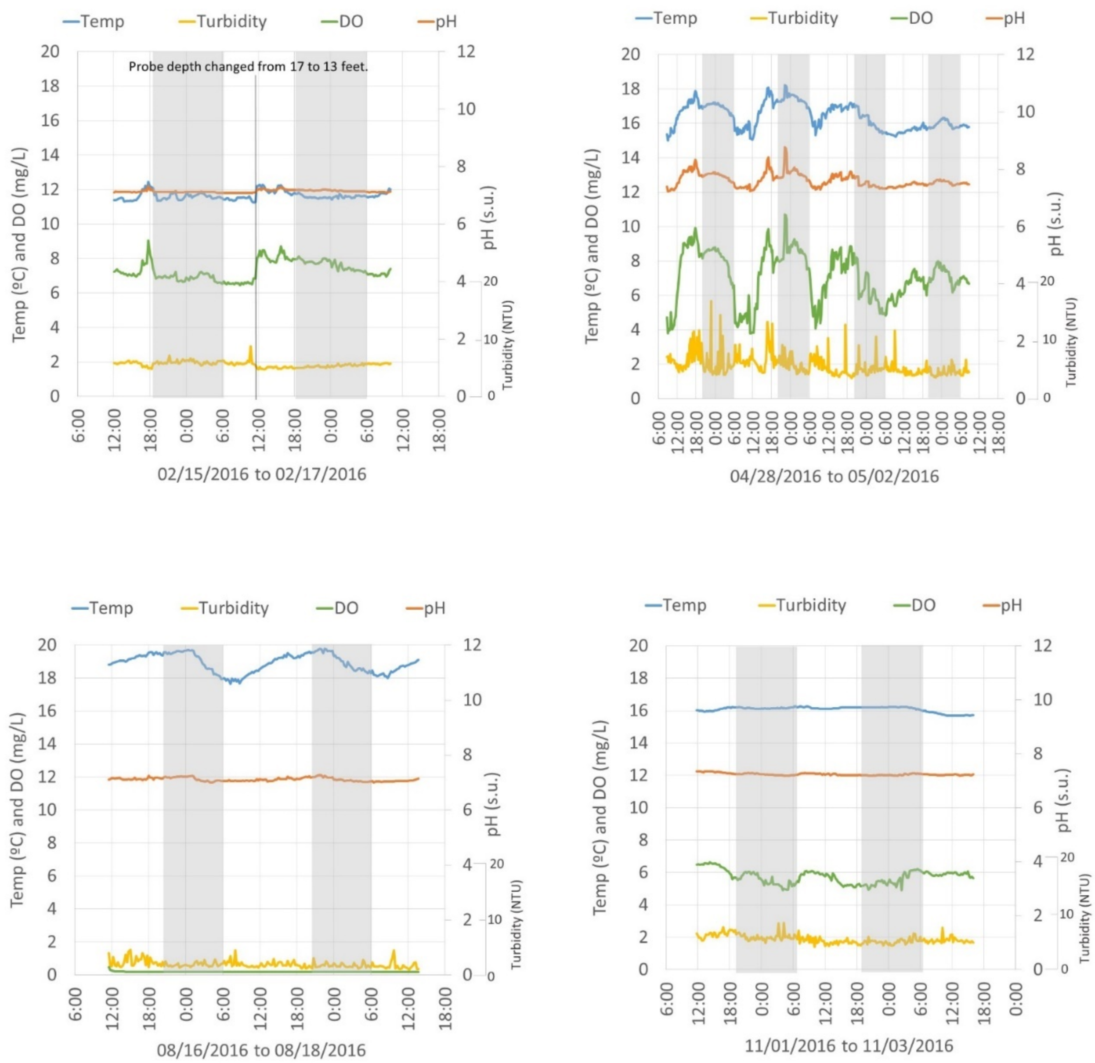


Figure 12. Seasonal trends in water temperature (temp), dissolved oxygen (DO), pH and turbidity in bottom waters of the Eastern Arm. Shaded bars indicate the period between dusk and dawn.

3.2.2 Sediment release of methylmercury and other redox-sensitive compounds

Results of the Soulajule Reservoir sediment chamber experiments confirmed that under anoxic conditions, the sediments tended to release a range of redox-sensitive compounds including ammonia, phosphate, manganese, iron, ionic mercury and methylmercury (Beutel 2016b). In general, the onset of anoxic conditions accelerated release of these compounds with a higher magnitude from sediments at the deeper sites near the dam as compared to sediments from the shallower Eastern Arm and Western Arm sites (Figure 13). Positive fluxes (i.e., sediment release) of total mercury and methylmercury did not occur exclusively under anoxic conditions; sometimes a large negative flux (i.e., sediment uptake) of methylmercury occurred during the last anoxic phase of the chamber experiment. Two possible mechanisms for sediment uptake of mercury species involve scavenging by ferrous sulfide (FeS) formation and repression of methylmercury production by sulfate-reducing bacteria at high sulfide concentrations, either through sulfide toxicity to the bacteria themselves, or a decrease in the bioavailability of ionic mercury to be methylated under high-sulfide condition (Beutel 2016b).

Results from the sediment chamber studies, along with results of sediment oxygen demand testing in Soulajule Reservoir sediment cores (Beutel 2016a), indicate that deeper sediments in the main portion of the reservoir are organic rich and have the greatest potential for release of redox-sensitive compounds under anoxic conditions, as compared with sediments located in the reservoir arms. While sediments in the shallow Eastern Arm of the reservoir exhibit relatively high mercury concentrations, and, based upon 2016 *in situ* water quality monitoring results, are likely to experience anoxic conditions during periods of reservoir stratification, these sediments do not exhibit great potential to release methylmercury. Peak methylmercury release rates measured in Soulajule Reservoir (near dam = 20−40 nanogram per meter squared per day ($ng/m^2{\cdot}d$), Eastern and Western arms = 2−8 $ng/m^2{\cdot}d$) were low compared to rates in sediments from hypereutrophic Lake Hodges Reservoir (California) and mercury-contaminated reservoirs in the Santa Clara Valley (California), both of which yielded anoxic methylmercury release rates on the order of 100−300 $ng/m^2{\cdot}d$ (Beutel 2015, Beutel 2016c).

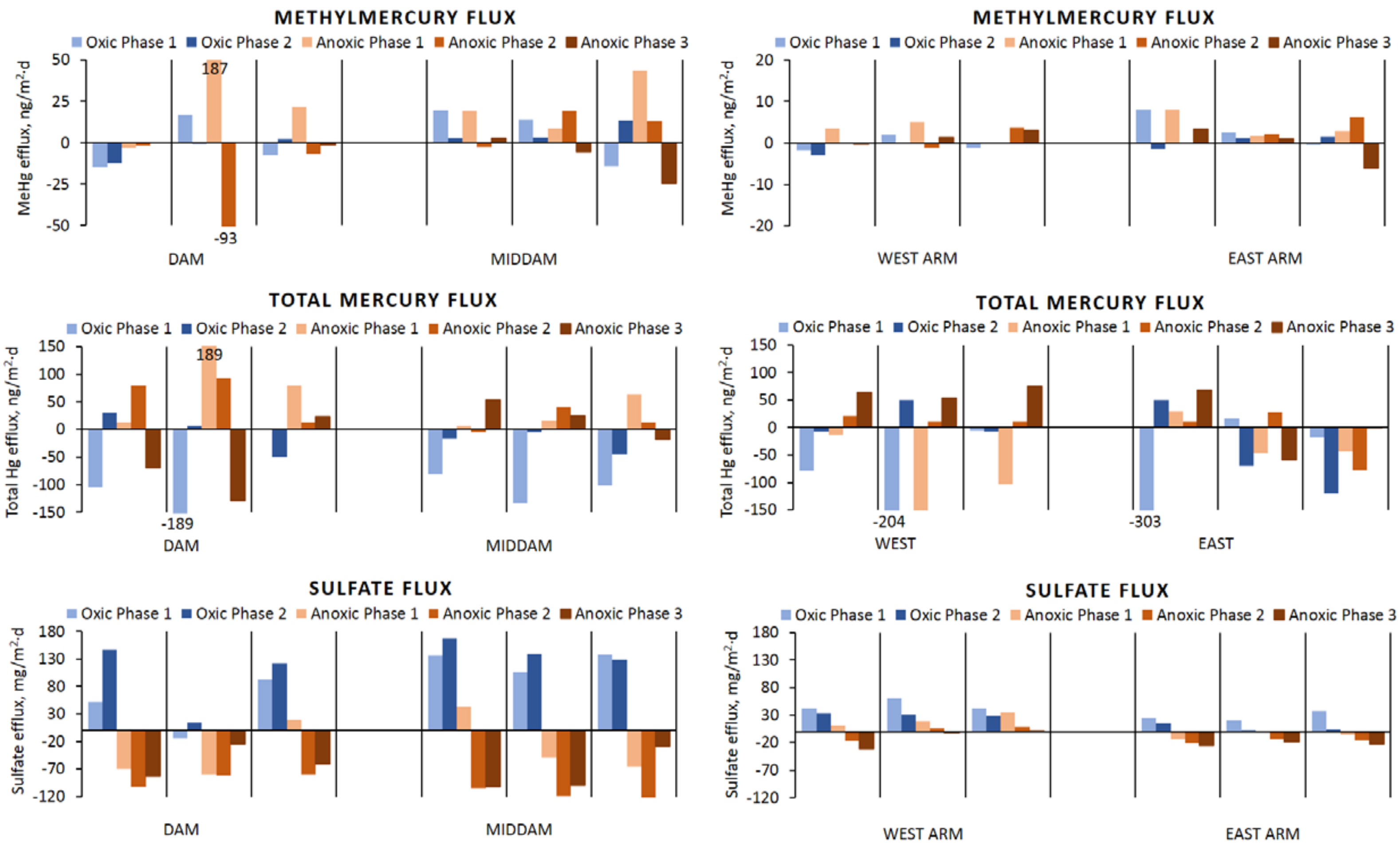


Figure 13. Methylmercury fluxes (top), total mercury fluxes (middle) and sulfate fluxes (bottom) in experimental sediment-water interface chambers collected at the deep "Dam" and "Mid-Dam" sites (left) and relatively shallow "West Arm" and "East Arm" sites (right). Each set of bars separated by vertical lines represents one of three chambers collected at each station. Blue bars are for the oxic period and brown bars are for the anoxic period. Note difference in scales on vertical axes on methylmercury figures. Also note that some large methylmercury and total mercury fluxes are labeled with values.

3.2.3 Methylmercury in reservoir bottom waters

Consistent with the relatively high methylmercury production rates observed in near-dam anoxic sediment cores, dissolved methylmercury concentrations collected from Soulajule Reservoir bottom waters in 2016 were the highest during sustained periods of low dissolved oxygen. Dissolved methylmercury concentrations steadily increased through the spring and summer, peaking in early fall at 5.2 ng/L (Figure 14). Dissolved methylmercury concentrations (0.1 ng/L–5.2 ng/L) measured in reservoir bottom waters were consistently greater than the Walker Creek Mercury TMDL allocation of 0.04 ng/L dissolved methylmercury (SFBRWQCB 2008) during the 2016 surveys (Figure 14).

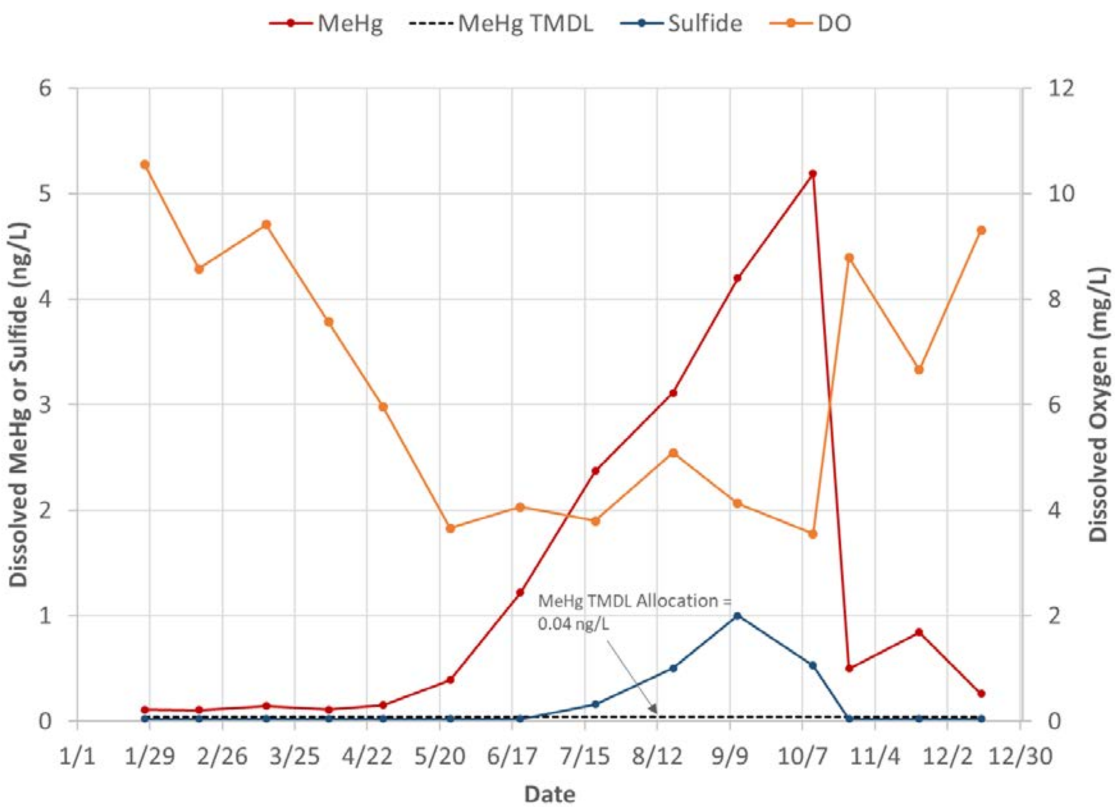


Figure 14. Monthly water concentrations of dissolved methylmercury (MeHg), dissolved sulfide, and dissolved oxygen (DO) at the dam outlet (Site AC-1).

3.3 Reservoir Primary Productivity

Phytoplankton are photosynthesizing microscopic organisms, which generally includes pelagic (open water) forms of algae and cyanobacteria (commonly referred to as blue-green algae). As primary producers, phytoplankton provide a source of organic carbon that fuels microbially-mediated mercury methylation in lakes and reservoirs. Additionally, as a TL1[1] organism that takes up dissolved methylmercury directly from the water column, phytoplankton serve as the primary entry point for methylmercury in the aquatic food web, concentrating methylmercury by two to four orders of magnitude or more compared with water column concentrations (Mason et al. 1996, Pickhardt and Fisher 2007, Gorski et al. 2008) (see also Section 3.3). To help determine the relative importance of phytoplankton in the Soulajule Reservoir food web relative to other forms of primary production (i.e., littoral zone primary productivity), the *Water Level Fluctuation Study* (Pilot Study 2) evaluated the littoral zone area and the percent cover of aquatic macrophytes and attached algae (e.g., benthic algae, periphyton) and considered whether changing water level management in the reservoir could affect the potential for mercury bioaccumulation by affecting primary productivity (Stillwater Sciences 2017b). The following hypothesis was assessed as part of the *Water Level Fluctuation Study* (Pilot Study 2):

> PS2-H1. The littoral zone in Soulajule Reservoir is relatively small and steep and supports low levels of benthic algal production and macrophytes.

Further, the *Additional Characterization of Methylmercury in Water and Biota Study* (Pilot Study 1) evaluated phytoplankton abundance and community composition seasonally and spatially in Soulajule Reservoir to help structure the investigation of potential patterns of methylmercury production and bioaccumulation (Stillwater Sciences 2017c). Like Hypothesis PS1-H2 regarding dissolved oxygen concentrations in bottom waters of the Eastern Arm (see Section 3.2), following hypothesis was developed to focus on reservoir conditions in the area where mercury sediment concentrations are the highest:

> PS1-H1. Seasonal algal blooms are concentrated in the Eastern Arm of Soulajule Reservoir and are dominated by blue-green algae.

The below sections summarize the Phase 2 pilot study results related to primary productivity in Soulajule Reservoir.

3.3.1 Low littoral zone primary productivity

The littoral zone in Soulajule Reservoir is typical of reservoirs in the Western U.S., which tend to flood steep-sided river valleys and experience seasonal water level fluctuations on the order of 5−20 feet annually due to steadily receding water levels during the dry summer and fall months (May–Oct) and rapidly increasing water levels following significant rain events in the winter and spring months (Nov–April). Consistent with Hypothesis PS2-H1, the littoral zone in Soulajule Reservoir is relatively small for wet and normal water year (WY) types (25−50 ac or 10−20% of total reservoir surface area), and increases by roughly a factor of two for the dry WY type (50−100 ac or 20−40% of total reservoir surface area) (Figure 15). While it is relatively narrow in the majority of the reservoir, extending 20−50 ft from shore along steep side slopes in wet and normal WY types and 40−100 ft in dry WY type, the littoral zone in the shallow eastern and western arms of the reservoir is significantly broader, extending up to 200 ft from shore in wet and normal WY types and up to 1,000 ft from shore in dry WY types (Figure 15).

The littoral zone in the main portion of the reservoir and the shallow arms appears to be characterized by small, submerged vascular plants with occasional clumps of filamentous benthic algae. While macrophytes exhibit a high percent cover (e.g., 80−100%) in the wetted portion of the littoral zone at most sites, the established species are almost exclusively limited to small, submerged vascular plants, which exhibit generally low productivity (<1,000 grams carbon per meter squared per year [g C/m^2/yr]; Mitsch and Gosselink 1993). While some degree of benthic algal productivity is evident in the Soulajule Reservoir 2016 survey data, the lack of any significant aquatic macrophyte community and low organic carbon in surface sediments (i.e., <50,000 parts per million [ppm] or 5% total organic carbon [TOC]) in the littoral zone is suggestive of relatively low littoral zone productivity for the reservoir and is generally consistent with Hypothesis PS2-H1.

3.3.2 Phytoplankton spatial distribution and seasonal composition

Soulajule Reservoir is eutrophic and has supported large algal blooms. Previous studies indicated that algal blooms typically occur between March and May, but blooms have also been observed in the fall during some years (Brown and Caldwell and Stillwater Sciences 2013). The analysis of six satellite images indicated that algal distribution patterns in Soulajule Reservoir are dynamic, and, contrary to Hypothesis PS1-H1, reservoir-wide blooms (or patchy concentrations of algae) can occur regardless of season. The distribution of chlorophyll-*a* is variable, with the highest concentrations being observed in the Eastern Arm (September) and near the dam (November), but also can be distributed evenly across the reservoir in the late-winter (March) and summer (June) (Figure 16). Dissipation of the blooms can occur quickly, as observed during spring, where the elevated and relatively evenly distributed chlorophyll-*a* concentrations largely dissipated within two weeks (May 2–May 18) (Figure 16).

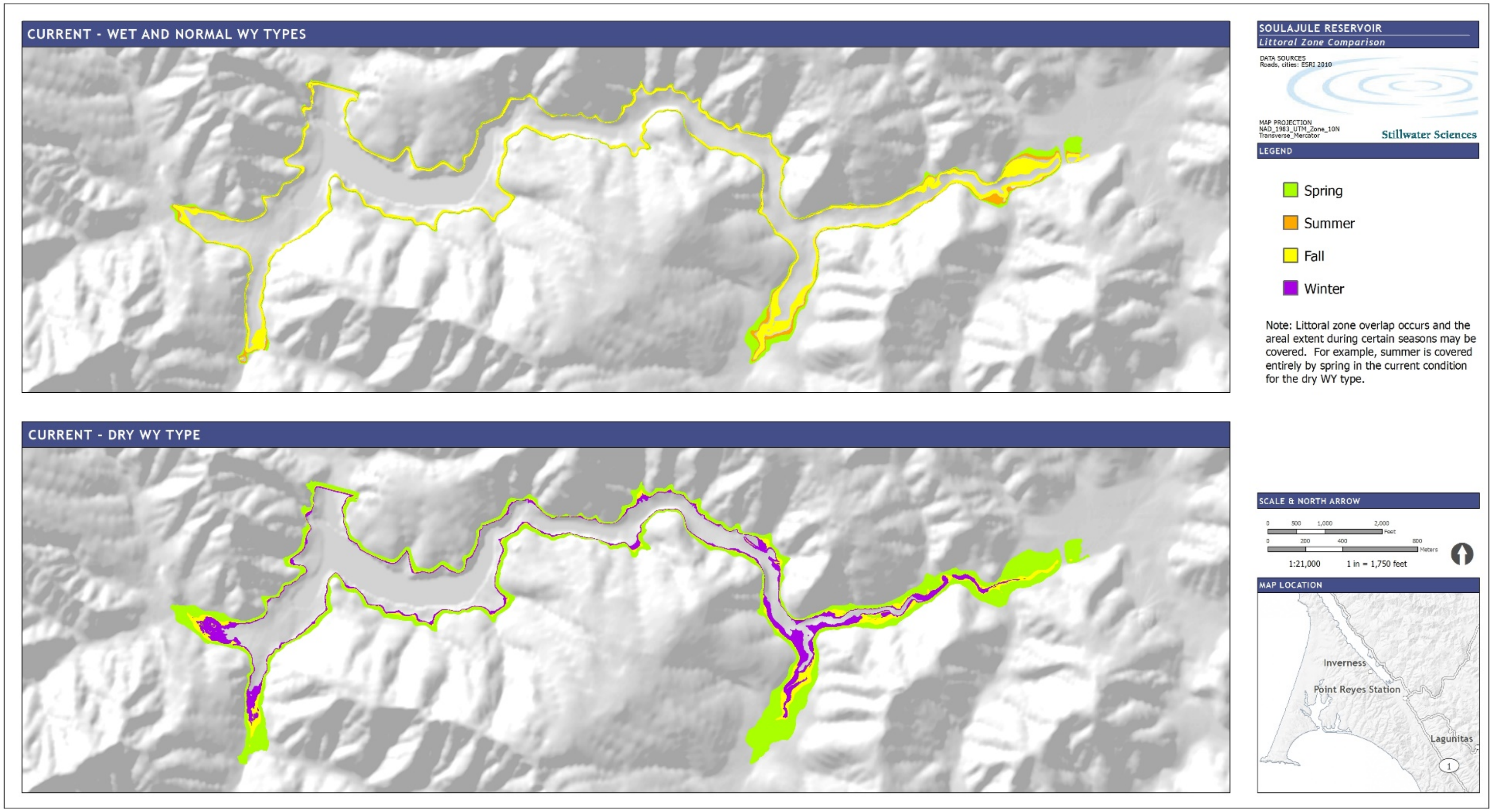


Figure 15. Spatial distribution and estimated area of the littoral zone in Soulajule Reservoir during wet and normal water year (WY) and dry WY types under current conditions.

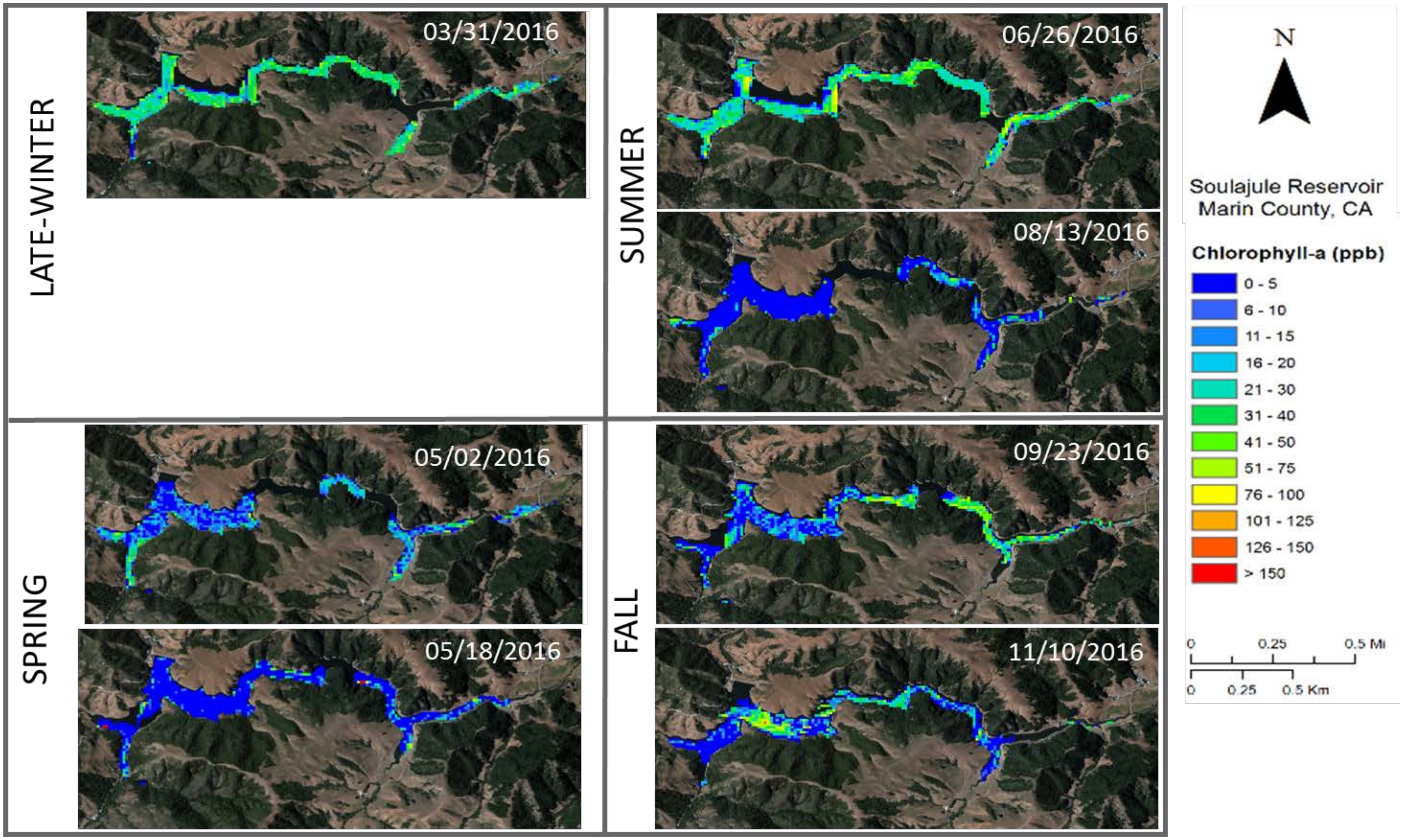


Figure 16. Chlorophyll-a concentrations on various dates in late winter, spring, summer, and fall 2016. Chlorophyll-a was measured using raw data collected by satellite (Landsat 7 and Landsat 8) imagery and processed by Satelytics (Toledo, OH).

Seasonally warm water temperatures, abundant phosphorus, and limited bio-available nitrogen in Soulajule Reservoir create conditions where nitrogen-fixing blue-green algae (cyanobacteria) thrive. Relative abundance data (collected as algae grab samples) indicate that cyanobacteria dominated at all sites and during all seasons (Figure 17). Similar to other California warm, eutrophic lakes such as Clear Lake, Lake County (Horne et al. 1979), *Aphanizomenon flos-aquae* was the primary algal species in spring (May) and summer (August) 2016, with other cyanobacteria (e.g., *Microcystis aeruginosa, Limnoraphis robusta* [*Lyngbya robusta*] *Woronichinia naegeliana*), as well as diatoms, green algae, dinoflagellates, and golden algae increasing in relative abundance in winter, summer and fall (Figure 18, Figure 19). The highest relative abundance of more edible phytoplankton (e.g., green algae, yellow-green algae, diatoms) exhibited the same pattern as overall diversity, with the highest abundance occurring during winter, particularly in the Eastern Arm. Relative abundance of edible phytoplankton was low during both summer and fall, with these species virtually absent during the summer (Figure 18, Figure 19).

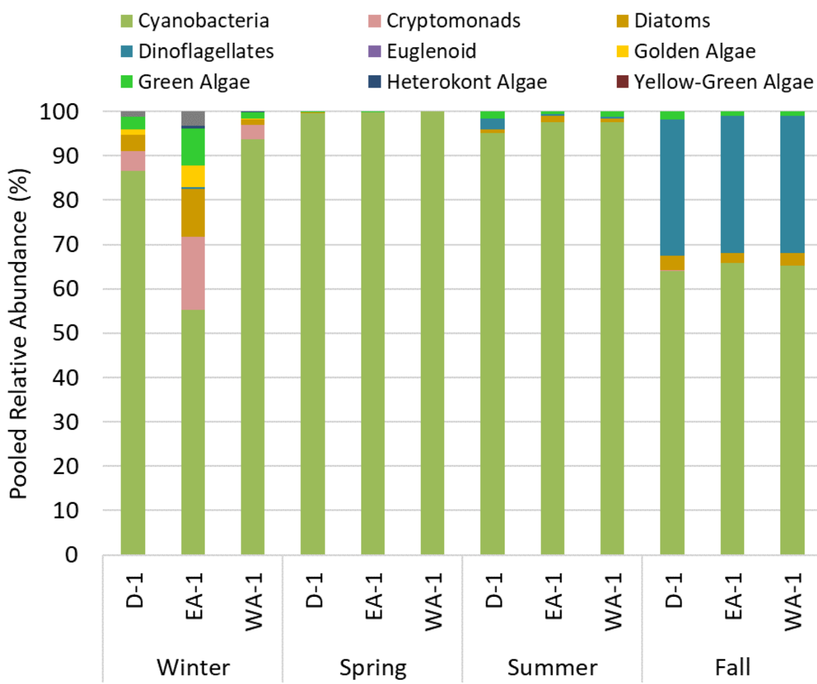


Figure 17. Seasonal relative abundance of all algal groups, pooled across triplicate samples at each site.

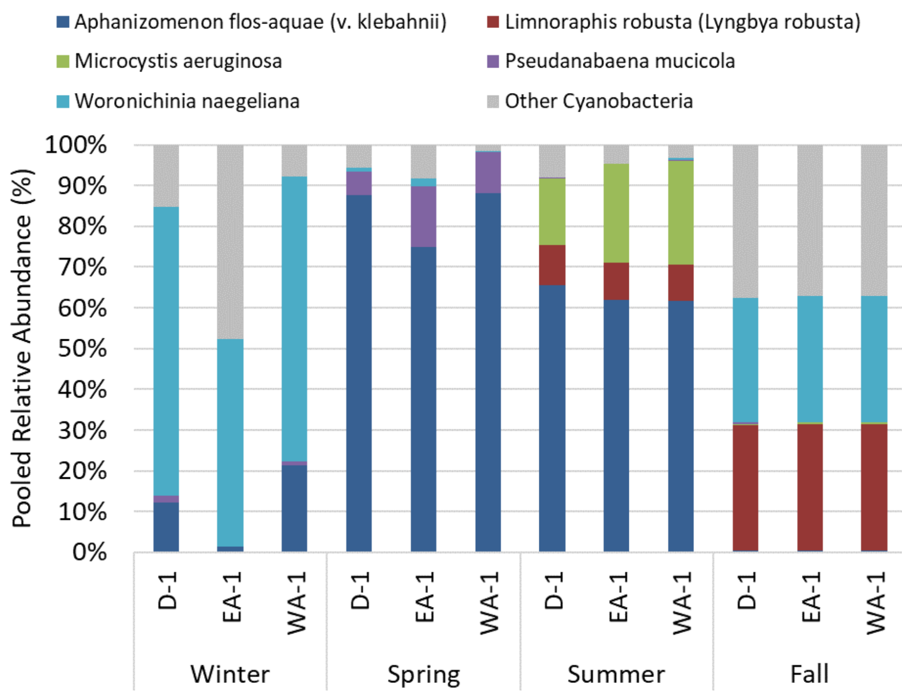


Figure 18. Seasonal relative abundance of species within the cyanobacteria group, pooled across triplicate samples at each site.

The 2016 survey results support Hypothesis PS1-H1 that blue-green algae (cyanobacteria) dominate Soulajule Reservoir algal blooms. However, results do not support the portion of Hypothesis PS1-H1 regarding a concentration of blooms in the Eastern Arm, where sediment concentrations of mercury are the highest. More broadly, the Phase 2 pilot study results indicate that littoral zone primary productivity in Soulajule Reservoir is very limited compared to pelagic (open water) primary productivity, where the latter is dominated by blue-green algae (cyanobacteria) across all seasons (Stillwater Sciences 2017c). Figure 19 illustrates a summer bloom of phytoplankton in the Eastern Arm, with a lack of littoral zone vegetation along the nearby shoreline.

Figure 19. Representative photo of a phytoplankton bloom and minimal littoral zone in Soulajule Reservoir, August 17, 2016.

3.4 Reservoir Food Web and Mercury Bioaccumulation

Methylmercury bioaccumulates, or biomagnifies, in the food web, which can result in biota concentrations that are orders of magnitude greater than ambient water concentrations (Weiner et al. 2003). Many environmental factors affect food web mercury bioaccumulation in lakes and reservoirs, including physical-chemical properties of habitat that affect the formation of methylmercury, proximity to point sources of mercury contamination, exposure time to methylmercury (via diet for higher tropic levels), potential for biodilution, and the growth rate of food web organisms. Phytoplankton (algae) is the primary entry point for methylmercury into the food web (see also Section 3.3), and methylmercury concentrations in phytoplankton can be 100−10,000+ times greater than concentrations in water (Mason et al. 1996, Pickhardt and Fisher 2007, Gorski et al. 2008). Methylmercury typically increases at each subsequent trophic level by a factor of two to five (Wood et al. 2010, Stewart et al. 2008, Cabana et al. 1994, Peterson et al. 2007).

As methylmercury exposure and subsequent bioaccumulation relate directly to food consumption, habitat and feeding niches for aquatic organisms at various life stages are important determinants of methylmercury tissue concentrations in upper trophic levels. Several literature studies have indicated that methylmercury concentration in aquatic organisms is often positively and significantly related to the trophic position of the organism (McIntyre & Beauchamp 2007, Gantner et al. 2009, Carrasco et al. 2011, Clayden et al. 2013). Consequently, identifying species' trophic position and feeding interactions within the Soulajule Reservoir food web was identified as an important step in evaluating management options to mitigate target species methylmercury bioaccumulation.

To improve understanding of mercury bioaccumulation patterns in the Soulajule Reservoir food web, data from multiple Phase 2 pilot studies were considered:

- Methylmercury concentrations in water, zooplankton, and small fish from *Additional Characterization of Methylmercury in Water and Biota* (Pilot Study 1) (see also Section 3.2);
- Distribution and relative abundance of primary producers (i.e., aquatic macrophytes, phytoplankton) from the *Assessment of Littoral Zone Extent and Productivity as Related to the Potential for Increased Water Level Fluctuation in Soulajule Reservoir* (Pilot Study 2) (see also Section 3.3);
- Fish community composition from *Soulajule Reservoir Fish Community Composition* (Pilot Study 3); and,
- Fish diet via through gut content analysis from the *Soulajule Reservoir Food Web Structure* (Pilot Study 4).

Additional data collection, beyond that already described for Pilot Study 1 (Section 3.2) and Pilot Study 2 (Section 3.3), included seasonal collection of phytoplankton, zooplankton, and small fish at sites EA-1, WA-1, and D-1 (Figure 9) and subsequent analysis for methylmercury content. Stillwater Sciences (2017a) provides additional details regarding sampling sites, frequency, and methodology for biota samples collected during Pilot Study 1. For seasonal fish community sampling, a variety of reservoir habitats were sampled in the general vicinity of seven reservoir sampling sites across three study reaches (E1, E2, E3, E4, M1, M2, W1) (Figure 9). Sampling methods included beach seine, boat electrofishing, adult gill net, and juvenile gill net. Collected fish were separated into TL3[1] and TL4[1] categories using size ranges established in the Walker Creek Mercury TMDL (SFBRWQCB 2008):

- TL3 size range: 5−15 cm FL
- TL4 size range: 15−35 cm FL

Gastric lavage (i.e., stomach pumping) samples were taken from larger TL3 (<15 cm FL) and TL4 (>15 cm FL) fish collected during the fish community composition survey; when possible, some fish <15 cm FL also were lavaged or collected for laboratory analysis of gut content. Stillwater Sciences (2017c) presents additional details regarding fish community sampling sites, frequency, and methodology.

Collectively, the data from Pilot Studies 1−4 address, to varying degrees, the following six hypotheses:

PS4-H1. The food web structure in Soulajule Reservoir is dominated by pelagic rather than littoral primary productivity.

PS4-H2. The relative dominance of blue-green algae in Soulajule Reservoir phytoplankton limits mercury bioaccumulation in the pelagic portion of the food web because the blue-green algae are disproportionately not consumed by planktivorous biota (e.g., zooplankton).

PS4-H3. The relative dominance of blue-green algae in Soulajule Reservoir phytoplankton exacerbates mercury bioaccumulation in the profundal portion of the food web (e.g., crayfish, catfish) because detritivores do not selectively exclude senescent blue-green algae from their diets.

PS1-H3. Algal, zooplankton, and small (5−15 cm FL, TL3) prey fish methylmercury concentrations and bioaccumulation factors (BAFs) are higher in the mining-impacted shallow, Eastern Arm of Soulajule Reservoir than the main body of the reservoir or the Western Arm.

PS1-H4. Algal, zooplankton, and small (5−15 cm FL, TL3) prey fish methylmercury concentrations and BAFs are higher in the fall than in the spring, independent of location in the reservoir.

PS1-H5. Measured BAFs are less than the 1,300,000 (L/kg) value assumed in the Walker Creek Mercury TMDL for development of the water column allocation (0.04 ng/L annual mean dissolved methylmercury) (SFBRWQCB 2008), such that an allocation based upon measured BAFs would be greater than 0.04 ng/L.

The below sections summarize the Phase 2 pilot study results related to reservoir food web and mercury bioaccumulation in Soulajule Reservoir.

3.4.1 Phytoplankton and zooplankton

The 2016 pilot study results indicate that pelagic (open water) cyanobacteria dominate primary production in Soulajule Reservoir across all seasons compared to other phytoplankton (green algae, golden algae, diatoms, and dinoflagellates) (Figure 17) and littoral zone productivity for the reservoir, consistent with Hypothesis PS4-H1 (see also Section 3.3). From a food web perspective, bloom-forming cyanobacteria have generally been considered a poor food source for primary consumers (i.e., zooplankton) due to the production of toxins, large and difficult-to-ingest morphology (Porter and Orcutt 1980, Lampert 1981, Webster and Porter 1978, Lynch 1980, Fulton and Paerl 1987, Pohnert et al. 2007), and a lack of essential lipids (Muller-Navarra et al. 2000, Dickman et al. 2008), hence the formulation of Hypothesis PS4-H2.

Each of the three major zooplankton groups (i.e., cladocerans, copepods, rotifers) were observed in the 2016 samples (Figure 20). Unexpectedly, copepods appeared to dominate zooplankton populations during winter 2016, which may be due to their ability to selectively prey on algal species (DeMott 1986, DeMott and Moxter 1991, Bouvy et al. 2001, Koski et al. 2002, Ger et al. 2016) such as the green algae, golden algae, diatoms, and cryptomonad algae present during this season (Figure 17). Selective predation pressure on these algal species would potentially suppress their relative abundance and help to facilitate a spring bloom of *Aphanizomenon flos-aquae* once water-column stratification occurs. Copepods were also dominant in terms of both abundance and biomass during the spring bloom of *Aphanizomenon flos-aquae*. Copepod dominance during the spring bloom could mean that these zooplankton are able to ingest some amount of the colonial, potentially toxic *Aphanizomenon flos-aquae* through internal detoxification mechanisms (Ger et al. 2016). Despite their higher relative abundance during seasonal zooplankton sampling, copepods were not identified in the gut contents of any fish samples in Soulajule Reservoir (Stillwater Sciences 2016b) suggesting that they are not an important food source for reservoir TL3 fish.

Like copepods, rotifers are also selective grazers and may be avoiding ingestion of cyanobacteria in Soulajule Reservoir, focusing instead on green algae, golden algae and diatoms, and contributing to overall low abundance of these other algae species. Rotifers also may be ingesting smaller cyanobacteria filaments created by copepod grazing (Fabbro and Duivenvoorden 1996). However, although typically high in abundance themselves, rotifers (0.1–0.5 mm) tend to be much smaller than either copepods (0.5–15 mm) or cladocerans (0.5–4 mm) and thus are typically less important grazers of phytoplankton. They are also less likely to be an important food source for TL3 fish due to their small size. Rotifers were not identified in the gut contents of any fish samples in Soulajule Reservoir (Stillwater Sciences 2016b), although due to their small size, rotifers are unlikely to be identified via this method.

Lastly, cladocerans (predominantly *Daphnia sp.*) exhibited low relative abundance and biomass in Soulajule Reservoir during winter 2016, and very low relative abundance and biomass in spring 2016 during the large bloom of *Aphanizomenon flos-aquae* (Figure 17, Figure 18). Cladocerans are generalist grazers with little ability to select among food particles (Kirk and Gilbert 1992, Ger et al. 2016) and several studies have noted Daphnia sensitivity to cyanobacteria toxins (Gilbert 1990, Gliwicz 1990a, Hawkins and Lampert 1989, Webster and Porter 1978, Ger et al. 2016). Despite their low relative abundance during seasonal zooplankton sampling, *Daphnia* were identified in the gut contents of TL3 and TL4 fish samples in Soulajule Reservoir (Stillwater Sciences 2016b) suggesting that they are both present and are an important food source for reservoir fish. The most likely explanation for the unusually high relative abundance of copepods as compared to cladocerans in Soulajule Reservoir is grazing pressure by abundant small fish. Copepods are more mobile than most cladocerans and thus they can more easily escape sight-feeding predators like fish. The lack of littoral vegetation and well-oxygenated waters below the euphotic zone (the zone between the water surface and the depth at which light levels reach 1 percent of photosynthetically active radiation [PAR]) may result in a concomitant lack of daytime predation refuges for cladocerans, such that these organisms are selectively eaten in Soulajule Reservoir.

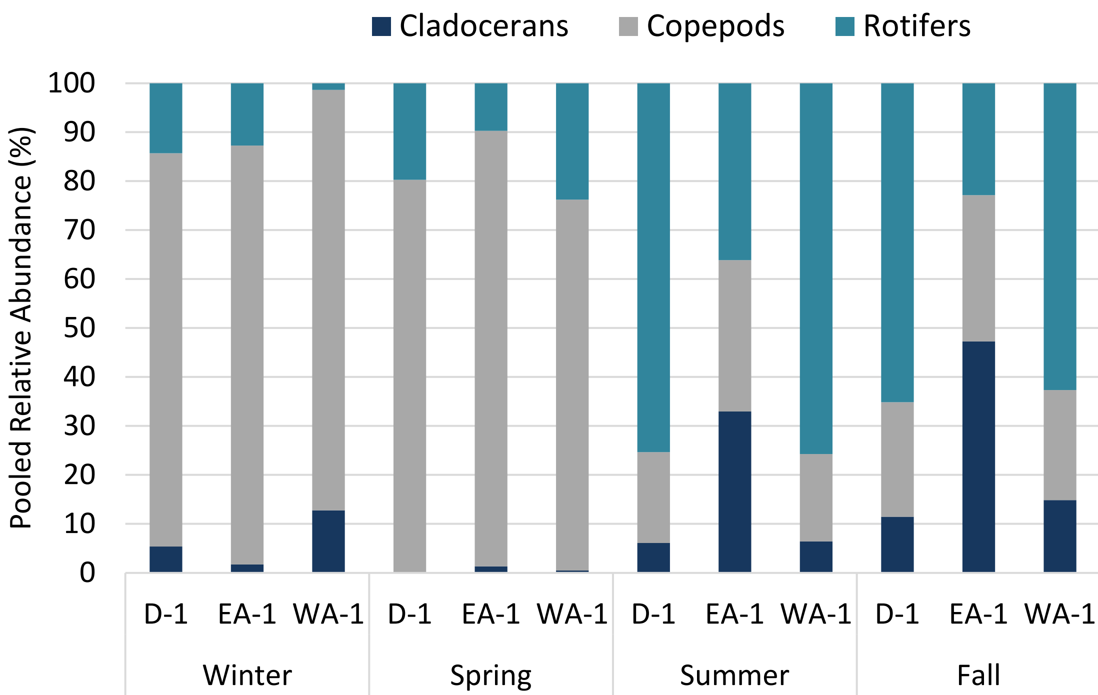


Figure 20. Seasonal relative abundance of zooplankton groups, pooled across triplicate samples per site.

3.4.2 Juvenile and adult fish

The results of the 2016 community composition study suggest that Soulajule Reservoir supports a productive fishery dominated by non-native centrarchids. Most fish collected were TL3, including juvenile bluegill, black crappie, golden shiner, and spotted bass. Adult largemouth bass and spotted bass comprised a majority of TL4 fish; smaller numbers of adult black crappie, bluegill, and golden shiner belonging to this trophic level (based on their size) were also observed. Fish abundance was highest near the dam (Sites M1 and M2) and in the Eastern Arm (Sites E1, E2, E3, E4) of the reservoir (Figure 21), where the latter is closest to the historic Franciscan and Cycle mines and has the highest concentration of total mercury in sediments (Figure 5).

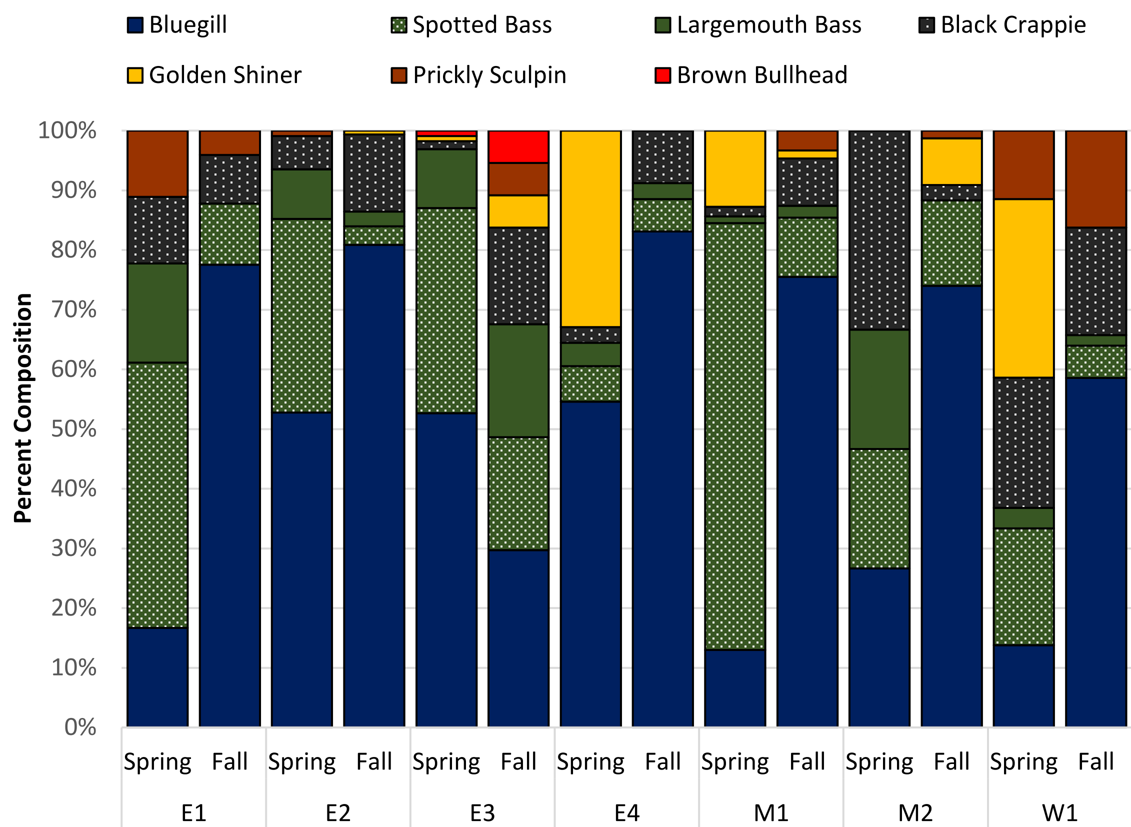


Figure 21. Fish composition in Soulajule Reservoir by reservoir study reach, spring and fall 2016.

The Soulajule Reservoir fish gut content results are consistent with results of other temperate latitude reservoir studies, whereby fish rely on diverse prey and exhibit seasonal diet switching to capitalize on prey abundance variation (Godinho and Ferreira 1994, Godinho et al. 1997, Garcia-Berthou and Moreno-Amich 2000, Mathur 1972). Although cladoceran consumption was apparent in several Soulajule Reservoir fish species as an important diet component, benthic chironomids also were consumed widely by TL3 and TL4 fish. The latter observation is consistent with benthic chironomid consumption as a primary energy source for fish in other freshwater eutrophic lakes (Forsyth and James 1988, Anderson et al. 2012, Eagles-Smith et al. 2008). While the contribution of benthic chironomids (TL2 primary consumer) to phytoplankton consumption is not known for Soulajule Reservoir, benthic chironomids are primarily filter feeders, creating burrows in a reservoir's littoral and profundal sediments and filtering particles (e.g., algae, bacteria, microplankton) near the sediment water interface. High abundance of benthic chironomids is typical in sediments of eutrophic lakes and reservoirs, suggesting that, like copepods, they tend to co-exist with large cyanobacteria blooms rather than serve as an important consumer of cyanobacteria. The next most widely consumed prey for TL3 and TL4 fish included members of

Amphipoda, Corixidae, and Odonata, which although broadly classified as TL2 based on size and position as prey for small fish, are also predatory or omnivorous insects and are thus secondary consumers themselves. Other prey categories were relatively infrequently consumed (Stillwater Sciences 2017c).

The assessment of higher trophic level feeding interactions also reveals key differences in the way adult fish in Soulajule Reservoir interact with other trophic levels. TL4 fish, including bass and black crappie, appear to obtain components of their diet from both TL3 and TL2 organisms (Figure 22). In contrast, bluegill feed exclusively from the primary consumers of the TL2 organisms (i.e., cladocerans, snail [Gastropoda], and benthic chironomids) (Figure 22). Some species also exhibited pronounced seasonal diet shifts; largemouth and spotted bass diets transitioned from a reliance on fish and crayfish in spring to benthic chironomids (TL2 primary consumer) in fall. In contrast, black crappie diets shifted from reliance on benthic chironomids (TL2 primary consumer) in spring to cladocerans (TL2 primary consumer) in fall. Results of the fish community composition study suggest juvenile bluegill, golden shiner, and bass (TL3) are the most likely prey fish species consumed by piscivorous (TL4) fishes in the reservoir.

TL3 fish were found most commonly within the littoral zone, whereas TL4 fish were more abundant within the profundal zone. Relatively few fish were captured within the pelagic zone (Stillwater Sciences 2017c). Despite fish capture data indicating differences in habitat preference (e.g., littoral or profundal) among species and life stages, the food web analysis indicates feeding interactions that would require organisms to leave their predominant site of capture to feed in other habitat types. For example, TL4 bass captured in the profundal zone fed heavily on juvenile fish captured in the littoral zone. This observation indicates at least occasional movement between these zones, either by TL4 or TL3 fish, and suggests that management activities targeting the food web should consider the littoral and profundal portions of the food web as interconnected in Soulajule Reservoir.

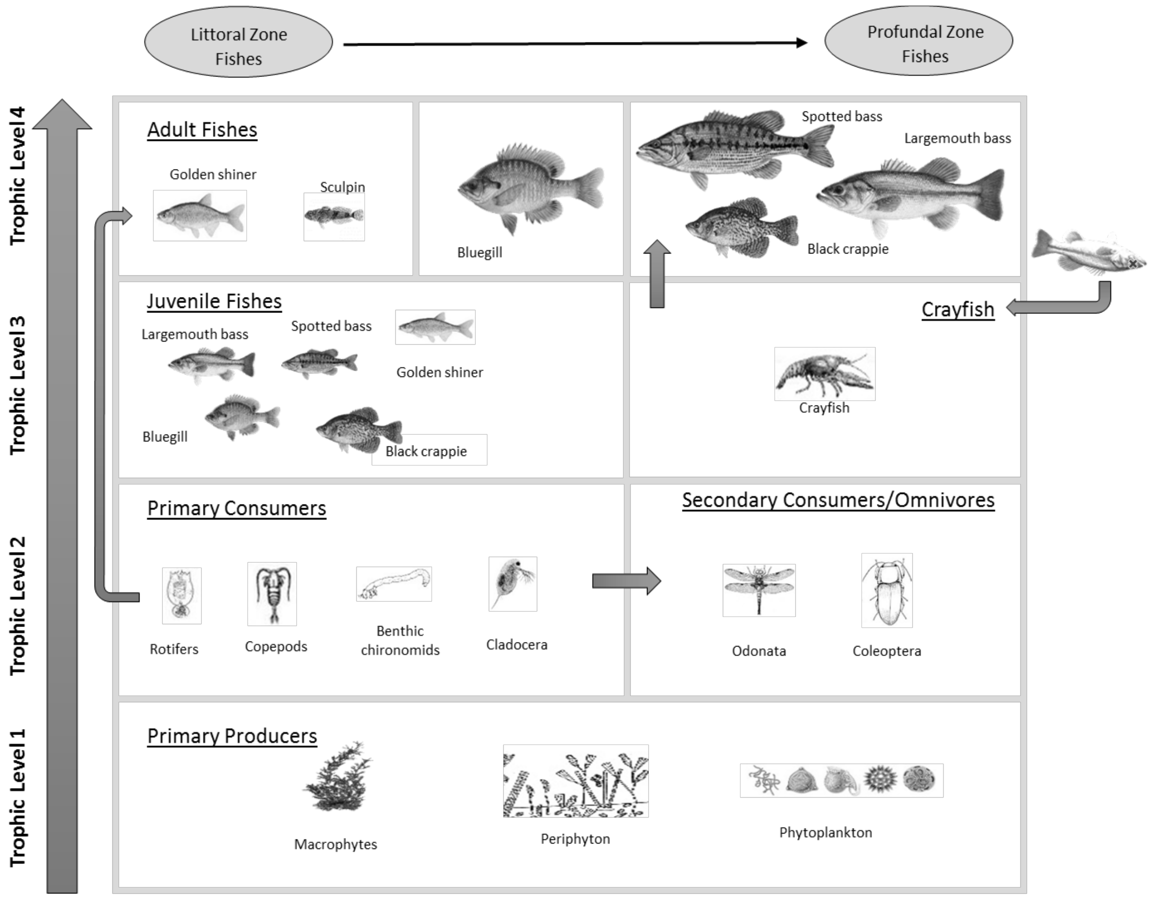


Figure 22. Conceptual food web for Soulajule Reservoir with arrows indicating the direction of feeding interaction

3.4.3 Methylmercury concentrations in biota

During 2016 mean methylmercury in phytoplankton ranged from 6.2–21.2 ng/g, with the highest concentrations occurring during spring in the Eastern Arm, and otherwise similar concentrations across sites during both summer and fall (Figure 23). Mean methylmercury in zooplankton ranged from 1.9–82.8 ng/g, with the greatest concentrations occurring during fall, and otherwise similar concentrations across sites within seasons (Figure 23). Overall, methylmercury concentrations in TL3 fish (5−15 cm FL) ranged 89–1,080 ng/g (wet weight), with concentrations generally increasing from winter to fall, particularly for Site EA-1 (Figure 23). Methylmercury concentrations in TL3 fish were consistently greater than the Walker Creek Mercury TMDL numeric target of 50 ng/g (wet weight) for TL3 fish (SFBRWQCB 2008). The California State Water Board sport fish water quality objective for TL3 fish is 0.2 mg/kg (200 ng/g) wet weight for skinless fillet collected within a calendar year, where TL3 is defined as 15−50 cm total length (State Water Board 2017). While the majority of the Soulajule Reservoir sampled TL3 fish (5−15 cm FL) were below the designated size range for State Water Board TL3 fish (15−50 cm total length), and Soulajule Reservoir TL3 fish were analyzed as individual whole body, homogenized fish rather than skinless fillet, there were five TL3 fish from Soulajule Reservoir that were 15 cm or greater total length (Table 4). All five individuals exceeded the California State Water Board sport fish water quality objective of 200 ng/g wet weight methylmercury.

Table 4. Methylmercury concentrations in Soulajule Reservoir sampled TL3 fish having total length 15 cm or greater.

Season	Total length (cm)	Fork length (cm)	MeHg (ng/g wet weight)
Spring	15.0	14.5	727
Spring	15.3	14.0	603
Summer	16.2	14.5	718
Fall	16.2	15.5	413
Winter	15.0	14.5	348

In general, concentrations in bass and golden shiner were greater than the other species (Figure 23), which was primarily driven by several young of year bass individuals exhibiting particularly high concentrations (>780 ng/g) in the fall and several Age 1+ golden shiner individuals exhibiting high concentrations (>700 ng/g) in spring, summer, and fall.

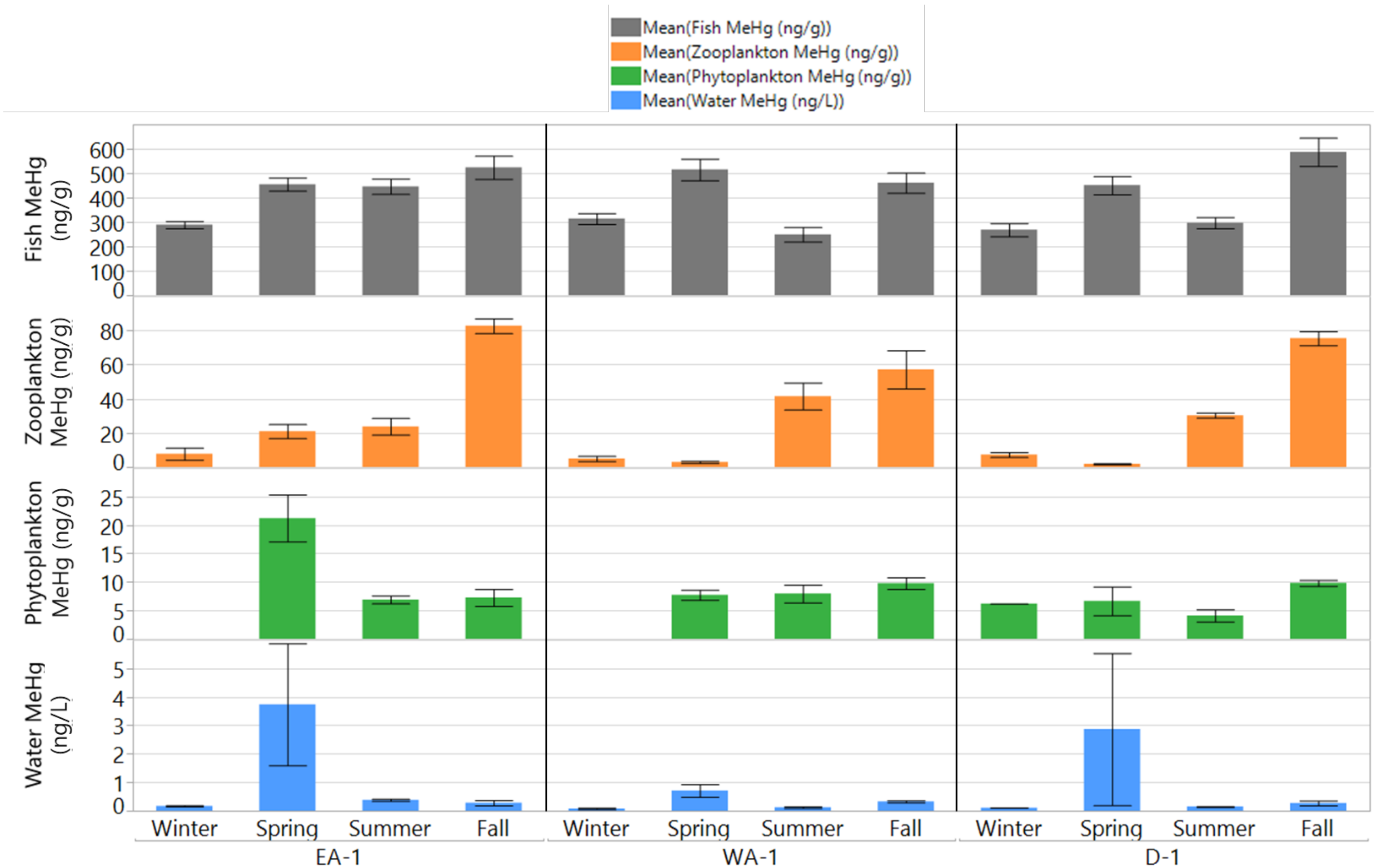


Figure 23. Seasonal methylmercury concentrations in surface water, phytoplankton, zooplankton and all TL3 fish (mean ± 1SE), pooled across replicate samples by site.

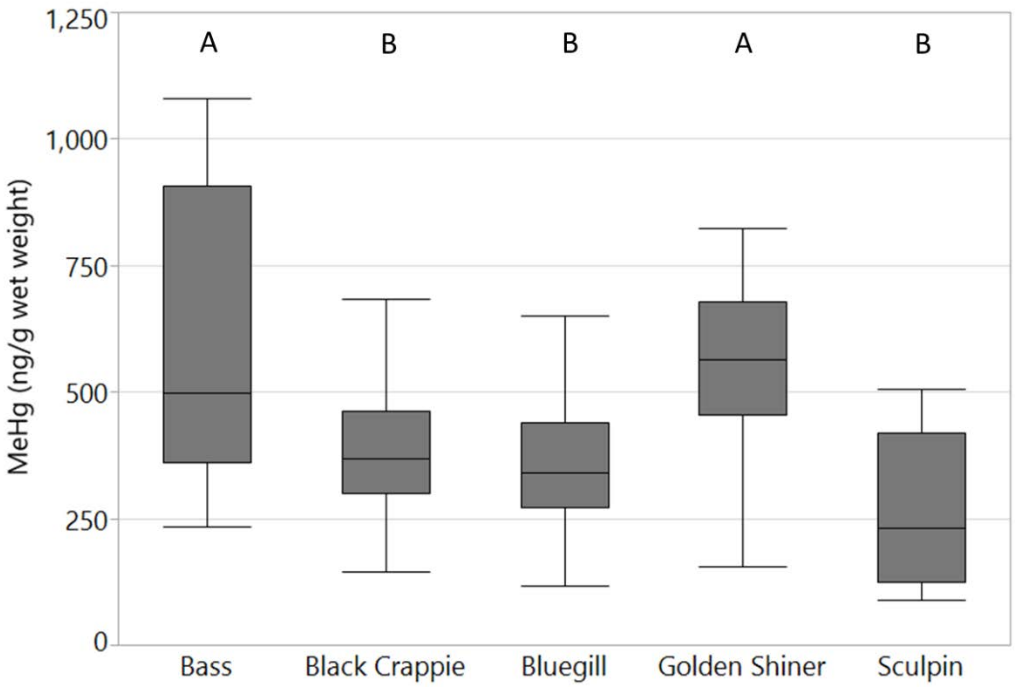


Figure 24. Methylmercury concentration in all TL3 fish and, Box and whisker plots show the data distribution across sites and seasons for each species. Median (50th percentile) = black line in the center of the two shaded boxes; upper quartile (75th percentile) = top of the boxes; lower quartile (25th percentile) = bottom of the boxes; whiskers drawn to the furthest point within 1.5 x interquartile range. Species with different letters are significantly different ($p<0.05$, Tukey's HSD test).

Consistent with Hypothesis PS1-H3, Soulajule Reservoir water, phytoplankton, and zooplankton methylmercury concentrations were highest in the shallow Eastern Arm during spring 2016 (Figure 23), co-occurring with variable, and occasionally low, dissolved oxygen near the sediment water interface in the Eastern Arm (Figure 12). These conditions suggest that fluctuating redox conditions near the bottom sediments (presumably caused by algae) may have promoted dissolved methylmercury formation and release from sediments that contain the reservoir's highest levels of mercury (approximately 0.6−6 mg/kg dry weight, see Figure 5). Although the results of Soulajule Reservoir sediment chamber experiments conducted as part of Pilot Study 5 indicate that mercury-enriched sediments in the shallow Eastern Arm exhibit a relatively lower potential to release methylmercury under anoxic conditions as compared with organic-rich sediments in the deeper portion of the reservoir near the dam (Beutel 2016a), the spring 2016 results suggest that water column mixing in the Eastern Arm may allow dispersal of methylmercury released from deeper bottom sediments to enter reservoir waters, resulting in elevated surface water and biota methylmercury concentrations. Further, the lack of distinct water column stratification in the Eastern Arm during spring (Figure 10) may have facilitated dissolved methylmercury uptake by phytoplankton located relatively near the shallow bottom sediments, during at least part of each diurnal period; alternatively, dissolved methylmercury may have mixed directly into the water column to be taken up by phytoplankton and subsequently zooplankton throughout the diurnal period.

Despite spring 2016 survey results, relatively higher methylmercury concentrations in the Eastern Arm were not evident in phytoplankton and zooplankton during other seasonal sampling events, and higher concentrations were not evident in TL3 fish (Figure 23). Thus, overall, 2016 study results support Hypothesis PS1-H3 with respect to spring methylmercury concentrations in phytoplankton and zooplankton, but not with respect to methylmercury concentrations in TL3 fish.

Consistent with Hypothesis PS1-H4, methylmercury in zooplankton and TL3 fish peaked during the fall (Figure 23), which corresponded to the period just after reservoir overturn, as evidenced by a well-mixed water column and little to no variation in water column temperature, dissolved oxygen, pH, and turbidity at all sites (Figure 10, Figure 11). During overturn, hypolimnetic waters containing elevated methylmercury were mixed into in surface waters, increasing fall zooplankton methylmercury concentrations and, through bioaccumulation, TL3 fish methylmercury concentrations. Elevated zooplankton and TL3 fish methylmercury concentrations observed in Soulajule Reservoir following fall overturn are consistent with results from other reservoir studies (e.g., Slotton et al. 1995). Although 2016 observed zooplankton and TL3 fish methylmercury concentrations generally support Hypothesis PS1-H4, phytoplankton results were not consistent with the hypothesis since phytoplankton methylmercury concentrations did not exhibit a significant increase at fall overturn relative to other seasons, and spring phytoplankton methylmercury concentrations in the Eastern Arm were 2−3 times greater than those observed at any other time during the study. Since winter methylmercury concentrations in phytoplankton were generally lacking in the dataset given the relatively low biomass present during the winter (February) survey (Stillwater Sciences 2017a), the data for the winter season cannot be used to either support or refute Hypothesis PS1-H4.

3.4.4 Bioaccumulation factors

Bioaccumulation factors (BAFs)[4] were calculated using surface water dissolved methylmercury concentrations and biota methylmercury tissue concentrations. In contrast to methylmercury concentrations, phytoplankton, zooplankton, and TL3 fish BAFs did not exhibit any spatial pattern (Figure 25). With respect to BAFs, the season and site location accounted for approximately 50% of the variation in surface water concentrations of methylmercury and approximately 80% of the variation in biota concentrations. Season was the more important factor, although this BAF pattern is largely due to an increase in spring dissolved methylmercury concentrations in surface waters at all sites, but particularly at sites EA-1 and D-1, which resulted in a measurable decrease in spring BAFs for phytoplankton, zooplankton, and TL3 fish (Figure 25). Excluding the decrease in spring

[4] *BAF =* $log_{10} (MeHg_{water}/MeHg_{tissue})$

BAFs resulting from elevated dissolved methylmercury in surface waters, BAFs were generally similar across seasons at all sites, and thus did not support either Hypothesis PS1-H3 or PS1-H4.

With respect to Hypothesis PS1-H5, all seasonal and annual phytoplankton and zooplankton measured BAF's were lower than 6.1, the value used in the Walker Creek Mercury TMDL (SFBRWQCB 2008), where 6.1 = $\log_{10}$ (1,300,000 L/kg). While 2016 fall and winter BAFs for Soulajule Reservoir TL3 fish were slightly above 6.1, spring and summer BAFs were slightly below this value, and the annual reservoir-wide mean for 2016 was 5.8. Converting the 2016 annual reservoir-wide mean to a dissolved water concentration target using the BAF relationship yields a slightly higher target value than included in the Walker Creek Mercury TMDL (0.04 ng/L dissolved methylmercury):

Dissolved MeHg (ng/L) = 0.05 mg/kg MeHg in TL3 fish x $\log_{10}(5.8)$ = 0.07

Thus, 2016 survey results are consistent with Hypothesis PS1-H5 that the annual average allocation for dissolved methylmercury throughout the water column in Soulajule Reservoir would be greater, albeit slightly, as compared with the 0.04 ng/L value included in the Walker Creek Mercury TMDL (SFBRWQCB 2008).

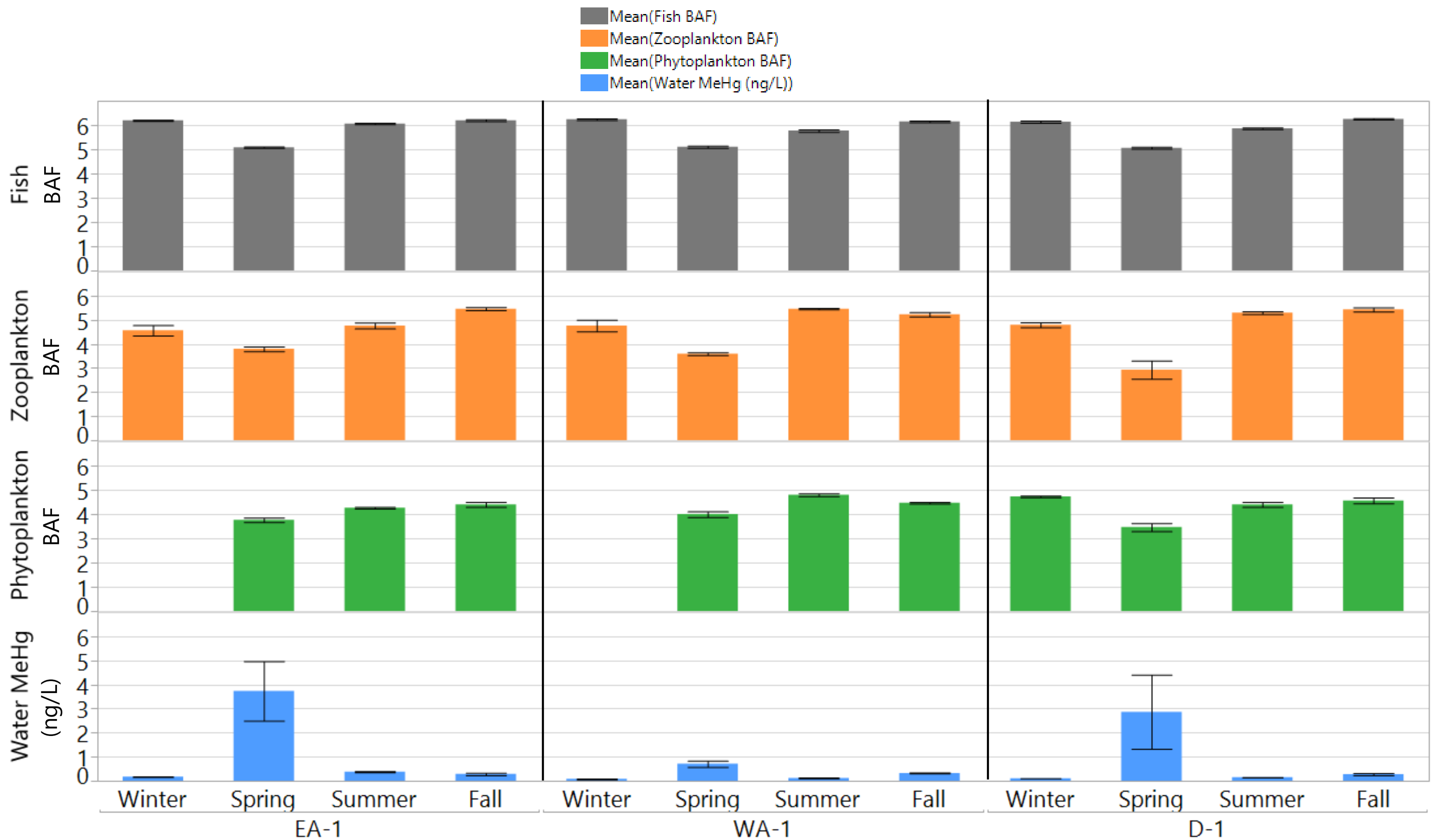


Figure 25. Seasonal dissolved methylmercury concentrations in surface water and BAFs for phytoplankton, zooplankton, and TL3 fish (mean ± 1SE), pooled across triplicate samples per site.

3.4.5 Implications of trophic level feeding interactions on methylmercury bioaccumulation

The trophic level feeding interactions described in Section 3.4.1 and 3.4.2 have potentially significant implications for methylmercury bioaccumulation in Soulajule Reservoir. Previous studies have demonstrated a significant and positive correlation between methylmercury concentration and trophic position in freshwater lakes and reservoirs, whereby higher trophic level organisms exhibit higher mercury concentrations (McIntyre and Beauchamp 2007, Gantner et al. 2009, Carrasco et al. 2011, Clayden et al. 2013). While results of the 2016 biota sampling do not address the portion of Hypothesis PS4-H2 definitively, stating that blue-green algae (cyanobacteria) are not consumed disproportionately by planktivorous biota (e.g., zooplankton), they are suggestive. Pilot study results suggest that the reservoir food web has adapted to cyanobacterial dominance through algal-grazing selection mechanisms (and potentially toxicity tolerance), as evidenced by TL2 primary consumers that are more heavily represented by highly selective grazers like copepods and rotifers, rather than non-selective cladocerans such as large Daphnia (Figure 20). However, cladocerans are in fact present in the water column, particularly in late summer and fall, and they are present in the gut content of small (<150 mm) TL3 fish (i.e., juvenile black crappie, adult and juvenile bluegill, golden shiner) in both seasons. This finding suggests that in addition to grazing on their preferred prey (e.g., green algae, diatoms), local populations of cladocerans have developed some degree of tolerance to cyanobacterial grazing defenses. Preliminary results from a study of zooplankton consumption of seasonal blooms of *Aphanizomenon flos-aquae* in Upper Klamath Lake, Oregon, indicate that during a large bloom, blue-green algae (cyanobacteria) may represent 98% of algal biomass and approximately 40% of Daphnia diet, while other algal species (e.g., green algae, diatoms) represent 2% of total algal biomass and approximately 60% of Daphnia diet (Brett, M. unpublished data, 2017). These results suggest that Daphnia can tolerate some level of consumption of blue-green algae (cyanobacteria) as long as they also have access to high quality food, such as green algae and diatoms.

Further, mercury bioaccumulation in Soulajule Reservoir may be more complex than a simple integer (i.e., TL1 → TL2 → TL3 → TL4) conceptual model would predict because many reservoir biota feed at intermediate and/or multiple trophic levels. For example, the fish gut content analysis exhibited a strong feeding interaction between TL3 and TL4 fish and TL2 organisms, where the latter include both primary consumers (e.g., cladocerans, benthic chironomids) and secondary consumers (e.g., predatory insects such as dragonflies [Odonata], water boatman [*Corixidae*], beetles [*Coleoptera*], amphipods) (Figure 22). Here, the secondary consumers within TL2 appear to serve as an intermediate trophic level between the more traditional TL2 primary consumers (i.e., cladocerans, copepods, rotifers) and TL3 fish. The position of predatory insects in the Soulajule Reservoir food web may represent additional potential for mercury bioaccumulation in the pelagic food web prior to consumption by TL3 and TL4 fish, because they feed across multiple trophic levels. Thus, some TL2 predatory insects (e.g., *Odonota*, *Corixidae*) appear to behave more like TL3 organisms, whereas others (e.g., *Coleoptera*, amphipods) are omnivorous and align better with the more traditional TL2 classification. In either case, because some insects appear to represent an average trophic level higher than TL2, they represent the potential for greater mercury bioaccumulation than other TL2 primary consumers (e.g., cladocerans, copepods, and rotifers).

Further, 2016 study results indicate that benthic chironomids are widely consumed by TL3 and TL4 fish. Benthic chironomids, which inhabit the sediment-water interface of the littoral and profundal zones, are non-specific filter feeders and thus represent a source of methylmercury to their TL3 and TL4 predators through uptake of algal particles. Also, benthic chironomids reside in reservoir sediments where methylmercury concentrations can be much greater than water column concentrations (Kainz et al. 2003). If methylmercury in benthic chironomids is largely a result of their proximity to methylmercury produced in reservoir sediments, then consumption of these TL2 organisms would largely bypass the pelagic primary production part of the food web, at least from a mercury perspective. Alternatively, if benthic chironomids are obtaining methylmercury from filter feeding of algal particles (Eagles-Smith et al. 2008), then they serve a more traditional TL2 bioaccumulation role, albeit in a profundal (or littoral) zone niche rather than a pelagic zone niche. Either way, benthic chironomids tend to be an important food source for fish in eutrophic lakes (Forsyth and James 1988, Anderson et al. 2012, Eagles-Smith et al. 2008), such that the link to cyanobacterial dominance in Soulajule Reservoir is generally consistent with Hypothesis PS4-H2.

Overall, the 2016 food web survey highlighted the relative importance of benthic chironomids as a prey source for TL3 and TL4 fish in Soulajule Reservoir, which, when combined with crayfish as another frequent benthic food source for TL4 fish (Section 3.4.2), further supports the notion of profundal (and littoral) contributions to methylmercury bioaccumulation in the food web. How strongly cyanobacterial dominance regulate the contributions of these benthic organisms is not discernible from the available information, such that Hypothesis PS4-H3 cannot be fully addressed based on the 2016 pilot study results.

3.5 Reservoir Water Level Fluctuation

In several reservoirs and natural lakes in the northern hemisphere the literature reports correlations between fluctuating water levels and increased fish tissue mercury concentrations (Selch et al. 2007, Sorensen et al. 2005, Evers et al. 2007, Verta et al. 1986). Generally, these studies found that periodic oxidation of sediments (e.g., when dried) enhances the release of inorganic mercury, sulfate, and dissolved organic carbon (DOC) into pore waters and promotes methylmercury production upon rewetting (Munthe et al. 2007). The increased methylmercury concentrations in water following sediment rewetting are then incorporated into algae and zooplankton that are eventually consumed by fish, increasing fish methylmercury tissue levels.

The *Assessment of Littoral Zone Extent and Productivity as Related to the Potential for Increased Water Level Fluctuation in Soulajule Reservoir* (Pilot Study 2) considered the potential implications of increasing water level fluctuations on mercury concentrations in fish and as a biomanipulation management strategy. The data collected as part of this study provided an initial evaluation of the following hypotheses:

- PS2-H2: Increasing magnitude and frequency of water level fluctuations will result in increased mercury concentrations in fish tissue.
- PS2-H3: Increased water level fluctuations will reduce spawning habitat for sport fish, thereby reducing the sport fish population and subsequently increasing prey fish populations, algal productivity, and, potentially, available carbon for mercury methylation.

For mercury methylation to be significantly promoted, the period of air-entry to the sediments must be sufficiently long to allow for the organic matter oxidation and conversion to sulfate (Branfireun et al. 2009). Equally importantly, the rewetting period must be sufficient to allow the maintenance of sulfate-reducing bacteria (Branfireun et al. 2009), which methylate mercury as a byproduct of normal cellular respiration. Results of Pilot Study 2 indicate that increasing the *magnitude* of water level fluctuations in Soulajule Reservoir to 20 ft in elevation each year (i.e., by an additional 2,000−3,000 ac-ft/yr) would result in a larger area of littoral sediments (i.e., by a factor of 2−3) that are periodically dried and rewetted, which could increase reservoir methylmercury production. Increasing the *frequency* of water level fluctuations in Soulajule Reservoir would increase the number of times each year that littoral sediments are periodically dried and rewetted; if the drying and rewetting periods last several weeks each, this change could increase methylmercury production in the reservoir as a whole.

However, given that primary productivity and total organic carbon (TOC) in surface sediments are relatively low throughout the reservoir's littoral zone (Section 3.3.1), the littoral zone does not appear to be the dominant pathway for mercury bioaccumulation under existing conditions. In contrast, mercury methylation occurring in deeper, profundal sediments that are perpetually saturated, receive carbon from abundant, senescent planktonic algae, and extend throughout the reservoir, is likely to be a larger relative contributor to bioaccumulation. Thus, under current conditions the magnitude and frequency of reservoir water fluctuations is likely to be less important for controlling mercury methylation in Soulajule Reservoir than reducing planktonic algal productivity and controlling rates of mercury methylation in deeper, profundal sediments. Thus, contrary to Hypothesis PS2-H2, study results suggest that increased withdrawals from Soulajule Reservoir would not increase mercury concentrations in fish tissue due to enhanced littoral zone mercury methylation. However, increased withdrawals from the reservoir would reduce water volume and could concentrate nutrients, potentially resulting in an increase in phytoplankton; the effect of increased planktonic algal growth on mercury methylation should be considered with respect to future management of the reservoir.

With respect to Hypothesis PS2-H3, the most effective water surface elevation (WSE) related control for spawning fish in Soulajule Reservoir (assuming current operational strategies) is likely to be the overall WSE itself, rather than the rate of WSE change. Based on the age-class distribution of species in the reservoir, and correlation to WSEs during the corresponding spawning years, WSEs below 326 ft may inhibit spawning of largemouth bass, spotted bass, and bluegill due to a reduced area of shallow reservoir margins. However, while trophic cascade theory would suggest that holding WSEs relatively low could reduce the number of small (TL3) fish and correspondingly decrease phytoplankton (TL1) populations in each year, the simultaneous reduction in water volume could concentrate nutrients and result in an increase of phytoplankton (TL1) populations, the primary carbon source for mercury methylation in reservoir sediments. Thus, contrary to Hypothesis PS2-H3, reducing reservoir WSEs as a top-down biomanipulation-based mercury management technique is not recommended.

3.6 Existing Conditions Conceptual Model

Based on the results of the Soulajule Reservoir Phase 2 pilot studies and information from prior investigations, several factors influence methylmercury production and bioaccumulation in Soulajule Reservoir, forming the basis of an existing conditions conceptual model (Figure 26). The key factors include the following:

- Upland soils surrounding Soulajule Reservoir exhibit total mercury concentrations ranging from less than the background TMDL allocation (200 ng/g dry weight) to relatively high levels (> 1,000 ng/g dry weight), where the latter occur in both undisturbed areas and soils proximal to the historical Franciscan and Cycle mercury mines. There is currently little to no evidence of erosion and transport of mercury-laden soils into the reservoir as surrounding hillslopes are currently stabilized by heavy vegetation cover.
- Although currently understood to be stable, thick graded fill located at the Cycle Mine site exhibits relatively high total mercury concentrations and is in contact with reservoir water.
- Reservoir sediments in the Eastern Arm directly adjacent to the Cycle Mine exhibit the highest total mercury concentrations in the reservoir; these sediments also appear to be stable.
- Methylmercury concentrations in reservoir sediments are 1,000-100,000 times lower than total mercury concentrations. Methylmercury release rates from reservoir sediments are approximately 10 times higher from sediments near the dam as compared with sediments in the Eastern Arm, where total mercury concentrations are the highest, and the Western Arm, where total mercury concentrations are at background levels.

- Soulajule Reservoir supports high levels of pelagic (open water) primary productivity and low littoral zone productivity. Primary productivity is dominated by blue-green algae (cyanobacteria). Reservoir-wide blooms (or patchy concentrations of algae) can occur regardless of season.
- Thermal stratification throughout the reservoir, including the shallow Eastern Arm, limits resupply of dissolved oxygen to bottom waters and reservoir sediments on a seasonal basis. Low to no oxygen for extended periods facilitates methylmercury production in anoxic sediments and/or bottom waters, as well as release of other redox-sensitive compounds like ammonium and orthophosphate that can contribute to overall internal nutrient loading and stimulate algal productivity in subsequent seasons.
- High planktonic algal productivity provides an ongoing source of organic carbon to fuel the microbial community in the reservoir sediments and the water column, which in turn deplete dissolved oxygen from the hypolimnion and reservoir sediments during stratification, and ultimately support mercury methylation.
- The littoral and profundal portions of the aquatic food web are interconnected, whereby TL3 and TL4 fish move between habitats and their prey include both pelagic (i.e., zooplankton, predatory insects) and benthic (i.e., chironomids, crayfish) forms.
- Methylmercury bioaccumulates in the Soulajule Reservoir food web, whereby tissue concentrations increase with trophic level, although patterns may be more complex than a simple integer conceptual model (i.e., TL1 → TL2 → TL3 → TL4) would predict because many reservoir biota are feeding at intermediate and/or multiple trophic levels.
- Spring-time water column mixing in the Eastern Arm may allow dispersal of methylmercury released from deeper bottom sediments to enter reservoir waters, resulting in water, phytoplankton, and zooplankton methylmercury concentrations that are 2−3 times higher than concentrations exhibited in other seasons and/or locations.
- Zooplankton (TL2) and small fish (TL3) methylmercury concentrations peak in the fall following reservoir overturn, due to a build-up of methylmercury in reservoir bottom waters and subsequent incorporation into the food web.

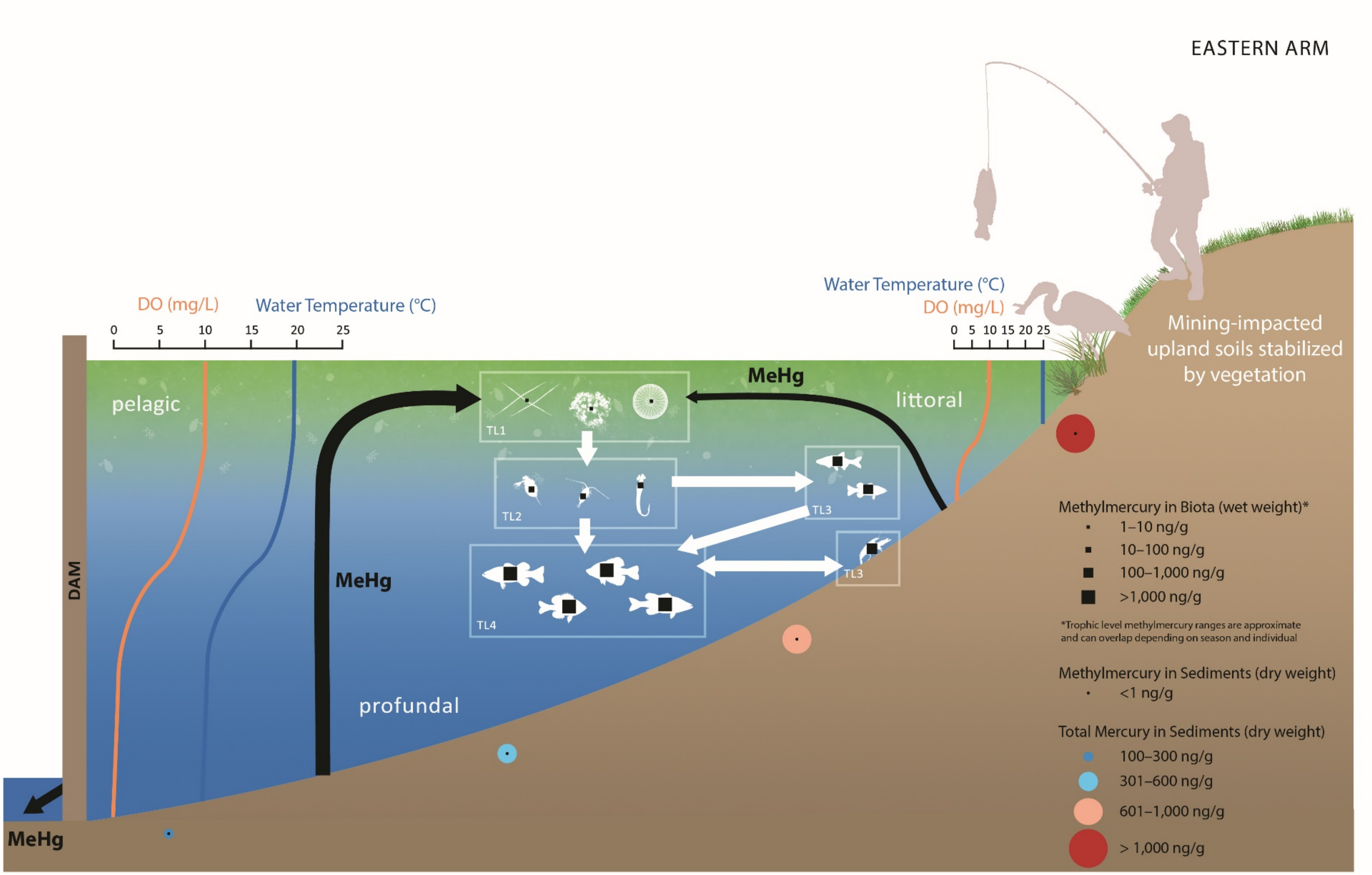


Figure 26. Existing conditions conceptual model of key factors controlling methylmercury production and bioaccumulation in Soulajule Reservoir.

4 PRIORITIZATION OF RESERVOIR MANAGEMENT MEASURES

4.1 Management Objectives

The District's water supply management objectives for Soulajule Reservoir currently include the following:

1. Provide storage and supply for municipal drinking water;
2. Achieve flow requirements in downstream Walker Creek (Table 5);
3. Support appropriate water quality objectives within Soulajule Reservoir and in downstream Arroyo Sausal.

Table 5. Minimum flow release requirements for Soulajule Reservoir.

Season	Normal water year	Dry water year	Critical water year
Winter	20 cfs	10 cfs	0.5 cfs
Summer	5 cfs	2 cfs	0.5 cfs

The Regional Board originally identified mercury management objectives for Soulajule Reservoir and the downstream Arroyo Sausal under "Next Action B," (see Section 0), and has considered development of the Study Plan (Phase 1) as well as implementation of the pilot studies (Phase 2). The mercury management objectives for Soulajule Reservoir are as follows:

1. Reduce loads of mercury from historical mining waste and mercury-laden sediment;
2. Manage water chemistry, especially redox conditions, to decrease methylation;
3. Decrease levels of harmful blue-green algae and increase zooplankton-edible green algae; and,
4. Manage fisheries to decrease methylmercury tissue concentrations.

4.2 Prioritization of Methylmercury Control Actions

As an initial activity under Phase 3 of the Project, District staff and the Stillwater Team reassessed potential methylmercury control actions and used this reassessment to develop priorities for the Reservoir Management Plan. Key assumptions for the seven in-lake and watershed management methods that were initially identified and ranked as potential methylmercury control actions for Soulajule Reservoir (**Error! Reference source not found.**) were then reassessed by District staff, the Regional Board, and technical team members using 'lessons learned' from the Phase 2 pilot studies and prior investigations (Table 6). The group subsequently selected and prioritized a subset of four methylmercury control actions for implementation under the Reservoir Management Plan (Table 6).

The four prioritized methylmercury control actions that will be carried forward into the Reservoir Management Plan include the following:

- Priority 1 − Low water survey of Cycle Mine thick fill as a one-time action in near term;
- Priority 2 – Biomanipulation – sport fish stocking permitting and implementation as a one- or few-times action that would occur prior to other in-lake approaches to address methylmercury production;
- Priority 3 – Hypolimnetic oxygenation system (HOS) design and implementation as a long-term in-lake approach to address methylmercury production; and,
- Priority 4 – Eastern Arm upland erosion control and/or localized in-reservoir sediment capping as a future action only if (a) mine site conditions change or (b) if actions 1–3 are not effective and additional studies indicate that either of these actions would be effective in reducing fish methylmercury levels.

Table 6. Summary of Project Phases 1–3 key assumptions, ratings, lessons learned and priorities for methylmercury control actions.

Phase 1			Phase 2 lessons learned	Phase 3 Reservoir Management Plan priorities
MeHg control action	**Weighted rating (Scale 1–100)**	**Key assumptions**		
Biomanipulation – sport fish stocking	81	• Results of fish community (Study 3) and food web (Study 4) studies would inform selection of appropriate annual stocking rates, species, and age-size class	• Based on fish community composition (Pilot Study 3), best sport fish to stock is adult largemouth bass and spotted bass • Stocking triploid (sterile) fish because stocking a non-native invasive species upstream of an anadromous reach is problematic • Stocking of low-mercury individuals • Initial annual stocking rate 15,000-20,000 TL4 fish (length to be determined)	<u>*Priority 2 – Sport Fish Stocking*</u> • Finalize estimated stocking costs and initial stocking numbers • Determine CDFW permitting requirements and obtain permit • Develop targeted monitoring plan to inform effectiveness and potential need for additional stocking events • Implement sport fish stocking and monitoring for 3–5 years
		• Results of spatial MeHg bioaccumulation study would indicate spatial distribution and whether any stocking strategy should account for a pattern	• MeHg bioaccumulation in TL3 fish tissue is not strongly spatially distributed (Study 1) • Better to base stocking strategy on stocked fish expected dispersal patterns within the reservoir	
		• Adaptive management approach on annual stocking rates, to demonstrate improvements in average sport fish tissue concentrations and reduced stocking over time	• Resulting trophic cascade from sport fish stocking is uncertain, but most likely TL4 fish population up, TL3 fish population down, TL2 cladoceran population up, TL1 algae biomass down • Need to monitor annual sport fish tissue mercury concentrations to determine success	
		• Would occur prior to other in-lake approaches to address MeHg production	• Stocking likely to be a better short-term (2-3 years) strategy than a long-term (> 3 years) strategy • If stocking is selected as a long-term management strategy, consider further elucidation of food web (i.e., uncertainties are currently chironomid MeHg concentrations and TL2 predatory insect importance as a food source for TL3 and TL4 fish)	

Phase 1			Phase 2 lessons learned	Phase 3 Reservoir Management Plan priorities
MeHg control action	**Weighted rating (Scale 1–100)**	**Key assumptions**		
Hypolimnetic oxygenation system (HOS)	79	• Results of Pilot Study 3 would confirm HOS feasibility and cost effectiveness	• Preliminary design indicates feasible options for HOS infrastructure	*Priority 3 – HOS Design and Implementation* • Undertake additional studies to inform HOS design – Geotech diver survey of near dam area for HOS platform – Tracer/mixing study for mixing of water in the hypolimnion and overlying waters moving away from oxygenated water releases (are there likely to be anoxic patches with HOS implementation?) • 30% HOS Design – Refine costs with ECO2 regarding cone and related equipment – Work with District to develop siting, power, pipeline/duct bank alignments, etc. – Develop targeted monitoring plan to include implementation, effectiveness and validation monitoring components for HOS • 100% HOS Design – Final cost and design refinements – Implement any additional baseline monitoring needed based on the targeted monitoring plan • Bid, construct and operate HOS and monitor
		• Results of Pilot Study 1 would indicate whether algae, zooplankton, and small fish near the dam exhibit highest levels of MeHg bioaccumulation and accordingly where to focus HOS	• Methylmercury tissue concentrations higher in spring in Eastern Arm for phytoplankton and zooplankton, but tissue concentrations in TL3 fish similar across Eastern Arm, Western Arm, and near dam for all seasons • Seasonal BAF pattern apparent, but mainly due to spring increase in surface water MeHg concentrations in the reservoir as a whole, and Eastern Arm in particular • Excluding the decrease in spring BAFs from surface water MeHg, BAFs were generally similar across seasons at all sites • HOS installation near dam still makes sense due to infrastructure needs and proximity to deepest, most anoxic waters	
		• Liquid oxygen (LOX) could be trucked, delivered and stored adjacent to the existing pump station on District property. If LOX delivery is not feasible due to space/transportation constraints, onsite oxygen generation is also feasible, though more expensive and involves more equipment and on-going maintenance.	• Pilot Study 6 provides 1.6 tons/day as conceptual sizing estimate to initiate preliminary discussions with oxygenation system manufacturers and liquid oxygen suppliers or on-site oxygen generation equipment suppliers • 2016 bathymetry indicates a slightly larger hypolimnion than estimated in Pilot Study 6, so THOD could be closer to 1.7 tons/day but this is within the uncertainty of the preliminary design calculations • HOS building and oxygen storage could be located on left side of dam or below the dam. Transferring oxygen and electric power into the reservoir from either location is quite feasible	
		• A Speece cone and supporting equipment would be located near the dam, with extensions into reservoir arms if results of Pilot Study 1 indicate that methylation and bioaccumulation in the arms is important	• Pilot Study 6 provides HOS Speece cone conceptual design with four diffuser manifolds to oxygenate hypolimnion in Western Arm, Eastern Arm, the area upstream of the old dam, and the area in between the old and new dams • Tracer study to refine understanding of mixing into the arms would be valuable for 30% HOS design • Pilot Study 6 flux chamber results indicate high degree of variability in methylation across sediments, with intermediate redox conditions producing the highest MeHg fluxes and low redox conditions reducing MeHg production • Pilot Study 6 flux chamber results highlight the importance of maintaining high dissolved oxygen at the sediment-water interface	

Phase 1			Phase 2 lessons learned	Phase 3 Reservoir Management Plan priorities
MeHg control action	**Weighted rating (Scale 1–100)**	**Key assumptions**		
Upland erosion control in Eastern Arm	74	• Results of Pilot Study 7 would indicate whether upland erosion is a significant and continuing external source of Hg	• No evidence of mining at two unnamed mining sites • Franciscan and Cycle mine sites have revegetated, not visibly eroding, not transporting sediment containing mercury into reservoir	<u>*Priority 1 – Low Water Survey of Cycle Mine Thick Fill*</u> • Conduct a low water survey of the Cycle Mine thick fill to provide visual evidence (or lack thereof) of active erosion of the fill materials into the reservoir • Regional Board to provide *in situ* x-ray fluorescence (XRF) measurements of TotHg along the thick fill shoreline to increase the number of data points available for characterizing TotHg levels in the thick fill <u>*Priority 4 – Eastern Arm Upland Erosion Control and/or In-reservoir Sediment Capping*</u> • Consider this action if stocking and HOS implementation do not significantly improve MeHg bioaccumulation in reservoir TL3 and TL4 fish
		• Landowners at mine sites would be amenable to recommended erosion control measures	• District can continue to work with landowners to minimize erosion	
		• Cattle grazing near mine sites would not interfere with erosion control measures	• Steep terrain and minimal forage would not attract cattle to the slopes around the old mine sites where cattle grazing could disrupt soils that apparently are stable. District would support exclusion fencing only if field observation and/or sampling for Hg indicates a problem, and at that point District would work with ranchers to address.	
		• Occurs in conjunction with other in-lake approaches to address MeHg production and bioaccumulation	• Managing reservoir chemistry is current priority and no additional BMPs recommended	
Vigorous epilimnetic mixing (VEM) in Eastern Arm	73	• Results of Pilot Study 1 would indicate whether algae, zooplankton, and small fish in the Eastern Arm exhibit relatively high levels of MeHg bioaccumulation • Note VEM has been successfully tested in the field for algae control but is yet untested for MeHg control	• Methylmercury tissue concentrations higher in spring in Eastern Arm for phytoplankton and zooplankton, but tissue concentrations in TL3 fish similar across Eastern Arm, Western Arm, and near dam for all seasons • Seasonal BAF pattern apparent, but mainly due to spring increase in surface water MeHg concentrations in the reservoir as a whole, and Eastern Arm in particular • Excluding the decrease in spring BAFs from surface water MeHg, BAFs were generally similar across seasons at all sites	• This potential control action will not be carried forward into Phase 3
		• *New:* Results of Pilot Study 1 would indicate whether seasonal algal blooms are concentrated in the Eastern Arm and are dominated by blue-green algae (cyanobacteria) such that a VEM located in this arm would be effective	• Seasonal algal blooms are dominated by blue-green algae (cyanobacteria) – mainly *Aphanizomenon flos-aquae* in spring/summer • Blooms are dynamic and reservoir-wide blooms (or patchy concentrations of algae) can occur regardless of season • VEM located in the Eastern Arm would not address reservoir-wide blooms or patchy concentrations of algae located elsewhere in the reservoir	

Phase 1			Phase 2 lessons learned	Phase 3 Reservoir Management Plan priorities
MeHg control action	**Weighted rating (Scale 1–100)**	**Key assumptions**		
Biomanipulation – prey fish stocking	71	• Results of fish community (Study 3) and food web (Study 4) studies would inform selection of appropriate annual stocking rates, species, and age-size class	• Based on fish community composition (Pilot Study 3), best sport fish to stock is adult largemouth bass and spotted bass (see above)	• This potential control action will not be carried forward into Phase 3
		• Results of spatial MeHg bioaccumulation study would indicate spatial distribution and whether any stocking strategy should account for a pattern	• MeHg bioaccumulation in TL3 fish tissue is not strongly spatially distributed (Study 1) • Better to base stocking strategy on stocked fish dispersal patterns within the reservoir	
		• Adaptive management approach on annual stocking rates, to demonstrate improvements in average prey fish tissue concentrations and reduced stocking over time	• Resulting trophic cascade from prey fish stocking is uncertain, but most likely TL3 fish population up, TL2 cladoceran population down, TL1 algae biomass up, TL4 fish growth rates up • Need to monitor annual sport fish tissue concentrations to determine success	
		• Would occur in conjunction with other in-lake approaches to address MeHg production	• Stocking likely to be a better short-term (2-3 years) strategy • If stocking is selected as a long-term management strategy, consider further elucidation of food web (i.e., chironomid MeHg and TL2 predatory insect importance)	
Dredging of reservoir sediments in Eastern Arm	65	• Results of Pilot Study 7 would indicate whether upland erosion is a significant and continuing external source of Hg	• Pilot Study 7 did not reveal active upland erosion problems but did not include the Eastern Arm location where Cycle Mine sediments (those submerged at higher reservoir elevation) might release Hg into the reservoir.	*Priority 1 – Low Water Survey of Cycle Mine Thick Fill* • Conduct a low water survey of the Cycle Mine thick fill to provide visual evidence (or lack thereof) of active erosion of the fill materials into the reservoir • Regional Board to provide *in situ* XRF measurements of TotHg along the thick fill shoreline to increase the number of data points available for characterizing TotHg levels in the thick fill *Priority 4 – Eastern Arm Upland Erosion Control and/or In-reservoir Sediment Capping* • Consider this action if stocking and HOS implementation do not significantly improve MeHg bioaccumulation in reservoir TL3 and TL4 fish
		• Results of Pilot Study 1 would indicate whether algae, zooplankton, and small fish in the Eastern Arm exhibit relatively high levels of MeHg bioaccumulation	• Methylmercury tissue concentrations higher in spring in Eastern Arm for phytoplankton and zooplankton, but tissue concentrations in TL3 fish similar across Eastern Arm, Western Arm, and near dam for all seasons • Seasonal BAF pattern apparent, but mainly due to spring increase in surface water MeHg concentrations in the reservoir as a whole, and Eastern Arm in particular Excluding the decrease in spring BAFs from surface water MeHg, BAFs were generally similar across seasons at all sites	

<table>
<tr><th colspan="3">Phase 1</th><th rowspan="2">Phase 2
lessons learned</th><th rowspan="2">Phase 3
Reservoir Management Plan
priorities</th></tr>
<tr><th>MeHg control action</th><th>Weighted rating (Scale 1–100)</th><th>Key assumptions</th></tr>
<tr><td rowspan="3">Capping of reservoir sediments in Eastern Arm</td><td rowspan="3">59</td><td>• Results of Pilot Study 7 would indicate whether upland erosion is a significant and continuing external source of Hg</td><td>• Available data do not support sediment capping now but capping would be feasible, albeit prohibitively expensive, if observed erosion at the location where Cycle Mine sediments (those submerged at higher reservoir elevation) might release Hg into the reservoir.</td><td rowspan="3"><u>Priority 1 – Low Water Survey of Cycle Mine Thick Fill</u>
• Conduct a low water survey of the Cycle Mine thick fill to provide visual evidence (or lack thereof) of active erosion of the fill materials into the reservoir
• Regional Board to provide in situ XRF measurements of TotHg along the thick fill shoreline to increase the number of data points available for characterizing TotHg levels in the thick fill

<u>Priority 4 – Eastern Arm Upland Erosion Control and/or In-reservoir Sediment Capping</u>
• Consider this action if stocking and HOS implementation do not significantly improve MeHg bioaccumulation in reservoir TL3 and TL4 fish.</td></tr>
<tr><td>• Results of Pilot Study 1 would indicate whether algae, zooplankton, and small fish in the Eastern Arm exhibit relatively high levels of MeHg bioaccumulation</td><td rowspan="2">• Methylmercury tissue concentrations higher in spring in Eastern Arm for phytoplankton and zooplankton, but tissue concentrations in TL3 fish similar across Eastern Arm, Western Arm, and near dam for all seasons
• Seasonal BAF pattern apparent, but mainly due to spring increase in surface water MeHg concentrations in the reservoir as a whole, and Eastern Arm in particular
• Excluding the decrease in spring BAFs from surface water MeHg, BAFs were generally similar across seasons at all sites</td></tr>
<tr><td>• Eastern Arm sediments are non-mobile</td></tr>
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MeHg=Methylmercury, Hg=Mercury, TotHg=Total Mercury, BAF=Bioaccumulation Factors, TL=Trophic Level, HOS=Hypolimnetic Oxygenation System

4.2.1 Low water survey of Cycle Mine thick fill

The highest priority methylmercury control action is to provide additional data related to the potential for active upland erosion of soil into the reservoir by conducting a low-water survey of the Cycle Mine thick fill (Table 5). Although there was little to no visual evidence of actively eroding upland soils at the Cycle Mine thick fill location due to well-vegetated slopes (Section 3.1), wave-action induced erosion of the thick graded fill located along the reservoir shoreline is possible. Since total mercury in the thick fill was highly variable (Figure 8, Table 3), further investigation of the fill at the reservoir water line during low water conditions would provide visual evidence (or lack thereof) of active erosion. Additionally, the Regional Board volunteered to provide *in situ* XRF measurements of total mercury along the thick fill shoreline to increase the number of data points available for characterizing total mercury levels associated with the historical Cycle Mine.

4.2.2 Sport fish stocking

Sport fish stocking was the only potential management method that received no negative ratings during the initial screening effort (Table 1). This is largely because adding sport fish un-impacted by methylmercury is the most direct way to reduce average sport fish tissue methylmercury concentrations, it carries a relatively low risk of failure with respect to disturbance of the existing food web, it is relatively inexpensive, and it requires little to no engineering or operation/maintenance requirements. Assuming that predation is the primary population control mechanism in Soulajule Reservoir, increases in sport fish (TL4) abundance would increase predation on smaller (TL3) fish and predatory insects (TL2), reducing their populations accordingly. Reducing the prevalence of bluegill and juvenile spotted bass, fish that are both abundant and prey heavily upon the largest cladocerans (TL2 primary consumers), may increase the abundance of these zooplankton and exert stronger predation pressure on the phytoplankton community in Soulajule Reservoir, inherently reducing blue-green algae (cyanobacteria) populations and reducing a source of carbon to the microbial community involved in mercury methylation (see also Section 3.6). For these reasons, sport fish stocking was identified as the second highest priority methylmercury control action to include in the Reservoir Management Plan.

Stocking Soulajule Reservoir with low-mercury sport fish would have the direct effect of diluting the average body burden of mercury in the broader sport fish population. Although mercury levels in individual sport fish residing in the reservoir before stocking would remain unchanged, overall average mercury concentrations in the sport fish population, taken as a whole, would be reduced. Stocking low-mercury sport fish at a ratio of 1:1 relative to resident sport fish would have the immediate effect of diluting the average sport fish mercury concentration by 50 percent. Increasing the ratio of “clean” to “contaminated” fish would dilute average sport fish mercury concentrations further. Because the Fish Population Study (Pilot Study 3) was not designed to provide an estimate of population size, the number of fish needed to achieve a 1:1 ratio cannot be precisely estimated given available information. However, an initial stocking of 15,000−20,000 adult bass is expected to provide a sufficiently large population of low-mercury sport fish that the effect on average tissue concentrations of mercury would be measurable. Subsequent stocking numbers would be adjusted based on the results of annual monitoring of mercury concentrations in sportfish tissue.

Sport fish stocking would occur in conjunction with another in-lake approach (or approaches) focused on controlling methylmercury production and water column concentrations. Sport fish stocking also would be implemented using an adaptive management approach with respect to annual stocking rates, with the goal of demonstrating near-term improvements in average sport fish tissue concentrations and reducing stocking over the long-term as other in-lake approaches successfully reduce methylation in the reservoir.

While stocked sport fish could be washed downstream and interfere with habitat beneficial uses in Arroyo Sausal by competing with or preying upon native anadromous fish and other aquatic species, this would represent an incremental effect, since largemouth bass are already in the reservoir. However, investigation of agency permitting requirements for sport fish stocking in Soulajule Reservoir would need to be undertaken in the planning stages for this control action (Table 5).

4.2.3 Hypolimnetic oxygenation system

HOS installation received the highest rating during the initial screening effort for compatibility with water supply objectives because, in addition to decreasing or eliminating methylmercury production, HOS would decrease blue-green algae (cyanobacteria) dominance and associated taste and odor compounds, as well as nutrient release (orthophosphate, ammonium, manganese, iron) from anoxic sediments during periods of stratification, and thus would enhance overall water quality in the reservoir (Table 5). By increasing dissolved oxygen in deeper waters, HOS also would increase the availability of daytime refugia habitat for zooplankton, which could increase grazing pressure on algae and further improve water quality. Fish habitat would extend into deeper waters, potentially enhancing sport fish recreational opportunities. In addition, HOS may provide a meaningful offset of the potential effects of changing reservoir stratification patterns (e.g., earlier seasonal stratification, longer period of stratification) associated with climate change projections for the northern coastal California region. Oxygenation of Soulajule Reservoir bottom waters that would be discharged downstream would also improve water quality conditions for salmonid habitat in Arroyo Sausal.

The *Evaluation of Reservoir Seasonal Oxygen Demand and Sediment Response to Hypolimnetic Oxygenation Study* (Pilot Study 4) included conducting further studies to evaluate hypolimnetic oxygenation system implementation feasibility, based on the hypothesis that adding oxygen to the hypolimnion would control methylmercury formation in Soulajule Reservoir. The initial steps of investigating HOS feasibility involved determining the approximate additional oxygen needed in the reservoir, particularly the hypolimnion, to maintain oxygenated conditions within the water column and surficial sediments.

HOS equipment must have capacity to deliver sufficient oxygen to the hypolimnion during periods of peak oxygen demand, which typically occur in spring and early summer on Soulajule Reservoir. The design parameter used for determining the equipment sizing is the total hypolimnetic dissolved oxygen demand (THOD), which is calculated as the sum of the water column oxygen demand (WCOD) and the sediment oxygen demand (SOD)[5], as indicated below. The WCOD accounts for the algal respiration and bacterial decomposition of suspended materials that occurs within the water column and consumes dissolved oxygen. The SOD accounts for oxygen consumption by both bacterial decomposition in the sediments and oxidation of reduced compounds within the sediments. Key factors that control the rate of SOD include temperature, water currents and dissolved oxygen concentration at the sediment-water interface, as determined by testing maximum oxygen demand at elevated water currents and dissolved oxygen concentration in laboratory bench-scale sediment testing (Beutel 2016a). When summed, the WCOD and SOD account for the total mass of oxygen that would be expected to be consumed (i.e., THOD).

For the purposes of calculating a conservative THOD, the Stillwater Team assumed that the WCOD was 0.08 milligram per liter per day (mg/L-d) throughout the hypolimnion, which was the highest depth-averaged dissolved oxygen depletion rate measured between quarterly profiles collected in 2014, neglecting the water just above the sediments[6] (Brown and Caldwell 2016a). The ’worst-case’ design SOD for Soulajule Reservoir” of 1.4 grams per square meter per day (g/m2/d), as determined by testing maximized oxygen demand in laboratory bench-scale sediment testing (Beutel 2016a), to the 173-ac surface area below the thermocline (i.e., hypolimnion surface area). The estimated THOD for Soulajule Reservoir at full capacity is approximately 3,100 pounds per day (lb/d), or 1.57 tons per day (tons/d). Table 6 presents the additional input and result assumptions used to calculate THOD.

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[5] $THOD = WCOD + SOD$; where THOD = total hypolimnetic oxygen demand (lb/d); WCOD = water column oxygen demand (lb/d); SOD = sediment oxygen demand (lb/d)

[6] The WCOD estimate for Soulajule Reservoir excludes the 10-foot layer of water immediately above the sediments to avoid double-counting the oxygen demand that is exerted by the sediments and influences the water overlying the sediments; this oxygen demand is accounted for separately in the SOD.

Table 7. Summary of Hypolimnetic Oxygenation System (HOS) sizing calculations.

Data/Calculations	Inputs/Results	Comments
Water surface elevation	332 feet	This elevation assumes the reservoir is full.
Hypolimnion elevation	312 feet	This elevation assumes the hypolimnion begins at 20 feet below the water surface.
Hypolimnion volume	4,500 acre-feet	Obtained from 2015 hypsographic data.
Hypolimnion surface area	173 acres	Obtained from District bathymetric data.
Hypolimnion DO depletion rate	0.08 mg/L-d	This average DO depletion rate was calculated from the Feb to May 2014 DO profile data, and verified with the 2013, 2015, and 2016 data.
WCOD rate	978 lb/d	Hypolimnion Volume x DO Depletion Rate = WCOD Rate
Design SOD rate	1.4 $g/m^2/d$	Empirical "worst case design SOD" (bench scale tests, Beutel 2016a).
SOD rate	2,200 lb/d	Design SOD Rate x Surface Area of Hypolimnion Layer = SOD Rate
THOD rate	3,100 lb/d	WCOD Rate + SOD Rate = THOD Rate

DO=dissolved oxygen, WCOD= water column oxygen demand, SOD= sediment oxygen demand, THOD= total hypolimnetic dissolved oxygen demand, lb=pound, d=day

Based on Soulajule Reservoir bathymetry and the THOD estimates presented above, Figure 27 provides an initial schematic for an in-reservoir Speece cone-based HOS and the associated equipment. The system would consist of an HOS equipment building, and it would have an oxygen delivery pipeline and electrical power provided to the submerged Speece cone. Although the HOS building is shown near the reservoir, alternatively, the HOS building might be located below the dam where the District has access to larger electrical service and has better truck accessibility. Piping from the Speece cone would convey oxygenated water to four diffuser manifolds positioned to oxygenate the reservoir's hypolimnion in the Western Arm, Eastern Arm, the area upstream of the old dam, and the area in between the old and new dams. Conceptual design would determine the relative fraction of the total oxygen delivered to each diffuser is the fractional oxygen demand depends on the mass of oxygen required to oxygenate the respective hypolimnion volumes in each of the four areas. Oxygen delivery to each diffuser manifold would be adjusted at start-up using valves on each manifold. Valves included on each individual diffuser port would allow further adjustment.

The primary areas of mercury contamination in the reservoir sediments are located near the Franciscan and Cycle mines, which are approximately 1.5 to 2.25 miles upstream of the proposed Eastern Arm diffuser. Although this seems like a long distance from the diffuser, natural reservoir circulation, e.g., wind-induced shear, likely will carry the oxygenated discharge upstream from the diffuser. For example, the HOS in Camanche Reservoir, operated by East Bay Municipal Utility District, achieved bubble plume transport up to 3 miles into the reservoir (ECO_2, 2014). It should be noted that Camanche Reservoir is relatively straight, whereas Soulajule is more sinuous and protected by adjacent hills. A tracer study during conceptual design would examine further the predicted oxygenated water plume upstream penetration.

Based on the Soulajule Reservoir THOD calculation, the Stillwater Team recommends conceptual sizing of the HOS to deliver approximately 3,100 lb/d (1.57 tons/d). Going forward, the District can use the 1.57 tons/d oxygen demand estimate to initiate preliminary discussions with liquid oxygen suppliers or oxygen generation equipment suppliers to determine approximate sizes of storage tanks, mechanical equipment, electrical demands, and other design requirements. Overall, the industry's experience has shown that under-sizing an HOS is a bigger risk than over-sizing an HOS. The incremental cost for a slightly larger system is minimal. If the system ultimately has more capacity than needed after reaching a future steady state, the District has can fine-tune the operation of the system by turning down the oxygen feed rate or by running the system intermittently. The reader should note that one Speece cone supplier ECO2, fabricates cones with somewhat standard capacities. For example, the District may purchase a nominal 2 tons/d system as the next size larger than the calculated capacity.

Further work during design of the HOS should determine the relative fraction of oxygenated water to be delivered through each diffuser manifold, assess the oxygenated plume transport up the Eastern Arm of the reservoir, including whether oxygen delivery to the manifolds could be adaptively managed on an annual basis to provide additional dissolved oxygen to the Eastern Arm (as needed

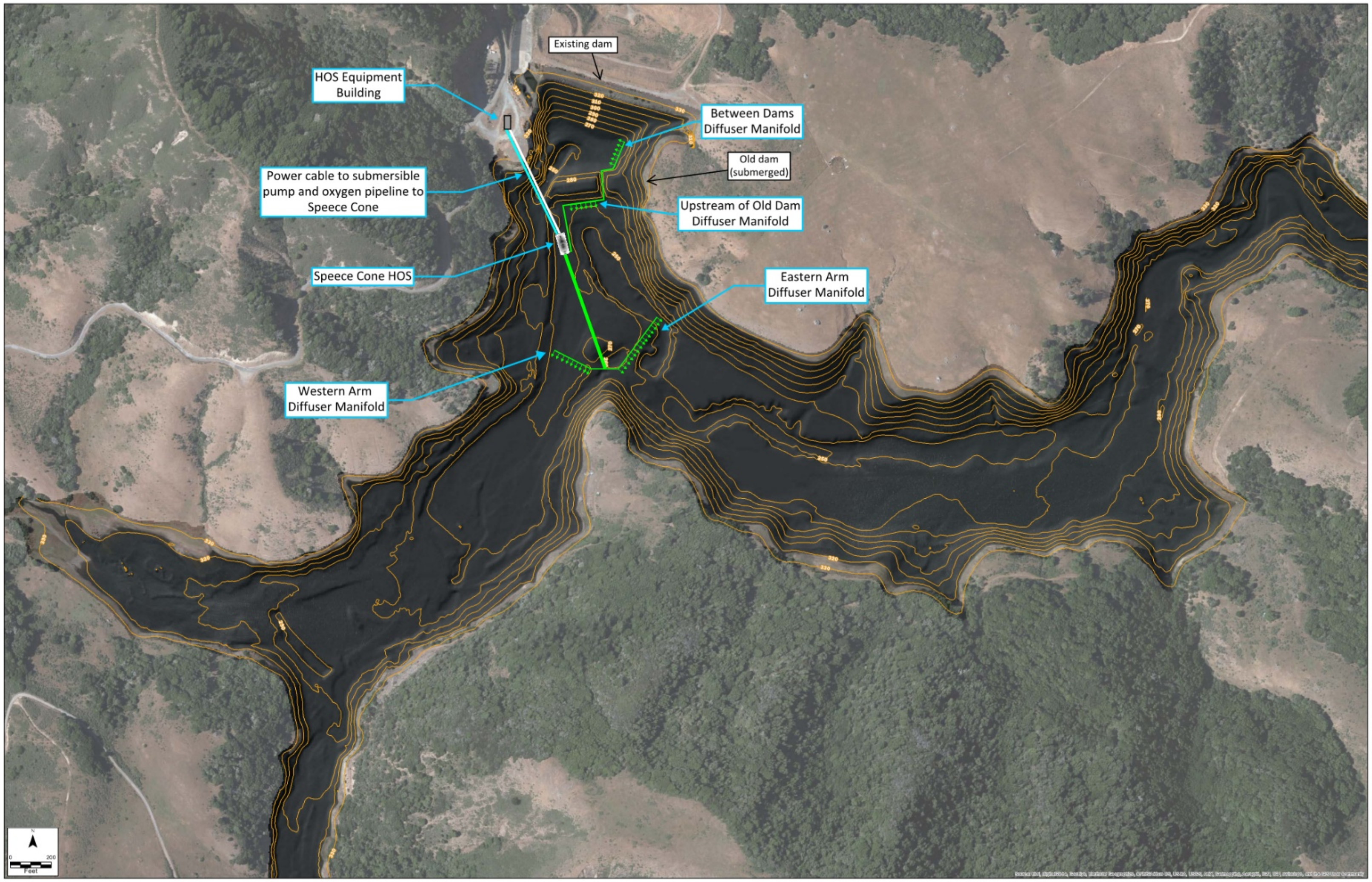

Figure 27. Preliminary in-reservoir HOS location and configuration.

4.2.4 Eastern Arm upland erosion control and/or in-reservoir sediment capping

The District later would further evaluate Eastern Arm Upland Erosion control or in-reservoir sediment capping if stocking and HOS implementation do not significantly improve methylmercury bioaccumulation in reservoir TL3 and TL4 fish.

4.2.5 Grazing best management practices

Cattle exclusion fencing for the Soulajule Reservoir shoreline is being considered as part of the Tomales Bay Watershed Conditional Waiver of Waste Discharge Requirements for Grazing Lands (2013 Grazing Waiver). The 2013 Grazing Waiver is a parallel but related process to the Walker Creek Mercury TMDL (SFBRWQCB 2008) in that the grazing waiver implements the mercury TMDL in specific locations within the Walker Creek watershed. The 2013 Grazing Waiver states that to avoid the inadvertent discharge of mercury-laden sediments to Walker Creek, grazing lands downstream of the Gambonini Mine and Soulajule Reservoir must incorporate grazing management practices to minimize the discharge of mercury or the production of methylmercury (SFBRWQCB 2013). Cattle grazing occurs within the uplands adjacent to Soulajule Reservoir, including along accessible portions of the reservoir shoreline such as the shallow Eastern and Western arms. Accordingly, the District is considering installing cattle exclusion fencing along the perimeter of Soulajule Reservoir to reduce shoreline erosion and related nutrient loading.

Since results of the Phase 2 pilot study characterizing upland mercury source loading indicated no visible signs of erosion at the historical mine sites or undisturbed background sites, cattle exclusion is not expected to directly affect external mercury loading to Soulajule Reservoir. However, the installation of exclusion fencing along the reservoir shoreline would indirectly address mercury management objective no. 2 (manage water chemistry) and no. 3 (decrease levels of harmful blue-green algae) (see also Section 4.1) by reducing a long-term source of external nutrients to the reservoir, where nutrients support excessive algal growth, low redox conditions, and ultimately fuel the microbial community engaged in mercury methylation. Given the greater relative importance of internal nutrient loading as a key methylation factor under existing conditions (see Section 3.6), exclusion fencing alone would not be an effective methylmercury control strategy. However, a reduction in external nutrient loading would help to maintain improvements provided by installation of an HOS. In summary, because cattle exclusion fencing along the Soulajule Reservoir shoreline would provide an additional indirect, long-term benefit with respect to mercury methylation and bioaccumulation control, it will be included as a related control action in the Reservoir Management Plan.

4.3 Phased Implementation of Priority Methylmercury Control Actions

In order to best achieve effective and fiscally responsible implementation of the priority methylmercury control actions, the District would implement the actions in additional Project phases, as described in Table 7. The preliminary schedule assumes that the District would implement the first control action (Phase 4a) in fall 2018. The Reservoir Management Plan will develop and discuss additional details of the phased approach.

Table 8. Reservoir Management Plan (RMP) priorities and phased implementation schedule.

RMP implementation[1]	**2018**				**2019**				**2020**				**2021**				**2021**			
Phase 4a – Low Water Survey of Cycle Mine Thick Fill																				
Phase 4b – Sport Fish Stocking																				
Phase 5a – Additional Studies to Inform HOS Design																				
Phase 5b – 30% HOS Design																				
Phase 5c – 100% HOS Design																				
Phase 5d – Construct and Operate HOS																				
Phase 6a – Eastern Arm Upland Erosion Control and Capping[2]																				

[1] Phase schedules are shown in dark grey shading. Pink indicates the planning phase prior to implementation. Light grey shading indicates possible implementation of management action based on the success of previous phases.

[2] Consideration of these management practices if stocking and Hypolimnetic Oxygenation System (HOS) implementation not significantly improving methylmercury bioaccumulation

5 SUMMARY AND NEXT STEPS

The next Phase 3 step of the Project involves development of the Soulajule Reservoir Management Plan, which would expand upon the methylmercury control actions described in Section 4. The Reservoir Management Plan will include the following: management objectives, prioritized control actions, implementation approach (e.g., spatial location, schedule), monitoring requirements based on a conceptual model of anticipated system response, success criteria, and responsibilities/roles for carrying out each of the control actions. For high priority control actions, the Reservoir Management

Plan will include refined cost considerations compared with those provided in the Study Plan (Stillwater Sciences and Brown and Caldwell 2013).

6 REFERENCES

Anderson. T. J., R. S. Stelzer, H. G. Drecktrah, and S. L. Eggert. 2012. Secondary production of Chironomidae in a large eutrophic lake: implications for lake sturgeon production. Freshwater Science 31: 365–378. http://www.journals.uchicago.edu/doi/abs/10.1899/11-042.1

Beutel, M. 2016a Soulajule Reservoir Methylmercury Control Measures Study - Phase 2, Task 6; Sediment Oxygen Demand Report. Prepared by University of California Merced, Merced, California for Marin Municipal Water District, Corte Madera and Stillwater Sciences, Berkeley, California.

Beutel, M. 2016b. Soulajule Reservoir Sediment Flux Report. Prepared by University of California Merced, Merced, California for Marin Municipal Water District, Corte Madera and Stillwater Sciences, Berkeley, California.

Beutel, M. W. 2016c. The other internal loading – a look at nitrogen and mercury. Lakeline 36: 13–16.

Beutel, M. W. 2015. Final Lake Hodges Reservoir Sediment Flux Study. Report for City of San Diego to Brown and Caldwell Consultants, Walnut Creek, California.

Bouvy, M., M. Pagano, and M. Troussellier. 2001. Effects of a cyanobacterial bloom (*Cylindrospermopsis raciborskii*) on bacteria and zooplankton communities in Ingazeira reservoir (northeast Brazil). Aquat. Microb. Ecol. 25: 215–227.

Branfireun, B. A., D. Fowle, and D. Krabbenhoft. 2009. Reservoir water level fluctuation and methylmercury cycling. Final Project Report for the Minnesota Power St. Louis River Hydroelectric Sediment Mercury Research Project. Submitted to R. Goldstein (EPRI) and A. Neudahl (Minnesota Power).

Brown and Caldwell and Stillwater Sciences. 2010. Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study Project No. 138402. Prepared by Brown and Caldwell, Walnut Creek, and Stillwater Sciences, Berkeley, California for Marin Municipal Water District, Corte Madera, California.

Brown and Caldwell and Stillwater Sciences. 2013. Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study Project No. 138402. Prepared by Brown and Caldwell, Walnut Creek, and Stillwater Sciences, Berkeley, California for Marin Municipal Water District, Corte Madera, California.

Brown and Caldwell. 2016a. Updated dissolved oxygen demand calculations for Soulajule Reservoir for conceptual sizing of a hypolimnetic oxygenation system. Prepared by Brown and Caldwell, Walnut Creek, California for Marin Municipal Water District, Corte Madera, and Stillwater Sciences, Berkeley, California.

Brown and Caldwell. 2016b. Upland soil mercury contribution to Soulajule Reservoir. Prepared by Brown and Caldwell, Walnut Creek, California for Marin Municipal Water District, Corte Madera and Stillwater Sciences, Berkeley, California.

Cabana, G., A. Tremblay, J. Kalff, and J. Rasmussen. 1994. Pelagic food chain structure in Ontario Lakes: A determinant of mercury levels in lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 51: 381–389.

Carrasco, L., L. Benejam, J. Benito, J. M. Bayona, and S. Diez. 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. Environmental International 37: 1,213–1,218.

Clayden, M. G., K. A. Kidd, B. Wyn, J. L. Kirk, D. C. G. Muir, and N. J. O'Driscoll. 2013. Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environmental Science and Technology 47: 12,047–12,053.

DeMott, W. R. 1986. The role of taste in food selection by freshwater zooplankton. Oecologia 69: 334–340.

DeMott, W. R. and F. Moxter. 1991. Foraging on cyanobacteria by copepods: responses to chemical defenses and resource abundance. Ecology 72: 1,820–1,834.

Dickman, E. M., J. M. Newell, M. J. Gonzalez, and M. J. Vanni. 2008. Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. Proc. Natl. Acad. Sci. U.S.A. 105: 18,408–18,412.

Eagles-Smith, C. A., T. H. Suchanek, A. E. Colwell, and N. G. Anderson. 2008. Mercury trophic transfer in a eutrophic lake: the importance of habitat-specific foraging. Ecological Applications. 18: 196–212.

Evers, D. C., Y-J. Han, C. T. Driscoll, N. C. Kamman, M. W. Goodale, K. F. Lambert, T. M. Holsen, C. Y. Chen, T. A. Clair, and T. Butler. 2007. Biological mercury hotspots in the northeastern United States and southeastern Canada. Bioscience 57: 29–43.

Fabbro, L. D., and L. V. Duivenvoorden. 1996. Profile of a bloom of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju in the Fitzroy River in tropical Central Queensland. Mar. Freshw. Res. 47: 685–694.

Forsyth, D. J., and M. R. James. 1988. The Lake Okaro ecosystem: Production of the chironomid Polypedilum pavidus and its role as food for two fish species. New Zealand Journal of Marine and Freshwater Research 22: 327–335. http://www.tandfonline.com/doi/pdf/10.1080/00288330.1988.9516305

Gantner, N., M. Power, D. Iqaluk, M. Meili, H. Borg, M. Sundbom, K. R. Solomon, G. Lawson, and D. C. Muir. 2009. Mercury concentrations in landlocked arctic char (*Salvelinus alpinus*) from the Canadian arctic. Part I: Insights from trophic relationships in 18 lakes. Environmental Toxicology and Chemistry 29: 621–632.

García-Berthou, E., and Moreno-Amich, R. 2000. Food of introduced pumpkinseed sunfish: ontogenetic diet shift and seasonal variation. Journal of Fish Biology 57: 29–40.

Ger, K. A., P. Urrutia-Cordero, P.C. Frost, L.A. Hansson, O. Sarnelle, A.E. Wilson, and M. Lurling. 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful Algae. 54: 128–144.

Gilbert, J. J. 1990. Differential effects of *Anabaena affinis* on cladocerans and rotifers: mechanisms and implications. Ecology 71: 1,727–1,740.

Gliwicz, Z. M. 1990a. Daphnia growth at different concentrations of blue-green filaments. Arch. Hydrobiol. 120: 51–65.

Gliwicz, Z. M. 1990b. Food thresholds and body size in cladocerans. Nature 343: 638–640.

Godinho, F. N. and M. T. Ferreira. 1994. Diet composition of largemouth black bass, *Micropterus salmoides*, in southern Portuguese reservoirs: its relation to habitat preference. Fisheries Management and Ecology 1: 129–127.

Godinho, F. N., Ferreira, M. T., and R. V. Cortes. 1997. The environmental bases of diet variation in pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, along an Iberian river basin. Environmental Biology of Fishes 50.

Gorski, P., D. Armstrong, J. Hurley, and D. Krabbenhoft. 2008. Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga. Environmental Pollution 154: 116–123.

Hawkins, P., and W. Lampert. 1989. The effect of Daphnia body size on filtering rate inhibition in the presence of a filamentous cyanobacterium. Limnol. Oceanogr. 34: 1,084–1,089.

Horne, A. J., J. C. Sandusky, and C. J. W. Carmiggelt. 1979. Nitrogen fixation in Clear Lake, California. III. Repetitive synoptic sampling of the spring *Aphanizomenon* blooms. Limnol. Oceanogr. 24: 316–328.

Kainz, M., M. Lucotte, and C.C Parrish. 2003. Relationships between organic matter composition and methyl mercury content of offshore and carbon-rich littoral sediments in an oligotrophic lake. Canadian Journal of Fisheries and Aquatic Sciences 60: 888–896.

Kirk, K. L., and J. J. Gilbert. 1992. Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. Ecology 73: 2,208–2,217.

Koski, M., K. Schmidt, J. Engstrom-Ost, M. Viitasalo, S. Jonasdottir, and S. Repka. 2002. Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. Limnol. Oceanogr. 47: 878–885.

Kreutzer, C., and W. Lampert. 1999. Exploitative competition in differently sized *Daphnia* species: a mechanistic explanation. Ecology. 80: 2,348–2,357.

Lampert, W. 1981. Toxicity of the blue-green *Microcystis aeruginosa*: effective defense mechanism against grazing pressure by Daphnia. Verh. Int. Ver. Limnol. 21: 1,436–1,440.

Lobanov, A. L. 2002. Feeding. *In* Beetles (Coleoptera) and Coleopterologist. Website http://www.zin.ru/Animalia/Coleoptera/eng/biol3.htm. [Accessed April 2017].

Lynch, M. 1980. Aphanizomenon blooms: alternate control and cultivation by *Daphnia pulex*. Am. Soc. Limnol. Oceanogr. Spec. Symp. 28: 299–304.

Marin Municipal Water District. (MMWD) 2010. Mercury Mines in the Soulajule Watershed.

Mason, R. P., J. R. Reinfelder, and F. M. M. Morel. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environmental Science and Technology 30: 1,835–1,845.

Mathur, D. 1972. Seasonal food habits of adult white crappie, *Pomoxis annularis Rafinesque*, in Conowingo Reservoir. The American Midland Naturalist 87: 236–241.

McIntyre, J. K., and D. A. Beauchamp. 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Science of the Total Environment 372: 571–584.

Munthe, J., R. A. Bodaly, B. A. Branfireun, C. T. Driscoll, C. C. Gilmour, R. Harris, M. Horvat, M. Lucotte, and O. Malm. 2007. Recovery of mercury-contaminated fisheries. AMBIO: A Journal of the Human Environment 36: 33–44.

Peterson, S. A., J. Van Sickle, A. T. Herlihy, and R. M. Hughes. 2007. Mercury concentration in fish from streams and rivers throughout the western United States. Environmental Science and Technology 41: 58–65.

Pickhardt, P. C., and N. S. Fisher. 2007. Accumulation of inorganic and monomethylmercury by freshwater phytoplankton in two contrasting water bodies. Environmental Science and Technology 41: 125–131.

Pohnert, G., M. Steinke, and R. Tollrian. 2007. Chemical cues, defense metabolites and the shaping of pelagic interspecific interactions. Trends Ecol. Evol. 22: 198–204.

Porter, K. G., and J. D. Orcutt Jr. 1980. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for Daphnia. Pages 268–281 *in* W. C. Kerfoot, editor. Evolution and ecology of zooplankton communities. University Press of New England, Hanover, New Hampshire.

Reiley, P., and T. K. McCarthy. 1990. Observations on the natural diet of Cymatia bonsdorfi (C. Sahlb.) (Heteroptera: Corixidae): an immunological analysis. Hydrobiologia, Volume 196: Issue 2.

SFBRWQCB (San Francisco Bay Regional Water Quality Control Board). 2008. Total maximum daily load for mercury in the Walker Creek Watershed, Staff Report. January 16, 2007 with minor revisions April 4, 2008.

SFBRWQCB. 2010. Surface Water Body Beneficial Use Documentation Tables. Attachment A to the Final Staff Report: San Francisco Bay Basin Water Quality Control Plan, Basin Plan Update, Addition of Water Bodies and Beneficial Uses. Adopted as a Basin Plan Amendment on July 14, 2010.
https://www.waterboards.ca.gov/sanfranciscobay/basin_planning.html

SFBRWQCB. 2013. Resolution No. R2-2013-0039. Renewal of Conditional Waiver of Waste Discharge Requirements for Grazing Operations in the Tomales Bay Watershed (Tomales Bay, Lagunitas Creek, Walker Creek, And Olema Creek) in the San Francisco Bay Region. https://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/agriculture/grazing/tomalesgrazing/R2-2013-0039.pdf

Selch, T. M., C. W. Hoagstrom, E. J. Weimer, J. P. Duehr, and S. R. Chipps. 2007. Influence of fluctuating water levels on mercury concentrations in adult walleye. Bulletin of Environmental Contamination and Toxicology 79: 36–40.

Shapiro, J., 1990. Biomanipulation: the next phase – making it stable. Hydrobiologia. 200/201, 13–27.

Slotton, D., J. E. Retuer, and C. R. Goldman. 1995. Mercury uptake patterns of biota in a seasonally anoxic northern California reservoir. Water, Air, and Soil Pollution 80: 841–850.

Sorensen, J. A., L. W. Kallemeyn, and M. Sydor. 2005. Relationship between mercury accumulation in young-of-the-year yellow perch and water-level fluctuations. Environmental Science & Technology 39: 9,237–9,243.

State Water Resources Control Board (State Water Board). 2017. Final Staff Report for Part 2 of the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California—Tribal and Subsistence Fishing Beneficial Uses and Mercury Provisions. Appendix A. https://www.waterboards.ca.gov/water_issues/programs/mercury/

Stewart, A. R., M. K. Saiki, J. S. Kuwabara, C. N. Alpers, M. Marvin-DiPasquale, and D. P. Krabbenhoft. 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. Canadian Journal of Fisheries and Aquatic Sciences 65: 2,351–2,366.

Stillwater Sciences. 2014. Addendum to the 2013 Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study Final Report. Technical memorandum prepared for Paul Sellier, Marin Municipal Water District. June 17, 2014.

Stillwater Sciences. 2015. Soulajule Reservoir and Arroyo Sausal Methylmercury Control Study Final Study Plan. Prepared by Stillwater Sciences, Berkeley, and Brown and Caldwell, Walnut Creek, California for the Marin Municipal Water District, Corte Madera, California.

Stillwater Sciences. 2017a. Characterization of Seasonal Mercury Cycling and Bioaccumulation in Soulajule Reservoir. Prepared by Stillwater Sciences, Berkeley, California for Marin Municipal Water District, Corte Madera, California. November 2017.

Stillwater Sciences. 2017b. Assessment of Littoral Zone Extent and Productivity as Related to the Potential for Increased Water Level Fluctuation in Soulajule Reservoir. Prepared by Stillwater Sciences, Berkeley, California for Marin Municipal Water District, Corte Madera, California. February 2017.

Stillwater Sciences. 2017c. Assessment of Soulajule Reservoir Fish Community Composition and Food Web Structure. Prepared by Stillwater Sciences, Berkeley, California for Marin Municipal Water District, Corte Madera, California. July 2017.

Tillyard, R. J. 1917. The Biology of Dragonflies. Cambridge University Press. London, England.

Tremblay, A., M. Lucotte, and I. Rheault. 1996. Methylmercury in a benthic food web of two hydroelectric reservoirs and a natural lake of northern Quebec (Canada). Water, Air, & Soil Pollution 91: 255–269.

Vanni, M. J., C. Luecke, J. F. Kitchell, Y. Allen, J. Temte, J. J. Magnuson. 1990. Effects on lower trophic levels of massive fish mortality. Nature 344: 333–335.

Verta, M. S. Rekolainen, and K. Kinnuenen. 1986. Causes of increased fish mercury levels in Finnish reservoirs. Publication of Water Research Institute National Board of Waters, Helsinki, Finland 65: 44–71.

Wade, S., T. Corbin, and L. McDowell. 2004. Class Crustacea. Critter Catalogue. A guide to the aquatic invertebrates of South Australian inland waters. Waterwatch South Australia.

Watras, C. 2009. Mercury pollution in remote freshwater lakes. Pages 100–109 *in* G. Likens, editor. Encyclopedia of inland waters. Elsevier, New York.

Webster, K. E., and R. H. Porter. 1978. Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. Limnol. Oceanogr. 23: 1,238–1,245.

Weiner J. G., D. P. Krabbemhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury. Pages 409–463 *in* D. J. Hoffman, B. A. Rattner, G. A. Burton Jr., and J. Cairns Jr, editors. Handbook of ecotoxicology. 2nd edition. CRC Press, New York.

Wood M. L., C. Foe, J. Cooke, and S. J. Louie. 2010. Sacramento–San Joaquin Delta Estuary TMDL for methylmercury: staff report. Regional Water Quality Control Board – Central Valley Region, Sacramento, Calif., April 2010.

Appendices

Appendix A

Summary of Data Collection Efforts

Table A-1. Summary of data collected in Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study (2012) and the Soulajule Reservoir and Arroyo Sausal Methylmercury Control Project (2016).

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Soulajule Reservoir Mercury Occurrence and Bioaccumulation Study (2012)					
Water	*In situ* – water temperature, dissolved oxygen, pH, conductivity, turbidity, ORP	Instantaneous, water column vertical profile	Seasonal (spring, summer, early winter)	Soulajule Reservoir Eastern Arm (S-WQ1, S-WQ2), Western Arm (S-WQ6) and near the dam (S-WQ3, S-WQ4); Arroyo Sausal (S-WQ5, AC-WQ1)	21
	TotHg, MeHg, TSS, chlorophyll-*a*, NH_4^+, NO_3^-, NO_2^-, OP, TP, SO_4^{2-}, total sulfide	Surface and bottom grabs			31
Sediment	TotHg, MeHg, percent fines, water content, TOC	Reservoir sediments (top 5 cm of sediment profile)	One-time (summer)	Soulajule Reservoir Eastern Arm (S-S1 through S-S21), Western Arm (S-S25 throughS-S29) and near the dam (S-S22 through S-S24); Nicasio Reservoir (N-S1 through N-S5)	33
	Reactive mercury (Hg(II)R)		One-time (summer)	Soulajule Reservoir Eastern Arm (S-S3, S-S8, S-S9, S-S11, S-S13 through S-S15, S-S17, S-S19 through S-S21), Western Arm (S-S25, S-S27, S-S29) and near the Dam (S-S22, S-S23, S-S24); Nicasio Reservoir (N-S1 through N-S5)	18

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Biota	Algae	Identification and enumeration	One-time (summer)	Soulajule Reservoir Eastern Arm (S-WQ1), Western Arm (S-WQ6) and near the Dam (S-WQ3, S-WQ4); Arroyo Sausal (S-WQ5, AC-WQ1)	7
	TotHg and MeHg in zooplankton	Multi-individual composites, homogenized	Seasonal (summer, early winter)	Soulajule Reservoir Eastern Arm (S-WQ2) and near the dam (S-WQ3)	2
	TotHg and MeHg in crayfish	Crayfish tissue	Seasonal (spring, summer, early winter)	Soulajule Reservoir; Arroyo Sausal	3
	TotHg in prey fish	Composite of 3–4 individuals, skinless filet	Seasonal (summer, early winter)	Soulajule Reservoir	7
	TotHg in piscivorous fish	Composite of 3–4 individuals, skinless filet	One-time (early winter)	Soulajule Reservoir	13

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Pilot Study 1—Additional Characterization of Methylmercury in Water and Biota (2016)					
Water	*In situ* – water temperature, dissolved oxygen	3 ft (1m) depth or valve sample[2]	Monthly[2]	Dam outlet to Arroyo Sausal (A-1)	36
	In situ – water temperature, dissolved oxygen, pH, conductivity, turbidity, ORP	48-hr continuous, 0.3–0.5 m from bottom sediments	Seasonal (winter, spring, summer, fall)	Soulajule Reservoir Eastern Arm (EA-1)	4
		Instantaneous, water column vertical profile	Seasonal (winter, spring, summer, fall)	Soulajule Reservoir Eastern Arm (EA-1), Western Arm (WA-1), near the dam (D-1)	12
	Dissolved MeHg, dissolved sulfide	Surface grab or valve sample, filtered to 0.45 um	Monthly[2]	Dam outlet to Arroyo Sausal (A-1)	16
	Dissolved MeHg	Surface grabs, filtered to 0.45 um	Seasonal (winter, spring, summer, fall)	Eastern Arm (EA-1), Western Arm (WA-1), near the dam (D-1)	36

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Biota	MeHg in algae	Multi-individual composites, homogenized	Seasonal (winter, spring, summer, fall)	Eastern Arm (EA-1), Western Arm (WA-1), near the dam (D-1)	36
	MeHg in zooplankton	Multi-individual composites, homogenized			36
	MeHg in prey fish	Individuals, whole body homogenized			180
	Algae, zooplankton, small fish	Identification and enumeration			36
	Algae	Satellite Imagery, chlorophyll-*a* (all algae) and phycocyanin (cyanobacteria)	Seasonal (winter, spring, summer, fall)	Soulajule Reservoir	7

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Pilot Study 2—Water Level Fluctuation (2016)					
Sediment	Nutrients (TP, NH_4^+, NO_3^-, NO_2^-), TOC, Grain size and texture	Sediment (upper 3 inches) from dry and wetted area of the littoral zone	One-time (summer)	Eastern Arm (EA-1-L, EA-2-L), Middle Reservoir (MR-1-L), Western Arm (WA-1-L, WA-2-L)	15
Biota	Macrophytes and macro algae	Percent cover of primary producers within the dry and wetted littoral zone littoral zone	Seasonal (spring and summer)		24

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Pilot Study 3—Soulajule Reservoir Fish Community Composition (2016) and Pilot Study 4— Soulajule Reservoir Food Web Structure (2016)					
Biota	Adult and juvenile fish community composition	Beach seine, littoral zone (<10 feet deep)	Seasonal (fall and spring)	Eastern Arm (E1, E2, E3, E4), Western Arm (W1), Main Body (M1, M2)	14
		Boat electrofishing, littoral zone			14
		Gill net - profundal; nearshore			14
		Gill net - littoral/profundal; nearshore			14
		Gill net - vertical array - pelagic/profundal	Seasonal (fall)	Main Body (M1)	1
	Gut content analysis of juvenile and adult fish	Gastric lavage and microscopic identification	Seasonal (spring and summer)	Eastern Arm (E1, E2, E3, E4), Western Arm (W1), Main Body (M1, M2)	76

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Pilot Study 5— Evaluation of Reservoir Seasonal Oxygen Demand and Sediment Response to Hypolimnetic Oxygenation (2016)					
Sediment	Sediment oxygen demand	Laboratory chamber experiments using sediment cores, alternating oxic and anoxic conditions	One-time	Soulajule Reservoir East Arm, West Arm, Dam and Mid-Dam	12
Sediment	Sediment release rates of redox sensitive compounds (NH_4^+, NO_3^-, PO_4^{3-}, Mn, Fe, TotHg, MeHg)	Laboratory chamber experiments using sediment cores, alternating oxic and anoxic conditions	One-time	Soulajule Reservoir East Arm, West Arm, Dam and Mid-Dam	12

Matrix	Constituents[1]	Sample type	Sample frequency (period)	Sample location	Total number of samples
Pilot Study 6—Upland Mercury Source Loading Characterization (2016)					
Soil	TotHg, soil texture	Upland soils (0-12 inches) near historical mine sites and undisturbed areas (background)	One-time	Franciscan Mercury Mine (FM-1 through FM-14); Franciscan Mine Background (FMBKG-9 through FMBKG-12); Cycle Mercury Mine (CM-15 through CM-21); Cycle Mine Background (CMBKG-17 through CMBKG-24)	29
		Upland soils (0-12 inches) near potential historical mines and undisturbed areas (background)	One-time	Unnamed Mine 1 (UNM1-1 through UNM1-10); Unnamed Mine 1 Background (UNM1-BKG-11 through UNM1-BKG-12); Unnamed Mine 2 (UNM2-1 through UNM2-10); Unnamed Mine 2 Background (UNM2-BKG-11 through UNM2-BKG-14)	28
		Sediments at major creeks entering the reservoir	One-time	Drainages near historical mines (EAD-1, EAD-2); Eastern Arm Inlets (EAI-1, EAI-2, EAI-3)	5
		Sediments downstream of the dam	One-time	Spillway (Spillway1); Arroyo Sausal (ArroyoS-Sed1)	2

[1] ORP = oxidation-reduction potential, TotHg = total mercury, MeHg = methylmercury, TSS=total suspended solids, NH_4=ammonia nitrogen, NO3-=nitrate, NO2-=nitrite, OP=orthophosphate, TP= total phosphorus, TOC=total organic carbon, SO42- = sulfate, PO43-=phosphate, Mn=manganese, Fe=Iron

[2] Sampling frequency increased to every two weeks surrounding the onset of reservoir stratification in spring and destratification in the late fall. Samples were collected at the dam pipe outlet in Arroyo Sausal from January to April, and at the sample box from May to November when the Howell-Bunger Valve was turned on.