RECLAMATION
Managing Water in the West

Final Feasibility-Level Eradication Plan: Zebra Mussels in San Justo Reservoir, Hollister Conduit, and Distribution System

Mid-Pacific Region

1.1.1

U.S. Department of the Interior
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DRAFT Eradication Plan: Zebra Mussels in San Justo Reservoir, Hollister Conduit, and Distribution System

Mid-Pacific Region

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NOTE: All units in this document are U.S.: U.S. liquid gallons, U.S. tons, etc.

1 Executive Summary

San Justo Reservoir is part of the San Felipe Division of the Central Valley Project in California and has been known to be infested with zebra mussels since 2008. Reservoir water is delivered for agricultural and municipal uses only; no surface water outflows from San Justo are connected to other water bodies, effectively creating an isolated zebra mussel infestation. The detection of invasive mussels at San Justo prompted closure of the area to public access in order to further prevent their potential spread. Because San Justo is essentially a terminal water body, it presents a relatively unique opportunity for eradication to eliminate the threat of invasive mussels and their spread.

This document details specifics for a zebra mussel eradication effort at San Justo Reservoir, initially investigated and presented in the Draft Finding of No Significant Impact (FONSI, Reclamation 2015). The selected alternative includes use of potassium solution (potash) to treat the reservoir and distribution system. Properties of potash such as toxicity and environmental impacts are reviewed and summarized in the draft FONSI. Basic components of the proposed eradication effort are as follows:

- Draw-down of the reservoir to 455 feet elevation, exposing shoreline mussels to desiccation and reducing the volume of water to be treated to approximately 3,000 acre-feet (97,755,000 gallons).
- Apply approximately 716,000 gallons of commercially acquired potassium solution (~ 12% potassium by weight) to San Justo Reservoir.
- Flush and charge the distribution piping system with treated water from San Justo Reservoir.
- Hold treated water within the reservoir and distribution system for approximately 45 days before resuming operations.
- Monitoring will be conducted to evaluate eradication treatment performance and results.

2 Introduction

2.1 San Justo Reservoir

San Justo Reservoir, located southwest of Hollister, California, is used primarily for off-stream water storage as part of the San Felipe Division of the Central Valley Project. The reservoir was built and is owned by the Bureau of Reclamation (Reclamation) and is operated by the San Benito County Water District (SBCWD). Water is primarily for irrigation and municipal uses and services 23,700 acres.

San Justo Reservoir receives water out of San Luis Reservoir by way of the Pacheco Bifurcation structure and through the Hollister Conduit. Water can be diverted at various points along the Hollister Conduit to recipients by way of the SBCWD distribution system and subsystems (distribution system). The conduit and distribution system are composed of more than 90 miles of total piping with various turnouts, flow control structures, and booster pumps.

During high summer demand, water stored in San Justo Reservoir is delivered to recipients through pump-assisted flows through the Hollister Conduit and distribution system. Water from San Justo does not flow past the Pacheco Bifurcation and does not re-enter San Luis Reservoir; all flows out of San Justo terminate at various end-use locations.

The invasive exotic zebra mussel (*Dreissena polymorpha*) was detected at San Justo Reservoir in 2008, and the reservoir has since been closed to recreational access to prevent potential spread. Adult zebra mussels were also found to infest the Hollister Conduit at multiple locations in 2009.

A multi-agency group, including members from the Bureau of Reclamation, California Department of Fish and Wildlife (formerly, Department of Fish and Game), California Department of Water Resources, San Benito and Santa Clara Valley Water Districts, and San Benito County have proposed and evaluated actions to eradicate zebra mussels from San Justo Reservoir, the Hollister Conduit and distribution system.

2.2 Open-Water Mussel Eradication Treatments

Mussel control or eradication treatments in large water bodies are inherently difficult and costly due to the scale of the treatment and volume of materials, potential impacts to beneficial organisms, and downstream use limitations. Various alternatives for eradicating invasive mussels at San Justo Reservoir are discussed in the Draft Finding of No Significant Impact (Reclamation 2015). Alternatives eliminated from further analysis included the use of copper-based pesticides, *Pseudomonas* bacterial derived biocide products, as well as a complete drawdown of the reservoir (desiccation) and programmatic mussel management (containment).

The proposed action for the eradication of mussels at San Justo Reservoir is the application of a potassium-based compound to the reservoir and distribution system. These compounds are commonly known as potash, which refers to a group of potassium-rich salts (primarily potassium chloride) that are commonly used as fertilizers. This method was selected based on the relatively low toxicity to non-target organisms, documented toxicity to invasive mussels, and previous successful use of this method for mussel eradication in other infested waterbodies.

2.3 San Justo Mussel Eradication

The scale of the effort to eliminate invasive mussels from San Justo Reservoir and the distribution system will be one of the largest yet conducted, with treated water volume estimated at 3,000 acre-feet and over 90 miles of associated pipe system. Successful mussel eradication at San Justo faces several intricacies: a substantial volume of water to be treated, extensive invasive zebra mussel populations, and a complex pipeline system. These aspects present a level of risk for eradication failure that is difficult to accurately quantify. Primarily, the ability to fully expose all mussels to water with sufficient potassium concentrations throughout the prescribed treatment period to cause complete mortality is unknown. Mussels will have the potential to “escape” treatment in various ways, including non-homogenous mixing and dispersal of the potassium solution, refugia areas within the drawdown zone, or incomplete charging of the distribution system.

Very small populations of surviving mussels would have the potential to completely re-infest the entire system in a relatively short period. For the eradication to succeed, treatments must ensure best possible concentration and contact times of treated water throughout the reservoir and connected systems. This includes San Justo Reservoir, the Hollister Conduit and distribution system as well as shoreline areas.

Timing of the eradication treatment has the potential to mitigate many of the potential risks described. Mixing could be improved by application of treatments shortly before the fall thermal stratification turnover, and/or extending the overall treatment duration. A longer, slow release of treated water through the distribution system would also reduce the potential for sub-lethal dose/exposure times. Drawdown of the reservoir earlier in the season would expose shorelines to hotter and drier weather, improving mussel desiccation and reducing potential refugia.

A feasible eradication effort must also coincide with ongoing water delivery and storage operations at San Justo Reservoir, which may necessitate sub-optimal timing and conditions in which to conduct the eradication treatments. This plan is based on the current consensus to conduct the eradication after seasonal water deliveries are completed, in the fall when the reservoir would typically be drawn down.

2.4 Eradication Plan Objectives

The objectives of this eradication plan are to:

- Provide the best feasible strategy for complete elimination of zebra mussels within San Justo Reservoir, the Hollister Conduit, and all distribution system components.
- Fully inform and describe known inherent risks for eradication failure for consideration in decision-making.
- Inform feasibility level cost estimation.

3 Reservoir Drawdown

The primary factor influencing eradication costs is the volume of potassium solution necessary to bring the reservoir water to the appropriate concentration. Drawing down the reservoir to reduce the total volume of water to be treated creates a more economically feasible - although still significant - amount of potassium solution necessary for mussel eradication.

Several drawdown scenarios for San Justo Reservoir were examined and indicated 455 feet as the minimum elevation below which adverse impacts would be seen by water users and stakeholders (Reclamation 2015) and is the current target elevation for the eradication treatment. If the eradication is conducted at higher elevations, accommodations would be necessary for increased treatment scale and could significantly increase costs as well as require modifications to equipment, number of personnel, and schedule.

Since January 2014 the reservoir elevation has fluctuated between 441 and 500 feet (Appendix B). Much of this history includes drought years where water was delivered out of San Justo from early spring through late fall/early winter, and then refilled during the winter off-season with water from San Luis Reservoir. High-water conditions such as seen in the 2016/2017 season may create challenges to drawing down San Justo reservoir for the mussel eradication effort as alternative storage may be limited. At present, conducting a drawdown to 455 feet is deemed to be feasible within 2 years of initiation.

The drawdown will be accomplished by delivering water to customers from San Justo and delaying inflows San Luis Reservoir; water will not discharge from San Justo Reservoir to rivers, creeks, or other open waters per the discontinued use of these turnouts (in effect since the discovery of invasive mussels at San Justo). Bathymetry indicates the capacity of the reservoir at the targeted elevation (455 feet) is approximately 3,000 acre-feet. The water treated in the reservoir will be used to flush the distribution system. The rate of drawdown will not exceed 1 foot of surface elevation per day to minimize stresses on the reservoir substrate. See Appendix G for San Justo Reservoir capacity and area by elevation tables.

Scheduling and implementation of the drawdown and reservoir refill will be conducted in coordination with San Benito County Water District and stakeholders so as to minimize the impacts to water users, costs of the eradication effort, and the risk of eradication failure.

3.1 Drawdown Holding Period

After the drawdown, the reservoir water will be treated with potassium solution, then flushed through the distribution system and held static for a minimum of 45 days after confirming all potassium concentrations are between 95-115 parts per million (ppm).

This holding period will meet the minimum requirements to reach complete mortality at historically low (down to 54° F) water temperatures (25 days, Reclamation 2016) as verified by on-site testing (Appendix A), the 60 day suggested minimum for shoreline desiccation (Chapman and Gruenhagen 2010), and fall within the available time-window between seasonal discontinuation of water deliveries and initiation of reservoir refill (generally October through December).

The 60-day minimum exposure time for shoreline mussel desiccation would be met by the combined duration (post-drawdown) of the active potassium solution dosing period (approximately 17 calendar days) followed by the 45-day minimum holding period. Lab tests (McMahon et al. 1993) imply adult zebra mussels may only require 10 days to desiccate to the point of complete mortality (extrapolated from McMahon et al. 1993 to typical air temperatures and relative humidity at San Justo during the eradication treatment), but these tests did not account for mussels in moist/muddy substrates or precipitation events. Ultimately the treatment duration should be adaptive based on frequent monitoring of potassium concentrations and mussel mortality observations.

At the conclusion of the holding period, assuming all short-term monitoring (see Section 5.1; Appendix E) indicates the eradication was successful, the reservoir will be refilled with water from San Luis Reservoir via the Hollister Conduit and resume typical operations, with additional long-term monitoring to confirm complete mussel extirpation from San Justo.

3.2 Exposed Shoreline

The reservoir drawdown will expose mussel populations established above 455 feet. Bathymetric analysis indicates the total area of exposed shoreline between 455- and 472-feet elevation is approximately 38.5 acres (472 feet was the maximum elevation the bathymetry was able to accurately capture surface contours due to the reservoir elevation at the time of the analysis). Zebra mussel colonization may extend on shoreline substrates up to 480 feet elevation or higher depending on reservoir operations, and the actual drawdown area may be larger depending on the seasonal starting elevation.

Desiccation is expected to cause mortality to the majority of the exposed mussels, but wet areas, particularly pockets within the reservoir armoring and along the perimeter of the immediate shoreline, may be able to support viable mussels through the eradication treatment period. The shoreline area will be monitored regularly as the drawdown progresses, by boat and/or on foot to determine the presence and extent of live mussels or viable refugia. Monitoring should continue until complete mortality is confirmed for all shoreline mussels (see Appendix E).

In the event of inadequate desiccation or incomplete mortality, contingency practices for treating shoreline mussels will be enacted (see Section 3.2.1). Best practices for shoreline treatments will depend on the topography of the exposed shoreline, weather conditions, and extent of observed presence/absence of live mussels or potential habitats.

3.2.1 Shoreline Treatments

Viable mussels found on the drawdown zone should initially be collected and placed in aquaria for use in live mussel assay monitoring (Section 5.1.2, Appendix E). Mussel-covered rocks can be gathered in small buckets or containers with reservoir water and kept alive for several hours. Placing containers in the reservoir and cutting holes in the buckets to allow water flow will increase the duration mussels may be temporarily stored before being moved to aquaria.

Live mussels required for monitoring should be collected from San Justo before the eradication treatment is initiated (see Appendix E). Remaining populations of viable mussels on the shoreline should be treated unless desiccation appears imminent.

Shoreline treatments will consist primarily of potassium solution application via backpack sprayer or high-pressure boat-mounted applicator. As a general guideline, assuming use of the same 12% by weight potassium solution applied for the reservoir treatment, 0.09 oz. of solution will need to be added to each gallon of untreated water to obtain 100 ppm potassium. Targeting treatment of wet or pooled shoreline areas at 2 to 3 times this rate is recommended to allow for a greater margin of error in visual field-estimations of the volume of water to be treated, provided this treatment is within limitations of approved permits for use of potassium as a pesticide.

Mechanical removal or physical destruction of adult mussels, such as using a blunt instrument or simply crushing them underfoot may be useful for small exposed colonies. Other methods to aid desiccation or prevent mussel survival may be acceptable upon review, provided the physical integrity of the reservoir will not be significantly affected.

4 Reservoir Treatment

4.1 Potassium Solution Supply and Storage

The proposed potassium solution for the eradication will be obtained from commercially available sources as a pre-mixed liquid consisting of muriate of potash, a mined potassium-rich salt, dissolved in water (solution = 12% potassium by weight). On a global level, potash is mined in significant quality and quantity in only a few countries. The continental U.S. is believed to contain one percent of the world's potash. In 2015, U.S. production was estimated at 815,700 tons, compared to global production of 44,753,800 tons. The largest known potash reserves are found in Canada (46% of global supply), with most mines located in central to south-central Saskatchewan (USGS 2017).

Fertilizer suppliers commonly deliver potassium for agricultural use in the form of potash solution, and various sources are available in the Central Valley of California. Off-site mixing and tanker-truck deliveries are services readily available in the vicinity of the project. However, due to the scale of product necessary for the eradication, acquisition of potash may require pre-planning and need to be initiated one or more years prior to conducting the treatment. For reference of scale, treating San Justo will require approximately 837 tons of potash, which equates to 0.1% of U.S. production in 2015 (0.002% of 2015 global production).

Specifications for the concentration of the potassium solution should be verified and calculations adjusted for any variations, as small changes in concentration may magnify to large alterations of the total volume of solution needed, delivery logistics and scheduling to treat the reservoir within the designated timeframe.

The eradication treatment will utilize solution-grade muriate of potash mixed with water off-site and delivered to the project area as a liquid (~ 12% potassium solution by weight) by way of 25-ton (5,000 gallon) tanker trucks. Multiple deliveries of potassium solution to the project site will need to occur on a daily basis.

Current estimates for potassium solution delivery minimum requirements:

- Total potassium solution at 12% potassium by weight: 716,000 gallons.
- Truckloads delivered per day: 12
- Total truckloads delivered: 144

Tanker-truck deliveries of potassium solution will be transferred to land-based storage tanks at several locations surrounding San Justo Reservoir, each equipped with spill containment, loading equipment/hookups, and liquid/slurry pump systems to deliver the solution to workboats. Storage tank volume should accommodate for a minimum of one full work-day of treatment.

Current estimates for potassium solution storage requirements include 4 storage tanks each with a minimum capacity of 15,000 gallons. Tank locations, as well as all staging areas and vehicle traffic will occur on existing paved or otherwise historically disturbed areas.

4.2 Potassium Solution Application

Various alternatives are available for the distribution of potassium solution to San Justo Reservoir. Basic requirements are workboats equipped with distribution/diffuser systems to dispense the potassium solution throughout various depths of the reservoir, or otherwise use practices to create a uniform distribution of potassium concentrations.

Storage, transfer, and delivery system will require specifications to meet the designated schedule obligations.

Workboat diffuser systems typically consist of one or multiple hoses, optionally perforated to some extent, and reaching at least 10 feet below the water surface. Longer hoses may be necessary to achieve full mixing of the potassium solution at depth, particularly below the thermocline (see Potassium Mixing and Distribution, Section 4.3 and Appendix C Figure C3 for water temperature profiles).

Connecting workboats directly to the land-based storage tanks with supply lines eliminates the necessity for separate chemical storage tanks and injection pumps on the boats, as well as time necessary to fill the workboat tanks from land-based storage. The Millbrook Quarry eradication effort found land-based tanks with ballasted/floatation-assisted supply hoses to workboats with diffuser systems to be an efficient method to deliver potassium solution to a water body (Dan Butts, personal communication, January 16, 2017). Workboat supply system specifications are expected to consist of 1-inch diameter by 2,000 feet long feed hoses, constructed of braided PVC or similar in multiple sections with quick connect cam-locks and shutoff valves. On-site fuel delivery and storage will also be necessary for workboat operation.

With supply hoses of 2,000 feet it is estimated that a minimum of 4 workboat distribution setups with 30 gallon per minute (gpm) chemical delivery pumps would be necessary to dose the reservoir within the prescribed timeframe (scheduling presented in Section 4.4; Table 4.1), as well as distribute the potassium solution over the entirety of the surface area of the reservoir (Figure 3.1).

Four stations are featured in Figure 3.1 as potential delivery locations; Station 3 may be unsuitable as it is located on a non-paved surface with more difficult access. Relocating Station 3 and use of longer supply hose (additional ~1,000 feet) will be necessary to reach the southeastern arm of the reservoir if the current location of Station 3 is deemed unacceptable.

Dosing rates and duration shall be within acceptable limits of those specified as follows:

- 4 workboats/delivery pumps at 30 gpm each, 8 hour working days = 57,600 gallons of potassium solution delivered to reservoir per day.
- 13 total working days to complete potassium application; approximately 17 calendar days.

Workboat entry to the reservoir under drawdown conditions will require temporary modifications below the existing boat ramp, as well as floating-dock/gangway installation for boat storage and personnel access.

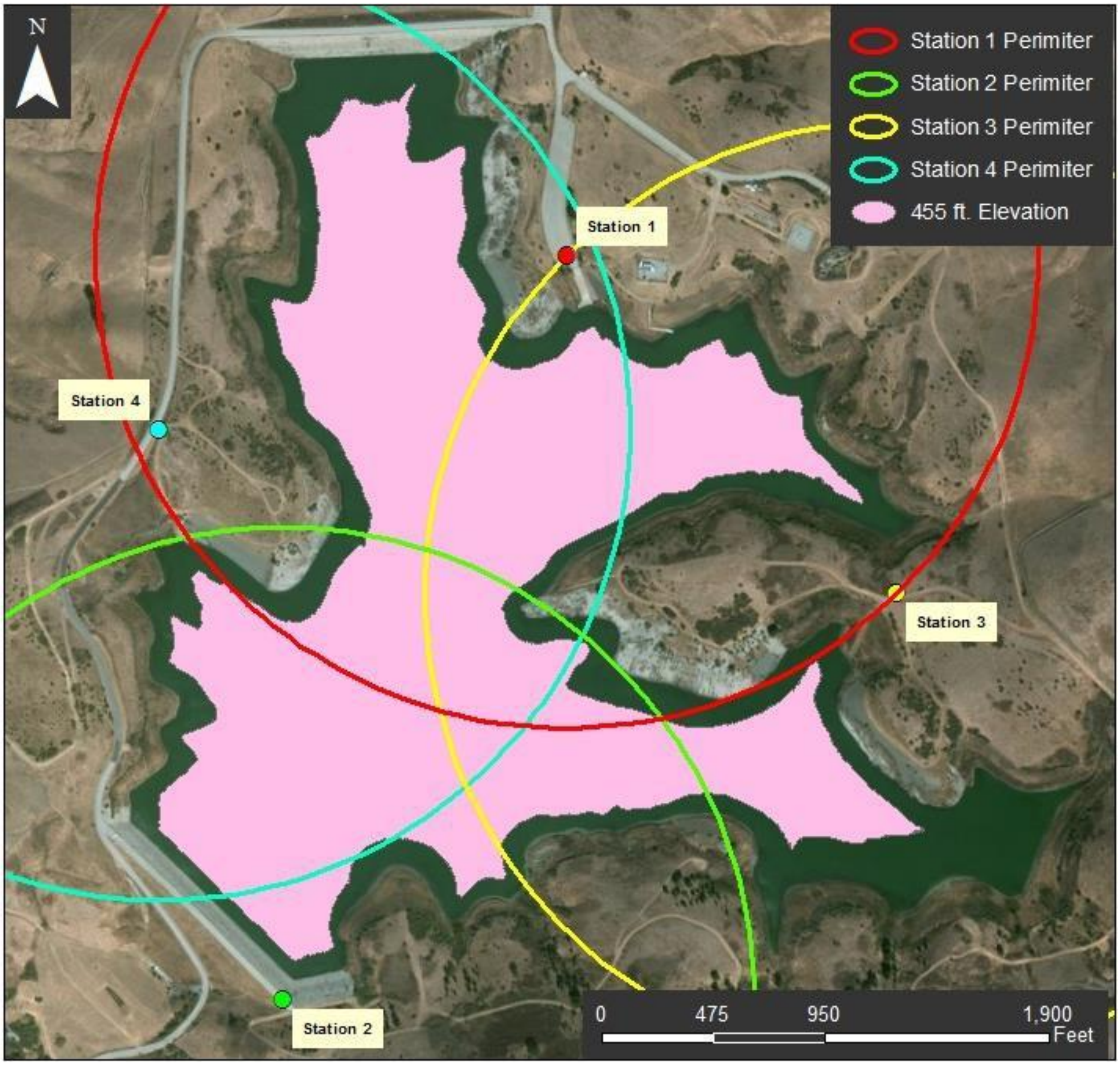

Figure 3.1. Potential location of potassium solution delivery stations and approximate range of workboats equipped with 2,000-foot supply hoses.

4.3 Potassium Mixing and Distribution

The ability to attain and maintain uniformly distributed concentrations of potassium throughout San Justo Reservoir is unknown. Previous eradication efforts using potassium solution have seen variable results: At Millbrook Quarry, complete mixing was verified within 24 hours of the treatment completion using only surface applications and distribution hoses less than 10 feet in length (ASI 1996).

Winter treatments were conducted at Christmas Lake by pumping potassium solution under surface ice. Monitoring results indicated vertical and horizontal stratifications of potassium concentrations, with “hot spots” accumulating at the deepest depths, attributed to the higher density of the potassium solution and low water temperatures. Efforts to mechanically mix the product under the ice were unsuccessful, and ultimately control of mussels was poor (Lund et al. 2017).

The timing of the potassium treatment at San Justo will coincide with cold but above freezing water temperatures, typically as low as 54º F (see Appendix C). Use of distribution hoses from workboats applying the potassium solution throughout the reservoir at various depths may not fully alleviate concentration of potassium at deeper parts of the reservoir, as was seen at Christmas Lake. Further investigation into mitigation measures to adequately mix potassium solution into colder temperature waters may be warranted.

Conducting the potassium treatment earlier in the season would present warmer water temperatures, and additionally the summer/fall temperature inversion could aid in mixing the potassium throughout the reservoir. However, the treatment timeframe is dictated by the San Benito County Water District operations and earlier-season treatments have been deemed impractical. Sufficient mixing of potassium at low water temperatures is therefore critical for the successful eradication of zebra mussels from San Justo Reservoir.

4.4 Holding Period and General Schedule

Reservoir applications are expected to begin in early October after the peak water-use season and completion of the drawdown. Total time required to apply potassium solution and bring San Justo Reservoir to the within acceptable concentrations is estimated at 13 working days or approximately 17 calendar days. General eradication preparation and reservoir treatment scheduling windows are presented in table 3.1.

It is recommended to conduct any preparatory staging and site setup work that will not interfere with ongoing operations in a timeframe that will allow the treatment to begin as soon as the drawdown target is reached. Modifications in number of storage/pumping stations and pump capacity may be explored to meet final budget and schedule requirements.

4.4.1 Contingency Timing and Duration

Resumption of normal operations should not be initiated until after all available data confirm no viable mussels exist within the reservoir or distribution system. This may require an extended time for potassium solution application and/or holding period beyond what is estimate in this plan. Potential interruptions to operations should be understood by all stakeholders and all contingency parameters determined.

Table 4.1 – Proposed schedule for San Justo Reservoir mussel eradication.

Milestone	Begin	End
Pre-Treatment Stage		
Reservoir drawdown	Fall/Winter Year -2	October Year 0
Mobilization and staging	~Year -1	Year 0
Treatment Stage (Year 0)		
Shoreline monitoring and treatments	Summer	Fall/Winter
Reservoir treatment	1-Oct to 15-Oct	19-Oct to 2-Nov
Reservoir holding period	20-Oct to 3-Nov	4-Dec to 18-Dec

5 Conduit and Distribution System Treatment

Once San Justo Reservoir is confirmed to be consistently within the target concentration range for potassium (95-115 ppm) throughout monitoring sites, the inlet/outlet valve will be opened to allow the conduit and distribution system to be flushed with treated water from the reservoir. Untreated water within the distribution system will need to be bled from multiple turnout locations to move full-concentration treated water to all wetted surfaces, and will be conducted so as not to cause any adverse impacts to water users or the environment.

The total volume of the distribution system is estimated at 80 to 90 acre-feet, or approximately 3% of the volume of treated water in the reservoir at 455 feet elevation. The San Benito County Water District estimates that charging the distribution system from 455 feet reservoir elevation would fully wet all but two of the subsystems. The remaining 2 subsystems will require injections of potassium solution at several locations. Two chemical feed stations are anticipated with roughly 500-gallon storage tanks necessary at each station. Chemical feed systems will consist of storage tanks and feed pumps with temporary spill containment.

Smaller systems such as residential “blue meters” and piping are extensive, numbering in the thousands, and may be very difficult if not impossible to fully charge with treated water for the duration of the treatment. These systems should be verified to be kept at positive pressure and/or equipped with backflow preventers to safeguard against re-infestation. It should be noted that these are preventative measures, and the systems may continue to harbor live mussels for many years, potentially indefinitely after the conclusion of the eradication treatment. This presents an unknown level risk to re-infest the distribution system and consequently San Justo Reservoir with invasive mussels, as mussels are motile to some extent as well as the possibility of system outages or malfunctions that would move or allow live mussels into the distribution system. Mitigation of this risk would require inspection, treatment and/or removal of all mussels from thousands of small-diameter (~2 inch) piping systems, and is assumed to be impractical or unattainable to conduct at this time. This risk is therefore inherent to the eradication effort and should be weighed accordingly.

6 Monitoring

Monitoring for water quality and mussel mortality/survival will be conducted at various sites and depths throughout the reservoir and distribution system on a weekly basis, before, during, and after the treatment. Monitoring protocols are presented in Appendix E.

6.1 Short-Term Monitoring

Short-term monitoring will be conducted to evaluate the eradication effort at San Justo Reservoir and provide input for any necessary strategic modifications. These protocols will be conducted prior-to, during, and immediately after the eradication treatments are conducted. The duration of short-term monitoring is somewhat flexible dependent on field conditions and other project schedules, but should generally begin within one year prior to treatment, then transition to long-term protocols after the conclusion of the eradication.

6.1.1 Water Quality Monitoring

Water quality monitoring will be conducted before (baseline), during and after treatments to confirm adequate potassium concentrations and record potential changes in other water quality parameters that may affect the dose-exposure necessary for eradication or any potential undesirable effects.

6.1.1.1 Reservoir Water Quality Monitoring

Water samples will be collected from various locations and depths within the reservoir at minimum on a weekly basis. More intensive sampling may be necessary dependent on deviations from expected levels through the treatment process.

Potassium concentrations will need to be monitored closely from multiple locations and depths to determine concentration levels and consistency of distribution. Samples will be collected at least once prior to treatment, then weekly during the application of potassium solution to the reservoir and the holding period. Concentrations of no less than 95 ppm and no greater than 115 ppm potassium throughout the water column are required for the eradication treatment. Monitoring results should provide rapid feedback to guide any necessary corrective modifications to the application process.

Other water quality parameters including temperature, dissolved oxygen, conductivity, pH, and turbidity will be regularly monitored with field instrumentation in coordination with sample collection for potassium concentration analysis. See Appendix E for the complete water quality monitoring protocol.

6.1.1.2 Distribution System Water Quality Monitoring

Various taps and hose bibs will be used to collect water samples throughout the distribution system. Monitoring parameters and protocols will mimic that of the reservoir. Potassium concentrations will be important to closely monitor at as many points as possible to verify full charging of the system with sufficient concentrations of treated water. Any deviations from expected levels noted within the system should initiate corrective application of potassium solution to the nearest upstream (towards San Justo) injection point, to be bled off through all potassium-deficient terminal ends of the system until acceptable concentrations are reached.

6.1.2 Mussel Monitoring

Mussels will be monitored as a verification of an adequate dose-exposure for complete mussel mortality. This includes shoreline surveys, artificial settlement substrates within the reservoir, plankton tows, and live mussels assays.

6.1.2.1 Shoreline Mussel Monitoring

Shoreline monitoring protocols are adapted from those used by Chapman and Gruenhagen (2010); general parameters and methods are summarized in Appendix F. Monitoring should be conducted regularly throughout the drawdown and eradication treatment. A combination of ground and boat surveys will likely be necessary to sufficiently access the entirety of the shoreline. Steep slopes or areas where the substrate is unstable or otherwise prohibitive to foot traffic should utilize boat surveys; ground surveys should be conducted as much as possible as this method will allow closer inspection of mussels and habitat.

Charging the distribution system with San Justo water at the end of the potassium dosing will lower the reservoir elevation from 455.0 feet to 454.2 feet, exposing approximately 1.8 acres of shoreline (estimates may vary at treatment elevations other than 455 feet). This thin band of exposed area may contain significant numbers of newly exposed mussels that, although exposed briefly to treated reservoir water, will have a shortened time period for desiccation. If reservoir potassium concentrations are confirmed to be at the appropriate levels and evenly distributed, any mussel refugia in these areas would be expected to contain lethal levels of potassium. However, this area should be targeted for additional treatment (see Section 3.2.1) during the holding period and treated appropriately if and where viable mussels are found.

6.1.2.2 Reservoir Mussel Monitoring

Mussels of all life stages will be monitored regularly at various locations and over the course of the eradication treatment. Specific methods for monitoring mortality include live mussel bioassays, plankton tows, visual inspection, and artificial settlement substrates.

Mussel bioassays are live adult mussels placed in mesh bags and exposed to treated water. Live mussels will need to be collected from San Justo prior to potassium dosing. Sufficient quantities of live mussels may be acquired more easily if collected during the drawdown process as mussels settled at deeper depths become accessible from shallows over time. Live mussels can be kept in aerated reservoir water for several months if water is changed regularly. Using chilled water in insulated aquaria will slow mussel metabolism and may extend viable retention times and may reduce necessary frequency of water changes.

For reservoir assays, mesh bags with live adult mussels will be strung from buoys at several locations and depths throughout the reservoir. Mussels should be examined and noted weekly for signs of intoxication or mortality. All mussels suspected dead will be placed in untreated reservoir water under aeration and observed after 72 hours to observe potential recovery. A triplicate set of live mussels will also be kept in a recovery jar without exposure to treated water as a control to verify recovery conditions are suitable. Monitoring will require qualified technicians and may involve substantial labor.

Plankton tows are high-volume filtered water samples that concentrate particulates and can be used to detect the presence of larval mussels. Tows are collected vertically from the just off the bottom substrate to the surface, taken in sets to acquire a standard total volume. Filtered samples are transferred to sample bottles, preserved and sent for laboratory analysis. See Appendix E for detailed planktonic mussel monitoring protocols.

Artificial substrates deployed from docks or buoys can be used to capture mussel settlement within the reservoir. Substrates are to be deployed within one week of the initiation of the application and monitored every two weeks. Mussel settlement observations will transition into the long-term monitoring phase, at which point they will emphasize efforts during the typical June through October peak settlement window at San Justo. The protocol for artificial substrate monitoring is discussed in detail in Appendix E.

At the completion of the eradication treatment, provided all other monitoring results indicate complete mortality of mussels, visual surveys conducted by divers or Remotely Operated Vehicle (ROV) video will be used to further validate the eradication effort.

6.1.2.3 Distribution System Monitoring

The Hollister Conduit will be monitored using bioboxes, which are essentially flow-through aquaria plumbed directly into the system. Inflow and outflow valves are installed to regulate flow at 2 gallons per minute, which allows enough retention time for mussels to settle.

Both mussel settlement monitoring and live mussel assays will be conducted in the bioboxes. Settlement is monitored by placing substrate plates (plastic or PVC) within the biobox; data collection protocol is identical to the buoy-deployed settlement monitoring plates. Live mussel assays for the distribution system comprises adult mussels in mesh bags placed in bioboxes. Protocols for mortality assessment including recovery and data collection are identical to the assays conducted in the reservoir.

Biobox outflow should be considered to be contaminated with viable mussels and must be discharged so as not to spread to water bodies, streams or other open waters. Bioboxes will be located within turnout/valve vaults; outflow will be delivered to designated waste areas by the sump system. Waste areas should be verified acceptable for discharge of both potassium-treated and mussel-infested water.

The total volume of water discharged per biobox on the distribution system over the course of the 45-day treatment period is estimated at 129,600 gallons (0.4 acre-feet). A minimum of 2 bioboxes located at the extreme ends of the distribution system (turnounts 1 and 10) are recommended. Additional bioboxes monitoring should be used if safe disposal of water can be accommodated.

Potassium concentrations should be verified from samples collected at all available taps from the Hollister Conduit, laterals, and all minor subsystems to ensure biobox conditions are relatable to the entirety of the distribution system.

A final visual inspection of the conduit will be conducted at manholes and by remotely operated cameras for presence of live mussels. See Appendix E for the complete distribution system mussel monitoring protocols.

6.2 Long-Term Monitoring

Final determination of eradication success or failure will require at least 2 full consecutive seasons of monitoring for presence of live mussels. A preliminary determination will be made at the conclusion of the visual mussel inspections, and if favorable will initiate monitoring strategies to shift from short-term to long-term protocols. Long-term monitoring will largely mimic short-term protocols but with efforts focused during the peak mussel settlement period at San Justo, June to October.

6.2.1 Genetic Analysis

It is suggested that the genetic profile of zebra mussels from San Justo Reservoir be obtained prior to treatment. Should mussels be found to re-infest the reservoir after the eradication, genetic information would be used in assessing eradication failure versus re-introduction from an external source.

7 Quarantine

All vehicles and equipment used for the eradication effort that come into contact with water from the reservoir or the distribution system, regardless of whether it has been treated with potassium solution, will undergo inspection and decontamination before moving off site. Inspection/decontamination will follow procedures presented in the Bureau of Reclamation Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species and the California Department of Fish and Wildlife Aquatic Invasive Species Decontamination Protocol, available at:

https://www.usbr.gov/mussels/prevention/docs/EquipmentInspectionandCleaningManual2012.pdf.

https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=43333&inline

Use of a high-pressure hot-water decontamination equipment will be necessary. A trailer-mounted mobile unit for on-site use is preferred; alternatively, all equipment and vehicles may be transported directly to the nearest decontamination station.

Care should be taken to either avoid contact with water or decontamination of all sampling equipment, boats, waders, or any other equipment for the duration of the eradication effort until data confirm the complete absence of invasive mussels. This will likely include an extended timeframe after the eradication effort is complete, the duration of which will be determined by the outcome of the monitoring effort and a consensus from all stakeholders. It is anticipated to be 2 years at a minimum, but likely longer.

Reclamation and San Benito have also committed to prepare a zebra mussel re-infestation prevention program, consistent with Bay Area Consortium's Zebra and Quagga Mussel Coordinated Prevention Plan.

8 References

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Appendix A Dose Exposure Responses of Zebra Mussels to Potassium (potash), Ex-Situ Study Conducted at San Justo Reservoir

A-1. Methods

Study design was a two-factor experiment, with water temperature and potassium dose as main factors. There were two levels for water temperature (54 °F and 72 °F) and five levels for potassium dose (0, 25, 50, 100, and 200 ppm). The 54 °F and 72 °F temperature treatments were created via a water bath using flow-through chillers, aquarium heaters, and plastic wading pools. We used 0 ppm potassium as a negative control and 200 ppm potassium as a positive control, expecting little to no mortality over the experiment at the 0-ppm dose and relatively fast and 100% mortality at the 200 ppm dose. There were five replicates for each treatment combination (total of 50 experimental units).

Each replicate was a one-quart glass jar containing ten adult zebra mussels and aerated with aquarium bubblers. Mussels were collected from the infested reservoir, examined and confirmed live, then sorted into one of 3 size classes: approximately <0.2 inches (small), 0.2-0.6 inches (medium), and >0.6 inches (large). Mussels were assigned at random to each replicate at the ratio of 7 large: 2 medium: to 1 small mussel, and each replicate was in turn assigned at random to a treatment combination.

For the first 3 weeks of the experiment, the number of mussels in each replicate that were dead and the number that were alive was recorded daily. The water in each replicate was replaced daily with fresh reservoir stock solutions of water dosed with the appropriate amount of muriate of potash. Each mussel in each replicate was observed to determine whether they were dead or alive. If a mussel exhibited symptoms characteristic of a dead mussel (e.g., gaping and unresponsive), it was removed from the treatment jar and placed into a separate jar supplied with untreated reservoir water (i.e., no MOP, 0 ppm potassium), allowing it to recover. A recovery jar was created for each experimental unit that yielded mussels that appeared dead on a given day. Mussel(s) that appeared dead were allowed to recover for 3 days and then were then observed again to verify their status (dead or alive). Any mussel(s) that had failed to recover (i.e., exhibit symptoms of a living mussel) after 3 days in the recovery conditions were recorded as dead (on the date it was removed from the treatment jar and placed in the recovery jar) and they were removed from the experiment. If a mussel recovered, it was deemed an inaccurate mortality assessment and removed from the experiment. Mussels exhibit levels of “intoxication” when exposed to potassium, where their shells gape and are unresponsive, but will return to normal after being placed in untreated water. Two of the experimental treatments were initially suspected to be dead but recovered after 24 hours. These treatments were re-created with new mussels from the reservoir, and the protocol modified for determination of live vs. dead mussels. None of the mussels recovered after being assessed as dead following the initial misclassification.

In addition to the number of dead and live mussels in each jar, water temperature, dissolved oxygen content, and the pH at the time of the observation were recorded. Also, the length of the shell for mussels that were determined confirmed to be dead was either measured directly, or the shell was photographed for later size measurement.

After three weeks of daily observations, the time between observations and water changes for the remaining live mussels was increased from 1 to 4 days through the duration of the study (total of 45 days). Observations were continued in order to determine potential long-term effects of treatment at lower temperatures and doses where mortality was low.

A-2. Results and Discussion

Both concentration of potassium and water temperature had considerable effects on mussel mortality. Dose-exposure curves for MOP treatments are presented in Figure A-1, and days required to reach complete mortality by treatment are presented in Table A-1. Control treatments (0 ppm) at both temperatures did not exhibit any mussel deaths, confirming mortality in treatment jars was caused by the MOP treatment and not an artifact of jar confinement, low oxygen/nutrient levels, etc. The 25 ppm treatments at both temperatures reached nominal mortality (2%) by the end of the 45-day trial.

At concentrations at and above 50 ppm the effects of water temperature were more apparent, with lower temperatures exhibiting slower mortality, likely the result of a slower metabolic rate and less-frequent feeding by the mussels, as they were observed to be generally more tightly shut in their shells at 54° F vs. 72° F. The 50-ppm treatment reached 100% mortality in 36 days at 72° F, whereas the 54° F/50 ppm treatments topped out at 42% mortality at the end of the 45 day trial. All mussels treated with 100 or 200 ppm potassium attained 100% mortality within the study period. Mussels exposed to both these concentrations in the warmer water treatments died off more quickly than the cooler water treatments.

These results suggest that eradication of zebra mussels at San Justo reservoir is feasible at 100 ppm potassium concentrations and should be able to eradicate mussels within the reservoir in just under 30 days, even in the later part of the season when temperatures are low. However, these tests were conducted in artificial environment and may not translate directly to reservoir-scale treatments. Water currents, thermal stratifications, and varying depths may prevent complete mixing of the applied MOP and cause a much longer holding period necessary to achieve the desired concentration throughout the reservoir and result in mussel eradication. Treatment with MOP when water temperatures are higher (late summer-early fall) will likely distribute throughout the reservoir more quickly and hit the mussels while they are more active metabolically, resulting in quicker eradication and potentially less risk of escapes (eradication failure).

Table A-1. Days to achieve 100% mussel mortality by potassium concentration and water temperature. Treatments with missing values did not reach 100% within the study period.

Treatment Concentration (ppm K)	Treatment Temperature (°F)	Days to 100% Mortality
0	54	-
25	54	-
50	54	-
100	54	25
200	54	17
0	72	-
25	72	-
50	72	36
100	72	8
200	72	6

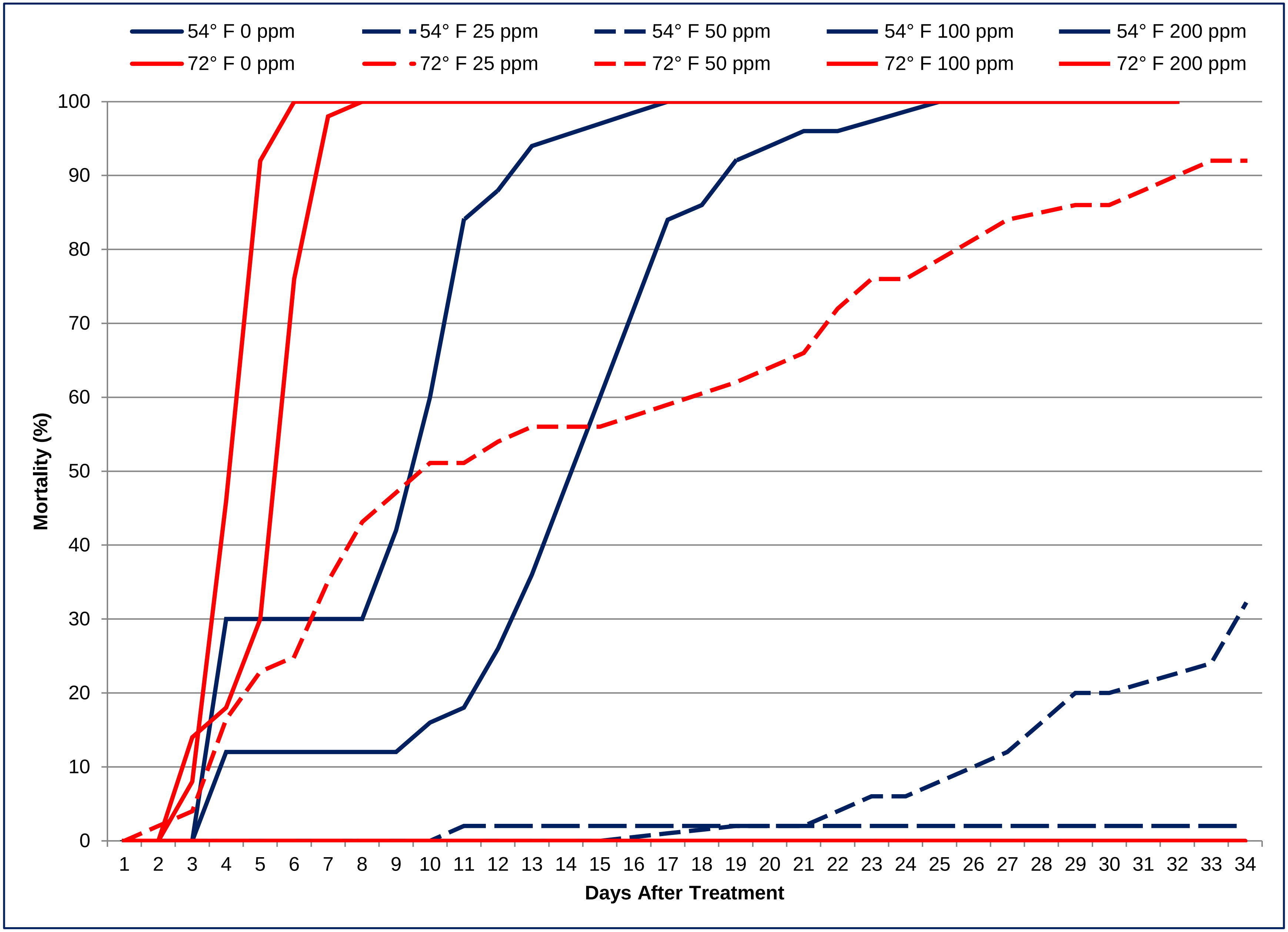
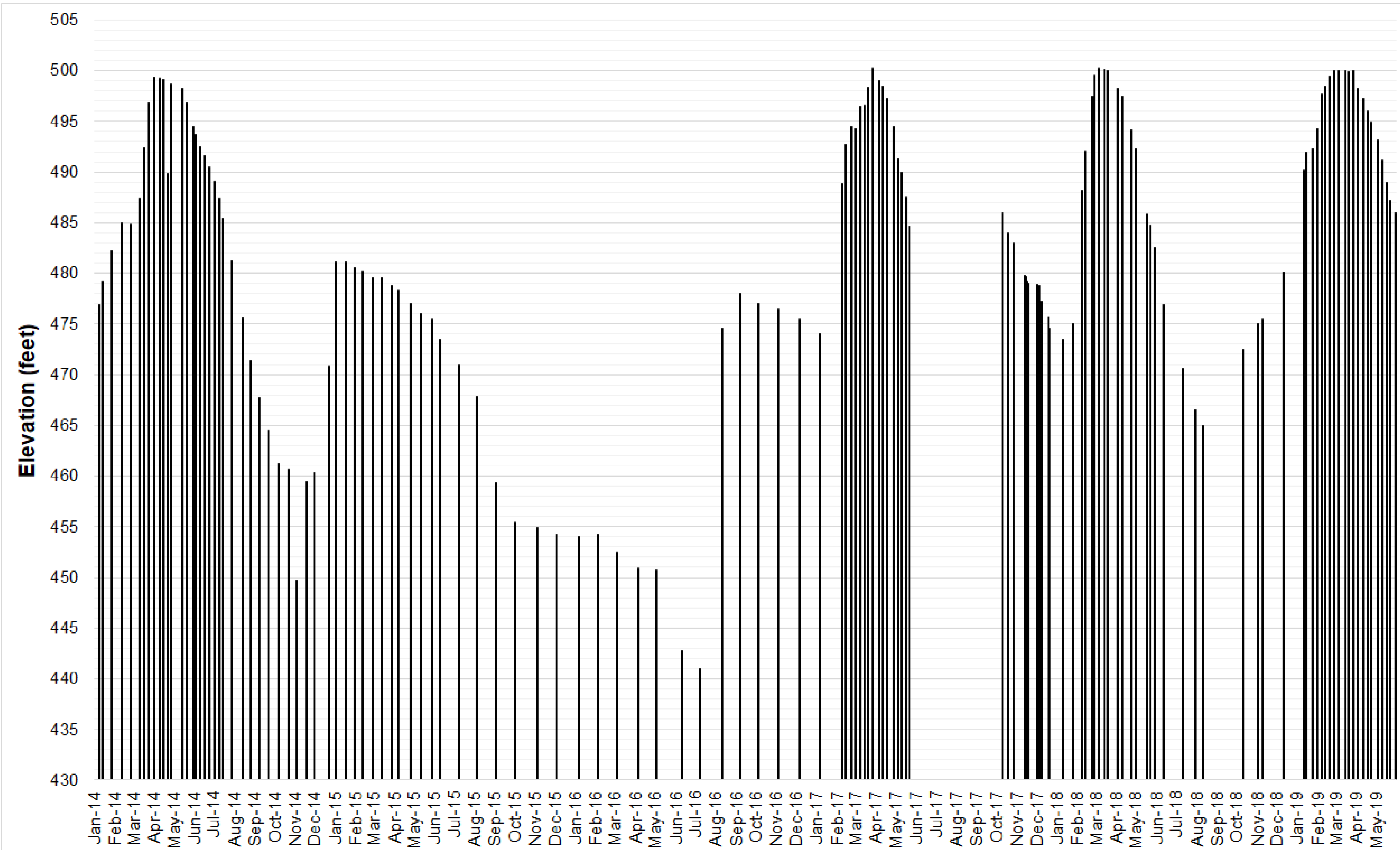


Figure A-1. Mean dose-exposure curves for mussels treated with potassium at 54° F and 72° F.

Appendix B San Justo Reservoir Elevations, January 2014 to May 2019



Appendix C San Justo Reservoir Water Quality Data

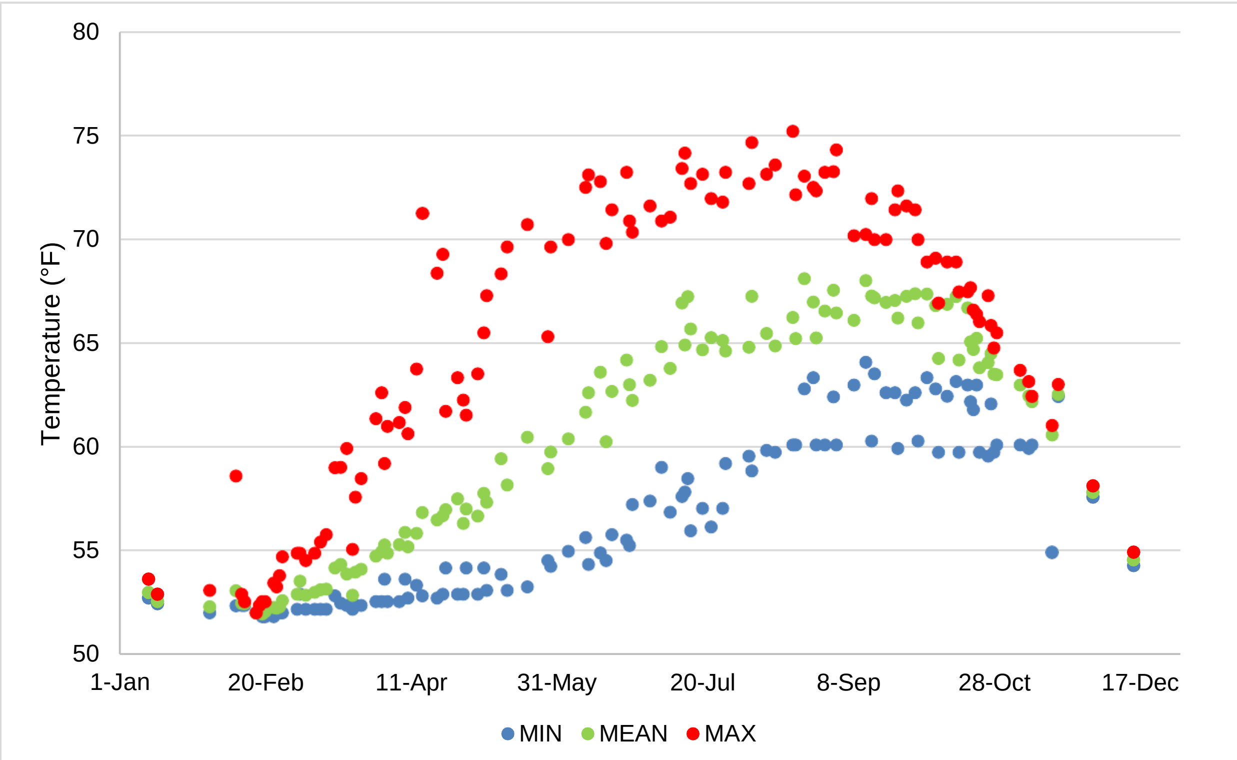
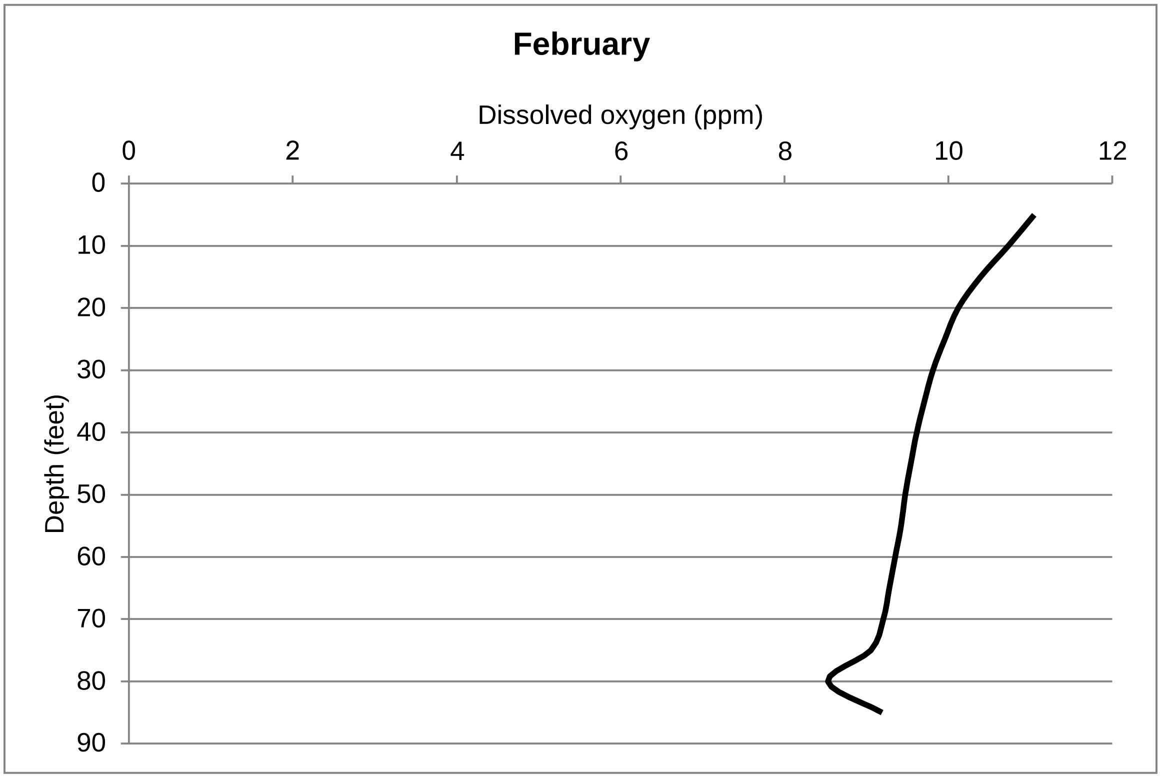


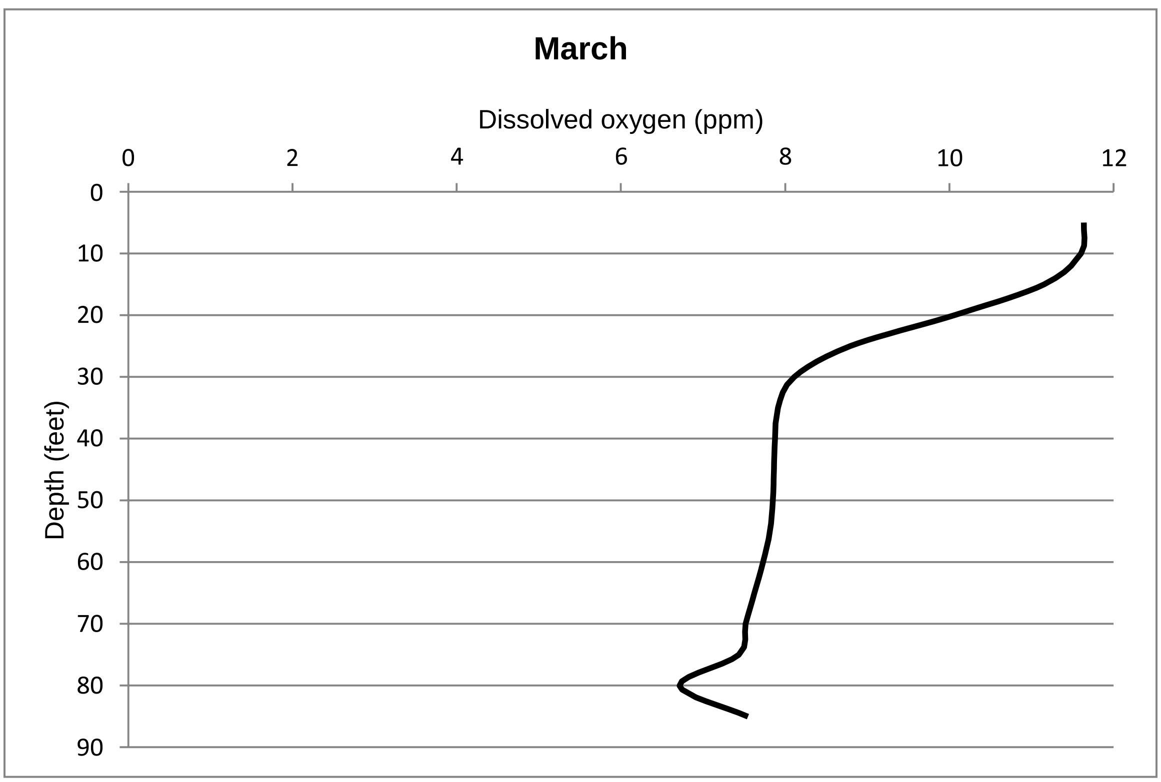
Figure C-1. San Justo Reservoir aggregate water temperature data compiled from vertical profiles and other available data, 2008-2011.

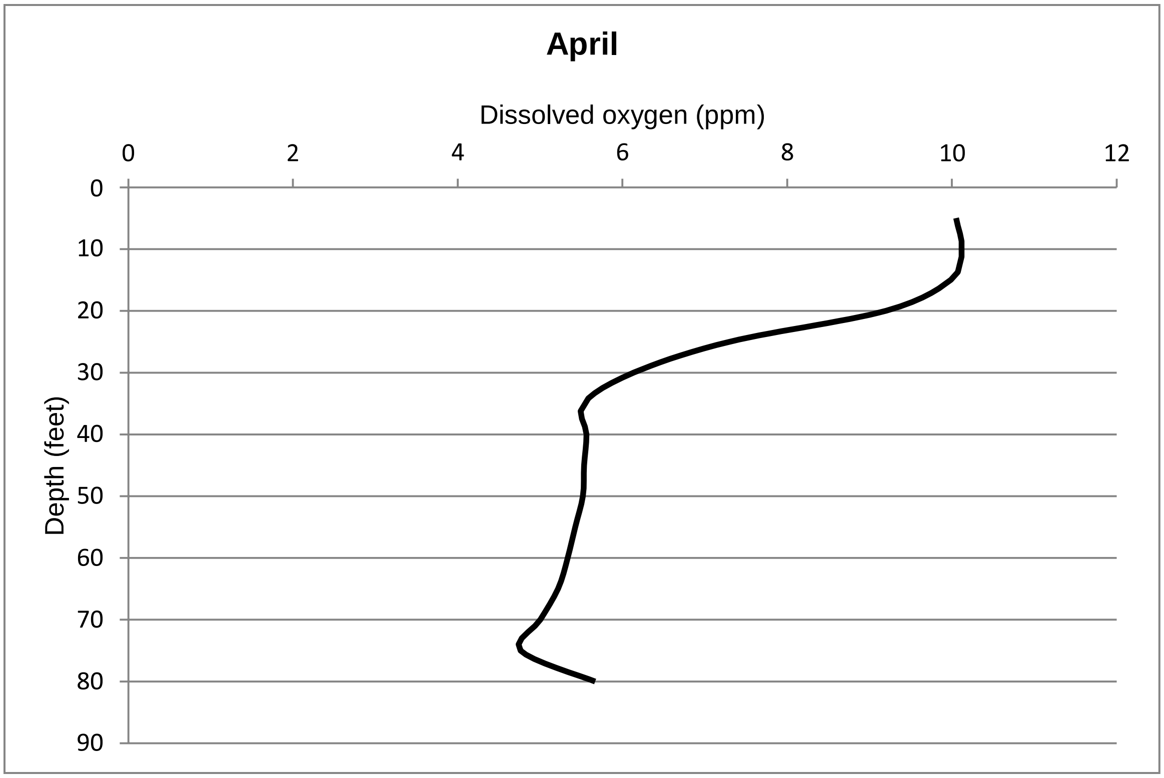
Table C-1. Existing water quality data, San Justo Reservoir

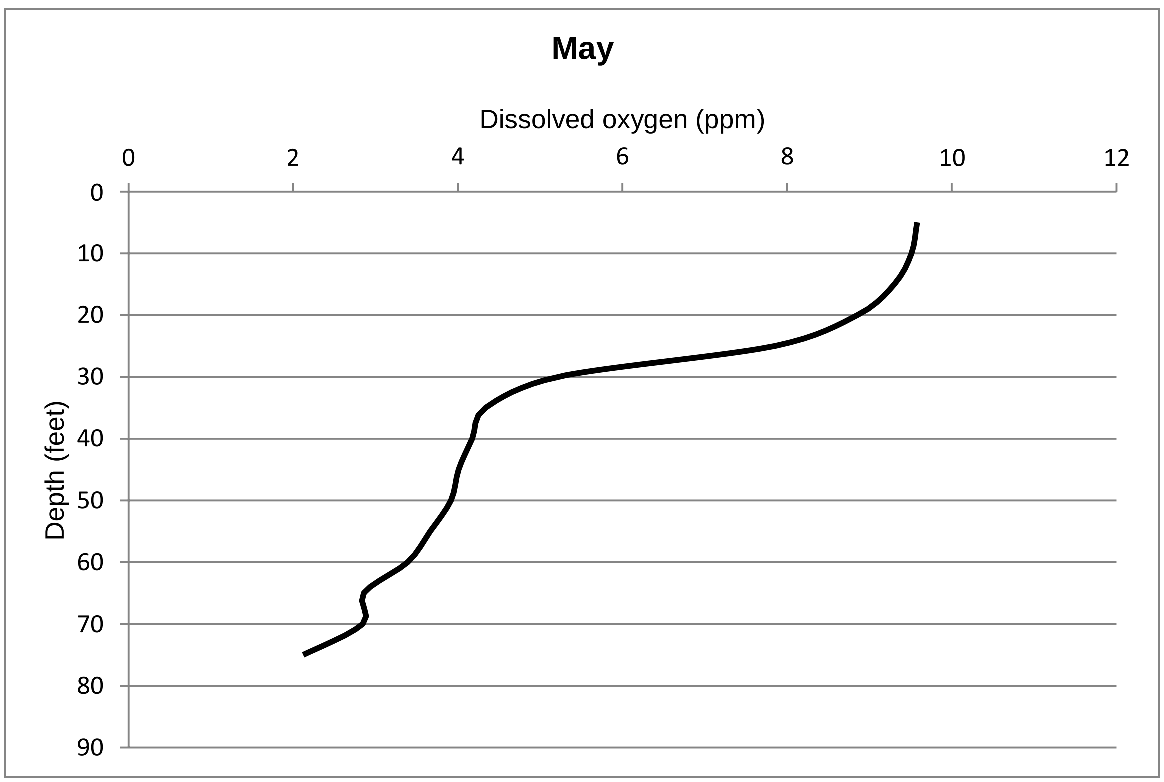
Parameter	Sample Date Range			Minimum	Average	Maximum	Units
Alkalinity	6/20/2012	-	7/27/2012	79	83	88	ppm $CaCO_3$
Calcium	5/19/2011	-	7/27/2012	9	16	21	ppm
pH	5/19/2011	-	7/27/2012	7.6	8.2	8.9	-
Turbidity	5/19/2011	-	5/19/2011	1.4	2.8	4.2	NTU

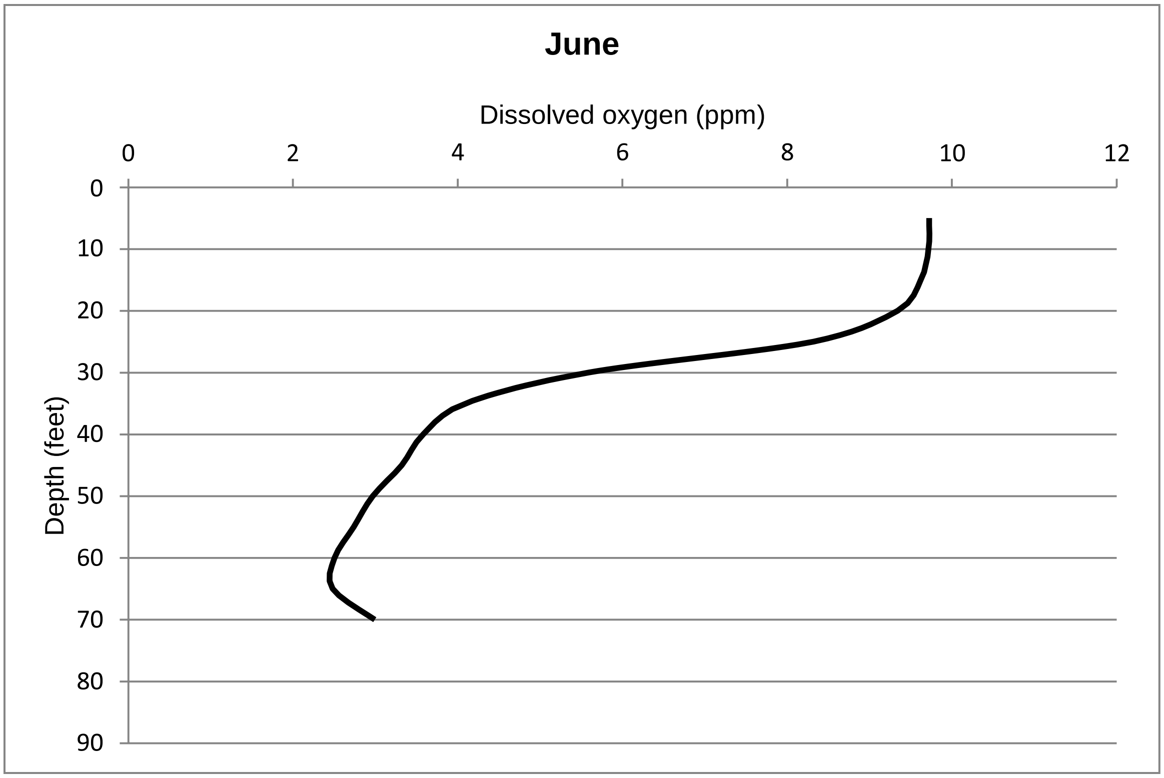
Figure C-2. Monthly dissolved oxygen profiles, San Justo Reservoir 2009-2010.

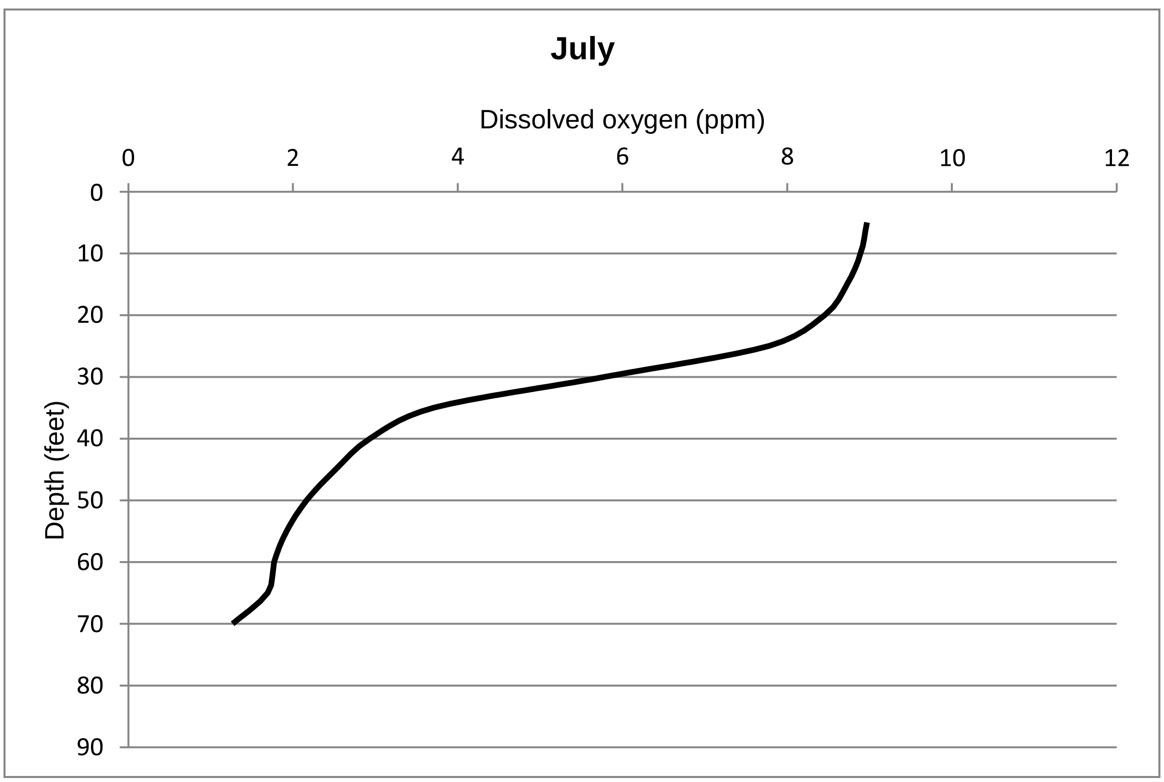


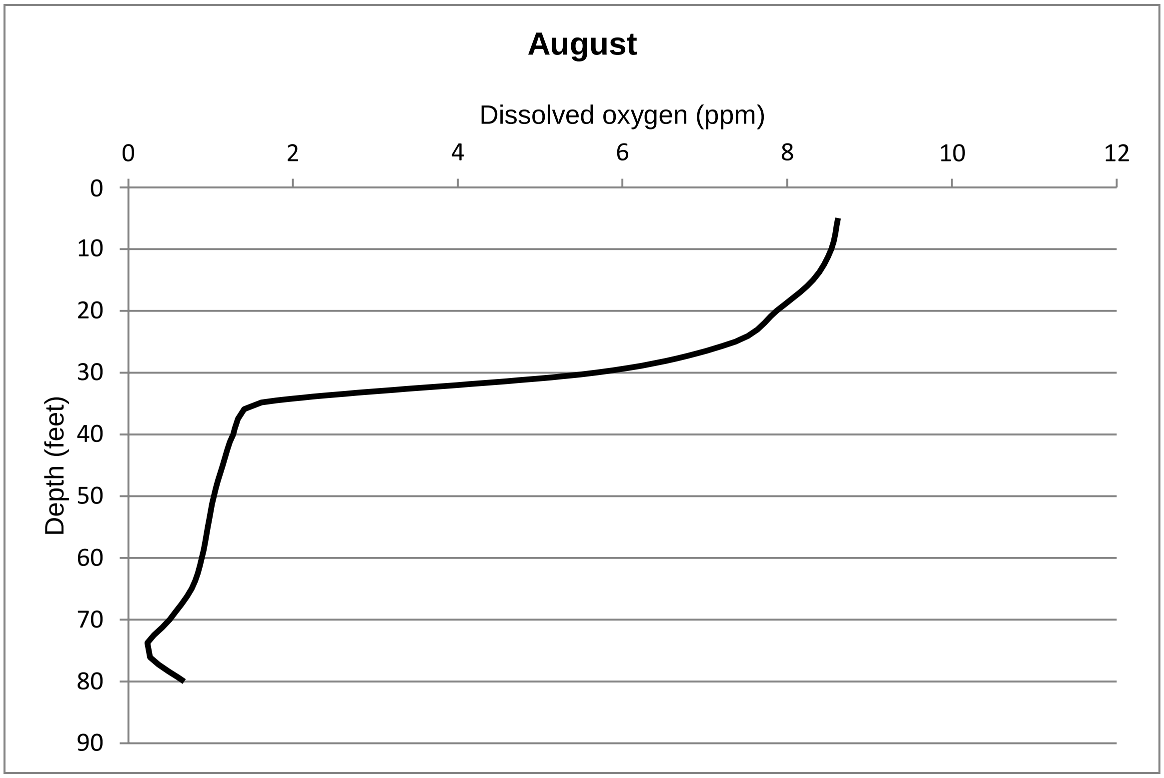


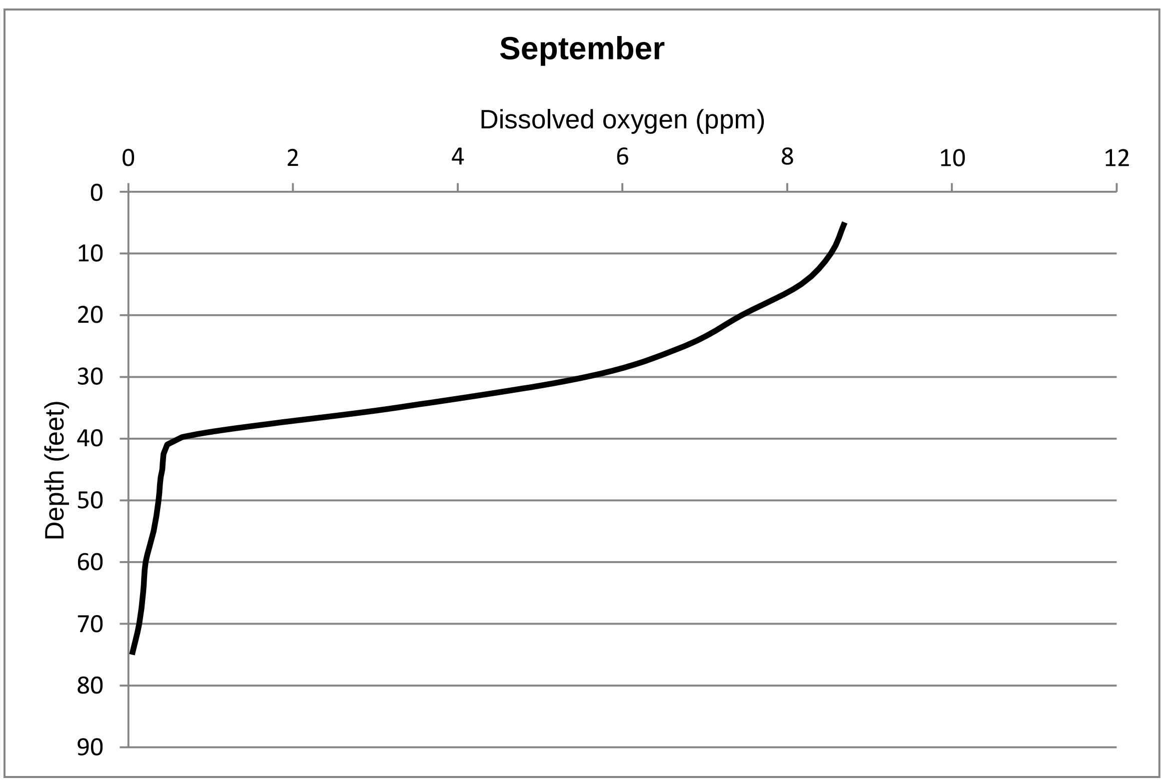

April
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90
Depth (feet)

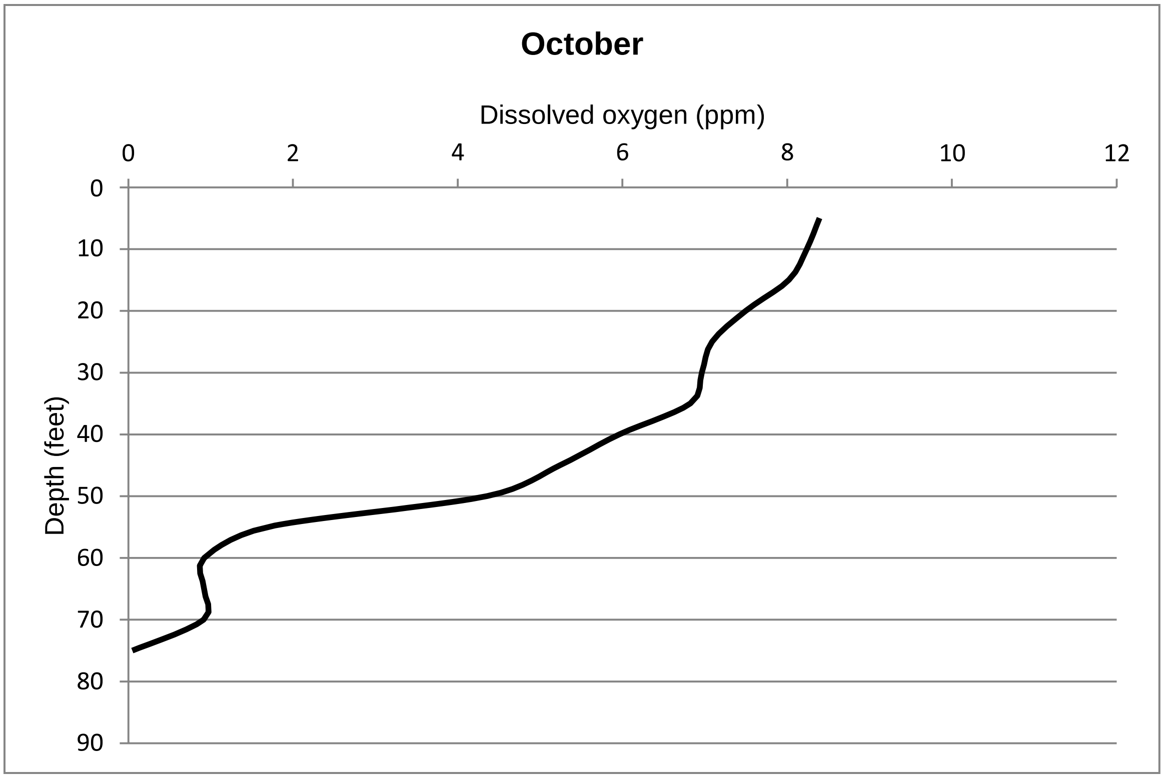

May
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
0
10
20
30
40
50
60
70
80
90
Depth (feet)

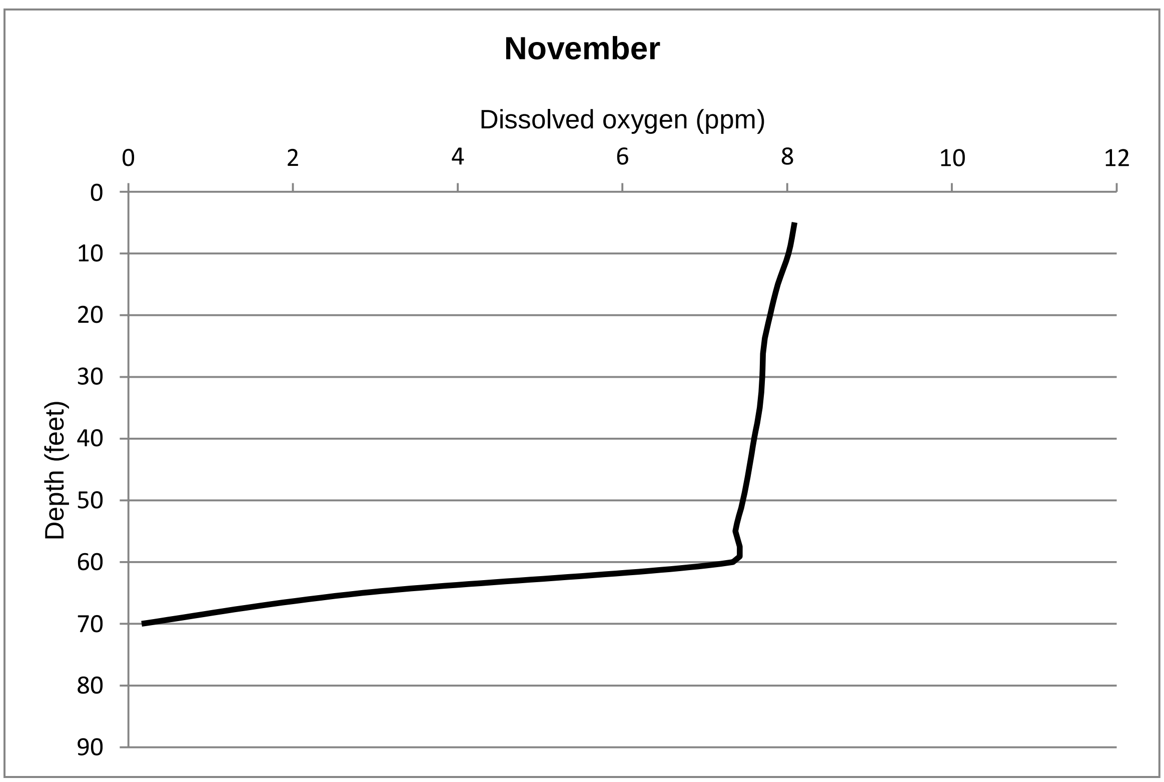

June
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90


July
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90


August
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90


September
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90


October
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90


November
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90

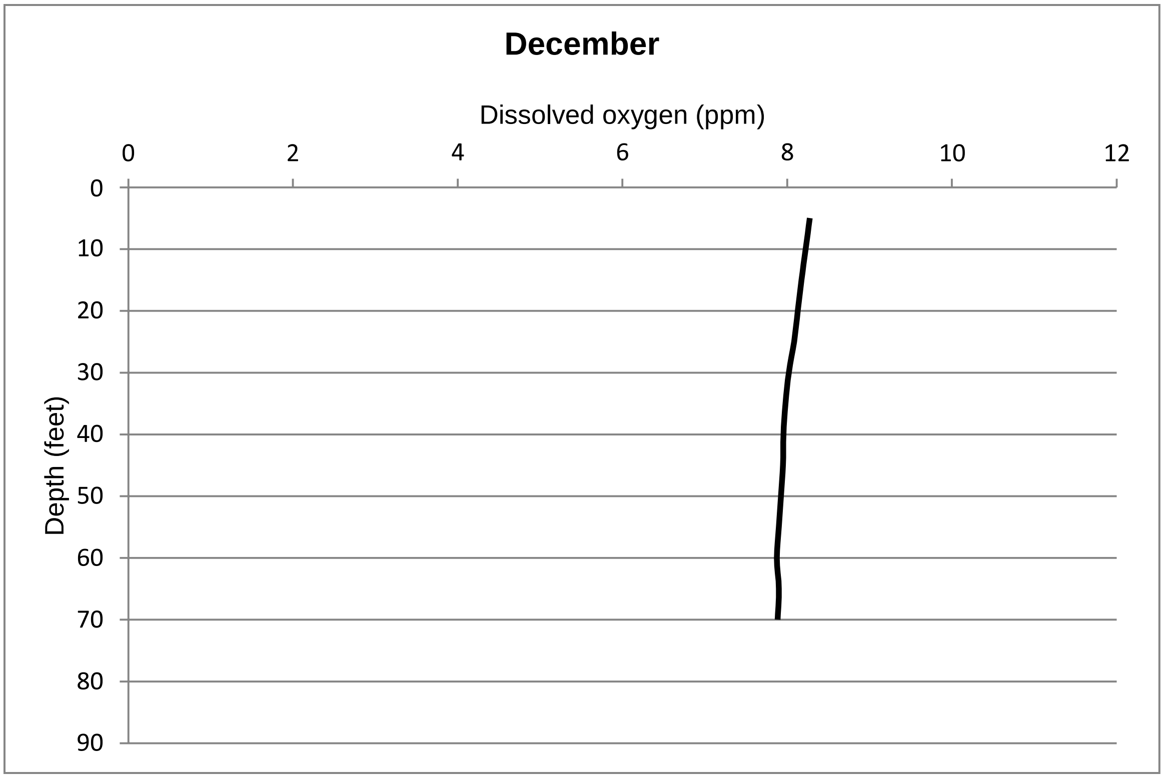
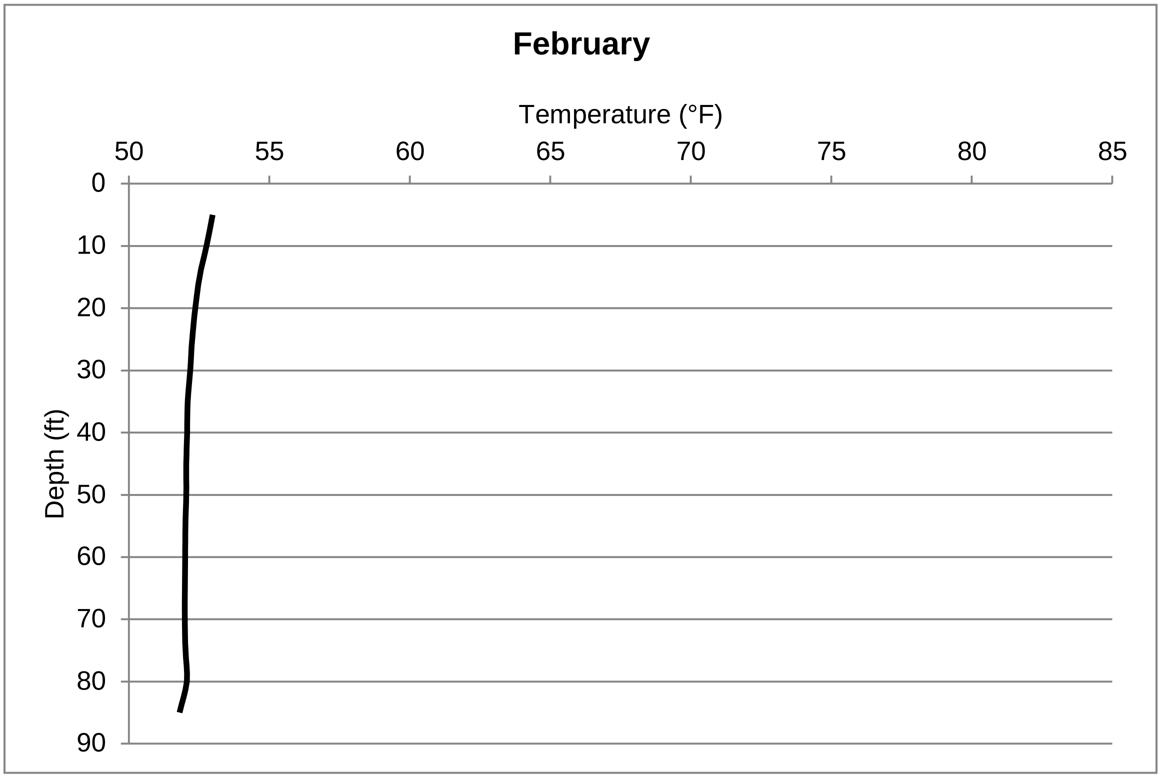
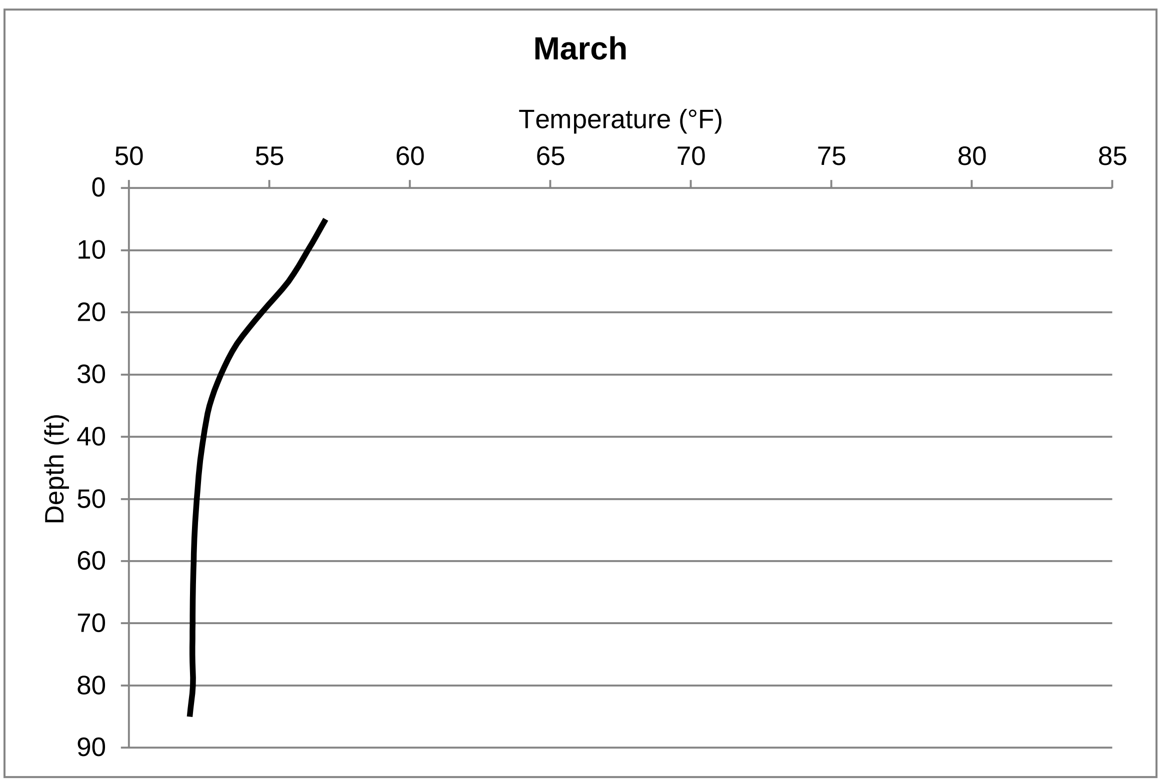
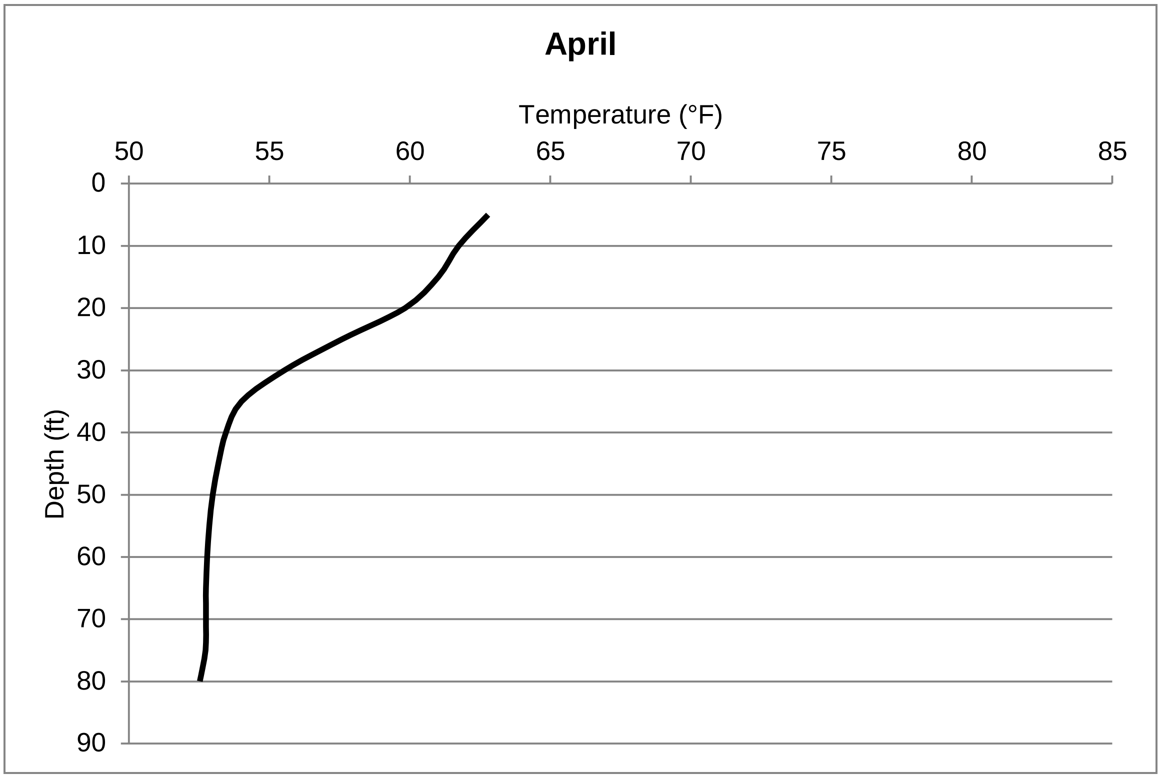

December
Dissolved oxygen (ppm)
0
2
4
6
8
10
12
Depth (feet)
0
10
20
30
40
50
60
70
80
90

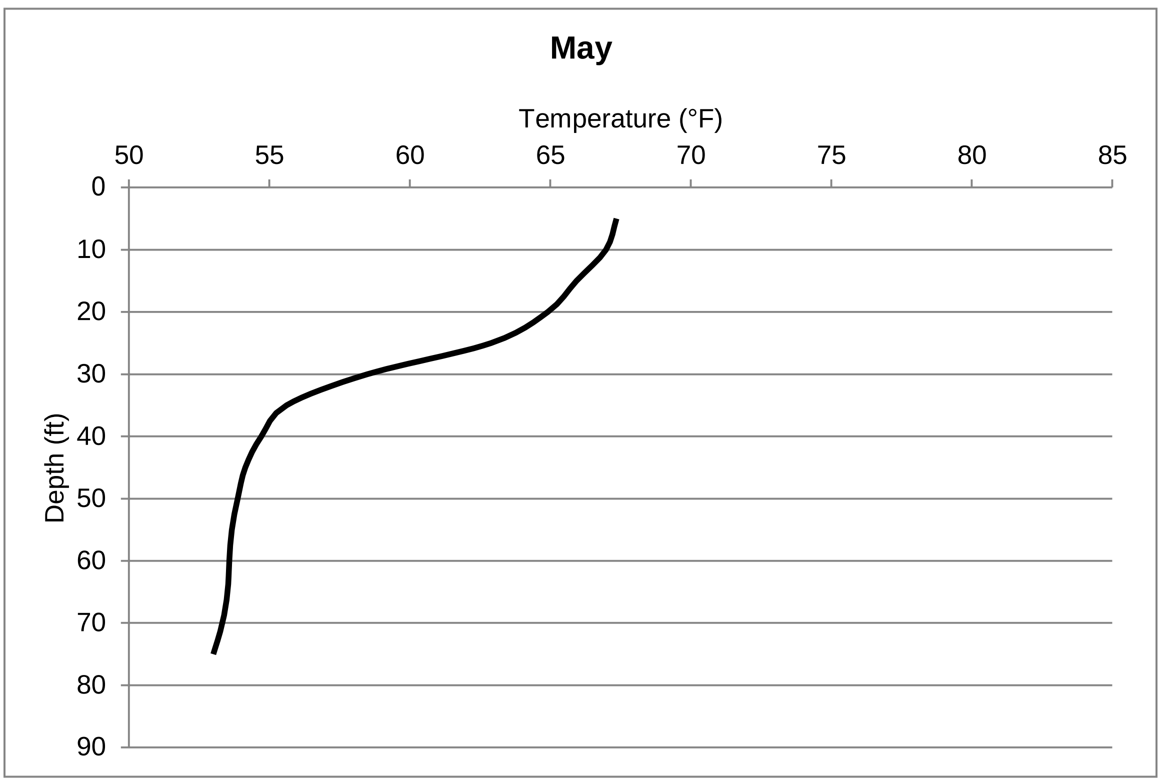
Figure C3. Monthly temperature profiles, San Justo Reservoir, 2009-2010



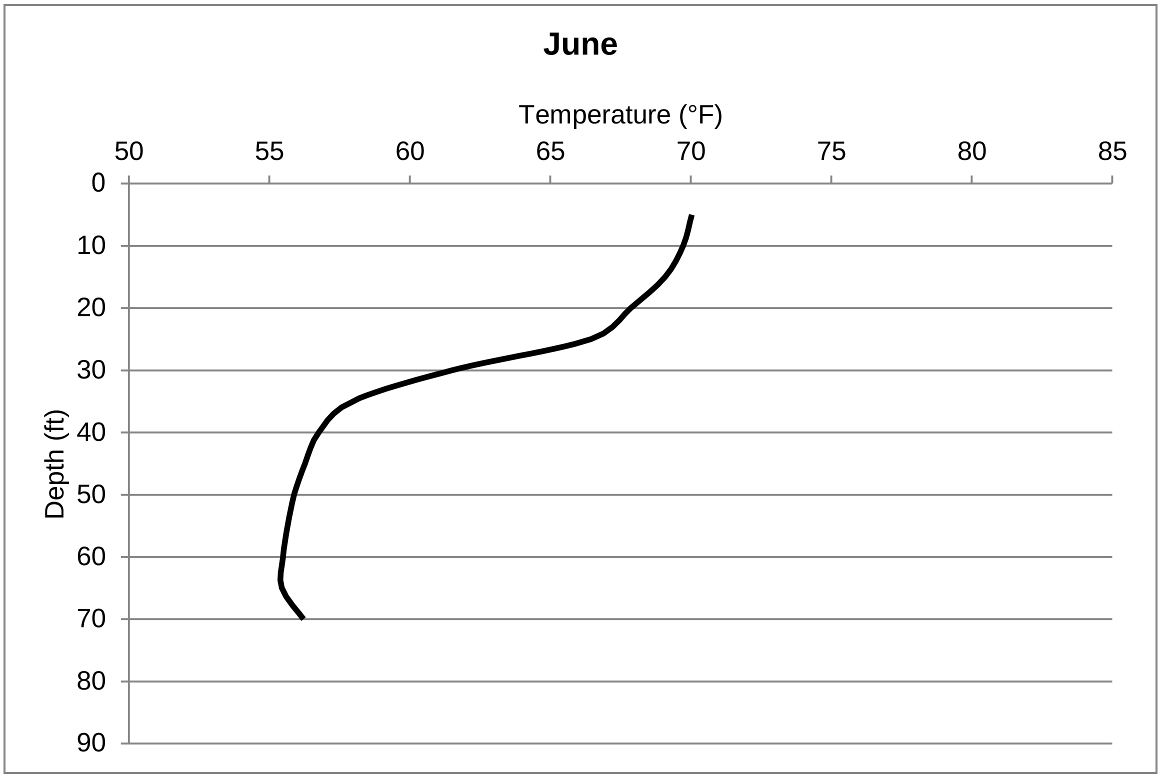




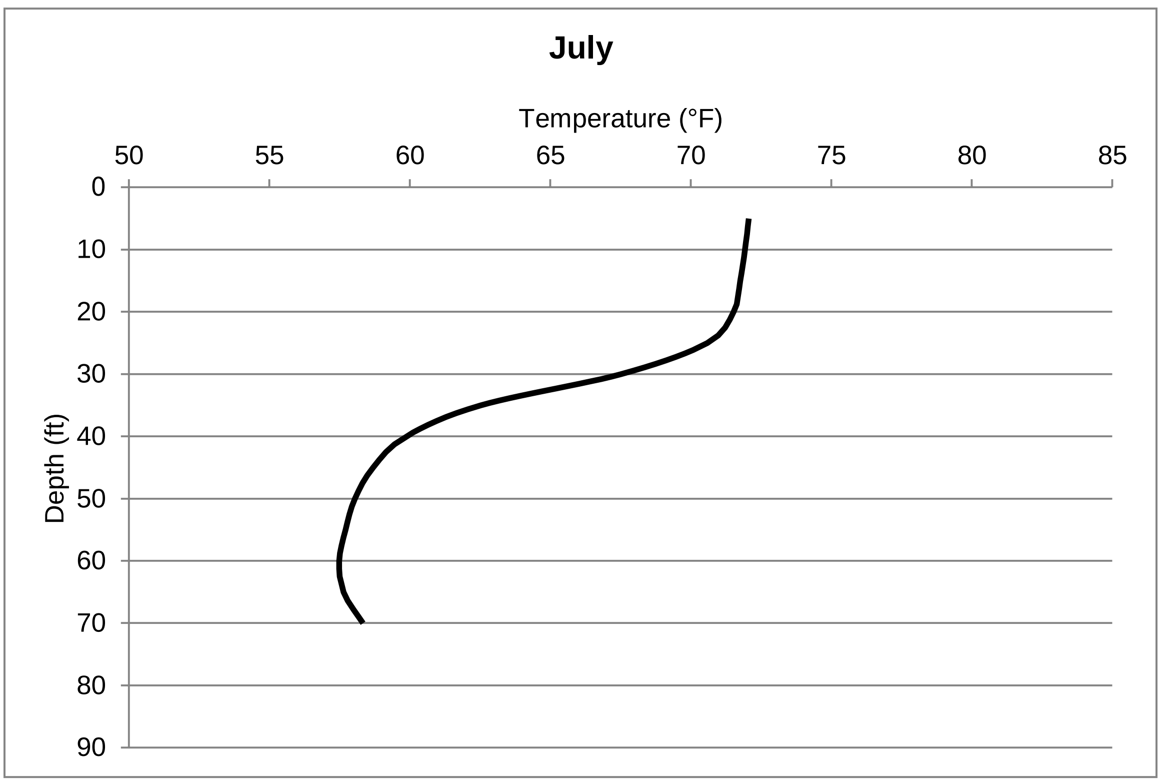
April
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)



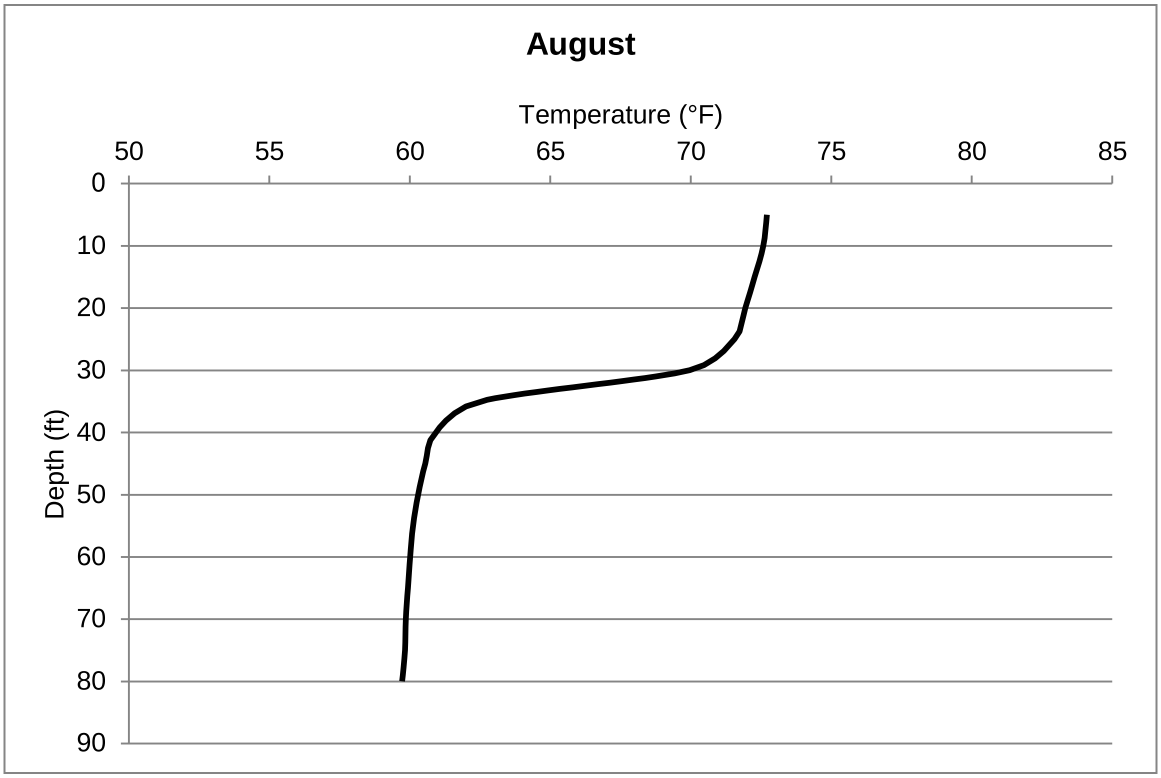
May
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)

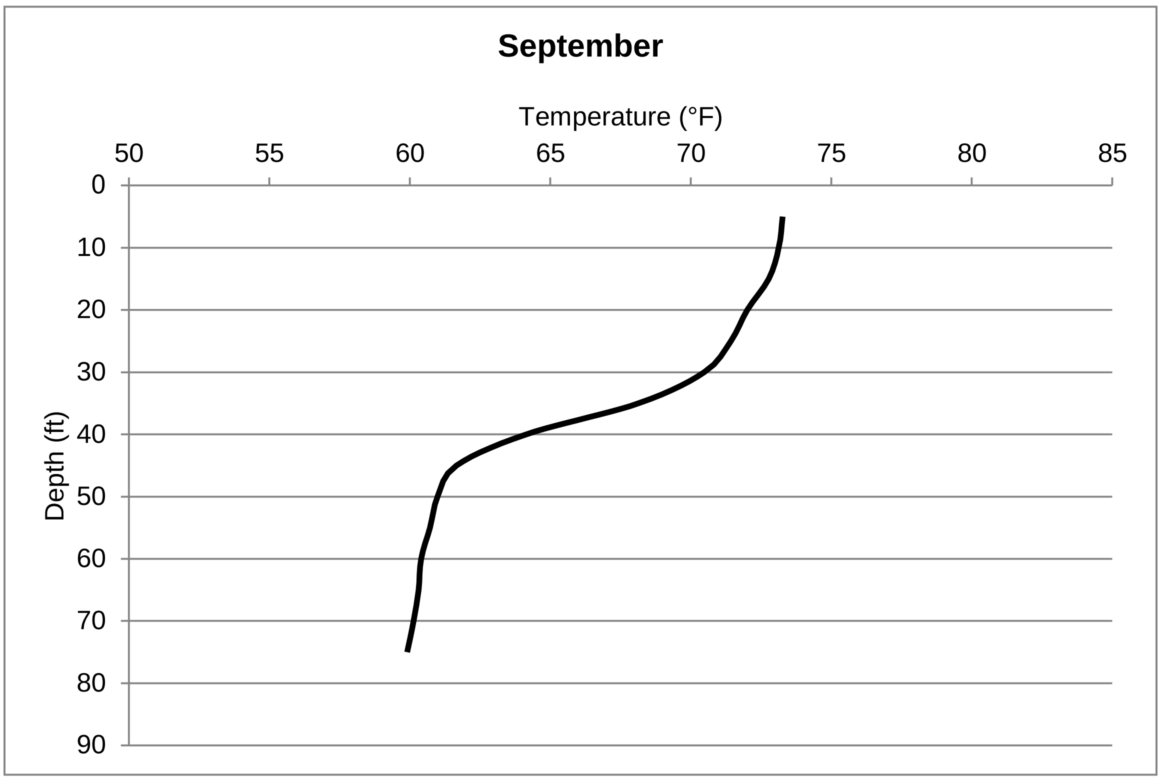


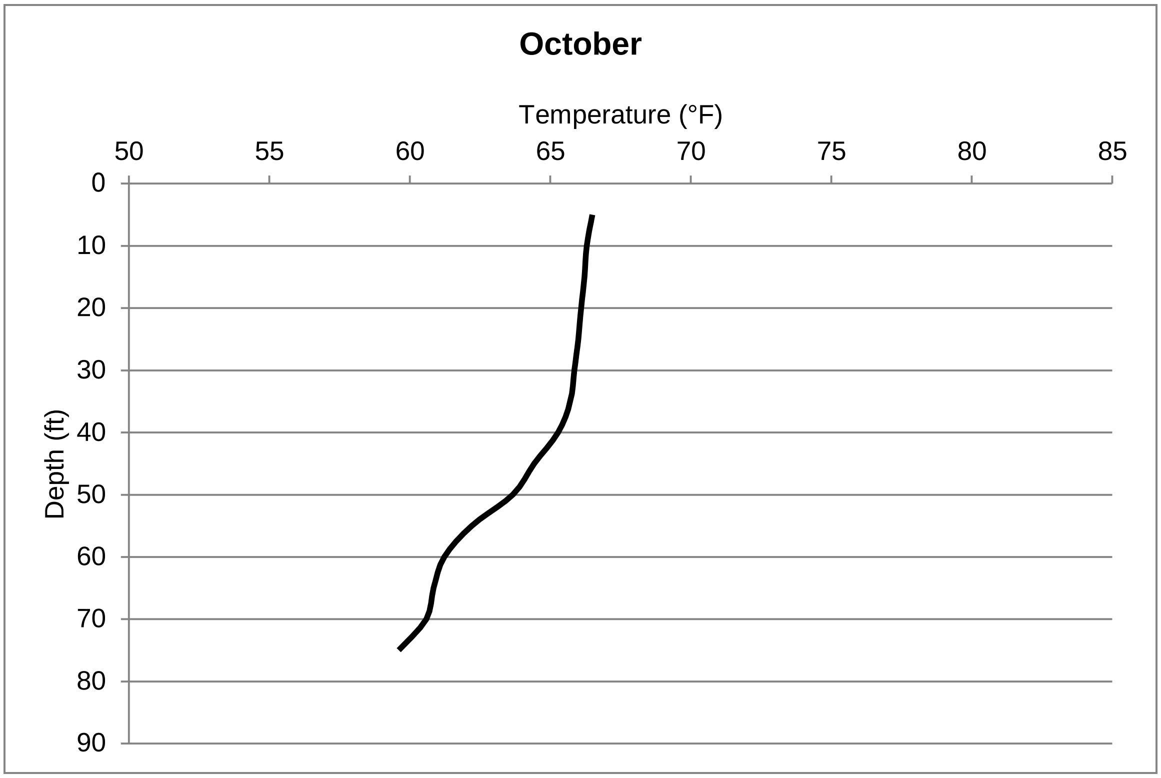
June
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)



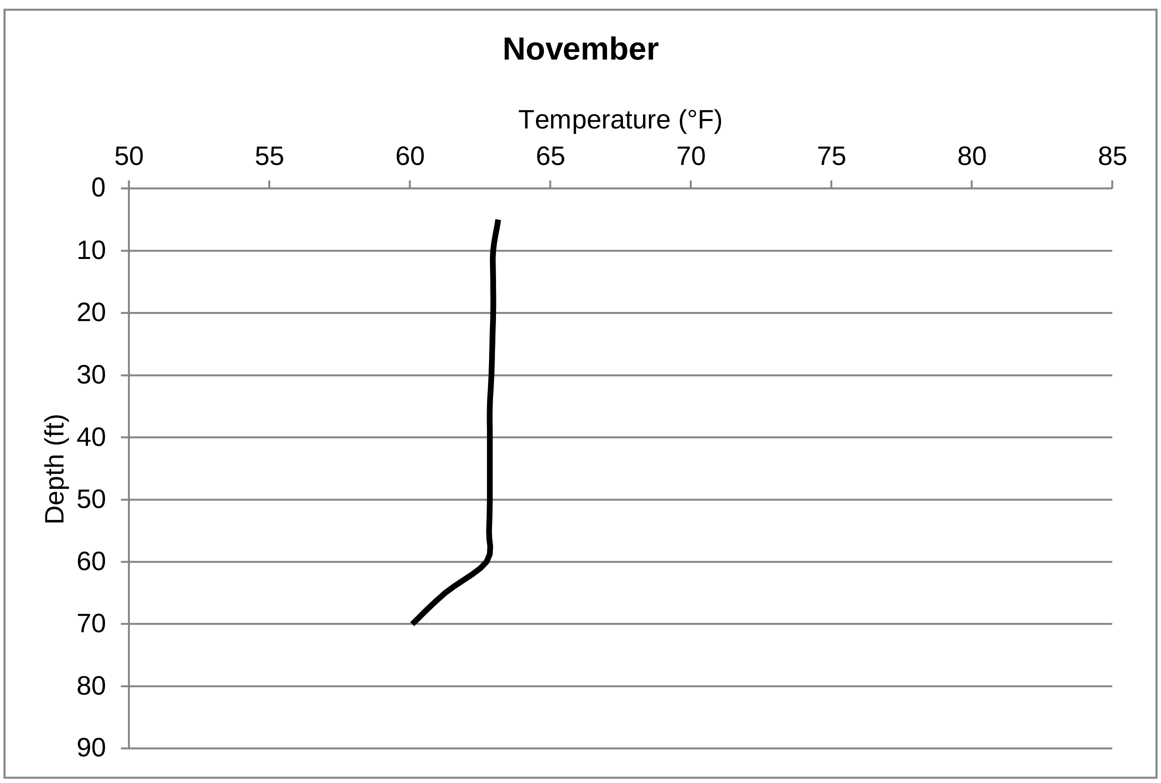
July
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)


August
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)

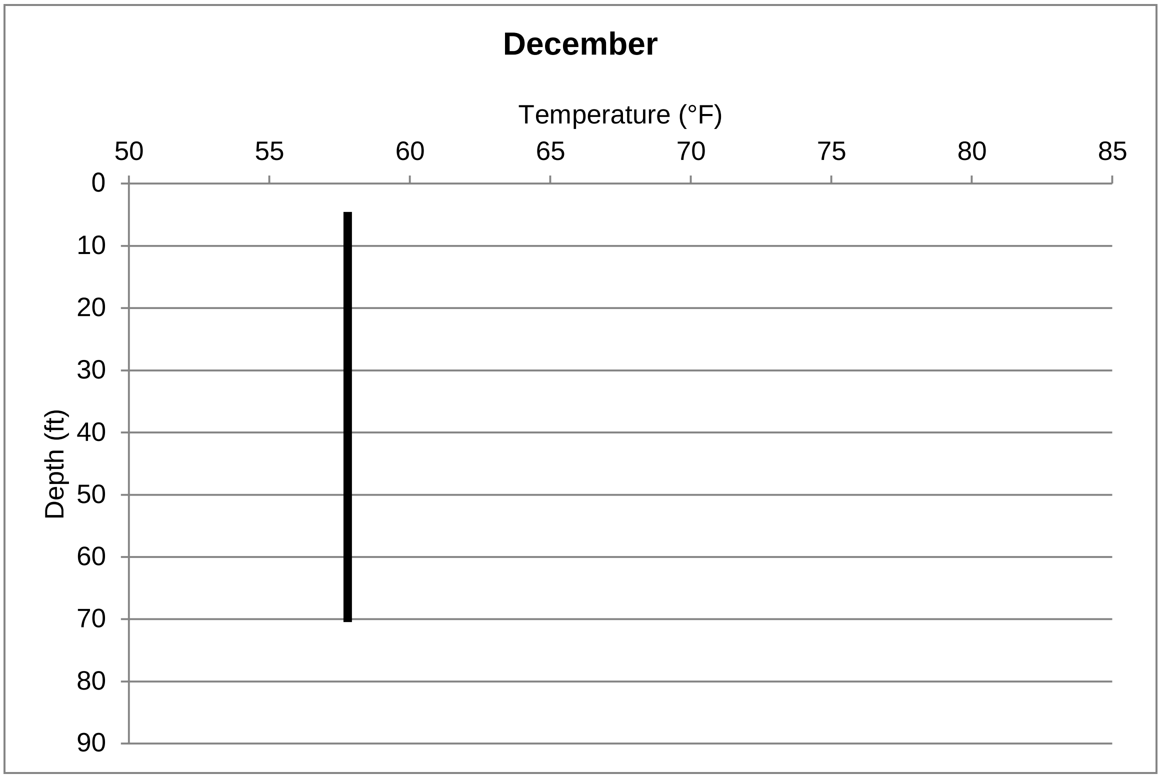

September
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)



October
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)



November
Temperature (°F)
50
55
60
65
70
75
80
85
0
10
20
30
40
50
60
70
80
90
Depth (ft)


December
Temperature (°F)
50
55
60
65
70
75
80
85
Depth (ft)
0
10
20
30
40
50
60
70
80
90

Appendix D. Historical Climate Data Summary, Hollister California

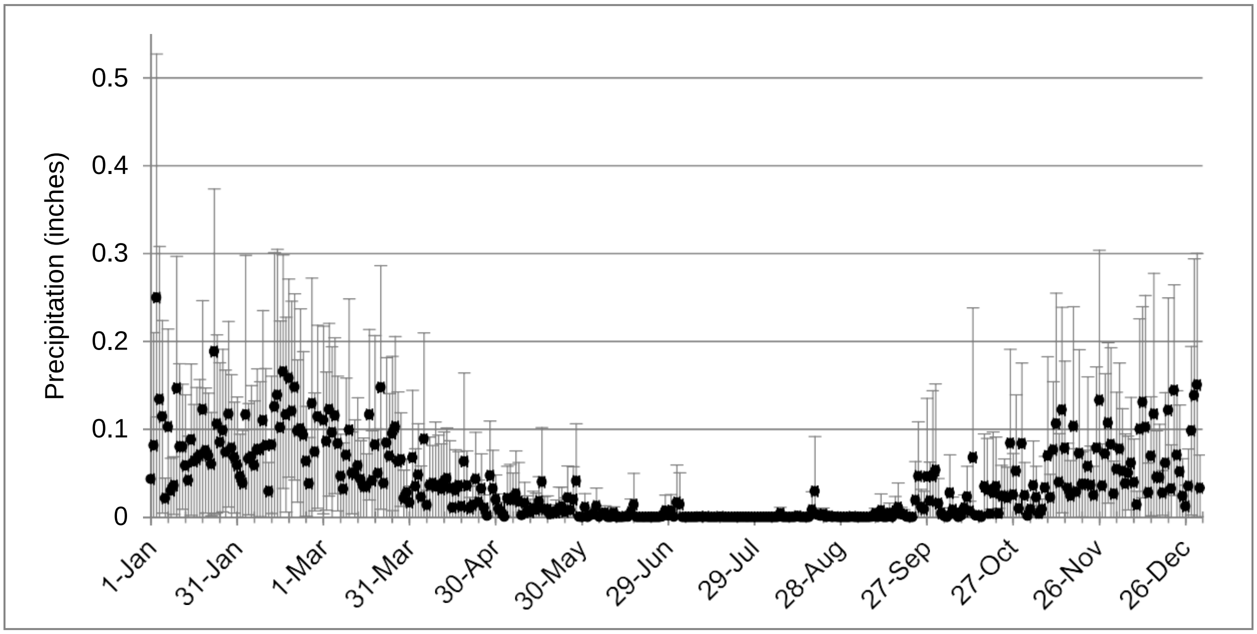


Figure D-1. 35 year daily precipitation means and 99% confidence intervals, Hollister weather station (2).

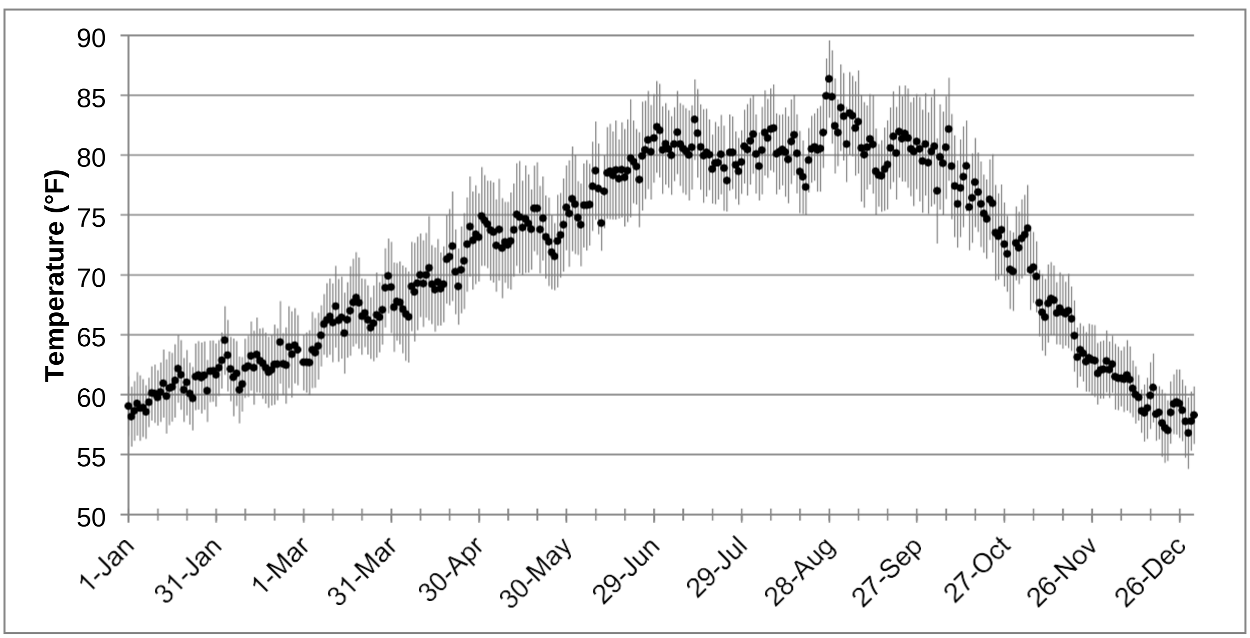


Figure D-2. 35 year daily temperature means and 99% confidence intervals, Hollister weather station (2).

NOTE - Further climate summary information for Hollister, CA can be found at:
https://weatherspark.com/y/1035/Average-Weather-in-Hollister-California-United-States-Year-Round

Appendix E. Eradication Treatment Monitoring

E-1 Decontamination

Sampling equipment will be dedicated for use at San Justo Reservoir for the duration necessary to provide sufficient data for the eradication, and will not be used for sampling other water bodies during this period. Dedicated equipment will alleviate any potential to spread mussels to new water bodies, but measures for decontamination should still be conducted regularly to provide accurate assessment of veliger distributions within the reservoir and over time.

E-2 Monitoring Overview

Eradication performance will be monitored using water quality parameters and zebra mussel presence, settlement, and mortality at multiple life stages. Parameters to be measured include:

- Water quality (field sonde)
- Potassium concentration (field and lab measurements)
- Shoreline adult mussel population surveys (visual)
- Shoreline temperature and humidity (sensors with data loggers)
- Adult mussel mortality (live mussel assays)
- Adult/juvenile mussel settlement (artificial settlement substrates)
- Larval mussel presence and viability (plankton tow net surveys)

Details regarding all sampling, timing, and duration is presented in table E-1.

E-3 Water Quality

Basic water quality data can be collected instantaneously in the field using a multi-parameter sonde and handheld meter. Water quality data should be collected from various locations throughout the reservoir at 5-foot depth increments. Suggested sampling locations and reservoir depths are presented in Figure E1. A full panel of water quality analysis should be conducted before, during, and after treatment to accurately describe treatment conditions.

E-3.1 Equipment

- Multiprobe water quality instrument (sonde) with probes for temperature, dissolved oxygen, pH, conductivity, and turbidity.
- Handheld readout/recorder with connecting cables.
- Protective cage and calibration solutions/cups.
- Additional miscellaneous supplies (batteries, electrical tape, buffer, etc).

E-3.2 Methods

- All probes should be calibrated before every sampling event per the specific multiprobe model's standard procedures.
- Calibrate DO and pressure/depth on site.
- Turn on handheld and activate per manufacturer's instructions.
- Enter sample information (location, date etc.) and collect the first sample just beneath the surface of the water.
- Lower sonde slowly, logging samples every 5 feet until the bottom is reached.
- Record the maximum depth.

E-3.2.1 Distribution System

Methods for the reservoir can also be used for point monitoring along the distribution system by collecting water samples from taps in a container and submerging the sonde. Suggested sampling points for distribution sampling are presented in figure E2.

E-4 Potassium Concentration

Field tests for potassium concentrations in the reservoir should be conducted daily during the potash application and weekly during the holding period. A Kemmerer or similar equipment should be used to collect water samples from different depths. These samples will be used for field testing and split for lab verification.

E-4.1 Equipment

- Kemmerer water sampler with drop-line marked at 1-foot intervals
- Samples bottles - cleaned, sanitized, and accurately labeled to avoid potential for contamination.
- Handheld field colorimeter and sample cells
- Reagent set for potassium up to ~115 ppm; dilution of sample may be necessary to achieve levels within suitable range for the field colorimeter
- 25 mL mixing cylinder with stopper
- Filtration setup (funnel, filter paper, vacuum pump, etc.)
- Squeeze bottles and DI water for rinsing Kemmerer.

E-4.2 Methods

- Dip a sample bottle from the boat to collect the surface sample.
- Lower the Kemmerer to the 5-foot mark on the drop-line and operate the sampler to collect the 5 foot depth sample.
- Pull up the Kemmerer and transfer to appropriate sample bottle.
- Rinse Kemmerer with deionized water
- Repeat Kemmerer sample collection and collect separate samples every 5 feet until the bottom of the reservoir is reached; collect a sample at the bottom if the depth is greater than 3 feet from the sample above it.

- All field test samples should be diluted as necessary, buffered and/or mixed with proper reagents and analyzed using the handheld colorimeter per the specific instrument specifications.
- Lab sample bottles should be sealed with electrical tape and prepared per specific instructions from the lab and shipped for analysis.

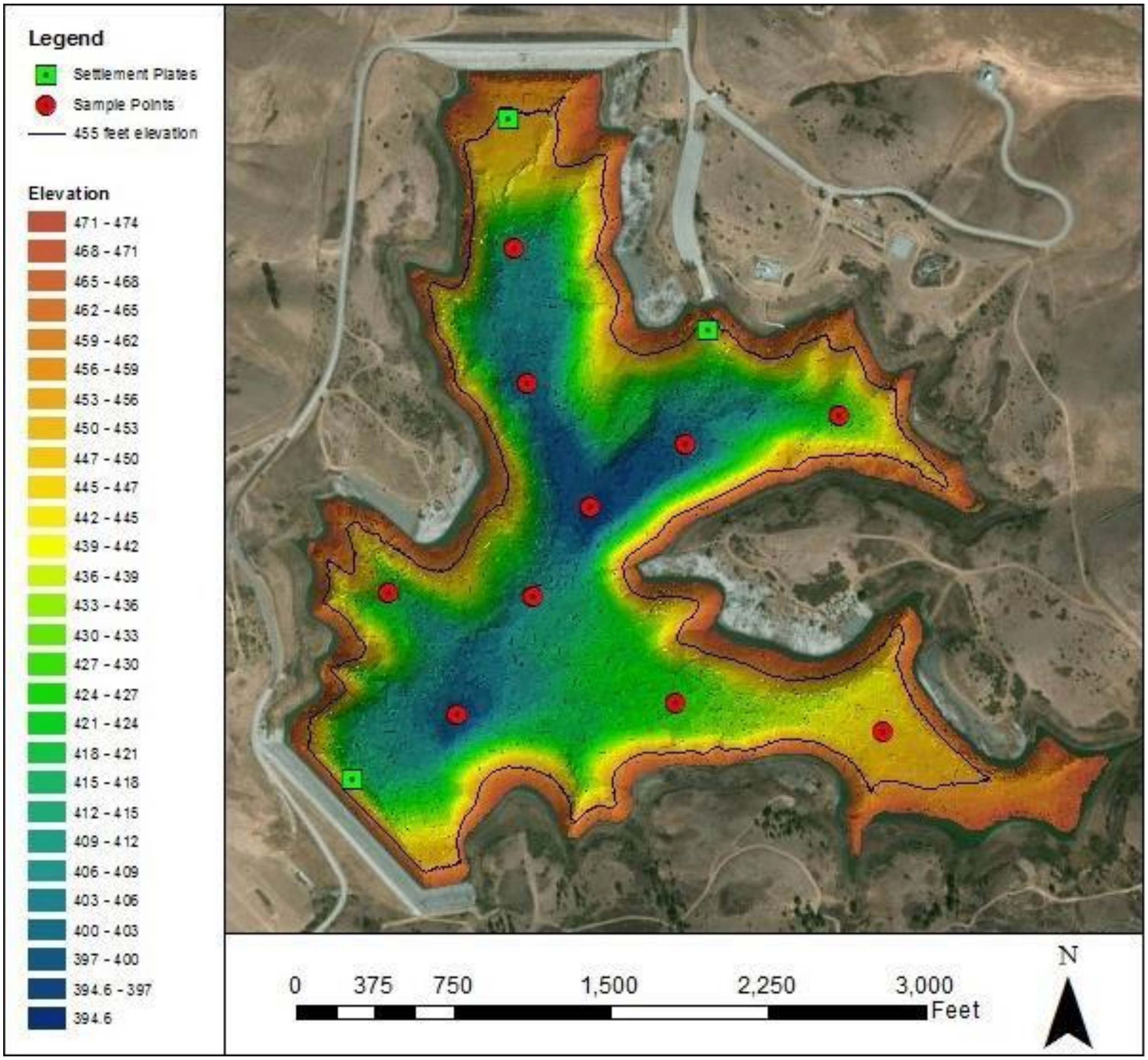


Figure E-1. Suggested monitoring locations, San Justo Reservoir.

E-5 Shoreline Monitoring

The shoreline of the reservoir should be visually inspected by a qualified biologist for live adult mussels or areas that could potentially provide isolated habitats. Data to be collected includes GPS points/polygons and ground marker or other physical on-site delineation of viable mussels and pools or wet areas that could harbor live mussels.

Shoreline surveys should be initiated approximately one month prior to the potash treatment and conducted every 2 weeks through the holding period. Variations in timing and schedule may be necessary to accommodate for drawdown rate, weather, or other factors.

Visual assessment of exposed shoreline area for the presence of mussels on a variety of substrates and potential habitat, including exposed crevices, pools, under-exposed or overhanging ledges, on and under loose rocks, attached to exposed vegetation, muddy surfaces, and any other substrates encountered. Surveys should be conducted a minimum of every two weeks.

- Pooled water or significantly wet areas should be physically marked and/or locations recorded by GPS for subsequent examination. Larger pools may be targeted for repeat monitoring and additional treatment.
- Subsets of the exposed shoreline mussel population should be checked for viability by prodding with a dull probe and noting any physical movement or response, and/or inspection of tissues.
- As a final check, one week prior to reservoir refill (post-treatment), any potential viable mussels (up to 100 viable individuals) should be collected, placed in aquaria with untreated reservoir water under aeration and checked daily for at least 72 hours to determine recovery/mortality. If mussels are found to be alive the duration of the treatment will be extended.
- Shoreline monitoring may be discontinued after the conclusion of the drawdown if four consecutive weeks of data collection show no live mussels or viable habitat.
- If shoreline treatments are deemed necessary, combining one or several of the final survey efforts with treatment may be desirable to improve efficiency and utilize real-time data.

E-5.1 Shoreline Microclimate Monitoring

Air temperature and humidity sensors with data logger will be placed in select areas to monitor potential refugia. High priority areas for microclimate monitoring include along armored faces of the dam and dike, and may be stratified by slope azimuth.

E-6 Mussel Settlement

Monitoring mussel settlement is a long-term strategy and may take several months for observable results due to the life-history of zebra mussels. In addition, the reservoir treatment will take place outside of the typical seasonal peak in mussel settlement. Nonetheless, settlement monitoring should be initiated during the short-term monitoring period as an additional metric for eradication performance.

E-6.1 Settlement Plates

Artificial substrates for settlement monitoring should be constructed using uniform-sized settlement plates made of plastic or PVC, strung together on rope or plastic-coated cable (figure E3). Plates should be spaced at 15-foot intervals, with the top plate approximately 5 feet below the surface and the bottom plate within 5 feet from the deepest depth. An anchor should be attached to the bottom of the line at a depth to ensure the sampling plates remain vertical in the water column. Settlement plates deployed from buoys will need anchors to reach to bottom with a slight amount of slack to keep the string of plates from wandering with water currents.

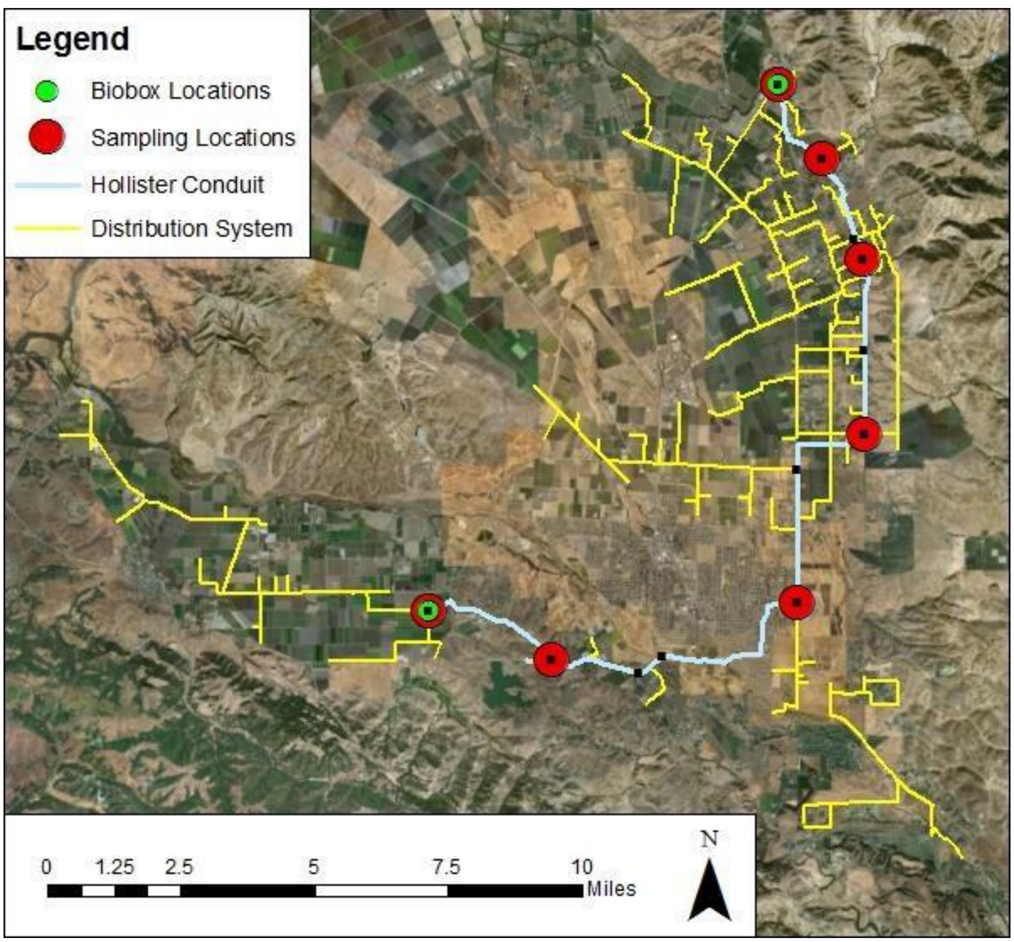


Figure E-2. Suggested monitoring locations, Hollister Conduit and Distribution System

Note that during the eradication treatment the maximum depth of the reservoir is estimated at 61 feet, but may be as much as 80 to 100 feet deep during the typical mussel settlement peak in June through October. Several options exist for accommodating elevation/depth fluctuations, including use of self-adjusting anchor buoys, regular relocation of the buoy and samplers to appropriate depths, or anchoring the stringer of settlement plates from above the water such as from the crest of the dam or a dock.

Recommended deployment sites and estimated depths for settlement plates are presented in figure E-1.

All settlement plates should be examined monthly during both the short and long-term monitoring phases.

E-6.2 Methods

- From a boat, pull one plate at a time out of the water. Plates should be handled carefully so as not to dislodge or crush any attached mussels.
- Visually inspect all sides and edges of each plate, as well as the rope/cable, anchor and any fasteners or hardware.
- Note and record any visible attached mussels.
- Lightly and methodically scrape the entirety of the surface of the settlement plate with a razor blade and collect all scrapings into a tray. Transfer to a water-tight sample bottle using multiple rinses with deionized water.
- Add baking soda and alcohol to preserve the sample and transport or ship to a qualified laboratory for microscopic analysis (see sample preservation under Mussel Veliger Monitoring).
- Replace the substrate stringer to its original location after all plates have been scraped.

E-6.3 Biobox Monitoring

Bio-boxes can be constructed from 35-L or larger coolers, which will help to maintain a consistent water temperature, and provide a dark location for settlement. The coolers are modified with inflow and outflow ports to allow continuous water flow through the biobox (Figure E4). The flow rate into each bio-box should be set at 7.6 L/ min (2 gal/ min), which will provide enough retention time for mussels to settle. A flow meter/ totalizer should be installed on the inflow to each bio-box to track the total flow into each box for comparison.

Settlement should be observed monthly during both short-term and long-term monitoring phases. Mussel settlement can be monitored by placing 10 settlement plates (approximately 15 cm x 15 cm) vertically in each bio-box. Settlement plates can be made of any material, but a smooth, plastic or PVC plates are easiest to scrape.

E-6.4 Methods

- Settled mussels can be detected by lightly scraping the surface of each plate with a razor blade and rinsing into a collection tray with DI water.
- The sample should be preserved and microscopically analyzed for total number of settled mussels.
- A water quality multi-probe can be used to monitor the temperature, dissolved oxygen, pH, and conductivity in each bio-box to detect water quality changes that might influence mussel survival.

Figure E3. An example of a stringer of settlement monitoring plates heavily colonized by quagga mussels.

E-7 Adult Mussel Bioassays

In order to directly observe adult mussel mortality resulting from potash treatment, captive live mussels will be exposed to treated water in the reservoir and distribution system, observed regularly for mortality and compared to mortality rates of untreated mussels kept in aquaria. Reservoir assays will suspend mussel bags from buoys at multiple depths. Bioboxes will be used to expose live mussels to treated water within the distribution system.

E-7.1 Methods

- Mussels should be collected prior to the potash treatment, verified live, and kept in aerated aquaria for use in the bioassays. Approximately 1,500 live mussels are desirable for the assays and controls, 500 from each size class; 2 to 3 times this number may need to be collected in order to acquire the appropriate number of viable individuals within each size class.
    - Size class ranges and quantity of mussels per bag may be modified to account for availability.
    - Classes are initially defined as small (<5 mm), medium (5-15 mm), and large (>15 mm).
    - The drawdown may kill off the majority of the larger mussel population, and smaller mussels (< 5 mm) may not be suitable to be remain within the mesh bags.
- Untreated water will also need to be collected and stored on site for maintaining controls.
- At the initiation of the potash treatment, mussels will be sorted into 3 size classes and 50 musses from each class placed into separate mesh bags.
- Clusters of 3 bags - one from each size class - will be suspended at three locations along a cable suspended from a buoy in the reservoir: 3 feet below the surface, middle of the water column, and 3 feet above the reservoir bottom.
- Anchors or tethers will be necessary to keep buoys in place. The reservoir elevation is expected to decline less than 1 foot over the course of the eradication treatment, so anchors at the end of buoy lines may be effective.
- Control bags (2 sets of the 3 size classes) should each be placed in separate aquaria or bioboxes, with either regular water changes or flow-through systems in place.
    - Chiller/heaters may be necessary to maintain water temperatures.
    - Daily water changes or 2 gal/min flow rate are recommended.
    - Water chemistry should be monitored to ensure conditions remain suitable to support viable mussels throughout the project.
- For observing mortality, bags should be collected from treated sited and kept in treated water throughout the observation and assessment process. Control mussels should be kept in untreated water at all times.
- Mussel assessments are to be conducted by a trained biologist, using stimulus response and/or tissue observations to discern intoxication/mortality condition.

E-8 Visual surveys

The reservoir bottom and underwater structures will be surveyed for mussel mortality using divers and/or underwater Remotely Operated Vehicles (ROVs) (USGS 2010). Groups of mussels potentially representing many size classes in a variety of locations and conditions in the reservoir can be checked in this manner for gaping and/or unresponsiveness, which are indicators of potential mortality. The use of divers has an advantage over ROVs because divers can perform both visual (gaping) and tactile (unresponsiveness to prodding) checks, and bring back groups of unresponsive gaping mussels to on-site aquaria containing untreated water to check for continued unresponsiveness. In contrast, ROVs can only offer a visual depiction of potential mortality (gaping mussels), but can also cover areas that may not necessarily accessible to divers.

- Inspections with divers and/or ROV can be conducted one month following the completion of potash treatment.
- Inspections should focus on areas where large masses of mussels are known to exist, such as indicated by previous sampling and surveys of the reservoir, and/or by observations of populations of stranded mussels along the shoreline exposed by lowering of the reservoir water level.
- Note specific locations where the surveys are conducted with GPS.
- Note if 100% of the mussels observed show a gaping response, and if not, estimate roughly what proportion are showing gaping response.
- Also note, in general, if the majority of gaping shells contain tissue or not. If divers are used, check for responsiveness and bring back samples of mussels for viability testing in aquaria containing untreated water.
- During the surveys, note if some mussels have closed shells. Subsequent surveys (weekly) may be required if a more than a third of the mussel shells are observed to be closed, or if some mussels are active and responsive.
-

Figure E-4. Example of a biobox setup in a modified cooler with mussel settlement substrates.

E-9 Larval Mussel Monitoring

Larval planktonic mussels (veligers) should be collected using net tows from various locations, likely a subset of the water quality monitoring points (Figure E1, total of 3 samples per day maximum). Collection of samples will be conducted in accordance with Bureau of Reclamation Technical Memorandum 86-68220-13-01, Field Protocol for Preparation of Water Samples for Dreissenid Veliger Detection, summarized below.

(https://www.usbr.gov/mussels/docs/FieldSOPPreparationandAnalysis.pdf, Revised 2013).

E-9.1 Equipment

- Plankton net with weighted COD-end piece made with 64 µm mesh; connected to rope reel marked every 1 meter up to 50 meters.
- Samples bottles
- Deionized water in squeeze bottle
- Bleach
- Vinegar
- Buckets or tubs for decontamination
- Baking soda (sodium bicarbonate)
- 70% isopropyl alcohol
- Sealable quart and gallon plastic bags
- Garbage bags
- Disposable diapers
- Insulated cooler
- Electrical tape
- Packaging tape

E-9.2 Field Prep

- Label bottles and lids with state (CA), water body (San Justo), sample site (see map), depth or length of tow, total number of tows, and date of sample collection.
- Inspect plankton tow net:
    - Look for holes, rips or tears, and make sure the mesh is firmly attached to the inside of cod end
    - Check metal/ PVC collar to make sure cod end screws on securely, and all knots securely tightened.
- Nets and cod ends should be decontaminated by submerging in vinegar between each sampling site, and soaked with bleach and rinsed after each sampling event.

E-9.3 Sample Collection - Reservoir

- Dip plankton tow net into the reservoir several times to rinse off the vinegar, making sure to keep the rim of the net hoop above the surface
- Refer to total depth from handheld multimeter, rounding down to nearest whole meter. This will be the depth to lower the plankton net for each tow. Record this depth
- Lower the plankton net slowly and straight down to the required depth; try not to disturb bottom sediment.
- Pull up the net slowly back to the surface; dunk the net two to three times again keeping the rim of the net above the surface – this will flush particles to the cod end. If the cod end is filled with sediment, discard the tow contents, rinse the net and resample at 0.5 meters above previous sample depth.
- Unscrew the cod end and gently swirl the sample to remove some of the water; pour the remaining water into the sample bottle.
- Repeat the plankton tow process five times.
- Using the squeeze bottle with deionized water, rinse the cod end and add rinsate to sample. Repeat for a total of three rinses. If there is too much water in the sample after any tow or after the rinse, pour some of the sample back into the cod end and swirl to reduce volume. Decant the sample back into the bottle and perform three rinses of the cod end. Make sure there is room in the bottle for alcohol to be added.
- Use a single sample bottle per sample location (5 tows), with 500 mL maximum sample volume including the alcohol preservative added at 25% of total sample volume.
- Add 0.2 grams of baking soda per 100 mL of sample and mix gently.
- Add alcohol to the sample to bring the sample to roughly 20% alcohol, mix gently.
- If shipping samples, seal sample containers with electrical tape
- Place sample in cooler with ice.

E-9.3 Sample Collection – Distribution System

- Larval mussel samples can be collected from the distribution system by running a standard volume of water (similar to plankton tows) from a tap through the net or simply the cod end piece.
- A catch-basin or flow meter should be used to determine actual volume of water filtered through the mesh.
- All other aspects of decontamination and sample preparation are the same as those conducted for the reservoir sampling.
- Dispose of all filtered water appropriately.

E-9.4 Sample Shipping

- Make sure the cooler is clean/dry, drain valve is taped closed and lined with a large trash bag to contain any leaks that may occur.
- Make sure that all labels are complete, easily legible and present on both the bottle and the lid. Lids should be wrapped tightly with electrical tape at the seam.
- Secure disposable diaper over sample bottle.

- Place samples in garbage bag lined cooler with blue ice packs to keep the samples chilled. Do not use regular ice, shipping companies will destroy any leaking packages.
- Close garbage bag tightly, and tie in a knot to close the bag
- Tape cooler lid and sides shut and affix shipping label.
- Overnight the cooler to the Reclamation lab for analysis; avoid shipping on Fridays if possible.

E-10 Long-Term Monitoring

Definitive confirmation of the success or failure to eradicate invasive mussels from San Justo will require long-term monitoring over a minimum of 2 consecutive years following the eradication and may be conducted indefinitely to monitor potential re-infestation should pubic access to the reservoir be reinstated. Long-term monitoring will be initiated at the start of the reservoir refill after the potash dosing, distribution system charging, holding period and final short-term assessments, and targeted during the June-October window when mussel settlement is most abundant.

E-10.1 Overview

- Water quality (field sonde)
- Adult mussel settlement (artificial settlement substrates)
- Larval mussel presence and viability (plankton tow net surveys)

E-10.2 Methods

All methods are identical to those conducted during the short-term monitoring phase.

E-10.3 Genetic Analysis

Genetic analysis of mussels at San Justo Reservoir can provide a valuable tool for evaluating the success of eradication efforts. Genetic tools developed during the recent sequencing of the zebra mussel genome by the University of Minnesota allows for differentiation of mussel population.

Genome by sequencing (GBS) techniques rely on fragmentation of genomic DNA by restriction enzymes, followed by massively parallelized DNA sequencing using widely available and inexpensive next generation sequencing (NGS). Sequences from numerous individuals are compared to identify differences in the DNA (single nucleotide polymorphisms/SNPs) that are unique to a population.

Should live mussels be detected in San Justo or the distribution system following eradication efforts, they could be compared to mussels collected before the eradication. This would allow determination of whether there had been incomplete mortality and a survivors from the original population, or a new introduction had occurred after eradication. Samples of mussels should be collected before the eradication effort occurs. These pre-eradication samples can be analyzed as part of the eradication effort or can be archived in a -112˚ F (-80˚ C) freezer for future use if needed.

E-11 Decontamination

It is recommended to treat all vehicles and equipment exposed to raw water at San Justo as if the reservoir was still infested until completion of the long-term monitoring phase. This means all boats, probes, nets, and other items used for monitoring should undergo appropriate decontamination procedures before entering or use in any other water body.

Table E-1. Summary of monitoring activities, duration and frequency for the San Justo Reservoir mussel eradication project.

Location	Target	Data Collected	Method	Duration		Frequency	
				Start	End	Short-Term	Long-Term
San Justo Reservoir Shoreline	Adult Mussels	Adult mussel and potentially suitable habitat presence/absence and location	Visual survey	At least one month prior to potash treatment	Until two consecutive surveys find no live mussels	Every 2 weeks	N/A
	Microclimate	Temperature and humidity	Deployed sensors with data loggers			Data logged hourly, loggers checked weekly	N/A
San Justo Reservoir	Water Quality	DO, temp, pH, conductivity, turbidity	Multiprobe/Sonde	At least one week prior to initiation of potash dosing	Through seasonal reservoir refill	Weekly	Monthly
	Potassium	Concentration	Field colorimeter	At least one week prior to initiation of potash dosing	Conclusion of holding period	Daily during potash dosing, weekly during holding period	N/A
			Lab	Initiation of potash dosing	Conclusion of holding period	Weekly	N/A
	Juvenile Mussels	Veliger counts	Plankton tow	After complete adult mussel mortality achieved	Through long-term monitoring phase	Every 2 weeks	Monthly
	Adult Mussels	Settlement	Settlement plates	At initiation of potash dosing	Through long-term monitoring phase	Every 3 weeks	Monthly
		Mortality	Bioassays	At initiation of potash dosing	Until complete mortality achieved	Every 2 weeks	N/A
Hollister Conduit and Distribution System	Water Quality	DO, temp, pH, conductivity, turbidity	Multiprobe/Sonde	At least one week prior to potash treatment	Through seasonal reservoir refill	weekly during holding period	Monthly
	Potassium	Concentration	Field colorimeter	Initiation of holding period	Conclusion of holding period	Weekly	N/A
			Lab	Initiation of holding period	Conclusion of holding period	Every 2 weeks	N/A
	Juvenile Mussels	Veliger counts	Filtered samples from taps	Initiation of holding period	Through long-term monitoring phase	Every 2 weeks	Monthly
	Adult Mussels	Settlement	Settlement plates	At initiation of potash dosing	Through long-term monitoring phase	Every 3 weeks	Monthly
		Mortality	Bioassays	At initiation of potash dosing	Until complete mortality achieved	Every 2 weeks	N/A

Appendix F. Potash Treatment Logistics

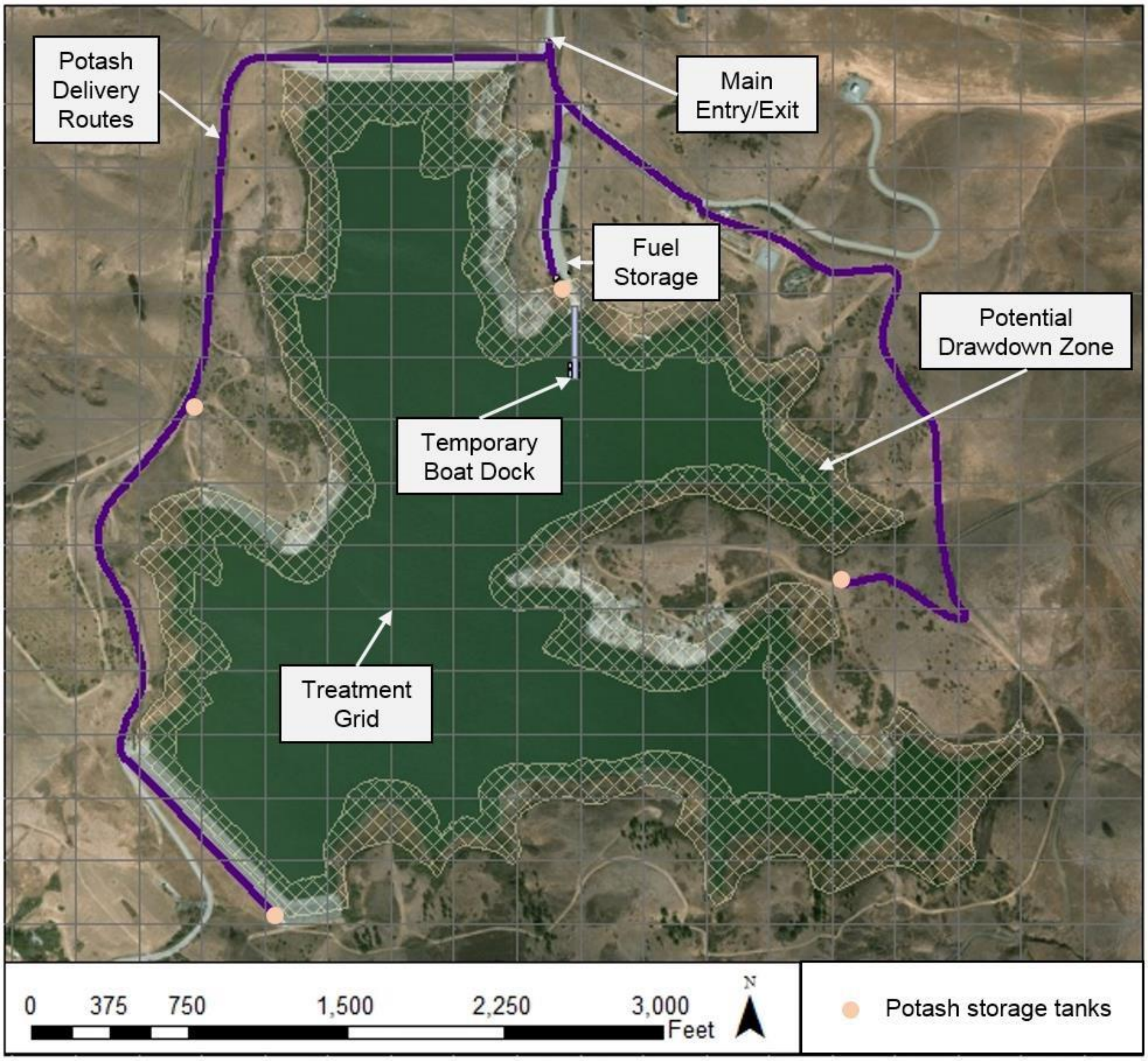
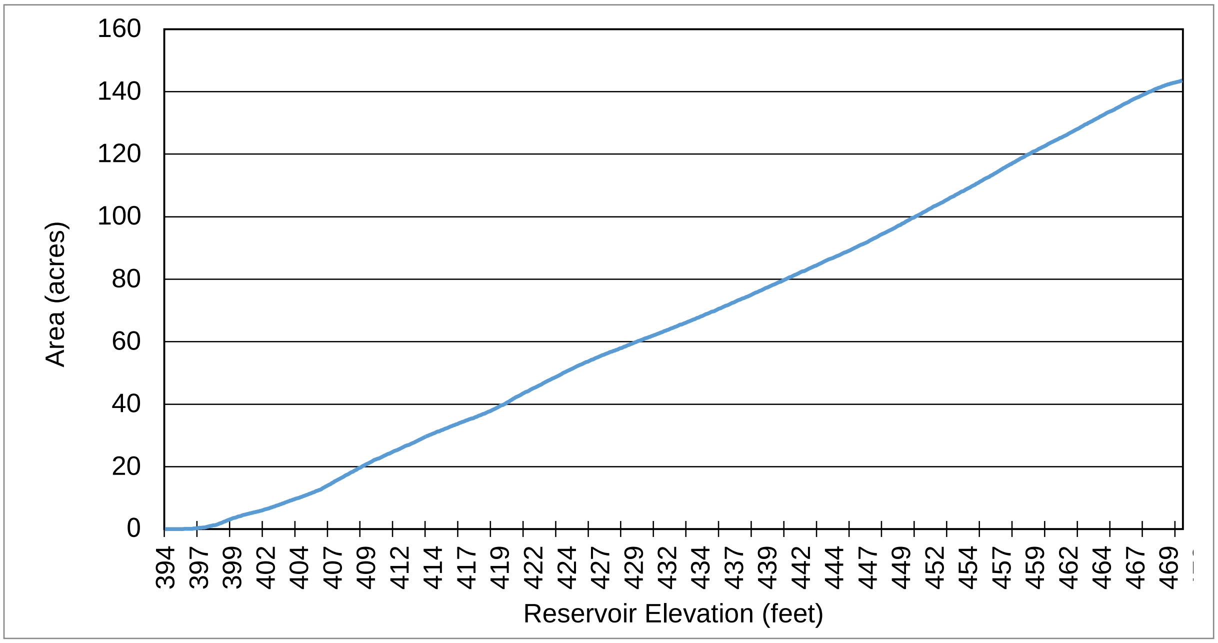
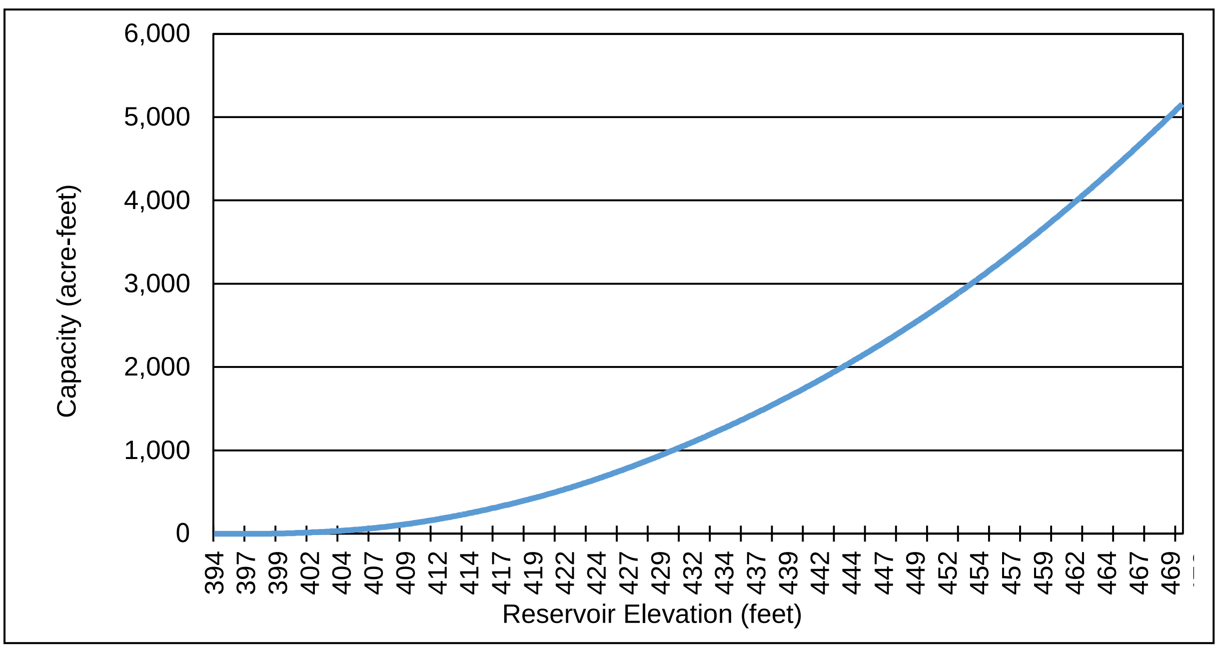


Figure F-1. Location map for potash storage tanks and delivery routes, fuel storage, and treatment grid. Treatment grid cells are 300 feet by 300 feet squares representing the approximate area to be covered per workboat per day during the potash treatment. Potential extent of the drawdown zone and temporary boat dock are also presented.

Appendix G San Justo Reservoir Area and Capacity by Elevation



Figrure G-1. San Justo Reservoir area by elevation.



Figrure G-2. San Justo Reservoir capacity by elevation.