Final Report

Modeling Mussels: Development of CE-QUAL-W2 *Dreissena* spp. mussel subcomponent (TI #320)

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The data generated during this project represents an unheralded effort by the field and laboratory assistants that toiled long hours in the rain, sun, and under the florescent bulbs of the laboratory to collect and analyze the data presented in this report. These field and laboratory assistants are listed below in reverse alphabetical order according to last name; many thanks to all of them!

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Introduction

Aquatic invasive species represent one of the most significant habitat alterations for fish, wildlife, and plants, and are rapidly becoming a dominant component of aquatic ecosystems within the Columbia River Basin (CRB) (Sanderson, Barnas and Rub 2009). Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. rostriformis bugensis*) are invasive freshwater mussels that cause extensive economic and ecological impacts in areas outside their native range (Dermott & Kerec 1997; Mann, Radtke, Huppert, Hamilton, Hanna, Duffield and Netusil 2010; Ricciardi, Neves and Rasmussen 1998). Zebra and quagga mussels, hereafter referred to as *Dreissena*, attach to hard submerged surfaces such as rock and concrete using byssal threads and this biofouling can create operational problems for hydroelectric and irrigation facilities (Boelman, Neilson, Dardeau and Cross 1997; Claudi & Mackie 1994; Jenner, Whitehouse, Taylor and Khalanski 1998; Neitzel, Johnson, Page, Young and Daling 1984). *Dreissena* can form large dense populations and through their collective filter feeding and deposition of feces and pseudofeces, they change the manner energy moves in an ecosystem, as well as increasing water clarity, light penetration and the growth of rooted macrophytes (Bastviken, Caraco and Cole 1998; Botts, Patterson and Schloesser 1996; Burlakova 1995; Caraco, Cole and Strayer 2006; Effler & Siegfried 1998; Effler, Matthews, Brooks-Matthews, Perkins, Siegfried and Hassett 2004; Fahnenstiel, Lang, Nalepa and Johengen 1995; Horvath, Martin and Lamberti 1999; Strayer 2008). If *Dreissena* become established in the CRB, the initial management costs at 13 hydropower facilities on the Columbia River was estimated to exceed $23 million with annual operational costs thereafter less than $100,000 per Federal Columbia River Power System (FCRPS) facility (Phillips, Darland, and Sytsma 2005).

In 2007, *Dreissena* mussels were found to have established populations west of the Rocky Mountains. In 2008, zebra mussels were discovered in San Justo Reservoir, California, and this remains the closest known established population to the Columbia River Basin. To date, established *Dreissena* mussel populations have not been detected in water bodies within Oregon, Washington, Idaho, and Montana. The continued interception of trailered watercraft with attached hitchhiking mussels in the aforementioned states, however, reinforces the high likelihood of mussel introduction into the CRB. Once established in a system, *Dreissena* populations can quickly spread within hydrologically-connected systems via planktonic larvae. The risk posed to the Pacific Northwest by the proximity of these new infestations is significant and increases the likelihood of the successful transport and introduction of these species into the CRB.

Dreissena mussels occupy different parts of a water body during their life cycle, and *Dreissena* larvae, or veligers, swim in the water column during larval development (Raven 1958). The planktonic veligers will likely be spatially and temporally clumped in the water column. *Dreissena* mass spawning is generally synchronized and ciliated veligers cannot swim horizontally towards specific objects (Boelman et al. 1997; Mackie & Schloesser 1996; Marsden 1992; Nichols 1996; Ram, Fong and Garton 1996; Sprung 1993). Water currents and wind conditions are likely to distribute veligers throughout the water column both vertically and horizontally, but may also concentrate veligers, for instance, in downwind bays (Kerley 1995; Kraft, Garton, Johnson and Hieb 1996). In low mixing conditions, veligers may concentrate near the thermocline (Mackie & Schloesser 1996). Larval density peaks when water temperatures are

16-19°C (McMahon 1996). Veligers have been found throughout the water column, ranging from near the surface to depths greater than 122-m (400-ft) (Sprung 1993).

Dreissena mass spawn gametes into the water, and larval development occurs in the water column following a developmental pattern. Larval development in the water column is relatively unique in freshwater bivalves, and *Dreissena* veligers can be differentiated from other freshwater bivalve larvae using size and morphology (Nichols & Black 1994; Wells and Sytsma 2013). The trochophore stage is the first larval stage that develops from the gastrula (Kume & Dan 1968; Raven 1958), but this larval stage is easily damaged during collection and preservation (Nichols and Black 1994). The straight-hinge larval stage is the second stage and the first shelled larval stage with a fully developed velum (Figure 1). In this stage, the hinge is straight and the umbo has not developed enough to protrude beyond the hinge line, resulting in a D-shaped shell. The velum develops from the prototroch and resembles a pair of semicircular folds with cilia along outer margin (Raven 1958). Bivalve larvae that have a velum are called veligers.

Figure 1: Veliger with velum (V) extended beyond shell margin (SM).

As the veliger grows, the umbo develops and protrudes beyond the straight hinge line, and the overall shape changes from D-shape to round, thus marking the transition to the umbonal larval stage (Figure 2). Sometime around this period, the mantle forms and new growth starts on the peripheral edges of the shell, resulting in commarginal growth lines (Carriker and Palmer 1979; Raven 1958). This new region of shell growth, characterized by growth lines, is the prodissoconch II (PII) (Carriker and Palmer 1979; Raven 1958). The shell then becomes umboned and asymmetrical with the anterior end of the valves becoming more pointed compared to the posterior end (Carriker and Palmer 1979).

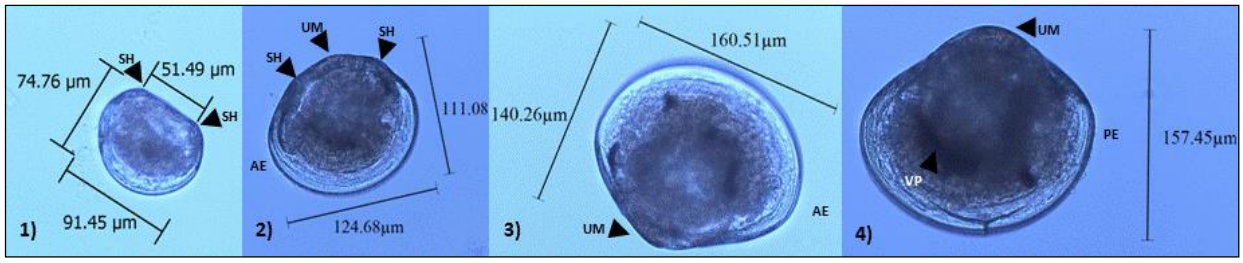

Figure 2: *Dreissena* veliger stages found in plankton including the 1) straight-hinge, 2) late-stage straight-hinge/ early umbonal (early umbonal), 3) umbonal, and 4) late-stage umbonal/ pediveliger. The hinge area is marked by the shoulders (SH) and umbo (UM). The velar pigment (VP) is marked. Overall shape includes symmetry of posterior end (PE) and anterior end (AE), umbo height beyond shell margin, and shell outline.

The development of a functional foot marks the transition to the pediveliger larval stage. The pediveliger stage is the settling stage and is defined by a distinct umbo, asymmetrical anterior and posterior ends, and well-developed foot (Carriker and Palmer 1979). Pediveligers still have the velum (Raven 1958), however the velum is broken down and shed during metamorphosis to the plantigrade larval stage.

Veligers in the pediveliger developmental stage actively settle out of the water column onto to a variety of submerged substrates including rocks, gravel, sand, bivalve shells, macrophytes, woody debris and submerged man-made objects (e.g., ceramic, glass, metal, and plastic) where they seek out a desired location, secrete a single byssal thread, and undergo metamorphosis (Ackerman, Sim, Nichols and Claudi 1994; Roe & MacIsaac 1997; Sprung 1993). After metamorphosis, and while it retains a humped clam-like shell, it is considered a plantigrade larvae, and it can secrete additional byssal threads or crawl around on its foot (Nichols 1996). Mussels are considered juveniles once the siphons are fully formed and the shell is mussel-shaped (Nichols 1996). Juveniles reach sexual maturity when they are between 5 and 12-mm in shell length (Nichols 1996). Juvenile and adult mussels translocate year-round to preferred areas, and are found in epilimnion, littoral and profundal areas (Claudi & Mackie 1994). Juveniles are more mobile than adult mussels, and older mussels are often immobilized by the byssal threads from proximate mussels (Wells 2014).

Dreissena do not thrive in all parts of a water body and hydro facility. Larval settlement and recruitment is reduced at oxygen concentrations < 2.0 mg/L (Karatayev et al. 2007; McMahon 1996). Settlement and recruitment are also reduced at higher water velocities > 1.8 m/s (Claudi & Mackie 1994) and in areas with large amounts of sediment (Sprung 1993). Mussel development is also affected by temperature and food availability (Sprung 1989).

The Bonneville Power Administration (BPA) recognizes the need to develop long-term management measures for the aforementioned aquatic invasive species in the region. BPA developed the Aquatic Invasive Species Prevention (AISP) Roadmap to guide research and development programs, and has funded numerous projects focusing on controlling and monitoring for *Dreissena* mussels, e.g., TI#233: Field evaluation of foul-release coatings under Columbia River field conditions. Because of the potential impacts, vulnerability assessments are being completed for FCRPS facilities that identify the structural components most at-risk for mussel colonization based on the flow velocity across the components and other factors. Current vulnerability assessments, however, utilize static templates (DeBruyckere and Phillips 2014), and it is difficult to account for spatial and temporal variation in the mussel propagule pressure, and distribution.

This project was funded by BPA under its Technology Innovation Initiative with the original aim to develop a *Dreissena*-specific subcomponent for the existing CE-QUAL-W2 model in order to allow the computation of the resultant spatial and temporal distribution and abundance of *Dreissena* mussels within a water body depending on particular environmental conditions and/or facility operations. Specifically, the original aim was to develop, calibrate, and validate a two dimensional model (vertical through the water column and longitudinal along the flow path) of *Dreissena* mussel growth and survival using population dynamics and habitat characteristics in San Justo Reservoir, CA as well as characteristics derived from the literature. The model would then be applied to habitat characteristics in Lake Roosevelt/Grand Coulee (US Bureau of Reclamation), WA and Brownlee Reservoir/Snake River (Idaho Power), ID to simulate the likely spatial and temporal distribution of *Dreissena* mussels if the waterbodies were infested. This information would benefit numerous BPA- and other agency-funded regional and Project-level activities within the CRB such as hydropower vulnerability assessments, cost/benefit analyses for various control technologies, and early detection monitoring.

CE-QUAL-W2 is a two-dimensional (longitudinal and vertical) hydrodynamic and water quality model developed by the US Army Corps of Engineers and the Water Quality Research Group at Portland State University. CE-QUAL-W2 consists of directly coupled hydrodynamic and water quality transport models. CE-QUAL-W2 simulates parameters such as temperature, algae concentration, dissolved oxygen concentration, pH, nutrient concentrations, organic matter and detention time. The current release of model is Version 3.71. The user manual and documentation can be found at the PSU website for the model: http://www.cee.pdx.edu/w2. The model is a public domain model available for download.

Between June 2004 and June 2012 there have been about 8,500 model downloads or about 1,000/ year from 159 countries. Of these downloads, 4,020 were for Version 3.6, the prior model version. Most downloads were from (in order): USA, Iran, Korea, China, Brazil, Canada,

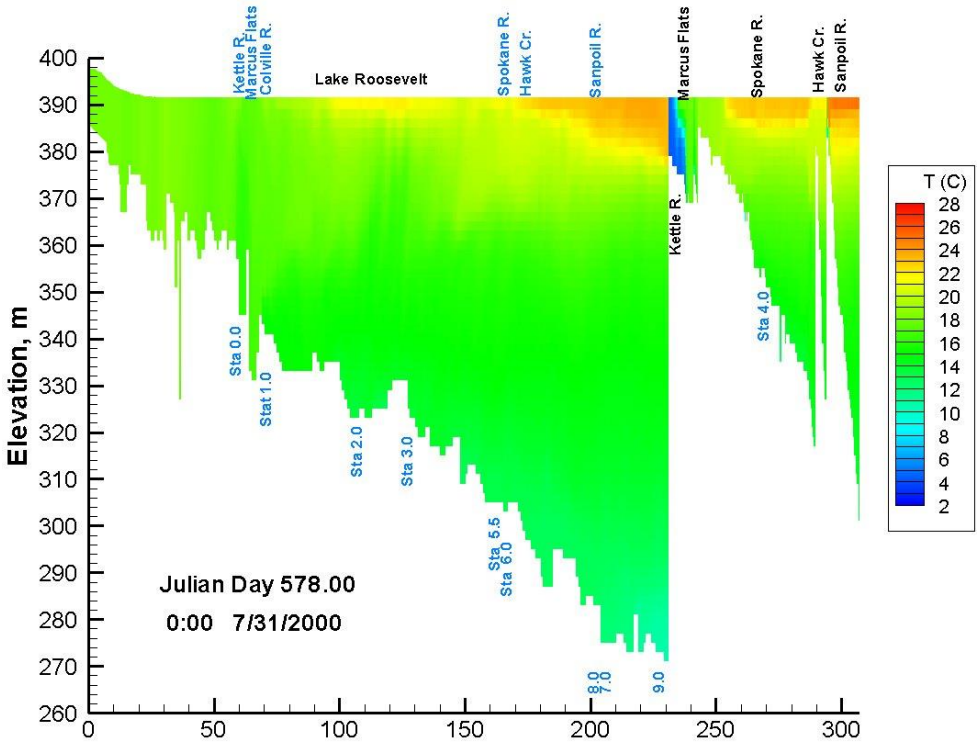

Figure 3: A CE-QUAL-W2 model simulation of temperature (°C) at different depths (m) in the Lake Roosevelt system.

Germany, India, Australia, Portugal, and Columbia. Version 3.7 was released at the end of 2012 that included a new post-processor from DSI. CE-QUAL-W2 has been used worldwide in reservoirs, lakes, rivers, estuaries, and pit lakes (Table 1). CE-QUAL-W2 has been applied in numerous CRB water bodies, e.g., Snake, Columbia, Spokane, Tualatin, McKenzie, N. and S. Santiam, Clackamas, Long Tom, and Willamette Rivers as well as many of the Snake, Columbia and Willamette Reservoirs, such as Brownlee, Oxbow, Hells Canyon , CJ Strike, Bonneville, The Dalles, Ice Harbor, Lower Monumental, Lower Granite, Dvorshak, Lucky Peak, Detroit, Cougar, and Long Lake (Wells et al. 2004, McKillip and Wells 2006, Berger et al. 2009).

Table 1: Applications of CE-QUAL-W2 model, world-wide.

Water body	Approximate # of known applications between 2000-2006
Reservoirs	>500
Lakes	>400
Rivers	>500
Estuaries	>100
Pit Lakes	>20
Countries	>116

The existing CE-QUAL-W2 model (Cole and Wells 2013), however, lacks a subcomponent specifically designed for the complex life cycle of *Dreissena* mussels. A better understanding of the timing and two-dimensional spatial distribution of veliger spawning and more importantly pediveliger settlement out of the water column, as well as the factors affecting juvenile and adult mussel survival and growth, could inform where and when particular control technologies are used to prevent veliger settlement within facilities, improve the effectiveness of early detection monitoring, discourage translocation of juvenile and adults to certain components, and kill and remove adults from facility components.

The ability of the CE-QUAL-W2 model to reproduce and predict system behavior is dependent on accurate input data. The original project proposed to develop and validate the *Dreissena* subcomponent using field collected data from San Justo Reservoir, which is the closest reservoir to the CRB that is infested with *Dreissena* mussels (22 sampling events were planned starting in January of 2015). Additionally, field data would be collected from two non-infested water bodies within the CRB, i.e., Lake Roosevelt and Brownlee Reservoir, in order to run simulations. The CE-QUAL-W2 model was previously developed for Lake Roosevelt and Brownlee Reservoir, but these models lacked a *Dreissena* subcomponent. The development of the *Dreissena* model subcomponent would require extensive sampling of the San Justo Reservoir *Dreissena* population to determine veliger periodicity, peak, and depth distribution over time; pediveliger and plantigrade larvae settlement patterns; and juvenile and adult mussel survivorship, growth, biomass, and distribution over depth and time. Calibrating and running simulations of the CE-QUAL-W2 model requires geometric, kinetic, hydrodynamic, and water quality data for the target systems.

The funding for this project was eliminated during the TI portfolio pruning process, and then an extension was granted that funded field collection and model conceptualization efforts through August 2015. A new proposal to complete the field collection efforts and to develop the model subcomponent was submitted to BPA for consideration in the FY16 TI Portfolio, but this proposal was not selected for funding. This final report details and describes the field sampling efforts in the period between January and August 2015, provides a brief note on mussel distribution patterns in space and time in San Justo Reservoir during this time period, and describes a conceptualization of the *Dreissena* model subcomponent.

Objectives

The goal of this project was to complete several key aspects of the originally planned research to inform future studies on how to model the life cycle of invasive mussels for management needs. Specifically, the goals of this project were to describe the *Dreissena* veliger spatial distribution and spawning timing in San Justo Reservoir, CA and to develop a conceptual framework for modelling mussel distribution using CE-QUAL-W2. The ultimate aim was that the model would allow the FCRPS and other water resource managers within the CRB and elsewhere to predict where and when the different mussel life stages would thrive within a water body given certain environmental parameters, determine how facility operations can affect colonization, aid cost/benefit analyses for various control technologies, and guide management to maximize effectiveness and reduce costs.

The specific project objectives were:

- Determine the *Dreissena* spawning periodicity as measured by the presence of straight-hinge veligers throughout the water column in San Justo Reservoir,
- Determine the timing of peak *Dreissena* spawning using the density of straight-hinge veligers throughout the water column,
- Determine the periodicity of veliger settlement out of the water column using the presence of pediveligers throughout the water column,
- Determine the timing of peak veliger settlement out of the water column using the density of pediveligers throughout the water column,
- Determine the vertical distribution of all *Dreissena* veliger stages in the water column using the density of veligers sampled at 1-m intervals,
- Determine the presence and depth of a thermocline using *in-situ* water quality data,
- Develop a conceptual framework describing how the *Dreissena* larvae, juvenile, and adult mussel survival and growth will be simulated in the CE-QUAL-W2 model,
- Prevent the unintentional introduction of *Dreissena* mussels and other AIS to other water bodies and/or the contamination of samples using appropriate equipment decontamination protocols, and adhering to field and laboratory standard operating procedures.

Methods

The ability of the CE-QUAL-W2 model to reproduce and predict system behavior is dependent on accurate input data. Development of the *Dreissena* subcomponent will require extensive sampling of the mussel population to determine veliger periodicity, peak, and depth distribution over time. Validating and running simulations of the CE-QUAL-W2 model requires geometric, kinetic, hydrodynamic, and water quality data for the target system.

The *Dreissena* veliger population in San Justo Reservoir was sampled along a vertical profile throughout the water column at one-meter depth intervals using a peristaltic pump and silicone tubing. The model conceptual framework was developed using literature searches, subject matter experts, and other modeling efforts.

Schedule

The project schedule was during the period between October 1, 2014 and August 31, 2015. The field and laboratory sampling and model framework development occurred in the period between January 1, 2015 and August 31, 2015. A total of eight sampling events were conducted during this period to describe the veliger spatial distribution and spawning timing (Table 2). The *Dreissena* sampling plan included tasks that occurred in the field in San Justo Reservoir, and tasks that were done in the PSU laboratory. The schedule and location of these tasks are identified in Table 2. This schedule ensured monitoring of *Dreissena* mussels throughout a portion of their life cycle, and the schedule of major tasks included the following:

- October, 2014 – August, 2015: Permitting, planning, and stakeholder coordination
- January, 2015: Field deployment of sampling stations and first sampling event
- February, 2015 – August, 2015: Field data collection approximately monthly
- February, 2015- August, 2015: Laboratory analysis
- January, 2015 – August, 2015: Quality assurance and quality control measures
- January, 2015 – August, 2015: Conceptual framework

Table 2: Schedule for mussel sampling and model conceptualization efforts.

FY2015		Oct				Nov				Dec				Jan				Feb				Mar				Apr				May				Jun				Jul				Aug			
TASK DESCRIPTION	Location	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Permitting & stakeholder coordination	PSU																																											
Field station deployment/ breakdown	Field																																											
Multi-probe profile	Field																																											
Plankton collection	Field																																											
Sample handling & shipping	Field																																											
Travel to/from field site	Field																																											
Plankton analysis	PSU																																											
QA/QC measures	PSU																																											
Literature search & data mining	PSU																																											
Conceptual framework	PSU																																											

Sampling locations

The field study area was San Justo Reservoir located in central California near the town of Hollister. San Justo Reservoir is an off-stream reservoir impounded by a US Bureau of Reclamation (USBR) dam and operated by San Benito County Water District (SBCWD). San Justo Reservoir is connected to SBCWD's distribution system with an inlet/outlet structure, and water is received from San Luis Reservoir via the San Felipe Unit of the USBR Central Valley Project (Cohen 2008). This water body is a warm monomictic reservoir infested with *Dreissena* mussels. San Justo Reservoir was closed to the general public in 2008.

Veliger mussel sampling was done at two locations in San Justo Reservoir, encompassing the deepest portion of the reservoir as well as the inlet/outlet structure (Table 3). The exact location of the deepest sampling site was determined in the field during the initial sampling event using a scientific echosounder and GPS unit. The sampling locations were marked with moored buoys, and during each sampling event, the boat was anchored to maintain position at each sampling location. Depth-specific plankton sampling was done at these two locations at one-meter depth intervals from the water surface to within one-meter of the reservoir bottom.

Table 3: Location of *Dreissena* veliger sampling locations.

	Location (dec. deg., WGS84)		
Site name	Latitude	Longitude	Site type
Deepest Location	36.817691	-121.446727	Plankton, profile measurement, oxygen/temperature logging
Inlet/Outlet Pipe Outfall	36.822177	-121.441536	Plankton, profile measurement, oxygen/temperature logging

Labeling

Each sample container (e.g., 50-mL centrifuge tube with screw-lid) was assigned a unique number (e.g., PPMS-2038), and this number was recorded on the sample container prior to field collection using permanent ink stickers. While in the field, the unique number was recorded on waterproof field datasheets during sample collection when that particular container was used. The unique number was used to track samples during field collection, shipping and receiving, laboratory analysis, and storage. The unique number was assigned according to the format PPMS-XX, where PP = pump plankton, MS = Mussel Squeeze, and XX is a running consecutive number starting at 01.

Each sample container also had a sample container label affixed to the outside of the container so that information was recorded on the label while in the field. The information recorded on the sample container label duplicated the field datasheet, albeit, the sample container label did not contain all information recorded. The information listed below was recorded on each sample container label using a pencil.

- Date of collection
- Sampling station ID (Deep or Inlet/outlet)
- Water depth

Field datasheets were used to record sample information in the field. Waterproof field datasheets were the primary means of recording sample information in the field, and a portion of the information recorded in the field datasheets was duplicated on the sample container label. The information listed below was recorded for each veliger sample on the field datasheet when applicable.

- Date of collection
- Time of collection
- Name of collectors
- Unique sample container number, e.g., PPMS-7000
- Water depth
- Sampling station ID
- Latitude and longitude (decimal degrees, e.g., 45.5678943 -122.345678)
- Notes

Sampling frequency

Plankton sampling was done approximately monthly over an eight month period starting in January, 2015. Depth-specific plankton sampling was done at each sampling location once during each sampling trip.

Dreissena mussel veligers

The objectives of the depth-specific pumped plankton samples were to determine the periodicity, density, size, and developmental stage of *Dreissena* veligers across depth and time in San Justo Reservoir. The sampling design utilized a peristaltic pump and silicone tubing to directly sample a specific volume of water from a specific location within the water column at 1-m depth intervals. This sampling effort was repeated approximately monthly over an eight month period, and yielded a large number of quantitative plankton samples (i.e., up to 111 samples per sampling trip).

Depth-specific plankton samples were collected along a vertical profile throughout the water column at two sample locations in San Justo Reservoir. Sample locations are identified in Table 3. The silicone tubing was attached to a weighted and marked rope that was used to lower the tubing into the water column and maintain vertical position during sampling at each depth. At each depth, 3-L of reservoir water were pumped through the tubing (approximately 2.5 times the volume of the tubing) to rinse the tubing with water at that particular depth, and then 5-L of reservoir water were pumped and passed through a 64-µm mesh filter cup. The filtered particulate collected on the 64-µm mesh was rinsed into 50-mL centrifuge tubes and preserved with ethanol (EtOH) to a final concentration of 70% EtOH. The EtOH was buffered using tris(hydroxymethyl)aminomethane (Tris) in order to maintain the pH of preserved plankton samples at a pH above 7.5. The screw cap of the preserved plankton samples was wrapped with electrical tape to minimize leakage, and samples were transported to PSU for analysis. The maximum veliger sample holding time prior to analysis was 365 days. Refer to Appendix A,

"Field Collection Protocols for Zebra Mussels", for complete details on *Dreissena* mussel veliger collection via pump.

Veliger plankton samples were collected and preserved in the field, and transported to the PSU laboratory for analysis using cross-polarized light microscopy (CPLM). In the PSU laboratory, plankton samples were analyzed using compound light microscopy to detect and count *Dreissena* veligers within the plankton matrix, measure the shell dimensions, and determine the larval developmental stage. Morphological features of the larval shell were used for freshwater bivalve identification (Conn, Lutz, Hu and Kennedy 1993; Kennedy & Hagg 2005; Nichols & Black 1994; Wells and Sytsma 2013). Light microscopy is an effective and established method for detecting and identifying *Dreissena* veligers in plankton samples (Frischer, Nierzwicki-Bauer and Kelly 2011). The use of cross-polarized light microscopy aids in the detection of bivalve larvae in plankton samples (Johnson 1995). Identification of preserved specimens via light microscopy was based on shell dimensions, overall shape, shell surface features and visible internal tissues, e.g., velum and foot (Ackerman et al. 1994; Nichols & Black 1994; Wells and Sytsma 2013).

Depth-specific plankton samples were processed and analyzed to examine 100% of the concentrated sample, and to quickly and accurately measure veligers and determine larval developmental stage. The plankton samples were collected in 50-mL centrifuge tubes, and the plankton samples were concentrated in the sample containers by centrifuging for 5 minutes at 1,000 RPM in the PSU laboratory. Veligers were relatively dense, and were pelleted into the bottom of the centrifuge tube. This pelleted particulate, or concentrated sample, was the actual portion of the plankton sample that would contain any veligers, and this was the portion of the sample that was analyzed (more than 75% of the plankton sample was reservoir water and ethanol).

The concentrated sample was carefully removed from the bottom of the centrifuge tube using a disposable pipette, and the aliquot was added to a Sedgewick-Rafter counting chamber (1-, and 2-mL total volume cells). The concentrated sample was diluted inside the sedgewick-rafter cell so that the matrix of algae, zooplankton and sediment allowed for the visual inspection of all objects. The supernatant in the centrifuge tube (70% ethanol) was used to dilute the concentrated sample unless the supernatant was heavily colored, in which case, fresh ethanol was used. Regular ethanol was the preferred liquid to dilute the concentrated sample because it reduced surface tension. Reducing the surface tension of the medium increased the likelihood of the particulate spreading out within the counting chamber. Distilled water was also an acceptable liquid for dilution. Efforts were focused on using a higher dilution factor (i.e., less concentrated sample per cell) to reduce the effects of the confounding matrix (e.g., obscuring veliger entirely or hiding key morphology). Additionally, several cells were prepared at one time to allow time for objects to settle to the bottom of the cell, thus reducing the three-dimensional character of the sample.

Binocular compound light microscopes with 10X lens pieces and 4X, 10X, and 20X plan achromatic objectives with a polarization kit, mechanical stage with Y- and X-axis travel knobs, sub stage condenser, and a trinocular-mounted digital microscope color camera equipped with digital micrometer were used to examine the sedgewick-rafter cell under cross-polarized light for

veligers. This microscope set-up allows a total magnification ranging between 40X and 200X with a numerical aperture up to 0.4.

A magnification of 40X was used for scanning plankton samples for the presence of veligers. The detection of suspect veligers was the objective of the scanning mode, and the initial scan of the counting chamber was done under lower total magnifications to capture more of the sample within the field of view and to reduce analytical time. Cross-polarized light microscopy was employed in scanning mode to increase the detection of birefringent objects within an interfering plankton matrix. The presence of birefringence and the characteristic "maltese cross" increases the likelihood of initially detecting veligers (Johnson 1995). Appearance of the maltese cross and birefringence under polarized light were not used for identification purposes, but rather served as a searching tool. Once the birefringent object was detected, the polarization was removed or reduced to allow more background light into the field of view and thus allowing greater visibility of morphological features used in identification. The sub stage condensers were used to concentrate the light illuminating the specimen, thus changing the optical characteristics. The condenser could be adjusted to help elucidate particular features such as internal tissues and hinge features.

Inspections, photomicroscopy and measurements were done as quickly as possible, and then the 40X scan under polarizing light was resumed to search for more veligers. If possible, veligers were identified, photographed, measured, and classified into a developmental stage under 40X total magnification with little or no polarization. In most cases, higher magnifications and resolving power were required to positively identify and/or measure specimens. The entire sedgewick-rafter cell was scanned in this way. Each veliger was identified and classified into developmental stage when possible. In some cases, veligers were positioned in three-dimensional space in a way that precludes accurate identification, measurement, and/or determination of larval stage; these veligers were counted as Unknown stage.

During microscopic evaluation, the planktonic *Dreissena* veligers were assigned to one of three larval developmental stages: 1) straight-hinge, 2) umbonal, or 3) late-umbonal/ pediveliger. Developmental stage classifications were based on hinge development, overall shape, size, and other morphology according to Nichols and Black (1994) and Wells and Sytsma (2013). In this study, straight-hinge veligers were defined as those veligers possessing a straight hinge where the umbo did not protrude beyond the shell line, and an overall shell shape resembled a capital D (Figure 4). Concentric growth lines on the periphery of the shell margin may or may not have been visible. Straight-hinge veligers possessed a velum although this was typically withdrawn within the shell and not visible using preserved specimens.

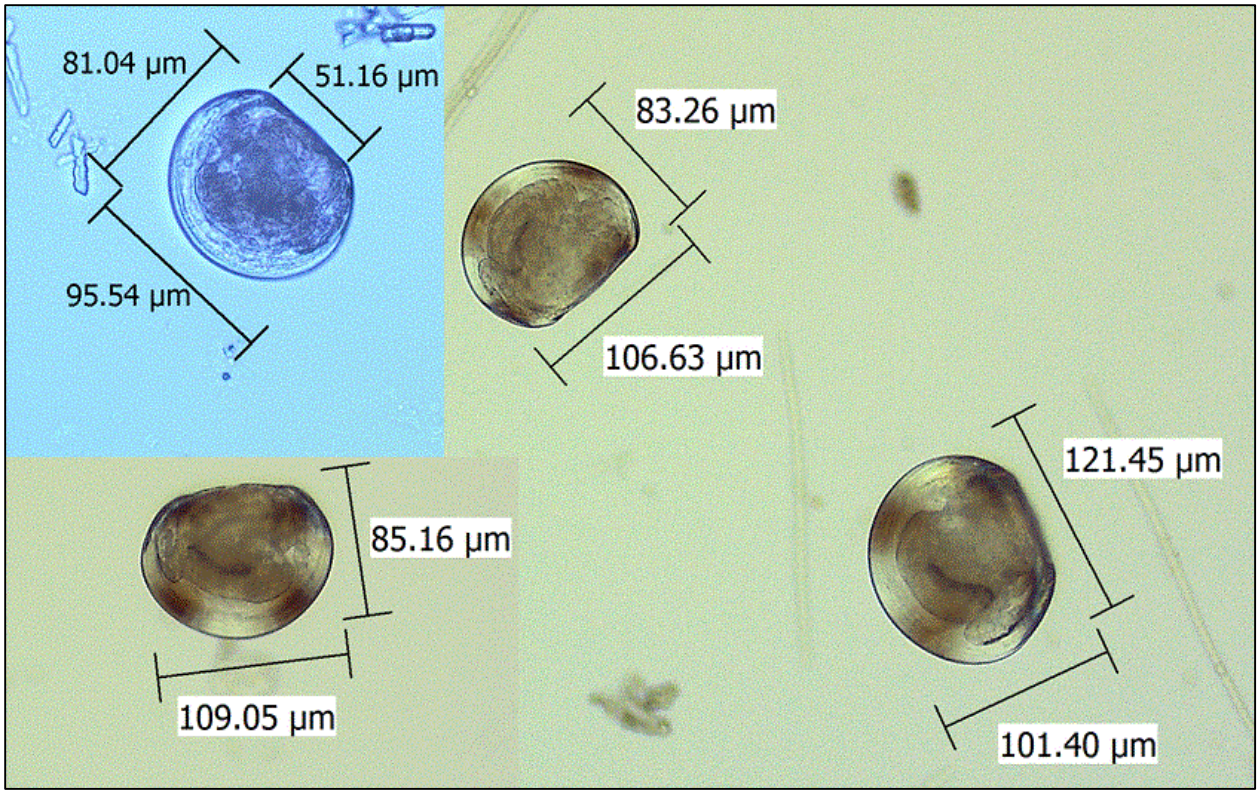

Figure 4: Straight-hinge stage *Dreissena* veligers.

Umbonal stage veligers in this study were characterized by an umbo beginning to protrude beyond the shell line giving the veliger an overall rounded shape, with both the anterior and posterior ends of the shell being relatively symmetrical. The umbo was low and rounded. Umbonal stage veligers may or may not have had concentric growth lines visible, and these veligers had a velum although it was generally not visible (Figure 5). Umbonal veligers did not possess a functional foot.

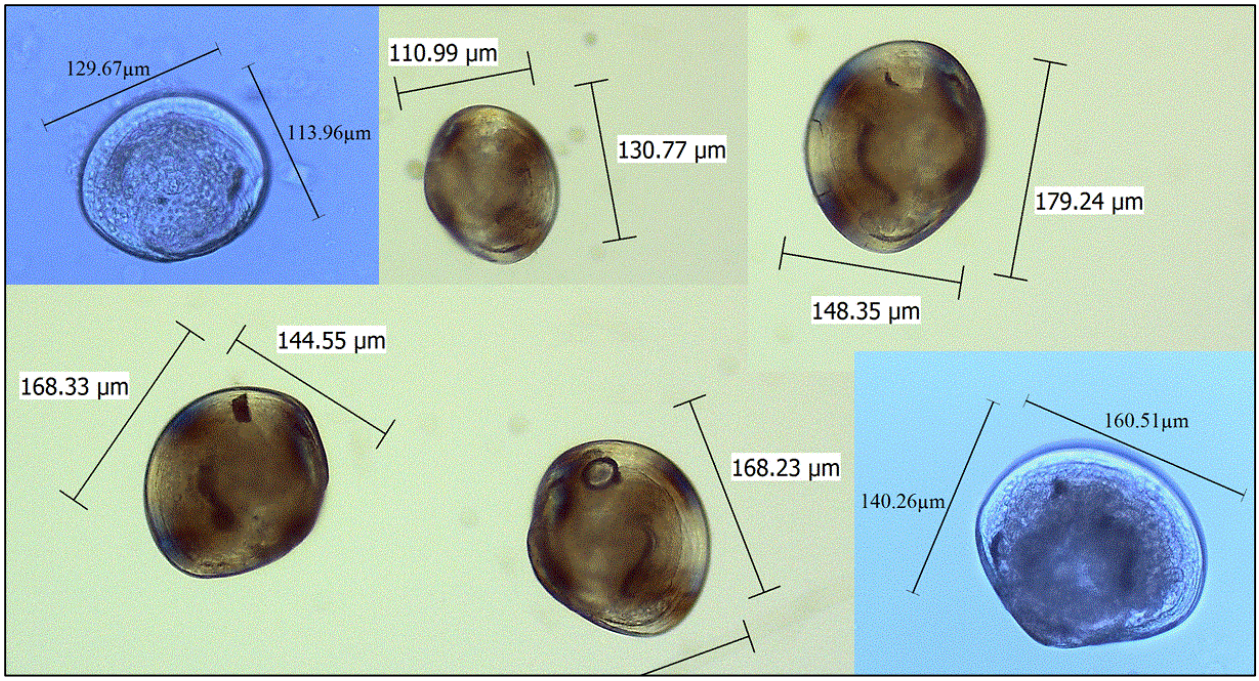

Figure 5: Umbonal stage *Dreissena* veligers.

Late-umbonal/ pediveliger stage veligers in this study were characterized by a pronounced umbo that protruded beyond the shell line, asymmetrical anterior and posterior ends, and well-defined concentric growth lines that converged in the shoulders (Figure 6). Both the umbonal and pediveliger stage veligers have a velum and can swim in the water column. The morphological difference between umbonal and pediveliger stage veligers is the presence of a functional foot in pediveligers, marking the veligers as ready to settle and colonize submerged surfaces. The foot was retracted on preserved specimens and was difficult to see; therefore, our classifications of veliger stage did not use the presence of a functional foot to separate pediveligers from older umbonal stage veligers. In this study, older umbonal veligers with a more pronounced umbo and asymmetrical ends were classified with pediveligers as shown in Figure 6.

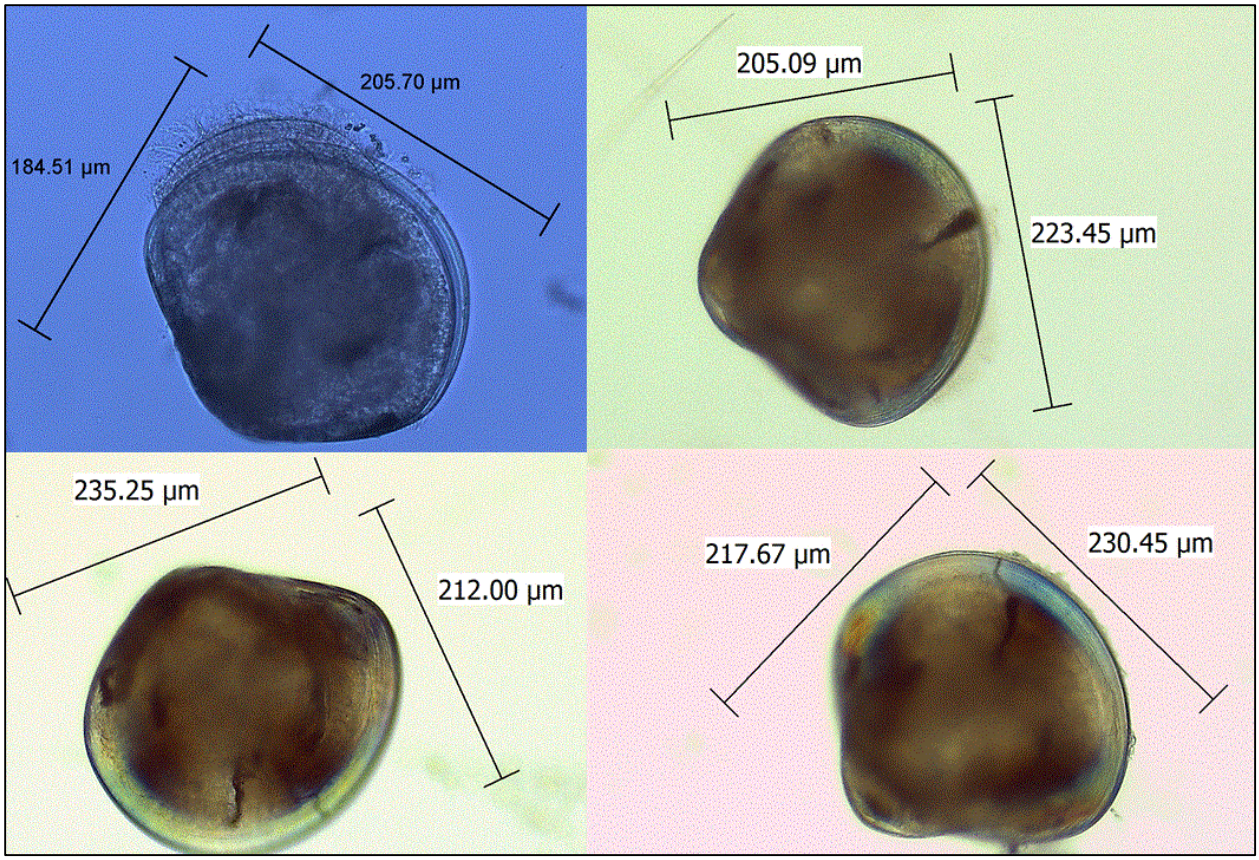

Figure 6: Late-umbonal/ pediveliger stage *Dreissena* veligers.

In this study, the presence of straight-hinge veligers in the water column was used to indicate adult mussel spawning. The straight-hinge veliger stage is the first larval developmental stage that can be reliably sampled in the water column following embryo fertilization in the water column. The rate of larval development varies but it can be assumed that the actual release of gametes from gravid adult mussels preceded the presence of straight-hinge veligers in the water column. In this study, the timing and peak of larval settlement out of the water column was measured by the presence and density of the settlement-ready veligers, classified as late umbonal/ pediveligers.

Plankton analysis was destructive, and the sample was destroyed following analysis and verification that quality control objectives were met. After a cell was scanned and its contents were measured and recorded, and appropriate QAQC measures were implemented, the aliquot was discarded. In between aliquots of the same plankton sample, the sedgewick-rafter cell was cleaned by rinsing with fresh water. Sedgewick-rafter cells were decontaminated using acid in between different samples, and at the end of a work shift.

Water quality

In-situ water quality data along a vertical gradient were acquired using a calibrated ProDSS multiparameter sonde deployed throughout the water column from a boat. Water temperature and dissolved oxygen were measured at 1-m depth intervals from the water surface to 1-m above the

reservoir sediment. Water quality data were collected concurrently with *Dreissena* veliger sampling at the two locations identified in Table 3. The presence and depth of a thermocline was determined from the 1-m vertical resolution *in situ* temperature profile data.

Measurement with the calibrated ProDSS multiparameter sonde was done by placing the sonde at one meter depth for at least five minutes or until readings on the handheld unit were no longer drifting in one direction, and variation was less than the precision criteria identified in the Quality Assurance Project Plan. Measurement values were logged on the sonde and were recorded on the waterproof field datasheets. The sonde was then lowered one meter and allowed to equilibrate for at least one minute or until there was no longer drift in readings and variation targets were met. Readings were logged and the process was repeated at one meter intervals to within one meter of the sediment surface. More time was required for equilibration after lowering between depths that were vertically stratified, i.e., more time was required to equilibrate when water chemistry was different than the previous strata. Measurements at two meters depth were repeated after the profile was complete as a measurement precision check.

Water quality equipment used during the project was calibrated according to methods and frequencies listed in Table 4. Calibration logs were recorded on field datasheets. Some of equipment listed in Table 4 are factory calibrated and thus, were not calibrated in the field, e.g., water temperature probe.

Table 4: Water quality equipment checks and frequency.

Equipment or sensor	Calibration method	Calibration frequency	Calibration check frequency
YSI ProDSS			
Barometric pressure	Factory calibration	none	Start and end of project
Optical diss. oxygen	100% water saturated air	Start of day	Start of day
Temperature	Factory calibration	none	Start and end of project

Model conceptual framework

The ability of the CE-QUAL-W2 model to reproduce and predict system behavior is dependent on accurately identifying the key input parameters to describe ecological processes, and then acquiring accurate data. The objective of the model conceptualization was to identify the key ecological processes influencing *Dreissena* mussel spatial and temporal distribution as well as the key input parameters to describe and simulate these processes.

This project relied on the Project Team's expert knowledge of zebra mussel biology and ecology, limnology, and two-dimensional hydrodynamic and water quality modeling to accurately describe the mussel responses to various environmental parameters and then develop model conceptualizations that capture these interactions to reproduce mussel distributions and abundance. This project also built upon efforts of several agencies conducting biological and water quality sampling in San Justo Reservoir since May 2008. Staff from USBR, California Department of Fish and Wildlife (CDFW), San Benito County Water District (SBCWD), and California Department of Water Resources (CDWR) have conducted biological and water

quality sampling in San Justo Reservoir including settlement substrates, veliger sampling, benthic grabs, turbidity, pH, temperature, dissolved oxygen, calcium, nitrate, and potassium (Cohen 2008; Meraz 2014; Veldhuizen and Janik 2010). Based on the available data from these efforts, it appeared that *Dreissena* survival and reproduction were limited at water depths below the thermocline in San Justo Reservoir (warm monomictic lake) due to low dissolved oxygen during stratification. There were potential confounding issues, however, due to water operations during the June through September period when water was pumped into San Justo Reservoir through an inlet/outlet structure located near the reservoir bottom during the night, and water was then pumped out during the day to meet water use demands (Cohen 2008). These water operations could be introducing oxygenated water into the hypolimnion. The aforementioned data collection efforts have produced valuable information, yet these data have not been synthesized into a management tool that can be directly applied in San Justo Reservoir as well as other water bodies. These other efforts were used to inform the development of the model conceptualization.

Equipment decontamination

Field survey personnel adhered to the decontamination protocols detailed in Appendix A, "Field Collection Protocols for Zebra Mussels".

Field equipment was decontaminated to prevent the transfer of organisms within and between systems and samples. Decontamination efforts were focused on zebra mussels, but efforts were also directed at other plant and animal species that may be encountered. For example, *Corbicula fluminea* (Asian clam) was also present in San Justo Reservoir. The decontamination procedures listed below were for all equipment leaving the San Justo Reservoir job site with the exception of the plankton sampling equipment and the YSI ProDSS. The plankton sampling equipment including the silicone tubing and the 64-µm mesh cups were decontaminated at PSU by soaking in 4% hydrochloric acid (HCl) or 5% acetic acid. The minimum soak time for acetic acid was 6 hours. The soak time for a solution of 4% HCl was 4 hours. Following the acid bath, equipment was rinsed with distilled water and air dried. The underwater YSI ProDSS unit sensors, which cannot be dried or bleached, were stored in an acidic environment to prevent survival of veligers or adult mussels.

Other sampling equipment and gear that came in contact with mussels or San Justo Reservoir was decontaminated using a combination of physical scrubbing to remove contaminants and a series of chemical soaks to kill, denature and dissolve shells. Deck brushes, long-handle bristle brushes, and fresh water were used to first physically scrub all surfaces of equipment. Equipment was then soaked in a 2% quaternary ammonium solution (Quat), e.g., Virkon Aquatic® or Sparquat 256®, for ten minutes. Quat cleaners are broad spectrum disinfectants and virucides widely used in aquaculture and janitorial service s (Wells 2014). Equipment was then soaked in solutions of 5% bleach (Clorox brand) for 15 minutes. Bleach denatures protein (Prince & Andrus 1992). Equipment was thoroughly rinsed with fresh water after chemical soaks. For equipment that could not be soaked in large plastic tubs, the chemical soaks were repeatedly applied using spray bottles.

Field equipment decontamination was done on level paved surfaces at least 122-m (400-ft) from open water. San Justo Reservoir was closed to the public, and there was ample space to conduct

equipment decontamination in the boat launch parking area. Mussels removed from boat and equipment were left on paved parking area of San Justo Reservoir boat launch. Anthropocentric waste materials from job site (that may be encrusted with mussels) were collected, bagged in plastic trash bags and disposed of in a dumpster that goes to a landfill.

Laboratory equipment and surfaces were decontaminated using a combination of physical scrubbing with soap and water, and both acid and bleach solutions to prevent the transfer of mussels and genetic material between samples in the PSU laboratory. Physical scrubbing removed most organisms. Bleach denatured protein, and acid solutions dissolved the calcite in the veliger shells (Prince & Andrus 1992). Laboratory equipment such as counting chambers, 500-µm filters, beakers, glass petri dishes, Imhoff settling cones, and centrifuge tubes were scrubbed with brushes using dish soap and warm tap water, and then thoroughly rinsed with tap water. Equipment was then soaked in solutions of 5% bleach for at least 15 minutes. Equipment was also soaked in a solution of 5% acetic acid or 4% HCl. The minimum soak time for acetic acid was 6 hours. The soak time for a solution of 4% HCl was 4 hours. Following the acid bath, equipment was rinsed with distilled water and air dried. Laboratory surfaces in contact with plankton samples, e.g. counters, plastic trays and microscope stages, were sprayed with a 5% bleach solution and wiped with disposable towels. Sample preparation and handling, including sample concentration, filtering, and adding aliquots into counting chambers, were done on plastic trays that have a rim. Plastic trays contain spills and were easy to clean.

Results

Thermocline/ water quality

San Justo Reservoir stratified by early April in 2015 as indicated by the water temperature and dissolved oxygen concentration across depth (Figure 7). The sampling event in late January was the only sampling period when the water column was mixed and was relatively isothermal. San Justo Reservoir strongly stratified as indicated by the slope of the profile in the metalimnion (Figure 7).

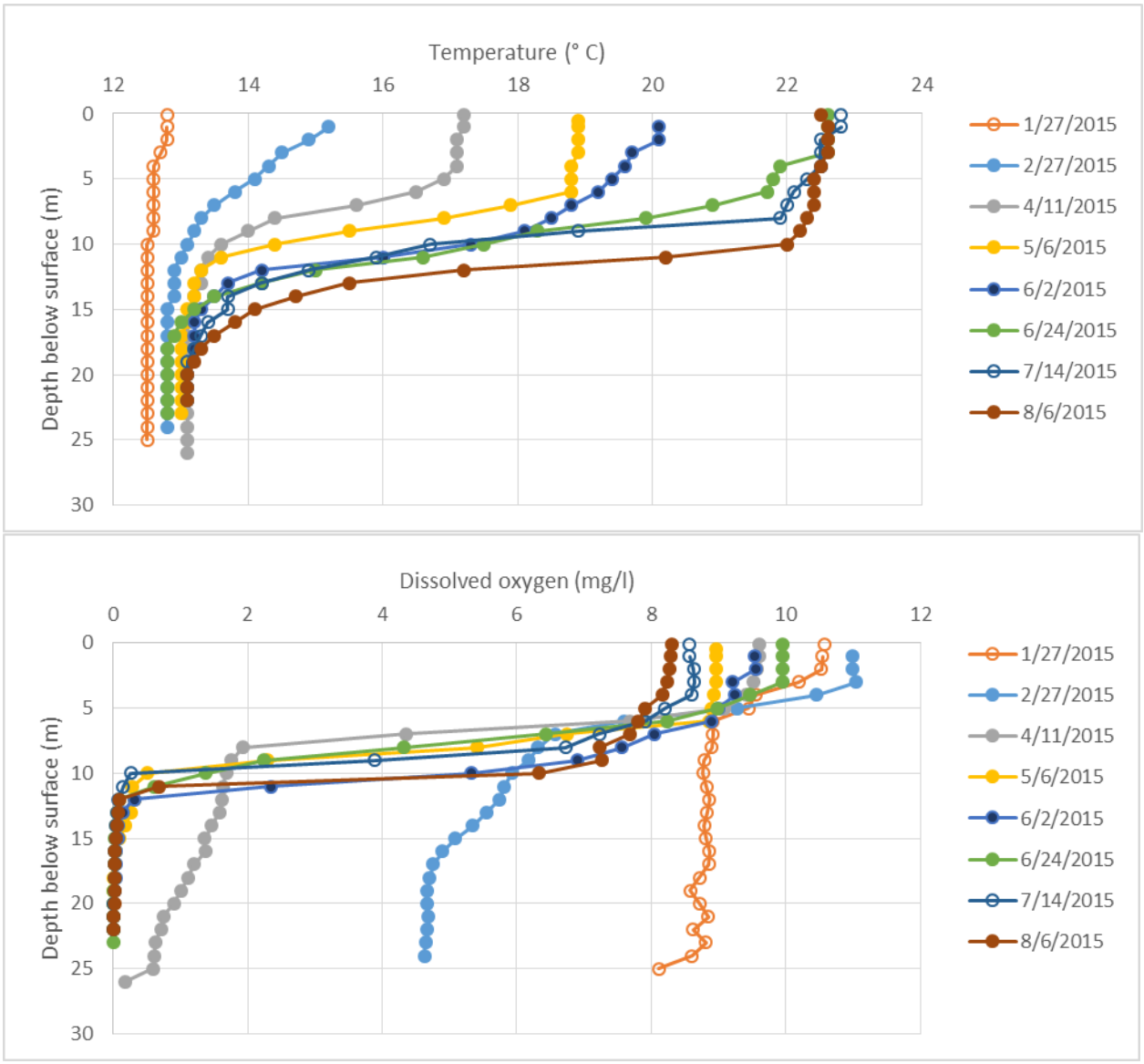

Figure 7: Water temperature (upper panel) and dissolved oxygen (lower panel) throughout the water column at the Deep location in San Justo Reservoir.

Dreissena population dynamics

Veliger spawning and settlement

Straight-hinge veligers were first collected in the water column during the 4/11/15 sampling event (Figures 8). Straight-hinge veligers were not collected in the water column in January and February, and the density of straight-hinge veligers collected during the period between April and May was low in comparison to early June, July, and August (Figure 8). Straight-hinge and umbonal stage veligers represented a large proportion of the veliger community composition in the late spring and summer periods (Figures 8 and 9).

The densities of both straight-hinge and umbonal veligers in early June at both sampling locations rapidly increased as compared to the two earlier sampling events that yielded straight-hinge veligers (i.e., 4/11/15 and 5/6/15) (Figures 8 and 9). The rate of increase in total veliger density observed during the 6/2/15 sampling event is indicated in Figure 8, and this is further highlighted by the different scales used for veliger density in late winter and early spring versus the late spring and summer sampling events in Figure 9.

The densities of younger veligers (straight hinge and umbonal stage) in the water column did not substantially decrease during the summer as the densities of older veligers increased (Figures 8 and 9). The densities of older veligers increased from the onset of spawning in the spring into the summer (Figure 9). The densities of older veligers, however, continued to increase at the Inlet/Outlet sampling location throughout the project period, whereas the density of older veligers appeared to peak in mid-July at the Deep sampling location (Figure 8). During the 4/11/15 and 5/6/15 sampling events when low densities of young veligers were collected, a concomitant low density of older veligers were also collected (Figure 9).

Straight-hinge veligers represented most of the veliger community in the water column during the 4/11/15, 5/6/15, and 6/2/15 sampling events at the Deep sampling location (Figure 9). The late umbonal/ pediveliger larvae represented all of the veliger community in the water column during the 1/26/15 sampling event. During the 2/27/15 sampling event, veligers were not collected in the water column (Figure 9). During the other sampling events, the veliger community composition was dominated by a combination of two larval developmental stages (straight-hinge & umbonal or umbonal & late umbonal/ pediveliger) (Figures 8 and 9).

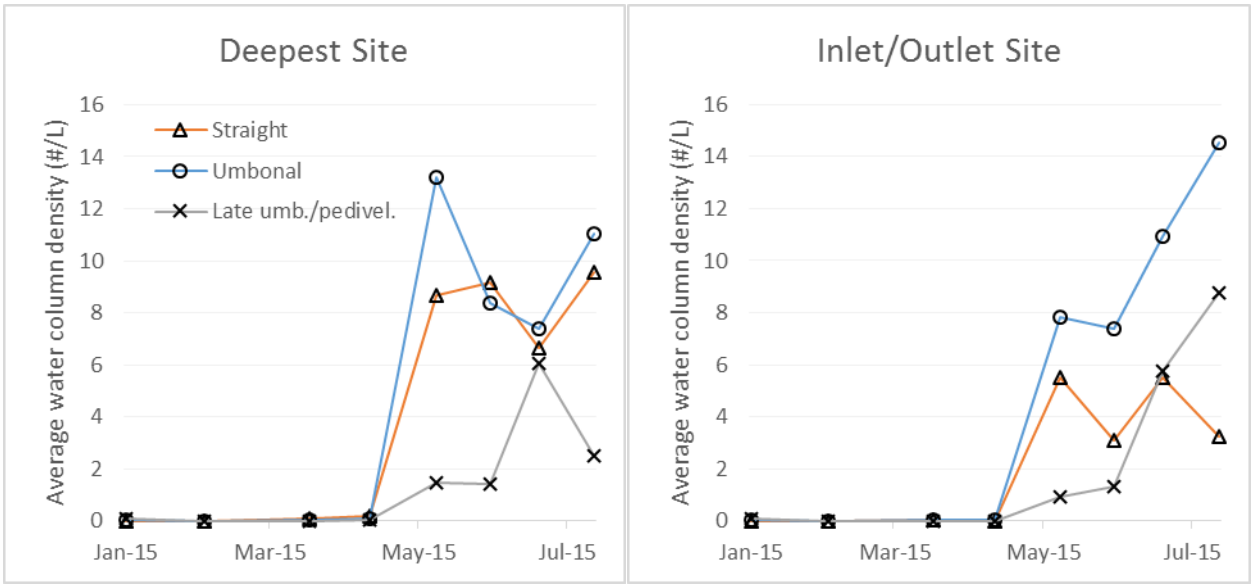

Figure 8: The presence and density of the *Dreissena* veliger developmental stages in the water column.

Veliger spatial-temporal distribution

Dreissena veligers were concentrated in the epilimnion of San Justo Reservoir when stratified, and veliger densities for all developmental stages peaked 5- to 8-m below the water surface

throughout the year. Densities of all veliger stages were relatively low near the surface (less than 1-m from the water surface) regardless of season (Figure 9). This pattern in the density of veligers across depth (i.e., peak densities between 5- and 8-m water depth, low densities near the surface, and very low densities in deep water) appeared to exist regardless of season and the presence of a thermocline. This pattern existed in late January (Figure 9) when the water column was not thermally stratified (Figure 7), as well as during sampling events during stratification (4/11/15, 5/6/15, 6/2/15, 6/24/15, 7/14/15, 8/6/15) (Figure 7).

Veliger densities for all larval stages were very low at water depths below stratification, and this pattern in veliger vertical distribution was especially apparent in regards to the temperature and dissolved oxygen concentration. The small dashed line in Figure 9 shows the lowest water depth at which there was no change in water temperature more than 1^{o}C per one meter depth. The large dash line in Figure 9 shows the depth of the dissolved oxygen concentration equal to or less than 0.1 mg/L. A small number of veligers were collected at water depths below these thresholds, but the status of these veligers upon collection was unknown, e.g., veligers could be dead or moribund and sinking out of water column. This pattern became clear in the summer (6/24/15, 7/15/15, and 8/6/15) when veliger densities plummeted to essentially zero and closely mirrored the dissolved oxygen and water temperatures profiles (Figure 9).

Larval development at the Deep sampling location peaked in late spring and summer in the epilimion. Straight-hinge veligers, presumably produced during spawning in the spring of 2015, were collected in low densities in the epilimnion in the spring (4/11/15 and 5/6/15) (Figure 9). Note the different scales used in Figure 9 for the veliger densities in the winter and early spring as compared to the scale used for veliger densities after early June when the number of straight-hinge veligers in the epilimnion rapidly increased (Figure 9). In early June, the number of straight-hinge veligers in the epilimnion rapidly increased, and the veliger community was dominated by straight-hinge larvae. By the end of June, however, there were high densities of both straight-hinge and umbonal stage veligers in the epilimnion. The increase in late umbonal/ pedivelgier larvae in the epilimnion from early June through August also indicates this continued larval development during the summer. By July and August, there were high densities of all larval stages in the epilimnion.

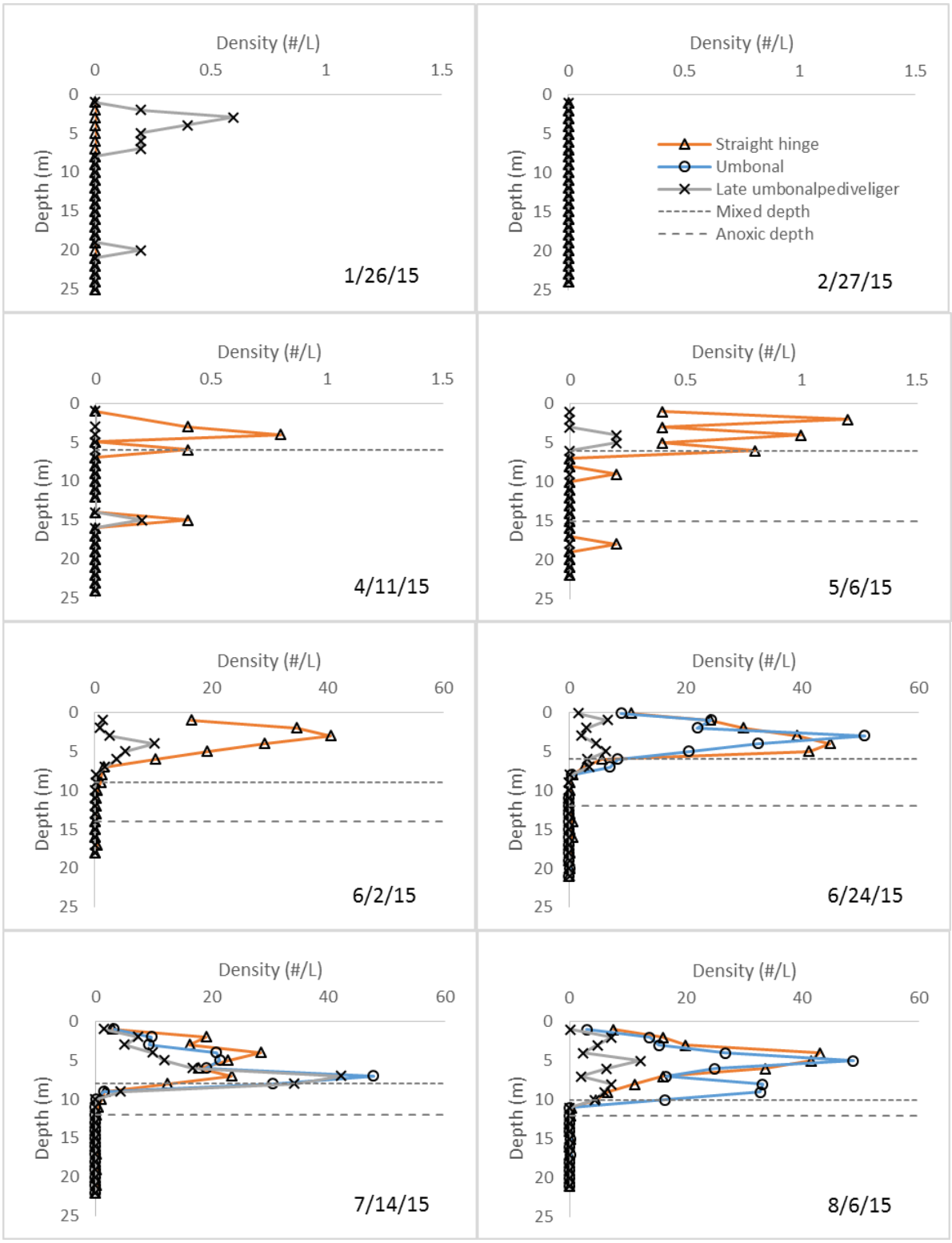

Figure 9: The density of *Dreissena* veliger stages according to water depth in the Deep sampling location. Anoxic levels are equal to or less than 0.1 mg/L.

Modeling mussel growth and movement

The conceptual model of mussel growth and transport is shown in **Error! Reference source not found.**0. In addition the modeling of mussels in the water column requires interaction between the mussel and the fluid domain for both transport and uptake/excretion (**Error! Reference source not found.**).

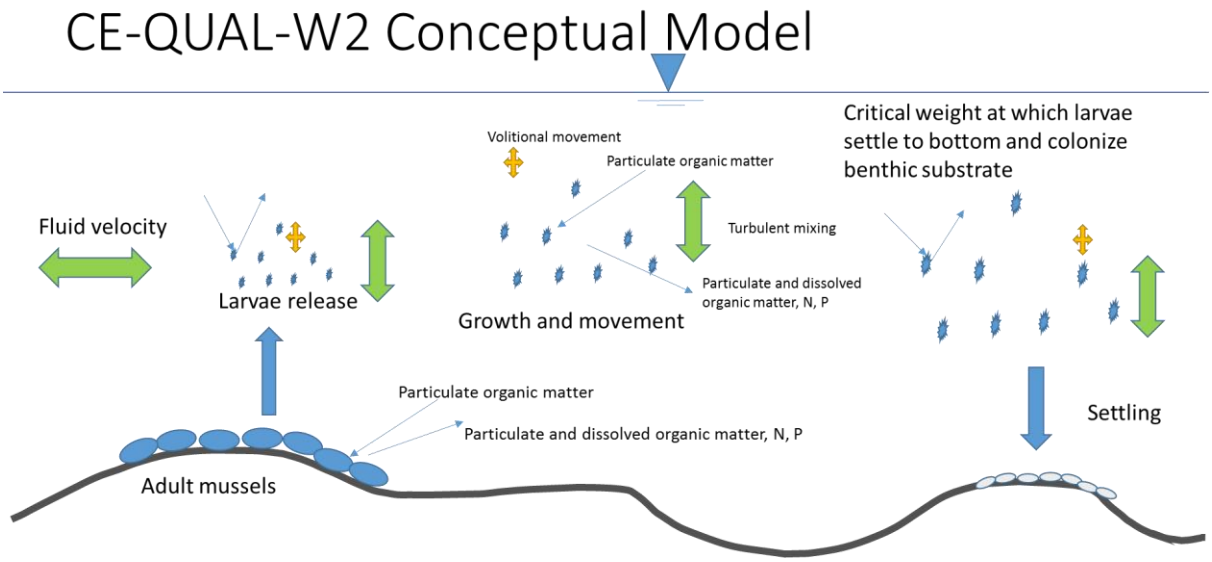

Figure 10: Conceptual model for the *Dreissena* subcomponent for CE-QUAL-W2.

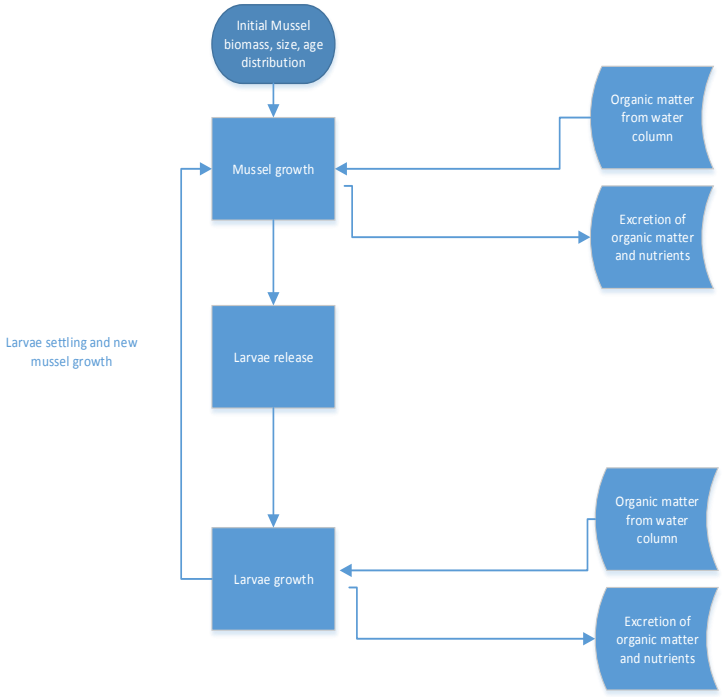

Figure 11: Overview of mussel life cycle and interaction with the water column.

Mussel equation

The wet mass of an individual *Dreissena* mussel will be modeled using a growth equation that includes consumption, respiration, egestion, excretion, and reproduction. The mussel will be simulated through its entire life cycle including veliger, juvenile, and adult stages. The location of a veliger will be calculated using particle tracking, and when it reaches a length of approximate 0.5-2 mm (USACE 2012), the veliger will settle out of the water column to a specific CE-QUAL-W2 model segment and layer. Similarly to Walz (1978a), Schneider (1992), Bierman et al. (2005), and Lindim (2015), the growth equation for a single mussel i will have the following form:

$$\frac{d\mathrm{M}_i}{dt} = \left(w_f C - w_r R - w_f F - w_f U\right)\mathrm{M}_i$$

where

M_i : wet mass of mussel soft tissue, g
C : Consumption or food ingestion (g food/g mussel/d)
w_f : conversion efficiency (g mussel/g food)
R : respiration (g O_2/g mussel/d)
w_r : respiration efficiency (g mussel/g O_2)
F : egestion (g food/ g mussel/d)
U : excretion (g food/ g mussel/d)
G : reproduction (1/d)

Consumption is a function of mussel size, temperature, food content, calcium concentration and dissolved oxygen concentration of the water:

$$C = a_c M_i^{b_c} \gamma_1 \gamma_2 \gamma_{Ca} \gamma_{DO} \text{MIN}\left(\frac{c}{K_c}, 1\right)$$

where

a_c : maximum ingestion rate (g food/g mussel/d)
b_c : exponent for weight effect on ingestion
γ_1 : ascending temperature rate multiplier
γ_2 : descending temperature rate multiplier
γ_{Ca}: calcium growth rate reduction factor
γ_{DO}: dissolved oxygen growth rate reduction factor
c : food content of water (mg dry weight/L)
K_c: saturation food concentration (mg dry weight/L)

The food content of the water c is the sum of palatable phytoplankton, zooplankton, and particulate organic matter concentrations. Water temperature affects mussel metabolism, growth and reproduction. The upper lethal limit has been found to be around 30° C (McMahon et al. 1995), but survival is possible for short periods above 30° C if the mussel is acclimatized to warm temperatures (Farr and Payne 2010). Temperature effects are modeled using the equations developed by Thornton and Lessem (1978), which are currently used in the phytoplankton compartment of CE-QUAL-W2. Calcium concentrations of 15 mg/l or less limits the distribution of zebra mussels (Mellina and Rassmussen 1994). The growth rate reduction factor γ_{Ca} will be modeled using an equation of the form:

$$\gamma_{Ca} = \frac{\Phi_{Ca}}{K_{Ca} + \Phi_{Ca}}$$

where Φ_{Ca} is calcium concentration (mg/l) and K_{Ca} is the half saturation concentration of calcium where mussel growth is half its maximum value without the limitation of low calcium concentrations. Low dissolved oxygen concentrations also can limit mussel growth in eutrophic systems (Far and Payne 2010). Zebra mussels are stressed in water with less than 40% to 50% dissolved oxygen saturation (Boelman et al. 1997), and 1 mg/l to 4 mg/l are lowest concentrations that survival is possible (Farr and Payne 2010). The ability of zebra mussels to withstand zero DO for short periods is related to size, with larger mussels able to tolerate anaerobic conditions longer (McMahon 1996 and Karatayev et al. 1998) The growth limitation factor γ_{DO} for low dissolved oxygen concentrations will be modeled with:

$$\gamma_{DO} = \frac{\Phi_{DO}}{K_{DO} + \Phi_{DO}}$$

Φ_{DO} is the dissolved oxygen concentration (mg/l) and K_{DO} half-saturation dissolved oxygen concentration when mussel growth is at half of its maximum without the limitation of low dissolved oxygen conditions.

Respiration is modeled as two components (Bierman et al. 2005; Lindim 2015). One component represents the base respiration rate the other corresponded to the energetic cost of feeding:

$$R = a_r M_i^{b_r} \gamma_1 \gamma_2 + \frac{w_f}{w_r} \times f_s \times (1 - F)$$

with

a_r : maximum base respiration rate (g O_2/g mussel/d)
b_r : exponent for weight effect on respiration
f_s: fraction of ingestion spent on feeding energy

Respiration is a function of temperature with optimal and maximum critical temperatures (Schneider 1992; Alexander et al. 2004). Equations developed by Thornton and Lessem (1978) will applied to model the dependence of respiration on temperature.

Egestion, or true feces, is a function of consumption (Schneider 1992 and Schol et al. 1999):

$$F = \alpha_f \exp\left(\gamma_f \times \min\left(\frac{C}{K_c}, 1\right)\right) C$$

where
α_f: intercept for the proportion of consumption egested versus proportion of maximum consumption realized
γ_f: coefficient of dependence of food availability

Excretion is a function of consumption and egestion will be modeled using an equation applied by Schneider (1992) and Lindim (2015):

$$U = a_u(C - F)$$

where a_u is the excretion fraction.

Typical coefficient values for consumption, respiration, egestion, and excretion from literature are listed in Table 5.

Table 5: Coefficient values for modeling zebra mussels.

Coefficient	Name	Value	Source
a_c	Maximum ingestion rate	0.031 g food/g mussel/day	Schneider (1992) and Walz (1978a)
b_c	Exponent for weight effect on ingestion	-0.39	Schneider (1992) and Reeders and Bij de Vaate (1990)
K_c	Saturation food concentration	5 mg dry weight/L	Sprung and Rose (1988) and Walz (1978a)
a_r	Maximum base respiration rate (g O_2/g mussel/d)	0.003 g O2/g mussel/day	Schneider (1992) and Walz (1979)
b_r	Exponent for weight effect on respiration	-0.25	Schneider (1992) and Walz (1979)

f_s	Fraction of ingestion spent on feeding energy	0.285	Schneider (1992 and Bayne and Scullard (1977)
α_f	Intercept for the proportion of consumption egested versus proportion of maximum consumption realized	0.315	Schneider (1992 and Bayne et al. (1976)
γ_f	Coefficient of dependence of food availability	0.88	Schneider (1992 and Bayne et al. (1976)
a_u	Excretion fraction	0.064	Bayne and Newell (1983)

Reproduction

Mussels can spawn one or more times a year and is dependent upon food availability, pheromones, temperature, and photoperiod (Ram et al. 1996; Cummings and Graf 2009; Gist et al. 1997). Up to 40,000 eggs can be produced in a single spawning event (Benson and Raikow 2012; Mackie and Schloesser 1996). Spawning has been shown to begin when water temperatures reach 12 °C and reaches a maximum at 15°C to 18°C (Claudi and Mackie 1994; McMahon 1996; Benson and Raikow 2012; Cummings and Graf 2009). Mass loss due to reproduction M_g will be modeled using the following equation:

$$\mathrm{M}_i = \mathrm{M}_i - \mathrm{M}_g$$

Egg production can be calculated using the approach of Akcakaya and Banker (1998) who used data provided by Sprung (1991) to develop the following equation:

$$E = 0.606 l^{4.42}$$

where E is the egg number in thousands and l is shell length. Shell length can be estimated from

$$\mathrm{M}_i = 0.071 l^{2.80}$$

or

$$l = \left(\frac{\mathrm{M}_i}{0.071}\right)^{-2.80}$$

Mass loss due to the production of eggs can be calculated with:

$$\mathrm{M}_g = E * 100 * m_e$$

where m_e is the mass of a single egg.

Zebra mussels are generally able to begin spawning before at an age of 1 year (McMahon 1996; USACE 2002; Cummings and Graf 2009; Benson and Raikow 2012). Probability P_s of a single mussel spawning will be calculated as a function a of mussel age t_i, mass M_i, water temperature T, dissolved oxygen concentration Φ_{DO} and food concentration c:

$$P_s = f(t_i, \mathrm{M}_i, T, \Phi_{DO}, c)$$

Veliger settling

The timing of settling for a veliger will be modeled using a critical threshold length or applying the following equation developed by Sprung (1989):

$$t_v = -2.25T + 68$$

where

t_v: Larval duration (days)
T: mean water temperature during development

Mortality

Adult zebra mussels generally grow to 6 to 45 mm in size and live 2 to 4 years. Causes of mortality that will be modeled include old age, settling of veligers onto unsuitable substrates, exposure to air, too warm of temperatures, predation and low dissolved oxygen concentrations. During settling stage of veligers the largest cause of mortality are unsuitable substrates (Benson and Raikow 2012). Zebra mussels prefer hard, stable substrata to attach using byssal threads (Farr and Payne 2010), and the SOD associated with silty bottoms can limit zebra growth and increase mortality (Karatayev, Burlakova, and Padilla 1998). When a veliger settles, the probability of a survival is dependent upon the area of suitable substrate A_s existing in the model segment. The probability of settling on to suitable substrate P will be calculated with:

$$P = 100 \times \frac{A_s}{A_T}$$

where A_T is the total bottom area of the model segment.

To account for mortality of larger, older adults, the following equation applied by Lindim (2015) will be used to calculate the mussel mortality rate m:

$$m = 0.0157 M_i^{-0.502} \text{ if } M_i \geq 0.025 \text{ mg-C}$$
$$m = 0.01 \text{ if } M_i < 0.025 \text{ mg-C}$$

The CE-QUAL-W2 simulates rising and falling water levels and can predict if a mussel is exposed to air. Mortality occurs within 15 hours if exposed to air temperatures ~1.5° C (Clarke 1993). When DO concentrations fall to a critical threshold, mortality of mussels due to low DO concentrations will also be simulated.

Filtration and Pseudofeces

Zebra mussels prefer phytoplankton and zooplankton, but bacteria and organic matter can be consumed (USACE 2002; Benson and Raikow 2012). Zebra mussels feed on particles between 0.7-1.2 um (Ten Winkel & Davids 1982; Horgan & Mills 1997; Baker, Levinton, Kurdziel & Shumway 1998; Cummings & Graf 2009), with unpalatable and large food expelled as pseudofeces (Ten Winkel and Davids 1982). Filtration rate is the amount of water cleared of particles per unit time (Fanslow et al. 1995). Walz (1978) showed that filtering rate was constant below a food concentration of 2.0 mg C/l, but then decreased at higher concentrations. Pseudofeces production F_p (d^{-1}) is the difference between the mass filtered Y and mass actually consumed C:

$$F_p = Y - C$$

Filtering rate and can be calculated using (Bierman et al. 2005):

$$Y' = \frac{C_{max}}{0.34 K_c} \quad \text{if } c < K_c$$
$$Y' = \frac{C_{max}}{0.34 c} \quad \text{if } c > K_c$$

where Y' is the filtration rate (l/g mussels/day) and C_{max} is the maximum consumption rate.

Fluxes to other compartments in CE-QUAL-W2

The internal fluxes of mass between *Dreissena* mussels and other water quality compartments will be accounted for in the CE-QUAL-W2 model. Mussel grazing of phytoplankton (algae), zooplankton, labile particulate organic matter and refractory organic matter will reduce mass in those compartments. Dissolved oxygen will be consumed through mussel respiration. Mussel mortality will contribute organic matter and nutrients to the CE-QUAL-W2 sediment module. Production of feces and pseudofeces will add labile and particulate organic matter to the water column and carbon, phosphate, and ammonium will be excreted. Fluxes between mussel compartment and other compartment in CE-QUAL-W2 are illustrated in **Error! Reference source not found.**. In order for mass fluxes to other compartments to be calculated, mussel wet mass M_i needs to be converted to dry mass. A dry weight to wet weight ratio of 0.15 was estimated by Walz (1979). Walz (1978b) also estimated that carbon content is 0.45 time dry weight, and zebra mussel length l (mm) and wet weight W (mg) were related using:

$$W = 0.071 l^{2.80}$$

Schol et al. (1999) used the following relationship between soft body weight carbon W_c (Mg) and shell length l (mm):

$$W_c = 0.0093 l^{2.80} * 0.5$$

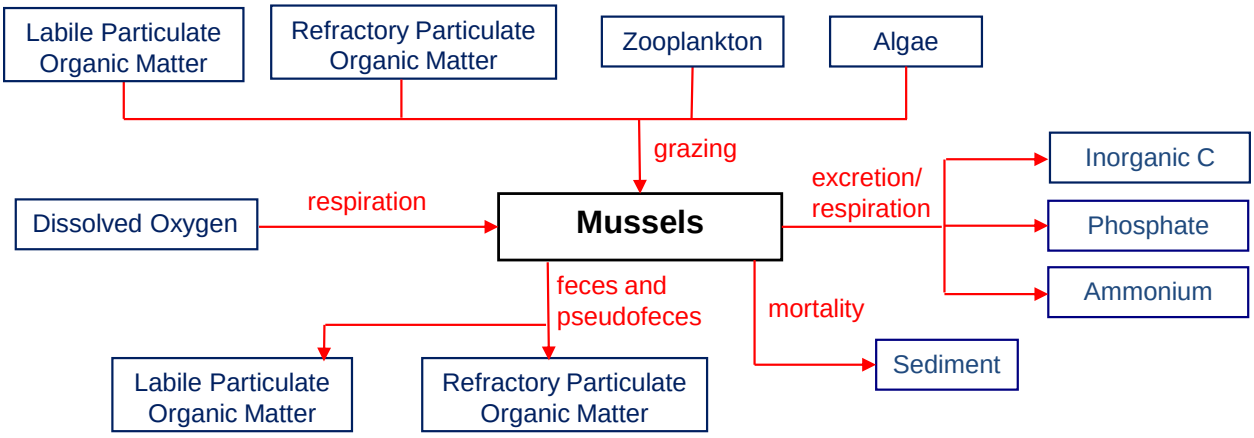

Figure 12: Fluxes between mussel compartment and other constituents in CE-QUAL-W2 model.

Modeling mussel transport in the water column

In modeling the movement of mussel larvae we will be using the approach of Goodwin et al. (2001). Since CE-QUAL-W2 is a 2D model, we will compute the position of the larval cell (or assemblage of cells) from these equations:

$$x_i^{n+1} = x_i^n + u_i^n \Delta t$$

$$z_i^{n+1} = z_i^n + w_i^n \Delta t$$

where i is the group of larvae, n is the time step, x is the longitudinal component and z is the vertical component, u is the longitudinal velocity component, and w is the vertical velocity component, and Δt is the model time step. The velocity components are determined from a

combination of the carrier fluid velocity and the "volitional" movement of the larvae (**Error! Reference source not found.**13). The "volitional" movement of larvae will be based on environmental gradients (such as temperature and dissolved oxygen) in the fluid and a random component of movement.

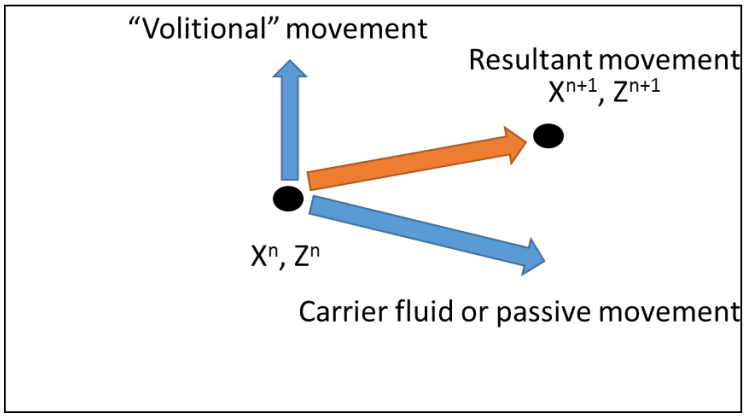

Figure 13: Resultant movement of mussel larvae.

The technique of Goodwin et al. (2001) will be used to determine environmental gradients and to prioritize "volitional" movement of the larvae. Also, movement of the attached mussel will be allowed as a result of low dissolved oxygen conditions.

Discussion

Water temperatures were above the lower limit for *Dreissena* spawning during all sampling events in San Justo Reservoir (i.e., above 12°C) (Figure 7). Water temperatures between 16° and 19°C are associated with peak *Dreissena* spawning (McMahon 1996), and epilimnion water temperatures during the April, May, and early June sampling events were in this temperature range (Figure 7). Water temperatures throughout the water column were not limiting to mussel growth and survival in San Justo Reservoir during the project period. Similarly, dissolved oxygen concentrations were well above lower limits in the epilimnion throughout the project period. Dissolved oxygen concentrations, however, became limiting at water depths approximately 10-m below the surface, corresponding with a lack of veligers collected at depth (Figure 9).

Dreissena mussel spawning in San Justo Reservoir was initiated prior to 4/11/15 as indicated by the presence of straight-hinge veligers collected in the water column, and the lack of straight-hinge veliger collections during the 1/26/15 and 2/27/15 sampling events. The exact timing of the onset of *Dreissena* spawning in San Justo Reservoir, however, was difficult to determine due to the temporal gap in sampling in late winter (e.g., March).

There was a distinct onset to a spawning period and this occurred in the late May to early June period (Figures 8 and 9). The densities of straight-hinge veligers collected during the 4/11/15 and 5/6/15 sampling events at both locations were much lower than the densities collected during the subsequent sampling events (i.e., 6/2/15, 6/24/15, 7/14/15, and 8/6/15). The onset of peak

spawning in San Justo and the initial rate of larval development was rapid and synchronized throughout the portions of the water body sampled (Figure 8).

The timing and periodicity of peak spawning and settlement could not be determined. Veliger settlement out of the water column appears to be possible in San Justo Reservoir when veligers of any developmental stage are present including the spring, summer, and winter. The peak of pediveliger settlement in San Justo Reservoir appears to follow the peak in total veliger density as well as the peak in younger stage veligers. Sampling, however, was not done in the fall and early winter. Additionally, the patterns in veliger composition by developmental stage varied between the sampling locations and summer sampling events (Figure 8).

There appears to be two distinct periods in the *Dreissena* veliger population during the sampling period between January and August of 2015: one period characterized by low densities of veligers in the water column (1/26/15 through 5/6/15), and another period characterized by much higher veliger densities for all larval stages (6/2/15 through 8/6/15). The period with low to no veligers could represent the transition from the prior year to the new spawning generation starting in the spring. The late umbonal/ pediveligers found during the 1/26/15 sampling event, and possibly the older veligers found during the 4/11/15 and 5/6/15 sampling events, represent overwintering veligers from the prior year that are finally settling out of the water column. Accordingly, the lack of young veligers in the water column during the 1/26/15 and 2/27/15 sampling events, and then the low densities of these stages collected during the 4/11/15 and 5/6/15 events, and the increase in density between 4/11/15 and 5/6/15 (Figure 9) also supports the notion that the *Dreissena* population ceased spawning for some period of time after August and was resuming spawning in the following spring.

It appears that *Dreissena* spawning in San Justo Reservoir occurs over a prolonged period and/or the rate of larval development is highly variable within the veliger community. The densities of younger veligers (straight hinge and umbonal stage) in the water column did not decrease during the summer as the densities of older veligers increased. This may be an artifact of sample size, a patchy or clumped spatial distribution, and other factors, but the apparent lack of a shift in the veliger population age structure with time indicates it is possible that new veligers were being continually produced during the late spring and summer periods. This finding corroborates other research indicating that *Dreissena* spawning can be prolonged and continuous under favorable environmental conditions. The temperature data collected from San Justo Reservoir indicates that thermal conditions in San Justo Reservoir were favorable for year-round reproduction and growth. Water temperatures remained above the lower thermal limits for *Dreissena polymorpha* gamete production of 12^{o}C (reference), and did not approach upper thermal tolerances of 30^{o} to 32^{o}C (reference). In water bodies where temperatures approach upper thermal tolerances in the summer periods such as the lower Colorado River, *Dreissena* spawning does not occur during these warmer periods. Additionally, in cold monomictic lakes that freeze over in the winter, the *Dreissena* spawning appears not occur during these colder periods.

The top of the hypolimnion represented a district threshold for the density of veligers in the water column, with veliger density plummeting to zero over a vertical distance of 2-m into the hypolimnion. The water depth corresponding to a dissolved oxygen concentration equal to or less

than 0.1 mg/ L dissolved oxygen represented a threshold for veliger presence, with no veligers collected in water depths below the dashed line in Figure 9.

It appears that *Dreissena* veligers have an active role in maintaining their vertical position within the water column. Veligers were limited to within approximately 10-m of the surface in San Justo Reservoir regardless of stratification, and veliger densities were consistently low near the water surface. *Dreissena* veliger density for all larval developmental stages peaked 5- to 8-m below the water surface regardless of season and stratification. The vertical distribution of veligers within the water column appears to be related to the physical density gradient at the thermocline, however, the same depth distribution was observed during periods when the reservoir was relatively isothermal (Figure 7). The dissolved oxygen concentration is likely a lower threshold for survival during stratification, and avoidance of ultraviolet light is one possible factor influencing the apparent avoidance of surface waters.

Veliger densities for all larval stages were essentially zero at water depths below the metalimnion, and this pattern in veliger vertical distribution was especially apparent in regards to the dissolved oxygen concentration. The small dashed line in Figure 9 shows the lowest water depth at which there was no change in water temperature more than 1^{o}C per one meter depth. The large dash line in Figure 9 shows the depth of the dissolved oxygen concentration equal to or less than 0.1 mg/L. A small number of veligers were collected at water depths below the upper thermally mixed layer, but essentially no veligers were collected below the oxygen threshold. The status of these limited individuals collected below the oxygen threshold was unknown, and it is possible these veligers were dead or moribund upon collection and were simply sinking out of the water column. This pattern was most apparent in the summer (6/24/15, 7/15/15, and 8/6/15) when veliger densities plummeted to essentially zero and closely mirrored the dissolved oxygen and water temperatures profiles (Figure 9).

Next Steps/ Recommendations

- Develop and validate the code for the *Dreissena* subcomponent for the CE-QUAL-W2 hydrodynamic and water quality model using the population dynamics and habitat characteristics in San Justo Reservoir, CA as well as characteristics derived from the literature.
- Apply CE-QUAL-W2 model with ZQM subcomponent to San Justo Reservoir to model the spatial and temporal distribution of mussel larvae, juveniles, and adults.
- Apply CE-QUAL-W2 model with ZQM subcomponent to Lake Roosevelt/Grand Coulee (US Bureau of Reclamation) and Brownlee Reservoir/Snake River (Idaho Power) to simulate the likely spatial and temporal distribution of *Dreissena* spp. if infested.

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Appendix A

FIELD COLLECTION PROTOCOLS
For
ZEBRA MUSSELS
2014

Steve Wells, Rich Miller, Mark Sytsma, Angela Strecker, Chris Berger, and Scott Wells

General comments for 2015

- San Justo Reservoir water surface elevation changes during the season. Maximum water depth of reservoir in study area is estimated to be 33-m (108-ft) during peak water surface elevation, which occurs in February. The lowest water surface elevation occurs between September and December.
- There are a total of 8 sampling trips. Sampling trips occur monthly during the period between January 1, 2015 and August 31, 2015.
- These field collection protocols are specific to *Dreissena* spp. sampling. Separate documents address water quality sampling and modeling.
- Each sample container is assigned a unique sample container number (e.g., MS-3005). These numbers are recorded on waterproof labels and covered with packing tape to resist effects of water, ethanol, and abrasion during sample handling. The numbers are recorded on clean empty containers prior to field work, and labeled containers are grouped by type and stored in clean plastic bags. In the field, when a particular container is selected for use, the sample container number is recorded on the field datasheet.
- If missing sample container number, assign one using following: MS- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add “a”, “b”, etc. as needed to end, e.g. MS-062515a.
- The field datasheet is the primary means of recording *Dreissena* sample information. The field datasheets are the responsibility of the field crew leader until they are relinquished to either Rich Miller or Steve Wells. Field datasheets are scanned upon receipt into PSU laboratory.
- A limited amount of sample information is also recorded on the sample container label while in field to duplicate the field datasheet.
- Write legibly.
- Work safely but quickly. Most field trips have two days scheduled for all sampling including *Dreissena* and water quality. Aim for less than ten minutes at each moored buoy station (i.e., retrieve and replace PVC coupons; measure survivorship and length on 20 mussels). Plan for 96-minutes to sample 33-m water column for depth-discrete pumped plankton samples. If you must eliminate activities due to time, reduce the

number of sample locations for depth-specific pumped plankton samples (two locations instead of three). The two trips that include PVC plate retrieval and grab sampler deployment have an additional day of field work scheduled.

DEPTH-SPECIFIC PLANKTON SAMPLES

Objective: determine the periodicity, density, size and developmental stage of veligers across depth and time in San Justo Reservoir.

Sample Locations:

- At each sample location, the water column is sampled at 1-m depth intervals from one-meter above the sediment to the water surface.
- At each plankton sampling location, two types of duplicate samples are collected. One type of duplicate sample is done for model validation, and these duplicate samples are collected at three depths: 1) 1-m above sediment, 2) in the middle of the water column, 3) and 1-m below the water surface. The other type of duplicate sample is done to evaluate sampling precision, and one field duplicate for precision is collected at a random depth.
- Depth-specific plankton sampling is done at three locations if time permits. If activities must be eliminated for time, eliminate the sample collection at the Deepest location (36.817691 -121.446727). Sampling locations are identified by the following GPS coordinates:

Latitude	Longitude	Name
36.822177	-121.441536	Inlet/outlet pipe
36.817691	-121.446727	Deepest location
36.820622	-121.444266	Deepest location near moored buoy stations

Horizontal distribution

At three sample locations.

Vertical distribution, i.e., depth

The entire water column at 1-m depth intervals from 1-m above reservoir sediment to water surface.

Frequency

- Bimonthly in the March to December period, and monthly in January and February. *Dreissena* sampling is done in the 2015 calendar year.

Equipment list for each sampling trip

- (111) 50-mL centrifuge tubes with lids
- (35+) ≤ 64-µm mesh filter cups
- Peristaltic pump with 40-m hose
- (2) car batteries
- field datasheet (Appendix B)
- GPS unit w/ (4) AA batteries
- 11.4-L (3-gal) 5% acetic acid
- duct tape
- (2) pencils and (1) sharpie pen
- 5.5-L regular EtOH buffered w/ Tris
- racks for centrifuge tubes
- watch or clock
- weight for hose
- (3) 5-gal bucket marked at 5-L
- 11.4-L (3-gal) fresh tap water
- Bucket w/ lid for acetic acid

- clamp for hose & cup on bucket
- Rinse bottle with distilled water or filtered reservoir water
- clamp for hose on boat gunwale
- Electrical tape or plastic paraffin film

Depth-specific plankton sampling using peristaltic pump

1. Secure one end of the flexible hose through the rollers of the peristaltic pump with 2-m of hose protruding from the pump (this end of hose will be used to direct water flow into filter cups on boat deck). The external casing on the peristaltic pump can be removed with screw pins to expose the rollers. The flexible hose is positioned into a "U" shape inside the pump with the rollers flattening the hose. Slightly rotate rollers to position the hose into U-shape under both rollers. Secure external casing. Attach car batteries to pump electrodes. Check pump operation.
2. Using duct tape, attach weight to end of hose to be lowered into water. When securing weight to hose, be careful not to restrict the hose opening.
3. Position and anchor boat at sample location.
4. Measure the two minutes average wind speed using a hand-held anemometer. Measure the wind within 1-m of water surface. Record on field datasheet.
5. Determine water depth at sample location using depth sounder and/or sounding line.
6. Lower the weighted hose to 1-m above reservoir bottom. Keep the hose off the lake sediment. Secure hose through clamp on boat gunwale to maintain hose at that depth. Make sure hose is not constricted by clamp.
7. Turn on pump, and pump 3-L of water through hose to flush it (63 seconds at maximum pumping rate). Collect the pumped water in a marked 5-gal bucket to measure the volume of water flushed through hose (buckets are marked at 3- and 5-L). If water doesn't begin to move through hose in several minutes, the pump may need to be primed. Prime pump by raising hose to water surface and pouring water into end of hose with weight attached. Once water is pumping through hose, continue pumping water as you lower hose to desired depth. Once at least 3-L of water has been pumped at a given water depth, the sample collection may begin. The pumped water used to flush hose is wasted overboard.
8. Position mesh filter cup inside a clean 5-gal bucket, and secure the mesh cup to the upper edge of bucket using a clamp. Feed the end of hose through clamp on bucket, and position end of hose above 64-µm mesh filter cup so that stream of water passes entirely through filter cup.
9. Start the timer. Pump 5-L of reservoir water through the 64-µm mesh filter cup. At maximum pump speed, it should take 1:45 minutes (105 seconds) to pump 5-L of water. Use the 5-L mark in the 5-gal bucket to measure the volume of water filtered, and use the timer to help monitor activities. The pump is battery powered, and the pumping speed (i.e., the volume pumped) may vary over time as batteries wear out.

10. Make sure that the entire stream of pumped water is passing through the 64-µm mesh filter cup, and no water is spilling over cup sides. Pumped water should be passing through 64-µm mesh filter cup and collecting in 5-gal bucket. If water is spilling over cup side, reposition the hose and/or reduce the pump speed.
11. Select a clean 50-mL centrifuge tube, and record the unique sample container number (e.g., MS-7956) on the field datasheet for the respective plankton sample location. On the field datasheet, also record the following: 1) Sampling date, 2) names of collectors, 3) and water depth.
12. On the sample container label, record the following: 1) "PP" for sample type code, 2) the sampling date, 3) sample location code (i.e., I/O, DB, or D), and 3) the water depth.
13. After 5-L of water has been filtered, remove the filter cup from the 5-gal bucket and set aside. Pour the filtrate in bucket overboard.
14. Remove hose from clamp on boat gunwale, move hose up 1-m in water column, and re-secure hose in clamp on boat gunwale.
15. Pump 3-L of water at the new depth to flush hose.
16. Using a rinse bottle with either distilled water or filtered reservoir water, rinse particulate in 64-µm mesh cup into the previously selected 50-mL centrifuge tube. Concentrate the sample as much as possible. Ideally the total volume of rinse water and particulate collected in the centrifuge tube is less than 15-mL. If more than 15-mL of rinse water is collected, divide the sample into multiple containers in order to achieve the desired concentration of preservative.
17. Add 95% regular ethanol (pre-buffered with Tris) to the 50-mL centrifuge tube containing the particulate rinsed off the mesh filter cup to achieve a final solution of 70% ethanol. Secure lid, and wrap closed lid in electrical tape or paraffin film (to reduce leakage).
18. Using the permanent marker, clearly mark the side of the centrifuge tube corresponding with the top of the preserved sample with a dark line. This line will indicate the total volume of the preserved sample to determine if leakage occurs during shipping and handling. If leakage occurs, fill with buffered 95% EtOH to reach line.
19. Place used 64-µm mesh cup into 5-gal bucket containing 5% acetic acid.
20. Once the hose is flushed, repeat steps #8-19 for the next water depth. Repeat collections until the entire water column has been sampled at 1-m depth intervals.
21. Remember that plankton collection is duplicated at three depths for model validation: 1) 1-m off the lake bottom, 2) in the middle of water column, and 3) 1-m below water surface. A field duplicate for precision is also collected at a random depth. Pump hose does not need to be flushed between duplicate samples at the same depth.

Sample preservation and handling

Plankton samples are preserved in a final solution of 70% regular ethanol immediately after collection to ensure sample integrity. After the particulate has been transferred to the 50-mL centrifuge tube from the mesh filter cup, 95% regular EtOH is added to the centrifuge tube to reach a final solution of 70% EtOH (e.g., 14-mL of particulate and rinse water + 32-mL of 95% regular EtOH). Centrifuge tubes are graduated to assist with calculations in the field. If there is more than 14-mL of particulate and rinse water in the centrifuge tube, gently swirl the tube to mix sample, and then carefully decant half of the particulate solution into a second centrifuge tube (i.e., do not add more than 45-mL total liquid into the 50-mL centrifuge tubes).

Regular ethanol buffered with tris(hydroxymethyl)aminomethane (Tris) is the preferred chemical preservative. In emergencies, denatured ethanol and isopropyl alcohol are acceptable chemical preservatives. When using alcohol as a preservative, use stock that is 95% alcohol or greater, and add enough preservative so that the final concentration is 70% alcohol. Other preservatives not mentioned here may be acceptable, but it is important to consider the effects of preservative on the crystalline structure of the bivalve veliger shell.

Tris is the preferred buffering agent for plankton samples. The preserved sample pH should be between 7.0 and 9.5, and the ideal sample pH is 8.0 to 8.5. If sample pH falls below 6.8, the bivalve shell will dissolve. Regular EtOH is buffered with Tris in the laboratory prior to field work by adding 7 drops of 4-M Tris to 500-mL of EtOH. In emergencies, sodium bicarbonate (i.e., baking soda) may be used to buffer preserved plankton samples. Add approximately 1-gram of sodium bicarbonate (the size of pencil eraser) to 500-mL of ethanol.

Samples preserved using a final solution of 70% buffered EtOH may be stored in a cool, dry place for up to one year. Avoid placing preserved samples in direct sunlight or freezing conditions. For long term storage, place samples in 70% buffered EtOH. Once the sample is preserved, and the centrifuge lid is secured and taped, invert the preserved sample to thoroughly mix sample. Plankton samples preserved in buffered 70% EtOH are stored in room temperature out of direct sunlight for up to a year.

Sample shipping

EtOH is a Class 3 flammable liquid, and there are restrictions regarding its transport. EtOH can only be transported on the ground/surface. Do not fly in an airplane with EtOH. EtOH can be shipped but there are training, certification, labeling and shipping requirements. Ship preserved samples to PSU laboratory via ground or surface mail using FedEx or USPS according to the protocols below, which allow exemptions for training and certification. Use FedEx when possible.

FedEx Ground

1. Ensure that each container lid is securely tightened, and lid is wrapped in electrical tape or paraffin film
2. Place each individual container into a sealable plastic bag (e.g., Zip-Lock bag).
3. Place individually bagged containers into a larger sealable plastic bag.
4. Place double-bagged sample containers into a cardboard box. Using an absorbent material (e.g., paper towels or newspaper), pack the remainder of space inside box. Secure box with packing tape.

5. Place sealed cardboard box inside a larger cardboard box. Add absorbent packing material to fill in space. Seal outer box with clear packing tape.
6. Affix FedEx shipping label to outside of larger box.
7. Affix the following warning sticker to three sides of the box, including the side with the shipping label.

This package conforms to 49 CFR 173.4

USPS Standard Mail or Parcel Post

1. Repeat steps #1-5 listed above for FedEx Ground.
2. Affix a recipient shipping address label and a return address label to one side of box.
3. Affix the following warning sticker to three sides of the box, including the side with the shipping label.

Surface Mail Only Consumer Commodity ORM-D Flashpoint = 55.6°F

Equipment decontamination

In order to prevent the cross-contamination of individual depth-specific plankton samples, separate 64-µm mesh filter cups are used when sampling the different depth intervals along the water column at a single sample location. After use, the individual mesh filter cup is placed into a plastic container for transportation to PSU for decontamination. At PSU, the 64-µm mesh filter cups are decontaminated by soaking in 4% HCl or 5% acetic acid. The minimum soak time for acetic acid is 6 hours. The soak time for a solution of 4% HCl is 4 hours. Following the acid bath, equipment is rinsed with distilled water and air dried.

All sample containers (e.g., 50-mL centrifuge tubes) are acid washed prior to use by soaking in 4% HCl or 5% acetic acid, and then rinsed 4X with distilled water.

The silicone tubing used with the peristaltic pump is flushed using reservoir water at each depth using at least 3-L of water prior to collecting a sample. The silicone tubing is decontaminated after each sampling event at PSU by filling the tubing with 5% acetic acid and soaking for a minimum of 6 hours, and then rinsing with distilled water.

DECONTAMINATION

Objective: remove and kill any plant and animals on gear and boat especially *Dreissena* mussels.

The pontoon boat will be dedicated to field collection efforts on San Justo Reservoir during the 2015 calendar year, and if possible, the boat will be stored in the San Benito County Water District storage area on the shoreline of San Justo Reservoir. The boat may also be stored in offsite storage facility (i.e., dry storage on trailer). The full decontamination procedures listed below are for equipment leaving the San Justo Reservoir job site.

Sample location

- San Justo Reservoir launch ramp parking lot at least 122-m (400-ft) from open water.

Equipment

- (> 5 gallon) 5% acetic acid
- (> 5 gallon) 5% bleach solution
- bottle of household bleach (appx. 6% NaOCl) (Clorox brand)
- (4) large plastic tubs with lids (> 10 gallons)
- (5 gallon) 2% quaternary ammonium compound, e.g., Virkon Aquatic® (Quat)
- deck scrub brushes
- large handle brushes
- Towels
- (13 gallons) fresh water
- spray bottle 5% bleach solution
- spray bottle 2% Quat
-

Decontamination

1. Rinse plankton net and cod-end in reservoir. Remove cod-end and lower net into water. Holding net ring, quickly pull net straight up and out of water. Repeat as needed to remove visible plankton and debris. Rinse out cod-end in reservoir. You may need to use fingers to gently clean mesh.
2. Soak plankton net, cod-end, plankton net rope, net anchor, and net anchor rope in 2% Quat solution for ten minutes, and then rinse with fresh water.
3. Soak plankton net, cod-end, plankton net rope, net anchor, and net anchor rope in 5% bleach solution for 15 minutes, and then rinse with fresh water.
4. Soak plankton net and cod-end in 5% acetic acid for a minimum of 6-hrs and up to 24-hrs. After acid, soak, thoroughly rinse net and cod-end with fresh tap water and hang to dry.
5. Using a brush, scrub the sediment grab sampler, sieve, boat anchor, tubs, mesh bags, and other equipment and gear that contacted San Justo Reservoir to remove visible contaminants. Soak equipment in 2% Quat solution for ten minutes.
6. Position an empty plastic tub under engine lower unit so that prop is inside tub with engine skeg flush with the bottom of the tub.

7. Add fresh water to tub until water surface reaches the bottom of the cavitation plates (approximately 13 gallons).
8. Put boat engine in neutral and start boat engine. Run engine for approximately two minutes. Do not allow water level to fall below cavitation plates. Do not return cooling water to the tub; you are flushing the cooling system with fresh water. Stop engine if cooling water temperature becomes hot and replace with fresh water.
9. Stop engine after two minutes. Raise engine unit out of tub.
10. Add approximately 2.5-L (0.7 gallons) of household bleach to tub containing 49-L (13 gallons) of tap water, and mix. Wear appropriate safety equipment. This is a 5% solution of bleach and is caustic.
11. Remove equipment from 2% Quat soak, rinse with fresh water, and then soak in 5% bleach solution for 15 minutes.
12. Remove equipment from 5% bleach solution, thoroughly rinse with fresh water, and allow to air dry.
13. Using deck scrub brush, remove mussels, plants, and debris from boat decking, gunwales, and exterior. Use hands if necessary to physically remove large debris from boat and trailer.
14. Spray boat hull and trailer with 2% Quat solution in spray bottle. Pay particular attention to screw holes, recesses, junctions between two surfaces, gaps in between floorboards, etc. Thoroughly soak areas where small mussels could be lodged.
15. Return to starting place, and scrub boat hull, trailer, through-hull fittings, engine casing, seats and floorboards with brush while spraying with 2% Quat again.
16. Spray the boat hull, trailer, fittings, engine casing, seats and floorboards with 5% bleach solution, and scrub with brush if necessary.
17. Soak the brushes in the 2% Quat solution for ten minutes and then rinse with fresh water.
18. If there is a bilge, plug the bilge drain (pontoon boat lacks bilge). Pour the 49-L of 5% bleach solution into the boat, washing down seats, flooring, and other surfaces. Pay close attention to cracks and crevices. Allow solution to soak in boat bilge for 30 minutes.
19. The 2% Quat, 5% bleach, and 5% acetic acid solutions are retained in plastic carboys and disposed of into drains connected to sewage treatment facility, e.g., toilets in hotel.
20. Mussels removed from boat and equipment may be left on paved parking area of San Justo Reservoir boat launch.
21. Waste materials from job site (that may be encrusted with mussels) are collected, bagged in plastic trash bags and disposed of in a dumpster that goes to a landfill.