Appendix D

Zebra Mussel Monitoring Plan for San Justo Reservoir and the Hollister Conduit and Distribution System

Zebra Mussel Monitoring Plan for San Justo Reservoir and the Hollister Conduit and Distribution System

PREPARED FOR: San Benito County Water District

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Introduction

Zebra mussels were first observed in San Justo Reservoir (SJR) in January 2008, and subsequently in the Hollister Conduit (HC) in January 2009. Since January 2008, zebra mussel infestation levels have increased dramatically in SJR. This potentially destructive mollusk is also presumed to be present in parts of the Distribution System (DS) that is connected to the HC via ten turnouts to provide Central Valley Project (CVP) water to agricultural and urban customers. SJR and HC are owned by the United States Bureau of Reclamation (USBR) and the San Benito County Water District (SBCWD) operates and maintains both these systems. SBCWD is currently working with the USBR on a plan to eradicate zebra mussels in SJR, HC and the DS.

The rationale and details of the plan for the eradication of zebra mussels from the SJR, HC and DS are being developed in SBCWD's Notice of Proposed Mitigated Negative Declaration for CEQA compliance (SBCWD 2011). To summarize, this eradication plan is two-fold and involves: (1) the treatment of San Justo Reservoir using 'muriate of potash' (potash or potassium chloride); and (2) the treatment of the Hollister Conduit in combination with District-owned water delivery conveyances (pipeline system) using potash. This two-fold eradication will be implemented concurrently, as eradicating zebra mussels in the reservoir without eradicating them in the HC and DS, and vice versa, would allow re-infestation of both appurtenant infrastructures.

For eradication treatment, the preliminary plans are to draw down SJR to a water surface elevation of between 455 and 470 feet, (personal communication with SBCWD staff), close the valve connecting SJR to the HC and DS, and treat the water in SJR, HC and DS with potash (typically beginning in November, when there is no water demand by agricultural customers) to achieve a potassium "kill" target concentration of 100 parts per million (ppm). Treatment of the HC and DS will require either treating SJR with potash and then opening the valve to charge the HC and DS, or treating HC and DS concurrently, but independently, using chemical feed stations. The reservoir drawdown has two purposes: (1) it exposes zebra mussels near the surface elevations to the air and causes desiccation-related mortality, and (2) it reduces the pool of water that needs to be treated with potash to kill the remaining mussels in the reservoir. Based on the results of a previous pilot study on desiccation of zebra mussels conducted at SJR by USBR (Chapman and Greunhagen 2010) and additional information provided by SBCWD, the length of drawdown-related desiccation and contact

time with the kill concentration of potash in the reservoir, HC and DS is assumed to be between 2-3 months (Treatment Period).

The purpose of this technical memorandum is to develop an implementable monitoring plan for confirming the eradication of zebra mussels from SJR, HC and DS during, and following the Treatment Period. Specifically, the purpose of the monitoring plan is to provide the monitoring methods needed to:

1. Confirm that eradication of mussels is occurring during the Treatment Period and that mussels have been eradicated immediately following the Treatment Period, and,
2. Verify the continued non-presence of zebra mussels in the longer-term after the Treatment Period.

Monitoring Plan for San Justo Reservoir

A multi-pronged monitoring and testing approach, that builds upon previous monitoring plans for mussels in SJR, and that targets various life stages of the mussels including veligers, newly-settled mussels and adult mussels, is recommended for a comprehensive assessment of the efficacy of the eradication treatment. The monitoring will be conducted for:

- Evaluation of mussel mortality along the shoreline exposed by the reservoir drawdown (desiccation-related mortality) and for,
- Mussels within the water body contained by the reservoir (mortality caused by potash treatment).

The monitoring will be supplemented concurrently by bioassays for the potash treatment to track mortality in a more controlled set of mussels exposed to similar treatment conditions.

Exposed Shoreline Survey for Desiccation Treatment

The reservoir drawdown will expose a significant portion of the SJR shoreline. Zebra mussels that have settled in this portion of the shoreline will be exposed to the air, and over time will be subjected to desiccation. The shoreline surveys will monitor for dead mussels in a variety of locations along the exposed SJR shoreline, including those areas where the pilot desiccation study (desiccation bioassay) was conducted by USBR in 2010 (Figure 1, Chapman and Greunhagen 2010).

The purpose of the USBR desiccation bioassay study was to assess the length of time the reservoir needed to be kept low during the cool and wet winter months (conditions that favor mussel survival) to ensure that the aerially-exposed populations of mussels were fully desiccated. Tests were conducted by exposing three size classes of zebra mussels (< 5 mm, 5-15 mm, > 15 mm) to a range of conditions (sheltered, unsheltered – to simulate shading and wind protection) at various locations along the SJR shoreline that typified various substrate habitats (Figure 1). Observations for mussel mortality were conducted at 1, 10, 20 and 40 day intervals and results indicated that some mussels appeared to be alive at 20 days, but following desiccation for 40 days, most mussels were dead. However, a small fraction of the

mussels (especially small mussels) observed at 40 days had flesh inside a tightly closed shell and it was uncertain whether these mussels would have revived if re-submerged. Based on these observations, it was recommended that the reservoir should be kept low for a period between at least 2-3 months, if possible, to maximize mussel eradication via desiccation.

Figure 1. Aerial image of the shoreline of San Justo Reservoir and locations (red numbered markers) where USBR conducted the desiccation bioassay study in 2010. The locations represent various habitat characteristics: (1) Dike Face with Large Boulders, (2) Geo-membrane Underlain Area with Small Rocks, (3) Point Area with Mid-Sized Rocks, (4) Muddy Area, (5) Reed Area, (6) Dam Face with Large Boulders. Adapted from: Chapman and Greunhagen (2010).

Shoreline Monitoring Protocol

- Shoreline surveys are conducted by walking as much of the exposed SJR shoreline as possible, close to the treatment water elevation, and will also include areas where the 2010 USBR desiccation bioassay study was conducted (Figure 1).
- Initial shoreline monitoring will commence one week after start of the potash treatment in the reservoir, following which monitoring will occur every two weeks.
- During these surveys, the exposed reservoir perimeter will be checked for any visibly-exposed mussels and for the presence of mussels on a variety of substrates

and "micro-habitats" including exposed crevices, pools of water, under exposed overhanging rock ledges, on and under loose rocks, attached to exposed vegetation on exposed mud surfaces, and any other exposed substrates that are encountered.

- When pools of water are encountered, their locations will be marked and recorded by GPS for closer examination in subsequent surveys. Small pools will be monitored for drying in subsequent surveys. Larger pools may be targeted for additional treatment (e.g., treated with kill concentration of 100 ppm potash, drained or pumped dry).
- Mussels detected during these surveys will first be quickly checked on-site for obvious signs of stress and/or mortality such as gaping and unresponsiveness to prodding of the exposed mantle with a dull probe, visible signs of desiccated tissue.
- At a particular location, when the numbers of detected mussels are relatively small (approximately < 100 mussels), all mussels are collected, placed in aquaria containing untreated reservoir water on-site and checked daily until they either recover (responsive to prodding) or show unmistakable signs of mortality.
- When the number of detected mussels at a location is large (> 100 mussels), the GPS location is noted and up to 100 mussels representing a range of mussel sizes (if possible) are collected, placed in aquaria and checked daily for either recovery or unmistakable mortality, as described above.
- Continue monitoring the exposed shoreline once every 2 weeks until two consecutive surveys find no live mussels, or up to the time when water level in the reservoir needs to be raised again.

Bioassay (for Shoreline Dessication Treatment)??

Complements the Pilot Test conducted by USBR in 2010; allows a more controlled assessment of mortality over time under a variety of aerial exposure conditions.

Reservoir Surveys for Potash Treatment

A key component of the reservoir surveys is the use of various monitoring and sampling techniques that target different life stages of the zebra mussel (veligers, newly-settled, and adult mussels) – this approach, used concurrently with a potash treatment bioassay in the reservoir, will allow for a robust assessment of whether, and how well the potash treatment is working to eradicate mussels from the reservoir.

Reservoir Monitoring Using Substrate Samplers

This monitoring method relies on deploying clean substrate samplers, or settling plates (Figure 2) to periodically check for newly settled mussels in the reservoir. If a new settlement of mussels is detected, it indicates the presence of viable mussels in the reservoir, and in particular, the presence of pre-settlement stages of veligers in the water column. Settlement of zebra mussels in San Justo Reservoir occurs mostly between June and October,

with little settlement occurring between November and May (Veldhuizen and Janik 2010; based on 2009-2010 data). During monitoring, caution therefore needs to be exercised in the interpretation of no new settlement detections during November to May as representative of eradication effectiveness. Long term monitoring, that includes the summer period when mussel settlement is known to peak, will be essential.

Figure 2. Substrate sampler used for monitoring zebra mussels. Photo Source: CDFG and CA DWR

Construction, assembly and deployment instructions for the substrate sampler, including an example datasheet for recording settlement information, are provided by the California Department of Fish and Game (CDFG 2011).

Protocol for Monitoring with Substrate Samplers

Short-Term – During Eradication Treatment

- One week after start of the potash treatment in the reservoir, deploy the substrate samplers at various locations in the reservoir (Figure 3), following which monitoring will occur once every two weeks.

- At each location (Figure 3), deploy two sets of substrate samplers, one at 5 ft. below the water level and the other adjacent to it, at 15 ft. below the water level. These depths are based on recent data from SJR indicating that mussel settlement in the reservoir peaks between 5 ft. and 15 ft. (Veldhuizen and Janik 2010). If the bottom of the reservoir at a certain location is at less than 15 ft depth, then deploy the sampler 1-2 ft. above the bottom, and record the actual sampling depth.

- After initial deployment of the samplers, monitor the samplers for mussel attachment once every two weeks. To check for newly settled mussels, carefully lift the sampler out of the water, taking precaution to not dislodge or crush any attached mussels. Carefully perform a visual inspection of each plate (top, bottom, and sides), the spacers, the cable and the weight. Note and record any attached zebra mussels if they are clearly visible (a field magnifying glass may prove to be helpful as newly attached mussels are very small).

- Along with the visual examination, perform a tactile examination of the sampling substrate, taking care not to press down too hard. This is necessary because when mussels first attach, they are very small, practically invisible and are easily crushed. A single mussel may feel like a grain of sand. If many mussels cover a surface, the surface feels gritty like sandpaper. Based on the visual and tactile examinations of the substrate sampler, it may be necessary to scape all suspected mussels off the samplers into a bag or vial for closer examination under a dissecting microscope to positively confirm the presence of newly settled zebra mussels. Record the presence (or suspected presence) of any mussels on the substrate samplers. Redeploy the cleaned sampler at the appropriate depth.

- If no mussels are detected, lower the sampler back into the water and check again during the next monitoring period. Zebra mussels are more likely to attach to a substrate that has some algal growth. If the substrate however becomes too heavily coated with algae, it may be unsuitable for mussel settlement. As necessary, gently remove heavy accumulations of algae to maintain suitable conditions for settlement.

- Monitoring will continue until two consecutive surveys find no live mussels, and the reservoir bioassay (see below) records 100% mortality.

Long-Term Post-Eradication Treatment Monitoring

- The post-eradication monitoring period begins after the 2-3 month treatment period when the reservoir water level is lowered and the potash is applied.

- The long-term monitoring specifically targets the warm summer period (June – October) when newly settled mussels are abundant in SJR (Veldhuizen and Janik 2010).

- Monitoring with substrate samplers will occur at the same depths and general locations, and as per the protocol for short-term monitoring, recognizing that sampler locations will have to be moved to deploy the samplers at 5 ft and 15 ft when the reservoir levels are raised.

- Monitoring will occur once every month, during June to October (5 separate sampling periods) in the same year as the eradication treatment is completed, and once every month during June to October (5 separate sampling periods) the following year. For example, if the 3 month treatment period ends in February 2012, the long term monitoring period for newly settled mussels will span a period of approximately 20 months, during which monitoring will occur on 10 separate occasions over 2 consecutive summers.

- Eradication measures are considered to be successful if no new settlements of mussels are detected after post-eradication monitoring over 2 consecutive summers.

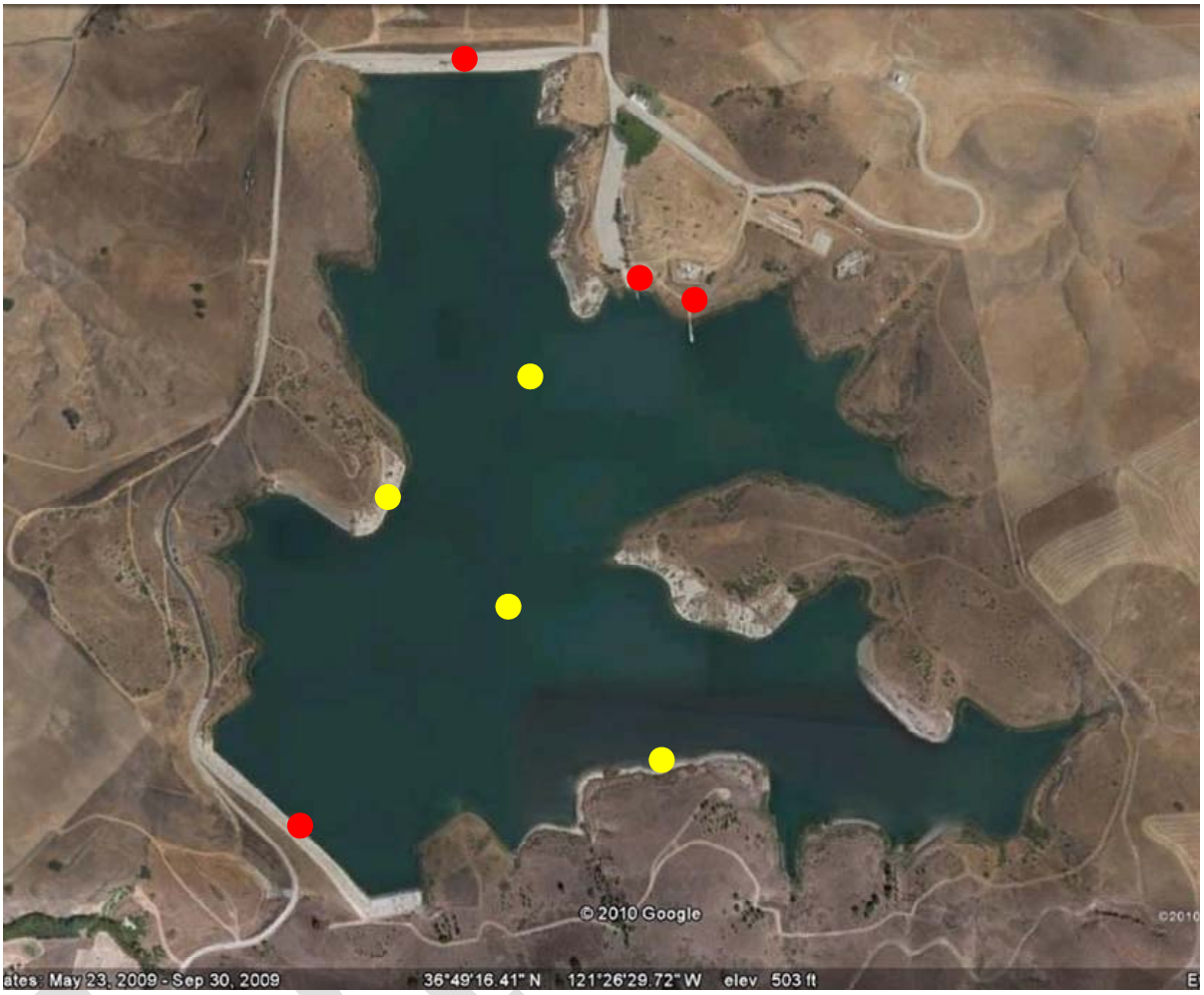

Figure 3. Proposed approximate locations for deployment of the substrate samplers for monitoring newly settled mussels in SJR. Red dots indicate obvious general locations such as the dike, dam, boat ramp and jetty areas from where substrate samplers can be easily deployed. Yellow dots depict other proposed locations that can be explored for sampler deployment. The two locations in the middle of the reservoir will require a float and anchor line to which the substrate samplers can be tethered at the required depths. If any of the proposed locations turn out to unsuitable for deployment, other locations can be explored.

Reservoir Monitoring Using Veliger Tows (Plankton Tows)

Plankton tows will be conducted to detect the presence of veligers in the SJR water column, which in turn is an indicator of the presence of viable and reproducing mussels in the reservoir. Based on data collected by the California Department of Water Resources (DWR) during June 2008- July 2010, veliger abundance peaks in May and June in SJR, drops of in July/August, with very few to no veligers present in the water column from September to April (Veldhuizen and Janik 2010). During monitoring, caution therefore needs to be exercised in the interpretation of no veliger detections during September to April as being

representative of eradication effectiveness, and in the longer term, veliger monitoring must incorporate the warmer peak summer period to adequately test for eradication.

This monitoring method uses a 63-µm plankton net (maximum mesh size) attached to a length of rope (that can be towed from a boat either vertically or horizontally through the water column (Figure 4).

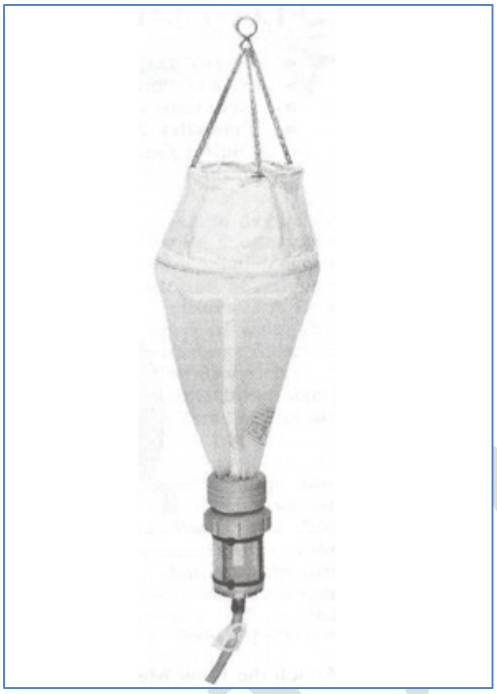

Figure 4. Plankton net for horizontal and vertical veliger tows. Photo Source: Wildco.

Horizontal tows sample for veligers present near the water surface, while vertical tows provide a depth-integrated sample of veligers that may be present in the water column at a certain location in the reservoir. In SJR, veliger abundance during the peak months of May and June was typically higher in the horizontal (surface) tow samples than in the vertical tow samples (Veldhuizen and Janik 2010), but the depths that veligers may be found may vary from year to year. Therefore, vertical tows are recommended for monitoring veligers in SJR at each proposed location.

Details on the plankton net and procedures for conducting the vertical tows, and preservation of samples for analysis, are provided by the Mussel Monitoring Program of the 100th Meridian Intiative (2009).

Protocol for Monitoring Veligers

Short-Term – During Eradication Treatment

- One week after start of the potash treatment in the reservoir, conduct vertical tows for sampling veligers at various locations in the reservoir (Figure 5), following which monitoring will occur once every two weeks.

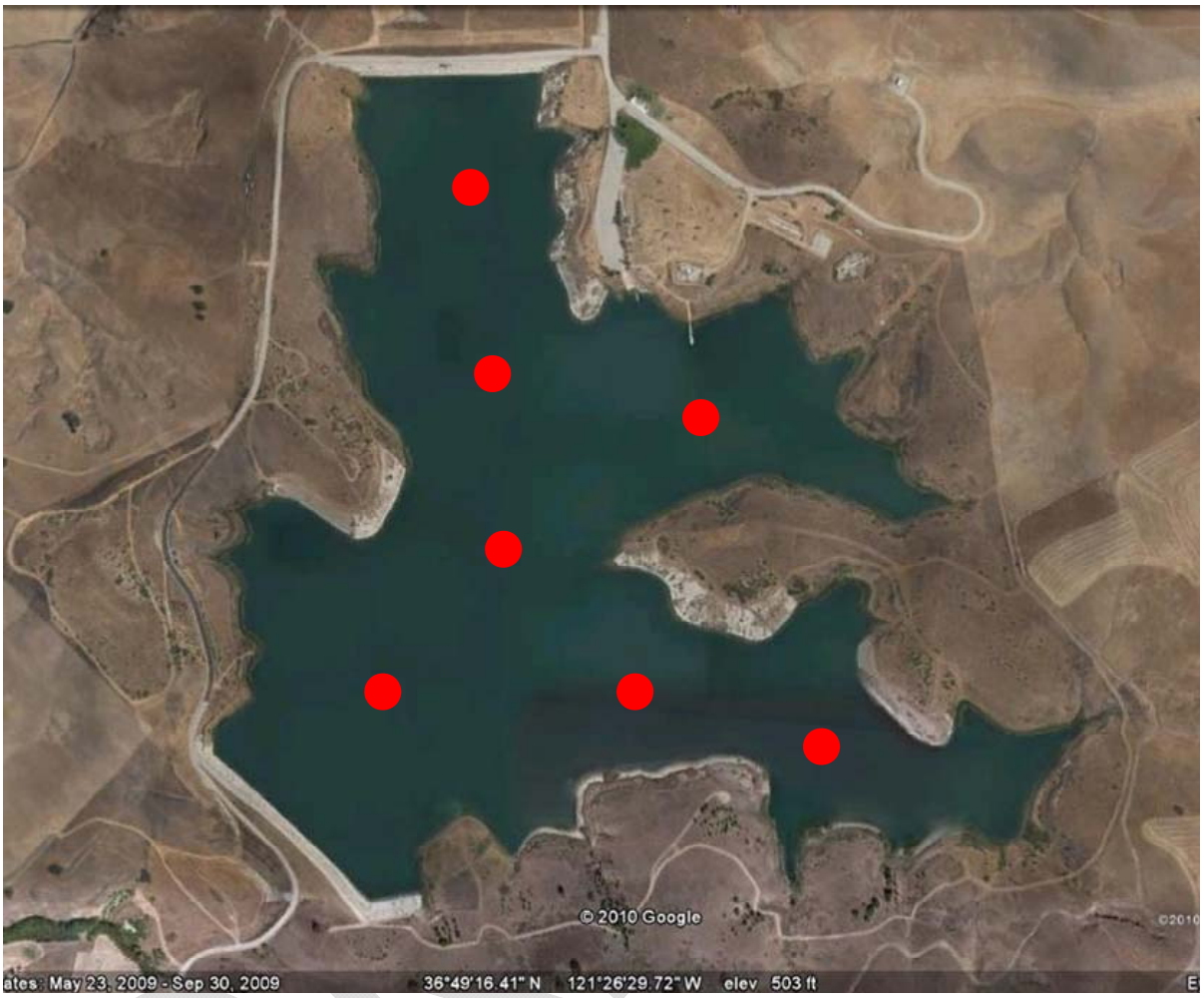

Figure 5. Proposed general locations of vertical plankton tows for monitoring veligers in SJR

- At each location (Figure 5), note the GPS coordinate, and conduct a vertical tow by lowering the plankton net to a depth of 20 m below the water surface, or to 1 m above the reservoir bottom, (whichever is deeper), and then slowly and steadily retrieving the net at a rate of 0.5 m/sec. Rinse the net, collect and preserve the veliger sample as per the referenced protocol (100th Meridian Intiative , 2009). Veligers in the samples are later detected using a Cross-Polarized Light Microscope (CPLM). With a CPLM, zebra mussels veligers can be easily distinguished from other plankton by the cross-hatch pattern they exhibit (Figure 6).

- Monitoring will continue until two consecutive surveys find no veligers, and the reservoir bioassay records 100% mortality.

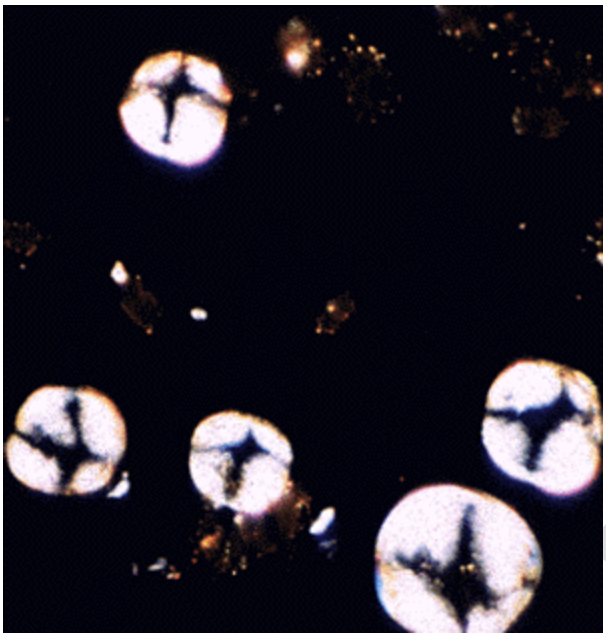

Figure 6. Zebra mussel veligers with the characteristic cross-hatch pattern observed with a Cross Polarized Light Microscope

Long-Term - Post-Eradication Treatment Monitoring

- The post-eradication monitoring period for veligers begins after the presumed 2-3 month treatment period when the reservoir water level is lowered and the potash is applied.
- The long-term monitoring specifically targets the warm summer period (May – August) when mussel veligers are relatively abundant in SJR (Veldhuizen and Janik 2010).
- Veliger monitoring will occur at the same GPS-marked locations, and as per the protocol for short-term monitoring.
- Veliger monitoring will occur once every month, during May to August (4 separate sampling periods) in the same year as the eradication treatment, and once every month during May to August (4 separate sampling periods) the following year. For example, if the 3 month treatment period ends in February 2012, the long term monitoring period for veligers will span a period of approximately 18 months, during which monitoring will occur on 8 separate occasions over 2 consecutive summers.
- Eradication measures are considered to be successful if no veligers are detected after post-eradication monitoring over 2 consecutive summers.

Reservoir Bioassay (for Potash Treatment)

A bioassay that allows for an assessment of post-treatment mortality of juvenile and adult zebra mussels placed at various depths and locations in the reservoir will be conducted. As such, this bioassay will allow for a more controlled tracking of the effectiveness of the potash treatment in the reservoir. The bioassay will compare mortality of bagged mussels

immersed in the reservoir immediately after potash application is completed (desired potash concentration is achieved), to mortality of mussels in control aquaria (at on-site facilities) with no potash treatment.

Reservoir Bioassay Protocol

- Just prior to reservoir drawdown and subsequent potash treatment, collect at least 20,000 – 25,000 zebra mussels (stock set of mussels) from San Justo Reservoir and place these mussels in aquaria (300 gallon aquaria) maintained in on-site facilities. Out of this stock set of mussels, a total of 9,900 viable mussels of various sizes will be needed for the reservoir bioassay test (9,000 mussels) and the control test (900 mussels), and an additional 3,600 viable mussels for the pipeline bioassay test. Sufficient mussels should be left over in the stock set to allow for natural mortality and other contingencies (e.g., disproportionate numbers belonging to one size class).

- During potash application to the reservoir, monitor the reservoir for potassium concentration at various locations and depths. When the concentration of potassium in the reservoir reaches the target concentration (100 ppm), sort the mussels (as described below) that were collected into 3 size classes (based on mussel length measured with calipers); < 5 mm, 5-15 mm, and > 15 mm length. While sorting into size-classes, check for the viability of these mussels; reject any gaping mussels that do not close when the mantle is prodded with a dull probe. Mussels will be sorted into 2 testing groups, one for the Reservoir Bioassay Test, and the other group for the Control Test.

- Reservoir Bioassay Test Mussels: Sort 100 viable mussels of each size class into mesh bags (1-2 mm nylon mesh material) that are marked to denote which mussel sizes and group (test) they contain. Prepare a total of 90 test bags; 30 bags for each size class (90 bags x 100 mussels per bag = a total of 9,000 viable mussels).

- Control Test Mussels: Sort 100 viable mussels of each size class into mesh bags that are marked to denote which mussel sizes and group (control) they contain. Prepare a total of 9 control bags; 3 bags for each size class (9 bags x 100 mussels per bag = a total of 900 viable mussels).

- The Reservoir Bioassay Test bags will be deployed at 3 different depths (below surface; middle of water column; just above reservoir bottom) at 10 different locations in the SJR (Figure 7), some in close proximity to the shoreline or structures such as docks, dikes, dams, ramps, and others towards the middle of the reservoir (hung from buoys). Bags will be tethered to an appropriate length of rope which will be tied to a shoreline structure (e.g., dock), or to a buoy, depending on location. At each location and at each depth, 3 bags will be deployed, each containing a different mussel size class (3 bags near the surface, 3 bags in the middle, and 3 bags near the bottom = 9 bags total at each location). Location will be marked (with a GPS if necessary) for repeated monitoring and retrieval of the bags.

- Control test bags will be suspended in an aquarium on site (3 bags, each of a different size class, near the surface, 3 bags in the middle, and 3 bags near the bottom) that contains untreated reservoir water, or water with 4 mg/l of potassium or less (approximately the background levels in San Justo Reservoir).

- On the day of the deployment of the bags in the reservoir and control aquaria, 100% of the mussels are assumed to be viable, based on the viability checks. After deployment, all bags (reservoir bioassay test and control test) will be inspected every other day (i.e., day 0 = deployment, then monitor on day 2, 4, 6, etc.) until all reservoir test bags indicate 100% mussel mortality. Also note the water temperature and potassium concentration, every time the mussels are monitored. During the monitoring, all gaping and unresponsive mussels will be immersed in recovery aquaria containing untreated water and checked for continued non-responsiveness (by prodding the mantle with a probe) after a 48-h period. Non-responsive mussels after 48-h will be marked as dead, and mortality counts will be noted.

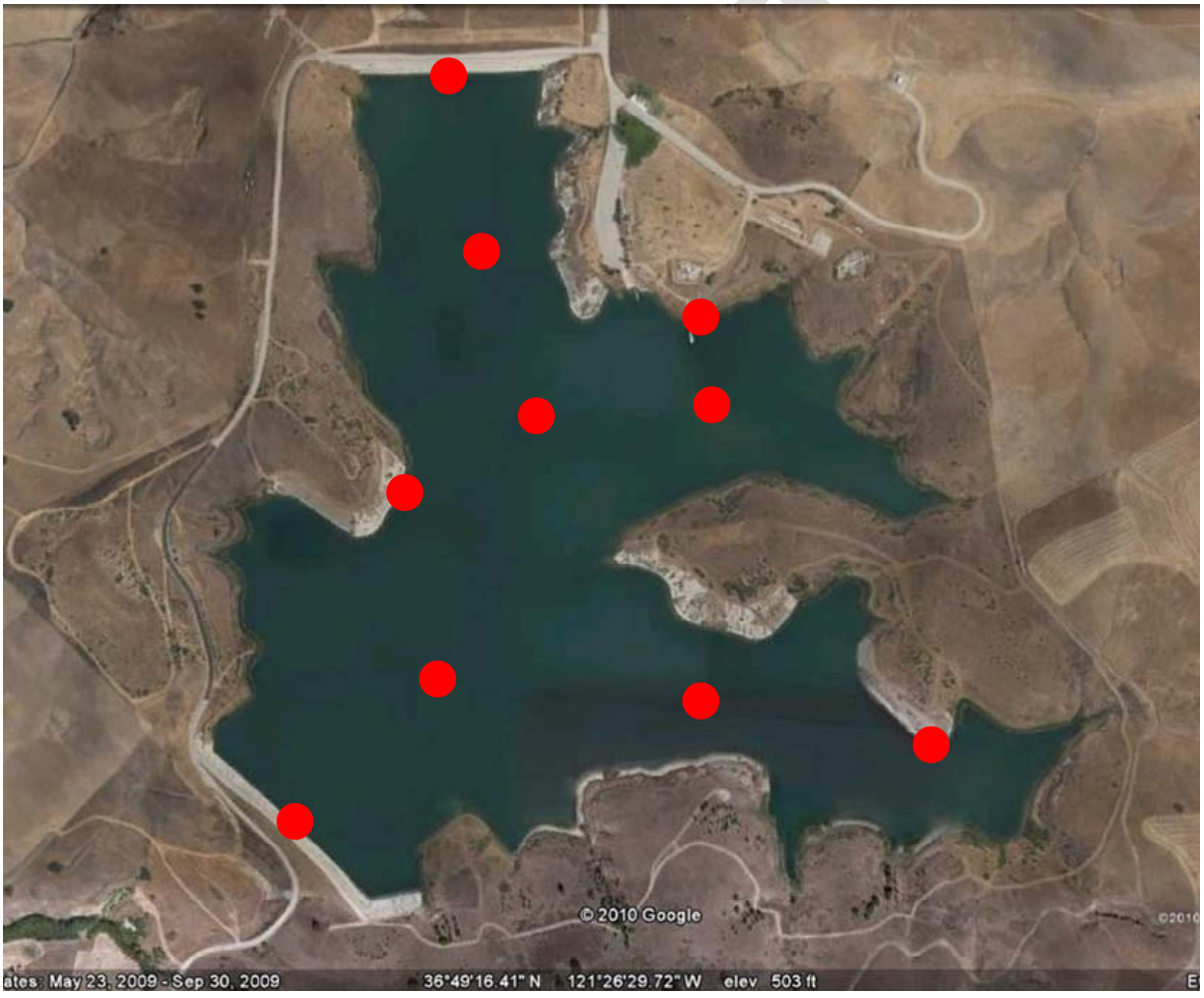

Figure 7. Proposed approximate locations for deployment of the Reservoir Bioassay Test Bags.

Divers/ROVs

Large areas of the reservoir bottom and underwater structures can be surveyed for mussel mortality with the use of 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 to accessible to divers.

Protocol for Divers/ROV

- Inspections of the reservoir with divers and/or ROV can be conducted one month following the completion of potash addition to the reservoir.
- Inspections can 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 the locations where the surveys are conducted. 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 unresponsiveness (by poking them?) and bring back samples of mussels for viability testing in aquaria containing untreated water.
- During the surveys, if some mussels have closed shells, note it. Subsequent surveys (weekly) may be required if a good proportion of the mussel shells are observed to be closed, or if some mussels are active and responsive.
- Monitoring will continue until two consecutive surveys find no live mussels, and the reservoir bioassay records 100% mortality.

Monitoring Plan for the Hollister Conduit (HC) & Distribution System (DS)

Treatment Plan Summary for HC and DS

Chemical eradication of zebra mussels in the Hollister Conduit and pipeline distribution system (Figure 8) will be implemented simultaneously with eradication measures in San Justo Reservoir. The eradication will involve dosing the Hollister Conduit and Distribution System with potash. Potash feed system(s) may be established at the Pacheco Bifurcation, and at various points in the distribution pipeline as appropriate, in the event that there is not enough hydraulic grade-line from San Justo Reservoir through the pipeline DS to move potash laden waters from SJR into the outer reaches of the DS. In such a case, said chemical feed system would likely consist of potash solution storage tanks and chemical feed pumps placed within temporary spill containment. Similar to treatment in the reservoir, the objective of the potash treatment will be to deliver and maintain a potassium concentration of 100 ppm (by volume) throughout the entire pipeline system.

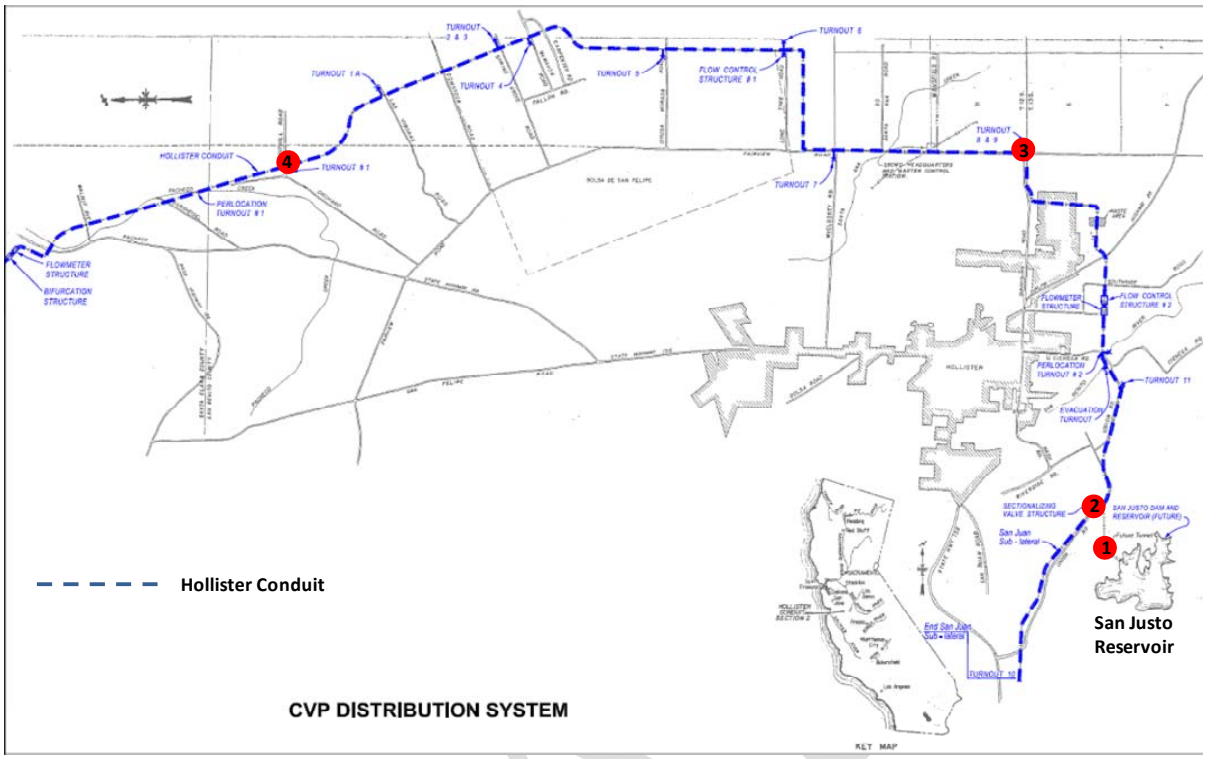

Figure 8. Schematic of the Hollister Conduit and its Distribution System showing locations of the 4 bioboxes (1-4) being used to monitor for zebra mussels. Bioboxes are located at, (1) the Valve House, (2) Sectionalizing Valve Structure, (3) Turnout 9L, and (4) Turnout 1.

Monitoring with Biobox

Four bioboxes were installed at various locations (Figure 8) in the HC in August 2009 and are being monitored for newly settled zebra mussels every one to three weeks since installation. It is recommended that these bioboxes continue to be used to monitor for conduit treatment effectiveness. To date, no mussels settlements have been observed in Bioboxes 2-4. In addition, Biobox 1 in the Valve House was seeded with live zebra mussels to test for the effectiveness of seasonal (summer-fall) hypoxia treatments (after the reservoir stratifies, pumping hypolimnetic water that is low in dissolved oxygen from the SJR) – therefore, this biobox is the only one that currently contains zebra mussels.

Biobox Monitoring Protocol – Short-term, During Treatment

- Prior to treating the HC and DS with potash, the bioboxes should be taken offline and thoroughly cleaned.
- Immediately following the treatment of the HC and DS with potash, the bioboxes are brought on line.
- Monitor the bioboxes at each location once every week for new mussel settlements. KCl concentrations and water temperature should also be recorded each time the biobox is monitored.

- Note any new mussels that are positively identified and that have settled and attached to the plates/sides of the biobox.
- After recording the findings, remove all mussels from the biobox, clean the plates/sides, replace plates in the biobox for subsequent monitoring the following week.
- Monitor on a weekly basis throughout the entire period that the HC and DC is treated with kill concentrations of potash.

Biobox Monitoring Protocol – Long-Term

- Veliger densities and new mussel settlement in SJR tends to peak in the warm summer months (Veldhuizen and Janik 2010). The long-term biobox monitoring should target the summer period (May to October) when new mussel settlements are most likely to be detected in the bioboxes.
- Monitor for new mussel settlements in the bioboxes on a bi-monthly basis during the first summer period (May to October) following treatment using the protocol established for short term biobox monitoring (during potash treatment), and again during the second summer period following treatment. (e.g., if treatment occurs in November 2011, then bioboxes will be monitored in the summers of 2012 and 2013).
- Bioboxes can also be kept on line (except when maintenance is needed) and monitored on a monthly basis from November to April, although new mussel settlements (if some mussels are still alive).
- No mussels settlements in the bioboxes for 2 years after the treatment will indicate that eradication measures were successful.

Pipeline Bioassay

- When the concentration of potassium in the pipeline system reaches the target concentration (100 ppm), sort the mussels from the bioassay stock set (see reservoir bioassay section) into 3 size classes (based on mussel length measured with calipers); < 5 mm, 5-15 mm, and > 15 mm length. While sorting into size-classes, check for the viability of these mussels; reject any gaping mussels that do not close when the mantle is prodded with a dull probe. These mussels will be used for the pipeline treatment bioassay.
- Pipeline Bioassay Test Mussels: Sort 100 viable mussels of each size class into mesh bags that are marked to denote which mussel sizes they contain. Prepare a total of 36 test bags; 12 bags for each size class (12 bags x 3 mussel size classes x 100 mussels per bag = a total of 3,600 viable mussels).
- Control Test Mussels: The control test described in the Reservoir Bioassay section above will apply to both the reservoir bioassay and the pipeline bioassay.
- The Pipeline Bioassay Test bags will be deployed at 3 different depths (below surface; middle of water column; just above the pipeline bottom) at 4 different locations in the pipeline system. Bags will be tethered to an appropriate length of

rope which will be tied to an appropriate structure at the access point of the pipeline. At each location and at each depth, 3 bags will be deployed, each containing a different mussel size class (3 bags near the surface, 3 bags in the middle, and 3 bags near the bottom = 9 bags total at each location). Location will be marked for repeated monitoring and retrieval of the bags.

- The results of the control test for the reservoir bioassay will also apply to the pipeline bioassay. There, the pipeline bioassay should be set up and occur concurrently with the reservoir (and control) bioassay.
- On the day of the deployment of the bags in the pipeline, 100% of the mussels are assumed to be viable, based on the viability checks conducted before they were bagged. After deployment, all bags (pipeline bioassay test and control test) will be inspected every other day (i.e., day 0 = deployment, then monitor on day 2, 4, 6, etc.) until all pipeline test bags indicate 100% mussel mortality. Also note the water temperature and potassium concentration, every time the mussels are monitored. During the monitoring, all gaping and unresponsive mussels will be immersed in recovery aquaria containing untreated water and checked for continued non-responsiveness (by prodding the mantle with a probe) after a 48-h period. Non-responsive mussels after 48-h will be marked as dead, and mortality counts will be noted.

Monitoring with ROV

- Inspections of parts of the HC and DC can be conducted with a ROV one month after completion of potash addition to the reservoir.
- Inspections can focus on areas where mussels are known to exist in the pipeline system such as indicated by previous visual inspections and information available about the presence of mussels in the pipeline system. Alternatively, an ROV inspection conducted before treatment begins would also point to areas with mussels where post-treatment inspections could be focused.
- During the ROV surveys, note if 100% of the mussels observed show a gaping response. Also note, in general, if the majority of gaping shells contain tissue or not.
- During the surveys, if some mussels have closed shells, note it. Subsequent surveys (weekly or bi-monthly) may be required if a good proportion of the mussel shells are observed to be closed, or if some mussels are active and responsive.
- Monitoring will continue until two consecutive surveys find no live mussels, and the pipeline bioassay records 100% mortality.

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