Zebra and Quagga Mussel Early-Detection Monitoring in High Risk Oregon Waters 2014

2014 Final Report

Submitted by:

S. Wells, M. D. Sytsma, and A., Strecker

Portland State University

To

Oregon State Marine Board

March, 2015

This page intentionally left blank

List of Acronyms

ABS	acrylonitrile butadience styrene
AIS	aquatic invasive species
BMS	blind matrix spike sample
BPA	Bonneville Power Administration
ODFW	Oregon Department of Fish and Wildlife
OSMB	Oregon State Marine Board
PSMFC	Pacific States Marine Fisheries Commission
PSU	Portland State University
PVC	polyvinyl chloride
Quat	quaternary ammonium compounds
Tris	tris(hydroxymethyl)aminomethane
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Service

Abstract

Zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*, respectively) were not detected by Oregon Department of Fish and Wildlife (ODFW) and Portland State University (PSU) during their early detection sampling in Oregon water bodies during 2014. Six high-risk Oregon water bodies were sampled for planktonic larvae and adult mussels during the August to September period. A total of 37 plankton samples were collected and over 520,000 liters of reservoir/lake water were filtered through 64-µm mesh nets during plankton sample collection. The greatest sampling effort occurred in Emigrant Lake, Lake Billy Chinook, and Prineville Reservoir but sampling also occurred in Howard Prairie Lake, Upper Klamath Lake, and Applegate Reservoir.

Non-native invertebrates and macrophytes were opportunistically collected during *Dreissena* sampling in 2014. *Corbicula fluminea* (Asian clam) adults were collected in Upper Klamath Lake. *Potamopyrgus antipodarum* (New Zealand mud snails) were collected in Lake Billy Chinook. *Potamogeton crispus* (curly leaf pondweed) was collected in Emigrant Lake, Howard Prairie Lake, Lake Billy Chinook, and Upper Klamath Lake. To the authors' knowledge, the *C. fluminea* in Upper Klamath Lake represents the first reported detection for these species in this location.

Introduction

Aquatic invasive species (AIS) are spreading rapidly through the western United States, and these non-native species degrade habitat for native species, interfere with hydro operations, recreation, and alter water quality (Sanderson et al. 2009). Many AIS are transported and introduced on trailered watercraft and on recreational gear (Haynes et al. 1985; Kelly et al. 2013). Some AIS are of particular concern because of the severity of their impacts, e.g., zebra and quagga mussels (*Dreissena* spp.).

Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostriformis bugensis*), hereafter referred to as *Dreissena* mussels, are invasive epifaunal freshwater mussels that cause extensive economic and ecological impacts in areas they are not native (Dermott and Kerec 1997; Ricciardi et al. 1998; Mann et al. 2010). *Dreissena* mussels attach to hard submerged surfaces such as rock and concrete using byssal threads and this biofouling restricts the flow of water through hydroelectric, irrigation, and fish facilities impacting component service life, system performance, and maintenance activities (Boelman et al. 1997; Claudi and Mackie 1994; Jenner et al. 1998; Neitzel et al. 1984). The annual cost to power plants and municipal drinking water systems in North America has been estimated at between $267 million and $1 billion dollars (Connelly et al. 2007). *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 et al.1998; Botts et al.1996; Burlakova 1995; Caraco et al. 2006; Effler and Siegfried 1998; Effler et al. 2004; Fahnenstiel et al. 1995; Horvath et al. 1999; Strayer 2008).

Dreissena larvae develop in the water column (i.e., planktotrophic) and hereafter are referred to as veligers. 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 because *Dreissena* mass spawning is generally synchronized and ciliated veligers cannot swim horizontally towards specific objects (Boelman et al. 1997; Mackie and Schloesser 1996; Marsden 1992; Nichols 1996; Ram et al.1996; Sprung 1993). In lakes and reservoirs with low mixing, veligers tend to be concentrated near the thermocline (Boelman et al. 1997; Gallager et al. 1996; Mackie and Schloesser 1996; Sprung 1993). *Dreissena* veligers, however, have been found throughout the water column, ranging from near the surface to depths greater than 122-m (400-ft) (Sprung 1993). Planktonic veligers may be concentrated by water current and wind conditions, e.g., eddies (Kraft et al. 1996). Veligers actively settle out of the water column onto to a variety of submerged substrates including macrophytes, rocks, gravel, sand, woody debris and submerged man-made objects where they undergo metamorphosis and become juveniles (Ackerman et al. 1994; Roe and MacIsaac 1997; Sprung 1993). *Dreissena* juveniles are generally found in the Midwest of North America between August and September (Thorp et al. 1994). Both juvenile and adult *Dreissena* mussels translocate year-round to preferred substrates and areas such as the bottom and sides of hard surfaces (Claudi and Mackie 1994). *Dreissena* adults are found year-round in epilimnion, littoral and profundal areas (Roe and MacIsaac 1997).

Dreissena mussels have established populations west of the Rocky Mountains in California, Nevada, Arizona, and Utah, and veligers have been detected in multiple water bodies (Benson 2015). Watercraft with attached, hitchhiking mussels are repeatedly detected being trailered into Oregon and the surrounding states at watercraft inspection stations (Phillips 2013). The risk posed to Oregon water bodies by the proximity of these infestations is significant.

Other AIS of concern for Oregon include, but are not limited to, macrophytes such as *Hydrilla verticillata* (water thyme/ hydrilla), and invertebrates such as *Potamopyrgus antipodarum* (New Zealand mud snail), and *Orconectes rusticus* (rusty crayfish). Invasive macrophytes, such as *Hydrilla* will have a detrimental impact to Oregon's natural environment and recreational use of water resources as well as economic impacts on irrigated agriculture (Langeland 1996). *Hydrilla* populations have been found in California, Washington and Idaho (USDA, NRCS 2014). *P. antipodarum* is an AIS snail found at high densities at a variety of sites in Oregon, including bays of the Columbia River Estuary, and heavily used recreational rivers like the Deschutes and Umpqua. These snails possess an operculum, which allows them to seal themselves inside their shells and resist desiccation as well as many fishes' digestive systems (Haynes et al. 1985). Once established, these snails can numerically dominate benthic biomass (Richards et al. 2001) and carbon and nitrogen fluxes (Hall et al. 2003), and out-compete native aquatic snails and insects that many species of fish depend on for food. *P. antipodarum* are small (< 5 mm length) and tolerant of desiccation, and are easily moved between water bodies by water users, such as fishermen and boaters. *O. rusticus* populations were first detected west of the continental divide in 2005 in the John Day River (Olden et al. 2009), and were detected in Magone Lake, OR in 2012 (Miller and Sytsma 2014). *O. rusticus* can displace native species and deleteriously affect macrophyte beds and fish (Wilson et al. 2004). *O. rusticus* populations have not been detected elsewhere in the Pacific Northwest (USGS 2014), and monitoring is important to track its status and spread (Larson and Olden 2011).

Monitoring and early detection are key to minimizing the risks posed to un-infested waters by these nearby potential seed populations. Prevention and containment efforts are dependent on accurate monitoring, early detection, and efficient information dissemination. Monitoring high-risk water bodies for early detection of incipient populations is key to implementing rapid response plans and managing the mussel, plant, snail or other AIS invasion. Combining early detection monitoring efforts to target several species increases the efficacy of effort using limited funds.

The Center for Lakes and Reservoirs at Portland State University (PSU) is well positioned to conduct early detection monitoring efforts in Oregon for *Dreissena* mussels and other AIS. PSU, in collaboration with 100th Meridian Initiative partners, currently directs a *Dreissena* spp. monitoring program throughout the western United States and have coordinated these efforts with many agencies and organizations in Oregon including Oregon State Marine Board (OSMB), Oregon Department of Fish and Wildlife (ODFW), Oregon Department of Agriculture (ODA), US Army Corps of Engineers (USACE), Pacific States Marine Fisheries Commission (PSMFC) and others. We have established a light microscopy laboratory to process plankton samples collected throughout the West for detection and identification of *Dreissena* veligers, and we have provided training to other laboratories on larvae identification. We completed a monitoring priority assessment for the Columbia River Basin (Wells et al. 2010) that identified 45 water bodies in Oregon that are at high risk for introduction or establishment of *Dreissena* mussels. PSU created a rapid response plan for *Hydrilla* in Oregon in cooperation with ODA, and has conducted numerous projects targeting AIS macrophytes throughout western United States. PSU conducted baseline monitoring for the presence and density of *P. antipodarum* in Oregon water bodies in 2006 – 2007, and has conducted opportunistic early detection monitoring for AIS since resulting in numerous new detections, e.g., *P. antipodarum* in Lake Billy Chinook and *O. rusticus* in Magone Lake.

PSU was contracted by the OSMB to conduct early detection monitoring in high-risk Oregon water bodies during 2014 for the presence/non-detect of *Dreissena* mussels. The primary project objectives were to conduct early detection monitoring for all *Dreissena* life stages at five high-risk Oregon water bodies, and to communicate to a large audience in a timely and effective manner, the results of the monitoring activities. Additionally, other AIS of concern were to be opportunistically sampled in the course of sampling for *Dreissena* mussels.

Project Objectives

- Develop Standard Field Protocols for targeted early detection of all Dreissena mussel life stages as well as the opportunistic sampling for submerged macrophytes and other invertebrates,

- Identify at least five Oregon water bodies to be sampled that are at high-risk for Dreissena introduction and/or establishment,

- Monitor for the presence of Dreissena larvae (veligers) in plankton community by collecting and microscopically analyzing samples,

- Monitor for the presence of settled juvenile and adult Dreissena mussels on man-made and naturally occurring submerged surfaces including, but not limited to, artificial

settlement substrates (Portland Samplers), rocks, macrophytes, gravel, dock floats, pilings, concrete, and other natural debris,

- Monitor for the presence of juvenile and adult Dreissena shells on shoreline areas,
- Prevent the unintentional transfer of organisms within and between water bodies and samples through field and laboratory equipment decontamination, and
- Communicate results of monitoring efforts to a large audience in a timely and efficient manner.

Methods

PSU staff conducted early detection monitoring at high-priority water bodies throughout Oregon during the time period most likely to contain all *Dreissena* life stages. At the priority Oregon water bodies, PSU staff sampled hard surfaces, macrophytes and sand/silt substrate for the presence of settled juveniles and adults, as well as collecting and analyzing plankton samples for the presence of veligers. Other species opportunistically encountered during *Dreissena* sampling were retained and identified at PSU.

Standard Field Protocols

Standard Field Protocols (SFPs) were developed specifically for PSU field crews (Appendix A) for the collection of all shelled *Dreissena* life stages (larval, juvenile, and adults), as well as the concomitant opportunistic collection of other AIS, e.g., invertebrates and macrophytes, and the collection of water quality data. The objective of these field collection efforts was the early detection, i.e., presence/non-detect of *Dreissena* mussels in water bodies in which *Dreissena* populations have not been previously detected. The target population, if present, was therefore assumed to be rare and spatially clumped. A targeted sampling design was employed to increase the likelihood of collection, i.e., sampling was conducted during time periods and in areas most likely to contain all target *Dreissena* life stages using methods that emphasized large sample size and qualitative data. Other AIS that were opportunistically collected during *Dreissena* sampling were identified and voucher specimens were retained. SFPs included equipment decontamination protocols to prevent the unintentional transport of organisms between water bodies and the cross contamination of samples. PSU developed these protocols based on previous experiences.

Coordination of Sampling

The Oregon water bodies that were targeted for early detection monitoring were identified from a monitoring priority assessment for the Northwest region (Wells et al. 2010) that identified 45 water bodies in Oregon that are at high to medium risk for *Dreissena* introduction and/or establishment. The water body list was further narrowed by identifying spatial gaps corresponding with the other early detection monitoring efforts occurring in Oregon water bodies during the summer of 2014, e.g., USACE and PSU, and through discussions with the OSMB and ODFW. PSU staff conducted *Dreissena* sampling for all shelled life stages at the following Oregon water bodies during the peak *Dreissena* spp. spawning period: Emigrant Lake, Howard Prairie Lake, Lake Billy Chinook, Prineville Reservoir, and Upper Klamath Lake (Table 1). ODFW field crews sampled for veligers in Applegate Reservoir (Table 1). PSU provided ODFW with sampling protocols and labeled sample containers with pre-buffered ethanol. The sampling

period also coincided with the peak boater recreational period (OSMB 2008), as well as the period when many AIS have distinguishing features used for identification, e.g., *P. antipodarum* brooding young (Richards and Lester 2003; Winterbourn 1970), and many macrophytes have reproductive parts such as flowers and seeds.

Table 1: Water bodies and schedule for early detection monitoring during 2014. Water bodies were sampled by PSU crews except for Applegate Reservoir, which was sampled by ODFW and is marked with an *.

Water body	August						September					
	5	10	15	20	25	30	5	10	15	20	25	30
Applegate Reservoir*								■				
Emigrant Lake	■											
Howard Prairie Lake	■											
Lake Billy Chinook	■											
Prineville Reservoir	■											
Upper Klamath Lake	■											

Sample Collection

PSU staff conducted *Dreissena* mussel sampling for all shelled life stages at the five high priority Oregon water bodies during the expected peak spawning period (Table 1). PSU collected plankton samples, deployed and inspected artificial settlement substrates, collected benthic grabs and substrate scrapings (e.g., pilings), and inspected natural substrates (e.g., submersed plants and rocks) at multiple locations in the water bodies according to protocols that were developed specifically for PSU field crews (Appendix A). The objective of these field collection efforts was early detection, i.e., presence/non-detect of *Dreissena* mussels. Therefore, a targeted sampling design was employed that focused efforts during times of the year and in areas most likely to contain the different *Dreissena* life stages using methods that emphasized large sample size and qualitative data. Other AIS that were opportunistically collected during *Dreissena* sampling were identified and voucher specimens were retained.

Plankton samples were collected using a 64-µm mesh simple, conical plankton net at a minimum of 15 locations within each water body using a boat in near shore and in the open water areas. Sampling was focused in areas near boat launches, marinas, dams, outflows, downstream and downwind positions, and other areas plankton collected (e.g., eddy) to increase the likelihood of collecting veligers. A combination of oblique/vertical plankton tows and trawling methods were

employed to collect plankton. Oblique/vertical tows collected plankton throughout the water column at discrete spatial locations. Trawling collected plankton at discrete water depths, i.e., near and above thermocline, across a large horizontal spatial area. The plankton collected from several tows or trawling events were composited into a sample container, labeled and preserved using pre-buffered regular ethanol to reach a final solution of 70% ethanol. Ethanol was pre-buffered using tris(hydroxymethyl)aminomethane (Tris) (8 drops per 500-mL sample), and additional Tris was added upon receipt into the laboratory to maintain sample pH above 7.0. GPS locations and lengths of tows were recorded on field datasheets for plankton samples as well as other metadata collected, e.g., water quality, secchi disk readings, weather, etc. During trawling, the boat speed and the time were recorded to calculate the length of the tow (i.e., distance = rate x time).

Adult *Dreissena* mussels were sampled using multiple methods including artificial settlement substrates (Figure 1A), tactile and visual inspections of existing submersed surfaces and shoreline areas, a surface scraper (Figure 1B), a sediment grab sampler (Figure 1C) and a thatch rake on a rope (Figure 1D). Suspect AIS specimens were retained in labeled sample containers with lake water, held on ice, and transported to PSU for identification. Collection data was recorded on field datasheets. Bivalve identifications were verified by Steve Wells at PSU and by using Thorp and Rogers (2011). Gastropod identifications were verified by Steve Wells at PSU and by using Frest and Johannes (1999); Harrold and Guralnick (2010), Lysne (2009), Perez et al (2004), and Thorp and Rogers (2011). Macrophyte identifications were verified by Steve Wells and Rich Miller at PSU, and by using Crow and Hellquist (2000, 2006), Hamel and Parsons (2001), and Brayshaw (2001). Following identification, invertebrate voucher specimens were placed in 95% regular ethanol buffered with Tris and stored at PSU. Selected macrophyte specimens were pressed and stored at PSU for each species encountered in a water body. Alive unionid mussels, e.g., *Anodonta* spp. and *Gonidea angulata*, if collected, were photographed and released; unionid mussels dead upon collection were retained.

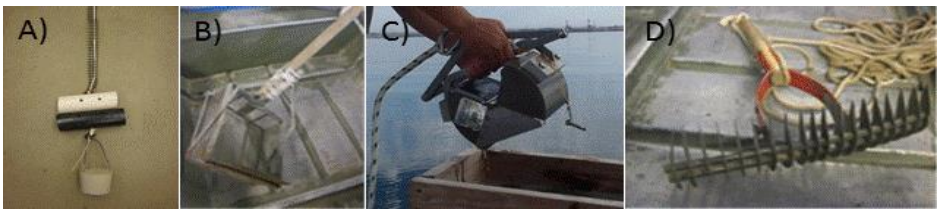

Figure 1: Sampling equipment for juvenile and adult *Dreissena* including A) Portland Sampler, B) surface scraper, C) grab sampler, and D) thatch plant rake on rope.

Portland Samplers were used for early detection and monitoring of newly-settled juvenile and adult *Dreissena* mussels that colonize substrate surfaces. Settlement substrates previously deployed in 2012 and 2013 were used when available, and new substrates were deployed during 2014 sampling as needed. Inspections of Portland Samplers were both visual and tactile.

Visual and tactile inspections of natural and other man-made submersed surfaces were done because these additional hard surfaces increased the surface area sampled for invertebrate colonization and thereby increased the likelihood of early detection. Existing submersed surfaces that were accessible, i.e., within arms' reach, that were sampled opportunistically using visual and tactile inspections for the presence of adult and juvenile *Dreissena* mussels included the

undersides of buoys and dam booms, buoy mooring chains, the undersides of dock floats, rocks, logs, shoreline areas and other items.

A surface scraper was used to sample submerged portions of hard, smooth surfaces including concrete walls, bridge abutments, pilings, channel markers, underwater booms, floating bathrooms, and dock floats. The surface scraper was attached to a long pole and lowered into the water, and it was then raised while dragging the metal rim along the surface. The dislodged organisms were collected in the attached mesh bucket for inspection at the surface.

Sediment dredge samples were collected using a Petite Ponar™ grab sampler to sample sand, silt, gravel, and small rock substrate for the presence of *Dreissena* adults. The grab sampler was deployed in water depths between 1- and 6-m. The collected material was rinsed in a 250-µm mesh sieve using lake water, and the sieve was inspected.

Submersed macrophytes were collected to sample for attached *Dreissena* juveniles and adults. Aquatic plants were collected from a boat by throwing a thatch rake attached to a rope, allowing rake to sink and then dragging for approximately 1- to 2-m along the sediment, which sampled an area of approximately 0.3-m^2. Macrophyte sampling occurred at locations with plant beds visible from water surface, in areas near marinas and boat launches, and in littoral areas likely to support macrophytes. The collected macrophytes were visually inspected for bivalves and then shaken in 5-gal buckets of water to detach smaller animals. Bucket water was poured through a sieve and the sieve and bucket were inspected for bivalves.

Water temperature, dissolved oxygen, pH and specific conductivity were measured near the deepest location of each water body using either a Eureka Manta™ or Hydrolab Quanta™ water quality multi-probe sonde. Accuracy of specific conductivity and pH sensors was assured by calibration at each water body using NIST certified 100-µS/ cm, pH 7 and pH 10 standards. The accuracy of dissolved oxygen was assured by calibration to 100% air-saturated water based on *in-situ* barometric pressure measurements at each water body. Accuracy of temperature probes was assured through factory calibration. Measurements were conducted at water surface, 1-m and at 1-m depth increments thereafter to within 1-m of the sediment or to 20-m depth. Values were recorded in field datasheets. Probes were held at each depth for at least one minute for equilibration with conditions at each depth. Precision was assessed by repeating the measurements at the 2-m depth after the profile was completed.

Sample Analysis

Plankton samples were analyzed at the Veliger Identification Laboratory at PSU for the presence of planktotrophic bivalve larvae (e.g., *Dreissena* veligers) using cross-polarized microscopy. Upon receiving the plankton samples into the PSU laboratory, the samples were logged into the sample tracking system. Plankton samples were inspected for leakage, proper preservation and pH. The water body, sample container number, collection date, date received at PSU, total sample volume, and the collecting agency were recorded. Additional regular ethanol was added to plankton samples when necessary as determined by leakage, the field datasheets and/or personal communication with the collector(s). The sample pH was measured using a pH meter in the laboratory, and additional Tris was added as needed to maintain sample pH above 7.0.

Plankton samples were prepared and analyzed using methods to increase the likelihood of detecting and identifying *Dreissena* veligers within the matrix of inorganic and organic particulates found in typical plankton samples (e.g., sediment, algae, and zooplankton). Large zooplankton were removed using 500-µm filters, if necessary. Plankton samples were concentrated using Imhoff settling cones and centrifugation. The settled particulate of the plankton sample was placed into multiple centrifuge tubes and covered with regular ethanol buffered with Tris. Twenty percent, at minimum, of the settled particulate, or concentrated sample, was microscopically analyzed in two- or three-mL Sedgewick-Rafter cells using Leica DME and DM750 compound light microscopes fitted with polarizing filters. Cross-polarized light increases veliger detection and reduces analytical time (Johnson 1995). The concentrated sample was diluted in the counting chamber using regular ethanol as needed to achieve a matrix density permissible to the visual inspection of all specimens. Microscopes are fitted with high-resolution, color digital Leica DFC295 and EC3 cameras for documenting suspect specimens. Shell measurements were made with digital micrometers to assist in identification.

Larvae of other freshwater bivalves such as the introduced *Corbicula fluminea*, are sometimes found in the plankton. Each bivalve larvae encountered during microscopic analysis for the presence of *Dreissena* veligers was identified, and the presence of other bivalves was recorded with plankton results, e.g., *C. fluminea* straight-hinge juveniles.

Microscopic analysis was non-destructive and large zooplankton, supernatant and concentrated sample were retained in original sample container and stored at PSU in 70% regular ethanol buffered with Tris. Following analysis, the contents of the Sedgewick-Rafter cell, were decanted and rinsed with regular ethanol into a secondary container, e.g., 250-mL beaker. After the entire sample was analyzed, and if no veligers were detected, the contents of the secondary container were deposited into the original sample container. If veligers were detected, these aliquots were kept separate from the other analyzed portion until it was verified whether the veligers were intentionally added as blind matrix spike samples.

Blind matrix spike samples (BMS) were used to evaluate the accuracy of microscopic analysis. BMS samples were prepared by adding a known amount of *Dreissena* spp. veligers to one of the centrifuge tubes containing a subset of the sample. For example, a typical plankton sample was divided into ten 50-mL centrifuge tubes with each tube containing 7-mL of pelleted particulate and 43-mL of ethanol and lake water. Each centrifuge tube was assigned a unique tracking number using the date of collection and a running consecutive number, e.g., 081114_101450. To make a BMS, one of these 50-mL centrifuge tubes was randomly selected, and 20-mL of the supernatant was removed and discarded. A 1-mL aliquot from a laboratory stock of *Dreissena* veligers was analyzed and the veligers counted. The 1-mL aliquot containing veligers was poured into the selected centrifuge tube, and the counting chamber was then rinsed into the tube using 19-mL of buffered ethanol. The spiked tube was capped, and the unique tracking number for the spiked centrifuge tube was recorded along with the total number of veligers, the proportion of veligers in each developmental stage, the sample tracking number, water body name and date of collection. The centrifuge tube contents were thoroughly mixed, centrifuged, placed with the other nine tubes for that particular plankton sample, and submitted for analysis. BMS were prepared by Steve Wells, the Laboratory Manager. One spiked BMS was analyzed in every batch of samples. Failure to detect the veligers in the BMS required reanalysis of all samples in the batch with a new BMS. If reanalysis was not possible (e.g., insufficient sample

volume) this was recorded with data results. The analyst was allowed to repeat analysis once. If after reanalysis, data still exceeded control limits (non-detect of veligers in spiked sample), the sample results were flagged and modifications were made to procedures and recorded with data results. BMS aliquots were discarded after analysis to avoid contaminating the rest of the plankton sample. In cases where aliquots from a spiked centrifuge tube may have contaminated the analyzed portion of the plankton sample in the secondary container, the contents of the secondary container were discarded. In cases where the entire sample may have been contaminated with a BMS, the original sample container was labeled with "CONTAMINATED".

Positive results for the presence of planktotrophic veligers, excluding BMS, would be verified with interlaboratory comparisons. Digital photographs and shell measurements would be immediately sent to a minimum of three separate laboratories for verification upon positive discovery of planktotrophic veligers. Sample splits would be sent to these laboratories if requested. Molecular tests will be performed if requested by BPA, PSMFC, USACE, or deemed appropriate by PSU. Cross-validated positive results for *Dreissena* mussels will result in the immediate notification of appropriate agency staff including Glenn Dolphin (OSMB), Rick Boatner (ODFW), Robyn Draheim (USFWS), and Stephen Phillips (PSMFC).

Field and Laboratory Equipment Decontamination

Field and laboratory equipment were decontaminated using a combination of physical scrubbing and chemical solutions to prevent the transfer of larvae and genetic material between samples and the unintentional transfer of any organisms to new water bodies. Physical scrubbing with a stiff bristle brush removed organisms and other debris. A 2% quaternary ammonium solution (Quat), e.g., Virkon Aquatic® or Sparquat 256®, was used to kill organisms. Quat solutions are broad spectrum disinfectants and virucides used in aquaculture and janitorial services. Genetic material was denatured using a solution of 5% household bleach (50-mL bleach in 1-L of water) (Prince and Andrus 1992). Acid solutions (5% and 10% acetic acid, and 4% hydrochloric acid) were used to dissolve the shells of veligers, which are composed of calcium carbonate. Additionally, multiple sets of field and laboratory equipment were used to allow complete air drying after decontamination and before future use.

Field sampling equipment and gear decontamination protocols employed by PSU field crews are detailed in Appendix A. Upon completion of sampling, and prior to leaving the water body, all equipment and gear were cleaned. Visible contaminants were physically removed. Sampling gear such as plankton nets, cod-end pieces, net rope, net anchors, boat anchors, thatch rake, sediment dredge, rope, sieve, surface scraper, and secchi disk were soaked in a 2% Quat solution for 20 minutes. The boat, including both interior and exterior surfaces, was sprayed with a 2% Quat solution and soaked for ten minutes, and then this process was repeated. Deck brushes, long-handle bristle brushes, were used to scrub down the boat surfaces including the hull, seats, flooring, engine case, through-hull fittings, etc., and then rinsed with tap water. A tub of tap water was used to flush the engine cooling line for at least two minutes. Bleach was then added to this tub of water to make a 5% solution of bleach, and all the sampling equipment previously soaked in the 2% Quat solution was then soaked in the bleach solution for 15 minutes. Following this soak, the equipment was rinsed with tap water and dried. The tub of 5% bleach water was then poured into the boat, washing down the sides, seats, and other areas focusing efforts to wash all organisms into the bilge area. The bilge area was soaked in 5% bleach solution for 15

minutes, and then drained on impervious surfaces at least 61-m (200-ft) from open water. The plankton net, cod end pieces, and net rope was then soaked in a 5% acetic acid solution for a minimum of 6 hours.

Laboratory equipment and surfaces were decontaminated using physical scrubbing, and both acid and bleach solutions to prevent the transfer of larvae and genetic material between samples. Counting chambers were physically scrubbed with dish soap and toothbrushes. Other laboratory equipment such as Imhoff cones and centrifuge tubes were physically scrubbed using large bristle brushes and dish soap. Laboratory decontamination for genetic material in microscopy laboratories was necessary because multiple analytical methods may be used to confirm identification made via light microscopy. Following physical scrubbing, the equipment used with veliger sample preparation and analysis (e.g., counting chambers, pipettes, filters, and settling cones) was soaked in a solution of 5% household bleach for at least 10 minutes, rinsed with fresh water and soaked in acid solution (i.e., 5% acetic acid, 10% acetic acid or 4% hydrochloric acid) for a minimum of 8 hours. The preferred acid soak time was 24 hours. Centrifuge tubes were soaked in bleach solution for a minimum of 24 hours, rinsed with fresh water, and soaked in acid solutions for a minimum of 24 hours. Laboratory surfaces in contact with plankton samples (e.g., counters, plastic trays and microscope stages) were sprayed with 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 contained spills and were easy to clean.

Data Management

Monitoring activities and results were summarized and communicated to OSMB, ODFW, and other stakeholders through meetings, email, and reports throughout and at the end of the season. At the completion of the sampling and plankton analysis, PSU provided monitoring data collected by PSU and ODFW staff to PSMFC and USGS in a spreadsheet format for inclusion in the following online map: http://crbans.psmfc.org/monitoring.html. Additionally, PSU displayed the *Dreissena* mussel early detection monitoring locations collected by both USACE and PSU in an online, interactive map maintained by PSU, which can be accessed from the following URL: http://mussels.geos.pdx.edu/.

Results

Sample Collection

A total of 37 plankton samples (e.g., 500-mL total sample volume) were collected by PSU and ODFW in six water bodies located throughout Oregon in 2014 (Table 2). More than 500,000 liters of reservoir/lake water were filtered through 64-µm plankton nets during sampling that occurred during the August to September period. The *Dreissena* veliger sampling effort was greatest in Emigrant Lake, Lake Billy Chinook, and Prineville Reservoir in terms of the number of plankton samples, and the amount of the river/reservoir water filtered (Table 2). PSU veliger sampling effort was concentrated in early August and ODFW efforts occurred in September.

Table 2: PSU and ODFW *Dreissena* veliger sampling effort in 2014. Water bodies sampled by ODFW are marked with an *.

Water Body	August		September		Total	
	#	vol fil (L)	#	vol fil (L)	#	vol fil (L)
Applegate Reservoir*	0	n/a	6	45,653	6	45,653
Water body total					6	45,653
Emigrant Lake	4	144,919	0	n/a	4	144,919
Water body total					4	144,919
Howard Prairie Lake	4	42,652	0	n/a	4	42,652
Water body total						
Lake Billy Chinook	9	183,415	0	n/a	9	183,415
Water body total						
Prineville Reservoir	6	77,026	0	n/a	6	77,026
Upper Klamath Lake	8	32,535	0	n/a	8	32,535
Total PSU effort	31	480,547	6	45,653	**37**	**526,200**

Adult *Dreissena* mussels were not detected by PSU during 2014.

Two non-native invertebrates were opportunistically collected by PSU during sampling efforts for adult *Dreissena* mussels (Table 3). Adult *Corbicula fluminea* (Asian clams) were collected in Upper Klamath Lake. *Corbicula fluminea* straight-hinge juveniles were not detected in any of the plankton samples in 2014 (Table 5; Appendix B). *Potamopyrgus antipodarum* (New Zealand mud snails) were collected in Lake Billy Chinook, Crooked River Arm. All other bivalves, gastropods and crayfish collected were native (e.g., *Pacifasticus leniusculus*) or unidentified species that belong to taxonomic groups that have many native species and few AIS (e.g., Physidae family snails) (Table 3).

Table 3: Invertebrates opportunistically collected by PSU while conducting adult *Dreissena* sampling in 2014. Non-native invertebrates are in bold.

Type	Family	Genus	Species	Emigrant	Howard	LBC	Prineville	UKL
snail	Hydrobiidae	*Fluminicola*						X
snail	**Hydrobiidae**	***Potamopyrgus***	***antipodarum***			**X**		
snail	Lymnaeidae						X	X
snail	Lymnaeidae	*(Fossaria/Lymnaea)*					X	
snail	Lymnaeidae	(*Stagnicola/Lymnaea*)						X
snail	Physidae			X	X	X	X	X
snail	Physidae	(*Physella*)			X			
snail	Planorbidae	G*yraulus*			X	X		X
snail	Planorbidae	*Vorticifex*				X		X
snail	Planorbidae	*(Helisoma/Planorbella)*		X			X	X
limpet	Ancylidae							X
bivalve	**Corbiculidae**	***Corbicula***	***fluminea***					**X**
bivalve	Sphaeriidae					X		X
crayfish	Astacidae	*Pacifastacus*	*leniusculus*		X			

One non-native macrophyte was opportunistically collected by PSU during sampling efforts for adult and juvenile *Dreissena* mussels in 2014 (Table 4). *Potamogeton crispus* (curly leaf pondweed) was collected in Emigrant Lake, Howard Prairie Lake, Lake Billy Chinook, and Upper Klamath Lake. Several plants were not identified to species level due to the lack of morphological features, e.g., seeds and flowers.

Table 4: Submersed macrophytes opportunistically collected by PSU during adult *Dreissena* sampling in 2014. Non-native macrophytes are in bold. Species identifications that are tentative are in parentheses.

Genus	Species	Emigrant	Howard Prairie	Lake Billy Chinook	Prineville	Upper Klamath Lake
Myriophyllum			X			
Myriophyllum	*(sibiricum)*					X
Potamogeton	***crispus***	**X**	**X**	**X**		**X**
Potamogeton	*filiformis*					X
Potamogeton	*pusillus*		X	X	X	X
Potamogeton	(*pusillus*/*foliosus*)			X	X	
Potamogeton	*perfoliatus spp. richardsonii*					X
Potamogeton	*natans*					X
Potamogeton	*praelongus*					X
Stuckenia	*pectinatus*			X		
Lemna						X
Lemna	*trisulca*					X
Elodea	*canadensis*		X	X		X
Callitriche					X	
Callitriche	*hermaphroditica*			X		
Ceratophyllum	*demersum*		X			X
Sparganium						X
Nitella		X	X		X	
Chara		X			X	
Sagittaria					X	X
Marsilea	*vestita*				X	
Ranunculus						X
Ranunculus	*trichophyllus*			X		
Fontinalus	*antipyretica*					X
Juncus					X	
Eleocharis/*Juncus*					X	X
Elatine					X	
Hippuris	*vulgaris*					X
Lycopodiella					X	

The water temperature, dissolved oxygen concentrations, and pH were conducive for *Dreissena* survival and spawning at all sampled locations during sampling in 2014 (Figure 2). Water quality was collected at the deepest location in each water body during each sampling trip in 2014. Sampling was conducted when water temperatures were suitable for mussel spawning; water temperatures above 16 and 19˚C are considered optimal for *Dreissena* mussel spawning, and mussels begin spawning at temperatures between 9 and 12˚C (Claxton and Mackie 1998; Garton and Haag 1993; McMahon 1996; Nichols 1996). Emigrant Lake, Howard Prairie Lake, Lake Billy Chinook, and Prineville Reservoir were thermally stratified during sampling. Upper

Klamath Lake was isothermal during collection. The dissolved oxygen in Howard Prairie Lake below an 8-m water depth was hypoxic, and mussels in these water depths would be stressed. The dissolved oxygen concentrations in Emigrant Lake below a 22-m depth were also hypoxic. The dissolved oxygen and pH concentrations were ideal for *Dreissena* throughout the water column in Lake Billy Chinook, Prineville Reservoir, and Upper Klamath Lake (Figure 2).

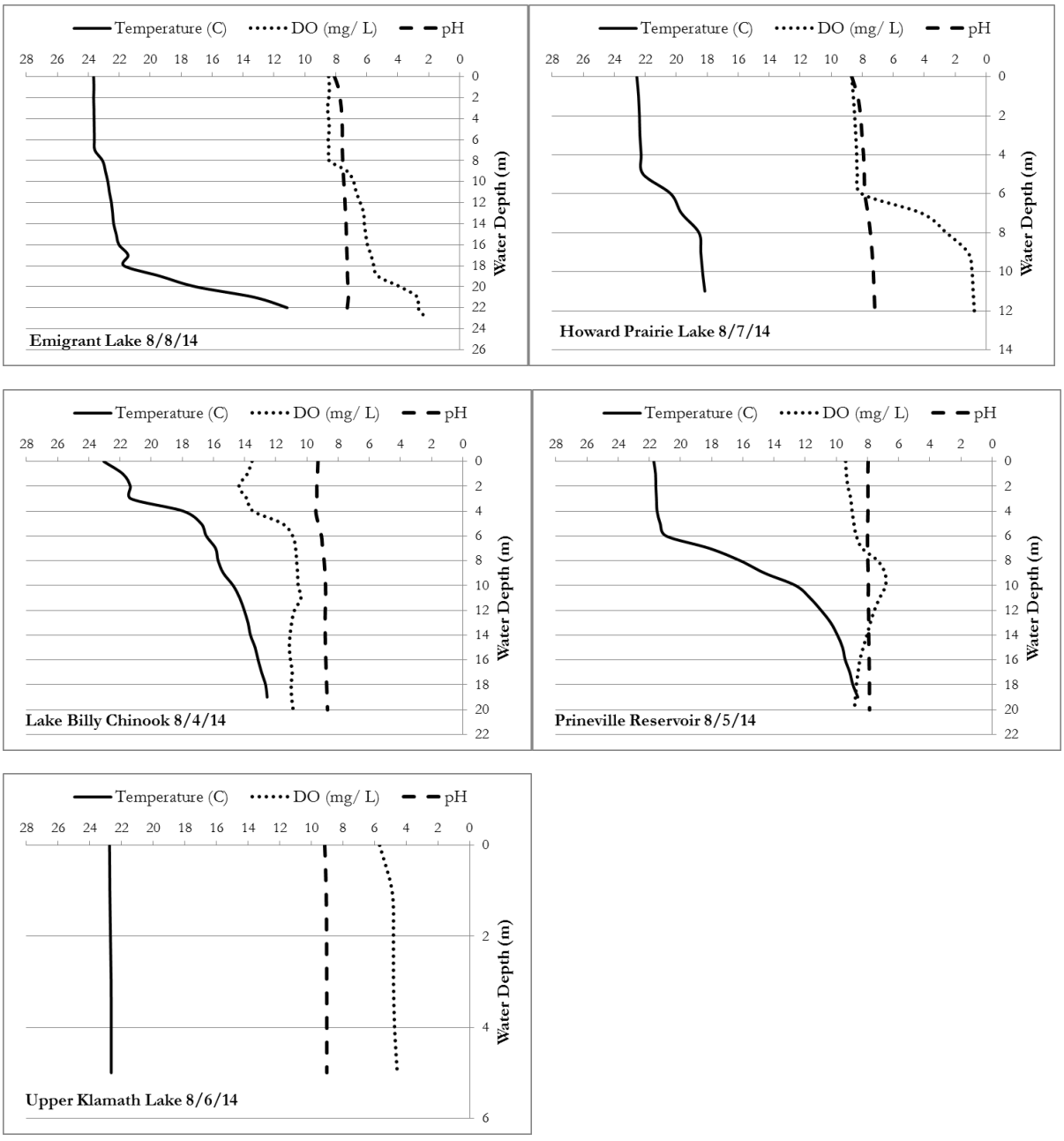

Figure 2: Water quality parameters collected across a depth profile by PSU during *Dreissena* sampling in 2014.

Sample Analysis

PSU microscopically analyzed a total of 37 plankton samples that were collected by both ODFW and PSU during 2014. *Dreissena* mussel veligers were not detected in these plankton samples. Approximately 34% of the concentrated sample of each plankton sample was microscopically analyzed at PSU in Sedgewick-Rafter counting slides (a minimum of 20%) (Table 5, Appendix B). The concentrated sample represented the pelleted particulate in the plankton sample that was isolated from the reservoir/lake water and ethanol using gravitational settlement and centrifugation. A total of 1,402 Sedgewick-Rafter counting chambers (2- and 3-mL slides) were analyzed under compound microscopes using total magnifications ranging from 40X to 200X (Table 5). A total of 351-mL of concentrated sample was analyzed in these counting chambers (Table 5). Ostracods were detected in all of the water bodies sampled. Ostracods detected in plankton samples were not identified beyond the Class Ostracoda.

A total of 11 BMS were submitted with these samples. The spiked *Dreissena* veligers were detected in all of the BMS during routine analysis in 2014. Details of the microscopic analysis including the total number of microscope slides analyzed, the amount of the concentrated sample analyzed, the total number of larvae and ostracods detected and the results of the blind matrix spike samples are provided in Appendix B.

Table 5: Summary of PSU microscopic analysis of plankton samples collected by PSU and ODFW in 2014 for early detection of zebra and quagga mussel planktotrophic larvae, veligers. *Dreissena* veligers were not detected in samples. Samples were analyzed in 2- and 3-mL Sedgewick-Rafter counting cells, and diluted in cells as needed with regular ethanol. Total amount of concentrated sample, or pelleted particulate, is shown (Conc sample). Total number of counting cells analyzed (# slide), the amount of the concentrated sample that was analyzed (mL conc sample), and the percent subsampled (% done) are shown. Number of planktotrophic bivalve veligers detected in samples is indicated (# ZQM). The number of *Corbicula* spp. straight-hinge juveniles (*Corbicula*) as well as ostracods (Ostracod) are shown.

Water body	CollectAgency	Con sample	# slide	mL conc sample	% done	ZQM	*Corbicula*	Ostracod
Applegate	ODFW	34	31	10.8	37	0	0	9
Emigrant Lake	PSU	365	435	82.8	23	0	0	122
Howard Prairie Lake	PSU	75	84	28.6	35	0	0	196
Lake Billy Chinook	PSU	263	297	62.8	39	0	0	67
Prineville Reservoir	PSU	313	328	96.9	43	0	0	103
Upper Klamath Lake	PSU	268	227	69.4	24	0	0	30
Total		**1,318**	**1,402**	**351**	**34**	**0**	**0**	**527**

Discussion

The monitoring efforts conducted by ODFW and PSU in 2014 under this project provided valuable early detection data for zebra and quagga mussels throughout Oregon. Monitoring was focused on water bodies with a high to medium likelihood of *Dreissena* mussel introduction and/or establishment according to Wells et al. (2010), and sampling occurred in six high priority Oregon water bodies. The targeted water bodies received large amounts of boater recreational use and/or exhibited dissolved calcium concentrations and pH values conducive for mussel survival and growth. PSU *Dreissena* sampling, targeting both veligers and adult life stages, covered a large geographical area in Oregon (Figure 3). Additionally, sampling efforts were distributed throughout the water bodies (Figure 4).

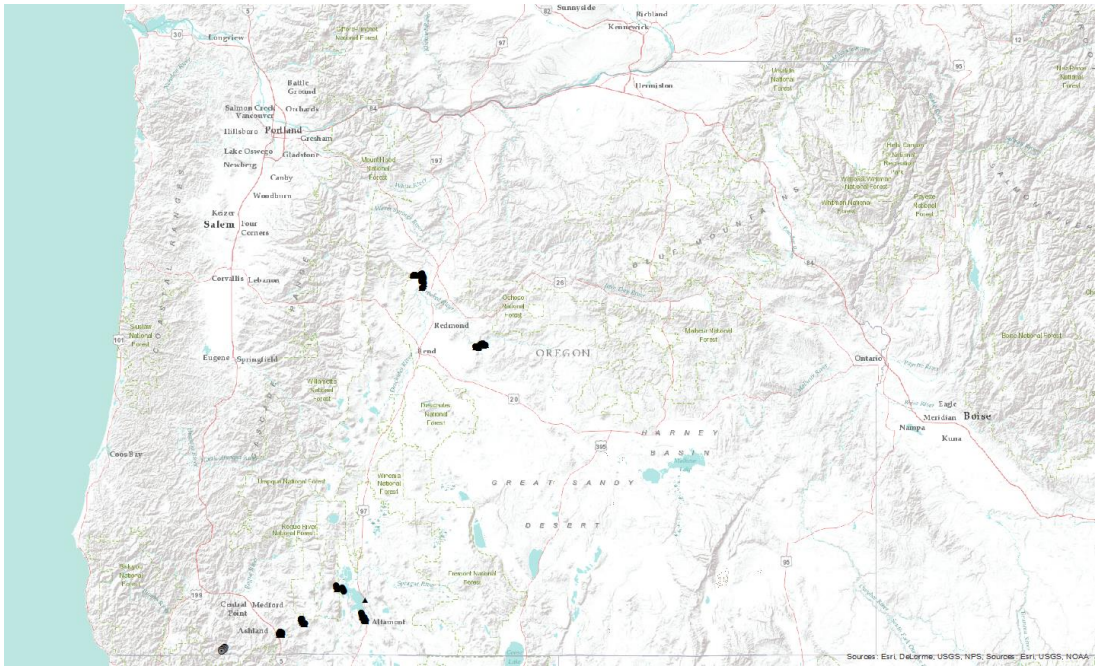

Figure 3: *Dreissena* early detection monitoring in Oregon water bodies by PSU (black) and ODFW (grey) in 2014. Sampling targeted both veligers (circles) and adults and juvenile mussels (triangles).

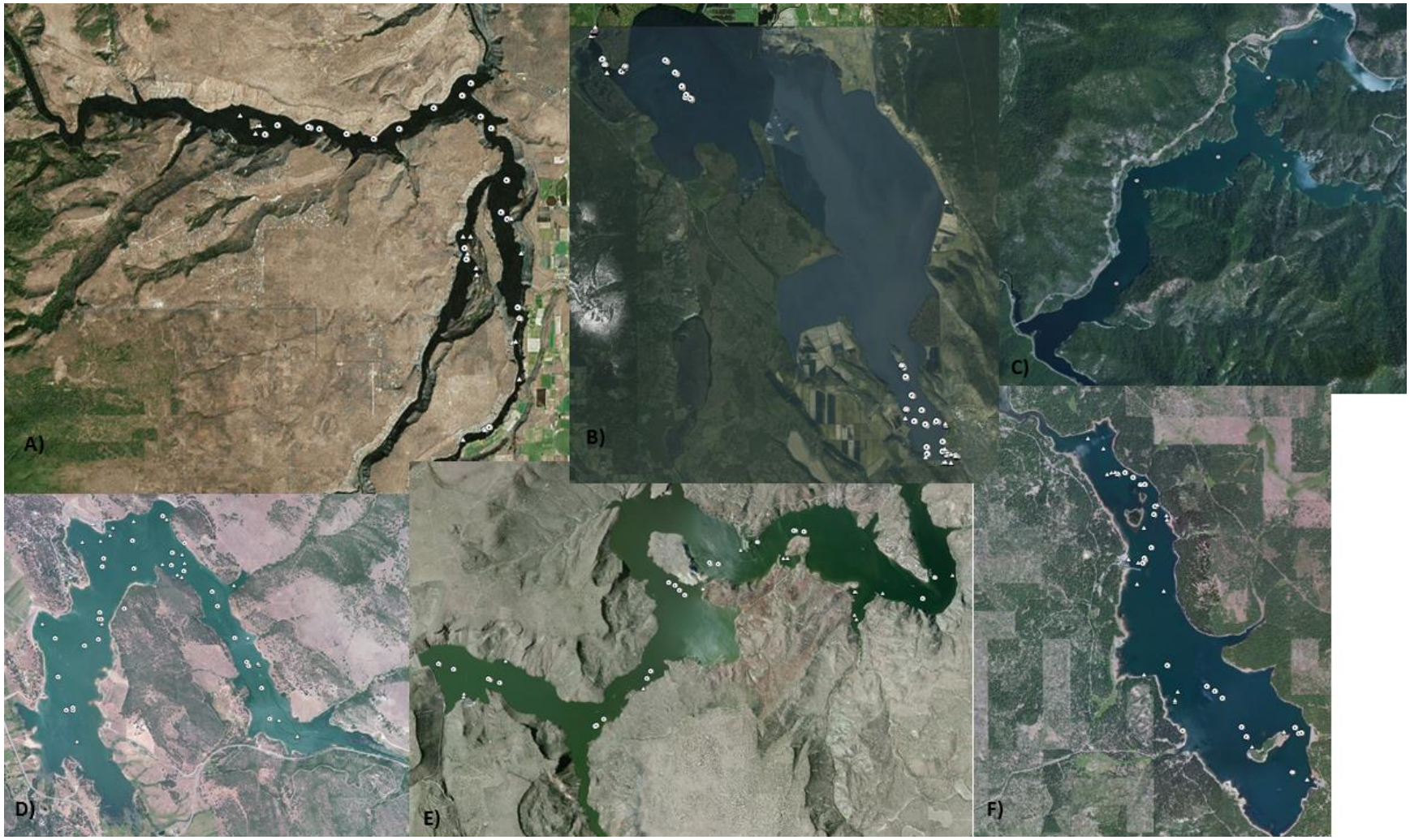

Figure 4: Locations of sampling in Oregon water bodies including A) Lake Billy Chinook, B) Upper Klamath Lake, C) Applegate Reservoir, D) Emigrant Lake, E) Prineville Reservoir, and F) Howard Prairie Lake in 2014. Veliger monitoring is indicated by circles, and adult/juvenile monitoring locations are indicated with triangles. PSU sampled Lake Billy Chinook, Upper Klamath Lake, Emigrant Lake, Prineville Reservoir, and Howard Prairie Lake. ODFW sampled Applegate Reservoir.

Sampling was focused during the period of expected peak mussel spawning (July – September) (Table 5) based on water temperature. Water temperatures above 16 to 19˚C are considered optimal for *Dreissena* mussel spawning, and mussels begin spawning at temperatures between 9 and 12˚C (Adrian et al. 1994; Claxton and Mackie 1998; Garton and Haag 1993; McMahon 1996; Nichols 1996; Roe and MacIsaac 1997).

The early detection sampling effort in 2014 was less than the level of effort done in 2013 regarding spatial and temporal coverage. Six Oregon water bodies were sampled throughout Oregon, and ODFW and PSU biologists sampled during the August to September period in 2014 (Table 6). Sampling in 2014 was focused during August, and the greatest sampling effort occurred in Emigrant Lake, Lake Billy Chinook, and Prineville Reservoir (Table 6). In comparison, nine Oregon water bodies were sampled in 2013 using OSMB funding, and sampling occurred in the July to August time period (Table 6). In 2013, the sampling was focused in East Lake, Paulina Lake, and Prineville Reservoir.

Table 6: Comparison of sampling efforts between 2014 and 2013.

Parameter	2014	2013
# Oregon water bodies	6	9
# plankton samples	37	113
Total volume filtered (L)	526,200	1,665,875
ODFW vol. filtered (L)	45,653	n/a
PSU vol. filtered (L)	480,547	1,665,875
Net efficiency (trawling vol.)	75%	60%
Sampling period	August-September	July-August
Peak sampling period	August	August
Focus water bodies	Emigrant Lake, Lake Billy Chinook, Prineville Reservoir	East Lake, Paulina Lake, Prineville Reservoir

The quality of veliger sampling in 2014 was less than the efforts done in 2013. The total number of plankton samples collected and analyzed decreased in 2014 (n= 37) as compared to 2013 (n = 113). The total volume of reservoir/lake water filtered through the nets during plankton sample collection in 2014 was less than the amount of water filtered in 2013 (526,200 liters; 480,547-L by PSU and 45,653-L by ODFW). Additionally, a more conservative estimate of the volume of water filtered via trawling was used in 2013 (60% net efficiency assumed) in comparison to 2014 (75% net efficiency), thereby reducing the calculated volume reported in 2013, and further increasing the disparity between the two years. Additionally, sampling efforts in 2013 were focused over a longer

time period to fill temporal gaps. The disparity between the sampling effort in 2013 and 2014 reflects the limited resources available for early detection monitoring.

Plankton sampling effort was best described by the volume of water filtered compared to the total number of samples collected. The total plankton sample volume ranged from 250-mL to 1-L, plankton tows were composited, and plankton was concentrated to varying degrees prior to being deposited into sample containers. Estimating the volume of water filtered is thus a more accurate and easy way to compare sampling effort. The amount of the water body filtered through the net was calculated by measuring the length of tows. In the case of trawling, the boat speed and trawling time were recorded and used to calculate length of tow. For trawling, a net efficiency was assumed to reflect the potential for net clogging. If the open spaces in the 64-µm mesh net become clogged, then the water can be pushed in front of the net reducing the actual amount of water passing through the net and being filtered. Efforts were also focused on preventing net clogging by reducing the length of the individual tows, collecting a greater number of smaller tows, and by reducing boat speed. In order to be conservative with volume estimates, however, a degree of net clogging was assumed to occur. Therefore, the calculated volume filtered for trawling was reduced by the assumed net efficiency. In 2014, a net efficiency of 75% was assumed (Table 9). Increasing the sample size, i.e., the amount of water filtered through the net, increases the likelihood of collecting veligers and decreases the likelihood of false negative results.

The level of effort expended on veliger laboratory analyses in 2014 was commensurate with the level of sampling as compared to 2013. More plankton was collected in 2013, and therefore, more plankton was analyzed. The average amount of the concentrated sample for each plankton sample that was microscopically analyzed during 2014 was approximately 34%, which was increased from the minimum of 20% (Table 8, Appendix B). The percentage of the sample analyzed was similar in 2014 and 2013. In 2014, a total of 1,402 slides were analyzed versus 5,355 in 2013. A total of 351-mL of concentrated sample was analyzed in 2014, as compared to 1,349-mL in 2013. The total number of *Corbicula fluminea* straight-hinge juveniles and ostracods reported in 2014 (0 and 527, respectively) was comparable to 2013 (12 and 2,758, respectively). In 2013, low densities of *C. fluminea* straight-hinge juveniles were detected in plankton samples collected in East Lake and Howard Prairie Lake (n= 1 and n=11, respectively), however adult *C. fluminea* were not collected. In 2014, several small *C. fluminea* adults were found in Upper Klamath Lake but no straight-hinge juveniles were detected in plankton samples from any of the sampled water bodies, including Howard Prairie Lake. Theses detections of *C. fluminea* appear to be the first detections of these bivalves in Upper Klamath Lake and Howard Prairie Lake. These data suggest *C. fluminea* populations in Howard Prairie Lake and Upper Klamath Lake are relatively small, isolated populations. These detections using different early detection methods over two years highlight the importance of long-term datasets as well as using multi-faceted approaches to early detection monitoring, i.e., use all the tools available.

The collaborative nature and the personnel involved with this project increased the effectiveness of the early detection monitoring in relation to other monitoring efforts

conducted by a single agency or a network of volunteers. More people were involved in these collaborative efforts, meaning more eyes on the ground and greater personal investment in the issue. Many of the ODFW biologists have been involved with these collaborative efforts for several years. The use of trained ODFW biologists and dedicated PSU field crews provided quality samples, which is invaluable when looking for an incipient population at presumed low densities. Both ODFW biologists and PSU field crews provided valuable outreach and education within their respective agencies and to the general public.

The effectiveness of the PSU early detection monitoring efforts are reflected in the opportunistic detections of other species during *Dreissena* sampling efforts in 2014. PSU collected two types of freshwater bivalves in 2014 sampling. Sphaeriidae adult clams were collected in both Lake Billy Chinook and Upper Klamath Lake, and *C. fluminea* adults were collected in Upper Klamath Lake (Table 3). It appears that these are the first detections of *C. fluminea* adult clams in Upper Klamath Lake (Benson 2015), however, the records for *C. fluminea* distribution are inaccurate, and it is possible the records do not reflect previous detections in this water body. A single *P. antipodarum* (New Zealand mud snails) specimen was collected in 2014 from Lake Billy Chinook in the location where a population was detected by PSU in 2010. The repeated collection of this species demonstrates the reliability of the employed sampling methods, while the collection of a single snail highlights the difficulty of sampling small populations. The lack of *P. antipodarum* in other sampling efforts distributed throughout Lake Billy Chinook suggests that this population is not widespread in Lake Billy Chinook. Other mollusk detections in 2014 also included snails in the Hydrobiidae, Physidae, Lymnaeidae, and Planorbidae families, as well as a freshwater limpet. Although native signal crayfish were previously detected by PSU in Howard Prairie Lake, the collection of native crayfish during 2014 sampling corroborated the sampling methods used. *Potamogeton crispus* (curly leaf pondweed) was found in Emigrant Lake, Howard Prairie Lake, Lake Billy Chinook, and Upper Klamath Lake in 2014. *P. crispus* was not collected from Emigrant Lake in 2013, although it was detected in the other aforementioned water bodies. PSU collected many types of macrophytes in 2014 (Table 4), and the variety of growth habits in the collected plants demonstrates the effectiveness of PSU field sampling efforts using multiple collection methods. For example, *Lemna trisulca* is a small floating plant that is not collected effectively using a plant rake as compared to *Myriophyllum* species that are large rooted plants with their biomass throughout the water column. Additionally, species such as *Chara* and *Nitella* are typically distributed in much deeper water and are not visible from the surface as compared to *Juncus* and *Eleocharis* species that grow in shallow shoreline areas, thus demonstrating the breadth of habitats sampled by PSU for *Dreissena* in 2014.

The use of trained personnel is important to collect quality veliger samples. Bias associated with veliger sample collection includes false-positive and false-negative results. False-positive results during sample collection are caused by cross-contamination of field sampling equipment. Multiple sets of gear and decontamination procedures were used to minimize these sources of bias. False-negative results during sample collection are caused by inadequate sample size, inappropriate location and frequency of sampling, and poor sample handling. Efforts were focused on collecting numerous plankton tows from multiple locations and water depths during several sampling events throughout the peak spawning period to maximize the likelihood of veliger collection. Samples were immediately preserved in solutions of 70% regular ethanol that was pre-buffered with Tris to maintain sample pH and specimen integrity. Sample pH was measured upon receipt into the PSU laboratory, and additional Tris was added as necessary to maintain pH above 7.0. The pH of ten plankton samples upon receipt into the laboratory, however, was below the acceptable threshold of 6.8, and these samples were discarded because of possible veliger shell dissolution and concerns about false negative results. Figure 5 shows the sample pH for the different water bodies sampled by PSU in 2014. The ten samples that were discarded due to low pH contained very large amounts of particulates, and smelled like decomposition. A properly preserved plankton sample in 70% regular ethanol should smell like ethanol. All water bodies received the same pre-buffered ethanol. It is therefore likely that these low pH samples were improperly preserved in the field by adding too much organic matter and not enough ethanol. Tracking the pH of plankton samples demonstrates the importance of proper field and laboratory protocols in addressing bias such as false negative results.

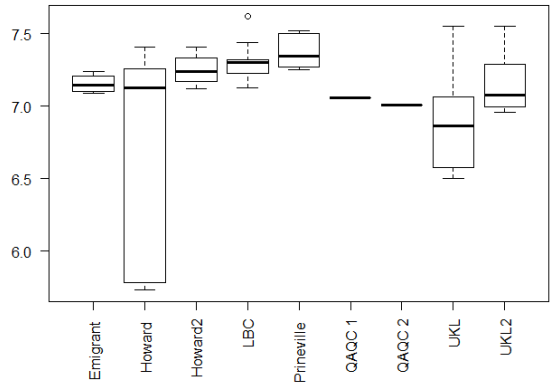

Figure 5: pH of plankton samples received into PSU laboratory. A total of ten samples were discarded from Howard Prairie Lake and Upper Klamath Lake because pH was below 6.8. The pH of the samples for Howard Prairie and Upper Klamath are presented again after discarding the low pH samples from these lakes (i.e., Howard2 and UKL2).

Laboratory quality assurance/quality control efforts are paramount for producing reliable veliger data that managers are willing to use to guide actions. Bias associated with veliger detection during light microscopy analysis includes false-positive and false-negative results. The sources of these biases are identified in Tables 7 and 8. Misidentification is addressed using appropriate equipment, laboratory control samples and other identification tools (e.g., veliger image database), using laboratory manager to inspect suspect specimens, using multiple experts to confirm identification, increasing subsample volume to locate additional specimens, and using molecular methods on sample splits. Contamination is addressed by laboratory decontamination procedures. Analyst error, matrix effects, and low abundances of target specimens cause false-negative results with light microscopy. Analyst error is addressed by using BMS and training with laboratory control samples including target organisms as well as look-alikes such as *Corbicula*

fluminea, *Gonidea angulata* and ostracods. Matrix effects are addressed by increasing aliquot dilution, thus reducing confounding matrix. Low abundance of veligers is addressed by sample handling procedures in the laboratory, sample concentration, and increasing the subsample volume.

Table 7: Sources of false positive errors (absent but detected) with veliger identification using light microscopy and corrective actions and quality control measures.

	Problem	Corrective Action/ QC Measures
Method	Misidentification	equipment (increase magnification) training (lab control samples, ID tools) duplicate sample analysis (two analysts, sample split) identification confirmed by Veliger Lab manager photomicrographs shared with independent experts molecular analyses on field split
	Contamination	field and lab decontamination
Process	other planktotrophic bivalve larvae	increase subsampling (multiple specimens) training (lab control samples, ID tools) identification confirmed by Veliger Lab manager photomicrograph confirmation by outside experts
	unusual/ poor/ limited # specimens	increase subsampling (multiple specimens) identification confirmed by Veliger Lab manager photomicrograph confirmation by outside experts

Table 8: Sources of false negative error (present but not detected) with veliger identification using light microscopy and corrective actions and quality control measures.

	Problem	Action
Method	analyst error	blind matrix spiked samples training (lab control samples, ID tools)
	matrix effects	increase aliquot dilution
Process	unusual/poor/limited # specimens	preservation/handling ([preservative], temp, pH) increase subsampling (conc. sample volume)
	low abundance, clumped spatial distribution	sample concentration increase subsampling (conc. sample volume)

The PSU laboratory met the acceptance criteria for sample analysis in 2014. A total of 11 BMS were submitted with PSU plankton samples during routine analysis for this project, and the veligers in the BMS were detected during routine analysis. It is noteworthy that the PSU laboratory analyzed a total of 102 BMS during 2014, and veligers in only two of these BMS were missed during routine analysis.

There is a high likelihood of false negative results (i.e., failing to find them when present) with early detection monitoring for zebra and quagga mussels. Although veliger detection is the acceptance criterion used for veliger quality control samples in the PSU laboratory,

it is useful to consider the percent recovery of spiked veligers during routine microscopic analysis. The percent recovery of veligers in the BMS in 2014 averaged 26%, but the entire concentrated sample in the BMS was not analyzed during routine analysis (Appendix B). Therefore, the adjusted percent recovery was calculated to determine the expected percent recovery if the entire BMS was analyzed based on the recovery in the portion that was analyzed (i.e., Adjusted percent recovery = % recovery/ % of BMS analyzed). Figure 6 shows the percent recovery and the adjusted percent recovery for the BMS analyzed during this project. The mean percent recovery during 2014 was 26% ± 10% (1SD; min= 8%, max= 43%, n=11). The mean adjusted percent recovery during 2014 was 112% ± 42% (1SD min=35%, max= 197%, n=11). The mussels exhibit spatial and temporal patchiness, the interfering matrix in typical plankton samples complicates the detection of veligers, and it is time consuming to analyze the entire plankton sample in a manner that is likely to detect all present veligers. Additionally, there is a human element of error with microscopy. These recovery rates demonstrate that microscopy is a reliable and effective method to detect *Dreissena* veligers in plankton samples. These recovery rates also demonstrate the inherent problems with detecting discrete organisms at low densities, and reinforce the importance of increasing the sample size of both collection and analysis efforts, implementing quality control and quality assurance laboratory procedures (e.g., blind matrix spike samples), properly training and equipping analysts, and developing and implementing a regional laboratory certification process.

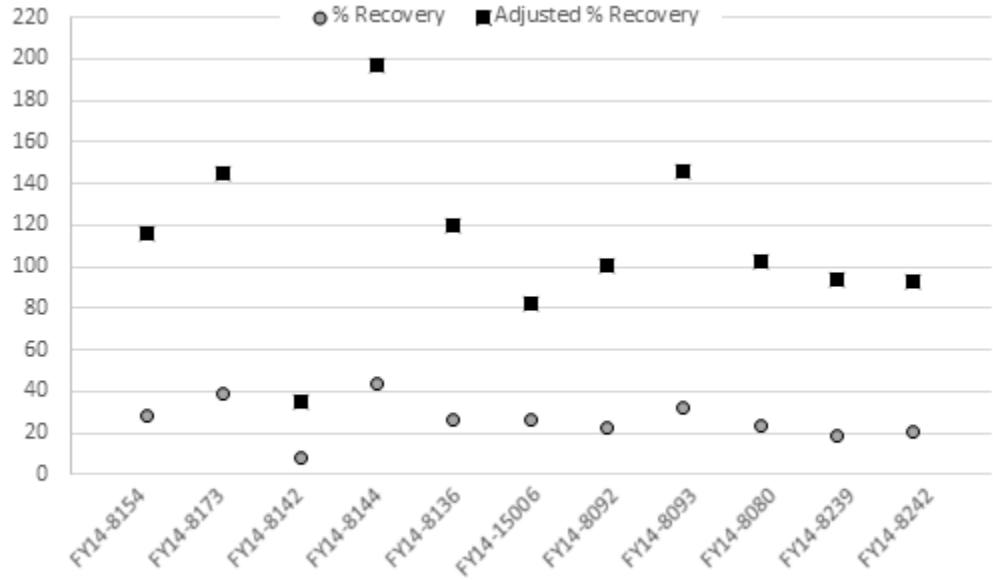

Figure 6: Percent recovery of blind matrix spike samples during 2014. The percent recovery of spiked veligers in the subsample recovered during routine microscopic analysis (% recovery) is shown; twenty percent, at minimum, of the concentrated sample was subsampled during routine analysis. The percent recovery adjusted to reflect the estimated recovery if 100% of the concentrated sample was analyzed, i.e., the actual percent recovery divided by the percent of the sample analyzed (Adj % recovery) is also shown. The ideal is an adjusted percent recovery of 100% or more. All BMS were detected during routine analysis

The risks posed by *Dreissena* mussels are significant, and early detection monitoring provides valuable information. These mussels are expected to survive and grow in many Oregon waterways, and watercraft contaminated with *Dreissena* mussels are continually

intercepted at watercraft inspection stations throughout Oregon and the region. If these mussels are present in Oregon water bodies, their early detection will provide the greatest amount of time to organize and mount rapid response efforts.

Next Steps/ Recommendations

- Continue collaborative early detection monitoring efforts for zebra and quagga mussels in Oregon waterways in 2015.
- Continue coordinating sampling efforts with other federal and state agencies to increase spatial and temporal coverage while reducing unnecessary overlap.
- Expand monitoring efforts targeting the juvenile and adult life stages. Adult monitoring methods are cheap, low-tech, and effective. The presence of juvenile and adult life stages provides more reliable evidence of an incipient or established population compared to the presence of the larval life stage.
- Quantify efforts targeting the juvenile and adult life stages to better communicate the on-the-ground effort to managers and the general public.
- Improve veliger sampling by collecting and processing more plankton, i.e., increasing the sample size. Increasing the sample size may involve increasing the amount of plankton collected during each sampling event, increasing the number of locations and sampling events, as well as increasing the subsample volume analyzed. Increasing the sample size, however, increases costs. Novel sample handling techniques should be explored to aid veliger analysis.
- Record the amount of water body filtered through plankton net to quantify the veliger sampling effort. The total number of plankton samples does not adequately capture the quality of the sampling effort.
- Continue proper field and laboratory decontamination to prevent cross-contamination of samples and the unintentional transfer of organisms between water bodies.
- Focus decontamination efforts on both the actual organism as well as genetic material and consider other target species in addition to *Dreissena*, e.g., New Zealand mud snails, plants like *Myriophyllum* and *Hydrilla*, Chytrid fungus, etc.
- Expand the use of blind matrix spiked samples during plankton sample analysis to check the accuracy of plankton analysis via light microscopy.

References

Ackerman, J.D., B. Sim, S.J. Nichols, and R. Claudi, 1994. A review of the early life history of zebra mussels (*Dreissena polymorpha*): comparisons with marine bivalves. *Can. J. Zool*. **72**:1169-1179.

Adrian, D.J., T. A. Ferro and H.T. Keppner, 1994. Annual and Seasonal Variations in Veliger Density in the Upper Niagara River. Proceedings of the Fourth International Zebra Mussel Conference.

Bastviken, D.T.E., N.F. Caraco and J.J. Cole, 1998. Experimental measurements of zebra mussel (*Dreissena polymorpha*) impacts on phytoplankton community composition. *Freshwater Biology*. **39**: 375-386.

Brayshaw, T.C., 2001. Pondweeds, bur-reeds and their relatives of British Columbia: aquatic families of monocotyledons. Victoria, B.C: Royal British Columbia Museum, 250 pg.

Benson, A. J., 2015. Zebra mussel and Quagga mussel sightings distribution. Retrieved March 23, 2015 from http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/maps/current_zm_quag_map.jpg.

Boelman, S.F., F.M. Neilson, E.A. Dardeau and T. Cross, 1997. Zebra Mussel (*Dreissena polymorpha*) Control Handbook for Facility Operators, First Ed. U.S. Army Corps of Engineers.

Botts, P.S., B.A. Patterson and D. W. Schloesser, 1996. Zebra mussel effects on benthic invertebrates: physical or abiotic. *Journal of North American Benthological Society.* **15**(2):179-184.

Burlakova, L., 1995. The Relationship between *Dreissena polymorpha* and other benthic animals. Proceedings of the Fifth International Zebra Mussel and Other Aquatic Nuisance Organisms Conference. Toronto, CA.

Caraco, N.F., J.J. Cole and D.L. Strayer, 2006. Top down control from the bottom: regulation of eutrophication in a large river by benthic grazing. *Limnology and Oceanography*. **51**:664-670.

Claudi, R. and G. Mackie, 1994. Zebra Mussel Monitoring and Control. CRC Press, Boca Raton FL.

Claxton, W.T. and G.L. Mackie, 1998. Seasonal and depth variations in gametogenesis and spawning of *Dreissena polymorpha* and *Dreissena bugensis* in eastern Lake Erie. *Can. J. Zool*. **76**:2010-2019.

Crow, G.E. and C.B. Hellquist, 2000. Aquatic and Wetland Plants of Northeastern North America. Volume One: pteridophytes, gymnosperms, and angiosperms: dicotyledons. The University of Wisconsin Press Madison, WI.

Crow, G.E. and C.B. Hellquist, 2006. Aquatic and Wetland Plants of Northeastern North America. Volume Two: A Revised and Enlarged Edition of Norman C. Fassett's A Manual of Aquatic Plants, Volume II: Angiosperms: Monocotyledons. University of Wisconsin Press.

Connelly, N.A., C.R. O'Neill Jr., B.A. Knuth and T.L. Brown, 2007. Economic Impacts of Zebra Mussels on Drinking Water Treatment and Electric Power Generation Facilities. Environmental Management **40**:105-112.

Dermott, R. and D. Kerec, 1997. Changes to the deepwater benthos of eastern Lake Erie since the invasion of *Dreissena*: 1979-1993. Canadian Journal of Fisheries and Aquatic Sciences. **54**:922-930.

Effler, S.W. and C. Siegfried, 1998. Tributary Water Quality Feedback from the Spread of Zebra Mussels: Oswego River, New York. *Journal of Great Lakes Research.* **24**(2):453-463.

Effler, S.W., D.A. Matthews, C.M. Brooks-Matthews, M.Perkins, C.A. Siegfried and J.M. Hassett, 2004. Water quality impacts and indicators of metabolic activity of the zebra mussel invasion of the Seneca River. *Journal of the American Water Resources Association.* **40**:737-754.

Fahnenstiel, G.L., G.A. Lang, T.F. Nalepa and T.H. Johengen, 1995. Effects of Zebra Mussels (*Dreissena polymorpha*) Colonization on Water Quality Parameters in Saginaw Bay, Lake Huron. *Journal of Great Lakes Research*. **21**(4):435-448.

Frest, T.J. and E.J. Johannes, 1999. Field Guide to Survey and Manage Freshwater Mollusk Species. Report No. BLM/OR/WA?PL-99/045+1792, 128 pp.

Gallager, S.M., C.S. Davis, P. Alatalo and A. Girard, 1996. Horizontal and Vertical Distribution of Zebra Mussel Larvae and Other Plankton in Saginaw Bay, Michigan in Relation to Hydrography Quantified Using the Video Plankton Recorder. Proceedings of the Sixth International Zebra Mussel and Other Aquatic Nuisance Species Conference.

Garton, D.W. and W.R. Haag, 1993. Seasonal Reproductive Cycles and Settlement Patterns of *Dreissena polymorpha* in Western Lake Erie, *IN*: Zebra Mussels: Biology, Impacts, and Control, Lewis Publishers. Boca Raton, FL, pg. 111-128.

Hall, R.O. Jr., J.L. Tank and M.F. Dybdahl, 2003. Exotic Snails Dominate Nitrogen and Carbon Cycling in a Highly Productive Stream. *Frontiers in Ecology and the Environment.* **1**(8):407-411.

Hamel, K. and J. Parsons, 2001. An Aquatic Plant Identification Manual for Washington's Freshwater Plants. Washington State Department of Ecology.

Harrold, M.N. and R.P. Guralnick, 2010. A Field Guide to the Freshwater Mollusks of Colorado. Colorado Division of Wildlife.

Haynes, A., B.J.R. Taylor and M.E. Varley, 1985. The influence of the mobility of *Potamopyrgus jenkinsi* (Smith, EA) (Prosobranchia: Hydrobiidae) on its spread. *Archiv für Hydrobiologie*. **103**(4):497-508.

Horvath, T.G., K.M. Martin and G.A. Lamberti, 1999. Effect of Zebra Mussels, *Dreissena polymorpha*, on Macroinvertebrates in a Lake-outlet Stream. *The American Midland Naturalist.* **142**:340-347.

Jenner, H.A., J.W. Whitehouse, C.J.L. Taylor and M. Khalanski, 1998. Cooling water management in European power stations: Biology and control of fouling. *Hydroécologie Appliquée*. **10**:1-255.

Johnson, L.E., 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. *Hydrobiologia*. **312**:139-146.

Kelly, N.E., K. Wantola, E. Weisz and N.D. Yan, 2013. Recreational boats as a vector of secondary spread for aquatic invasive species and native crustacean zooplankton. *Biological Invasions*. **15**:509-519.

Kraft, C., D.W. Garton, L. Johnson and J. Hieb, 1996. Spatial and Temporal Patterns of Veliger Abundance in Inland Lakes. Proceedings of the Sixth International Zebra Mussel and Other Aquatic Nuisance Species Conference.

Langeland, K.A., 1996. *Hydrilla verticillata* (L.F.) Royle (Hydrocharitaceae), "The Perfect Aquatic Weed". *Castanea*. **61**(3):293-304.

Larson, E.R. and J.D. Olden, 2011. The state of crayfish in the Pacific Northwest. *Fisheries*. **36**:60-73.

Lysne, S., 2009. A Guide to Southern Idaho's Freshwater Mollusks. US Fish and Wildlife Service, US Bureau of Reclamation, USDA Forest Service, Idaho Governor's Office, The College of Idaho and Orma J Smith Museum of Natural History, 43 pg.

Mackie, G.L. and D.W. Schloesser, 1996. Comparative Biology of Zebra Mussels in Europe and North America: An Overview. *Amer. Zool*. **36**:244-258.

Mann, R., H. Radtke, D. D. Huppert, J. R. Hamilton, S. S. Hanna, J. Duffield and N. R. Netusil, 2010. Economic Risk Associated with the Potential Establishment of Zebra and Quagga Mussels in the Columbia River Basin. Independent Economic Analysis Board, Northwest Power and Conservation Council, IEAB 2010-1, Portland, Oregon.

Marsden, J.E., 1992. Standard Protocols for Monitoring and Sampling Zebra Mussels. Illinois Natural History Survey Biological Notes 138.

McMahon, R.F., 1996. The Physiological Ecology of the Zebra Mussel, *Dreissena polymorpha*, in North America and Europe. *Amer. Zool*. **36**:339-363.

Miller, R. and M. Sytsma, 2014. Aquatic invasive species surveys of Eastern Oregon water bodies during 2013 and 2014. Interim report to the Bureau of Land Management.

Nichols, S. J., 1996. Variations in the Reproductive Cycle of *Dreissena polymorpha* in Europe, Russia, and North America. *Amer. Zool*. **36**:311-325.

Neitzel, D.A., K.I. Johnson, T.L. Page, J.S. Young and P.M. Daling, 1984. Bivalve Fouling of Nuclear Power Plant Service Water Systems. Correlation of Bivalve Biological Characteristics and Raw water system design. U.S. Nuclear Regulatory Commission, NRC FIN B2463 NUREG/ CR-4070, PNL-5300 Vol 1.

Olden, J., J.W. Adams and E.R. Larson, 2009. First record of *Orconectes rusticus* (Girard, 1852) (Decapoda, Cambaridae) west of the Great Continental Divide in North America. *Crustaceana*. **82**(10):1347-1351.

Perez, K.E., S.A. Clark and C. Lydeard, 2004. Freshwater Gastropod Identification Workshop: "Showing your shells" A Primer to Freshwater Gastropod Identification. University of Alabama.

Phillips, S. "Watercraft Interception Programs for Dreissenid Mussels in the Western United States". Pacific States Marine Fisheries Commission. Power Point. 24 April 2013. http://icais.org/pdf/2013abstracts/3Wednesday/Session%20B-7/1140_Phillips.pdf.

Prince, A.M. and L. Andrus, 1992. PCR: How to Kill Unwanted DNA. *Biotechniques*. **12**(3):358-360.

Ram, J.L., P.P. Fong and D. W. Garton, 1996. Physiological Aspects of Zebra Mussel Reproduction: Maturation, Spawning and Fertilization. *Amer. Zool*. **36**:326-338.

Raven, C.P., 1958. Morphogenesis: The Analysis of Molluscan Development. Pergamon Press, New York, 365 pp.

Ricciardi, A., R. J. Neves and J. B. Rasmussen, 1998. Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology* **67**:613-619.

Richards, D.C., L.D. Cazier and G.T. Lester, 2001. Spatial distribution of three snail species, including the invader *Potamopyrgus antipodarum*, in a freshwater spring. *Western North American Naturalist*. **61**(3):375-380.

Richards, D.C. and G.T. Lester, 2003. Survey of the invasive New Zealand mudsnail, *Potamopyrgus antipodarum* in the Silver Creek drainage in and around The Nature Conservancy's Silver Creek Preserve, Idaho USA. Prepared for the Nature Conservancy Silver Creek Preserve, 19 pp.

Roe, S.L. and H.J. MacIsaac, 1997. Deepwater population structure and reproductive state of quagga mussels (*Dreissena bugensis*) in Lake Erie. *Can. J. Fish. Aquat. Sci*. **54**:2428-2433.

Sanderson, B. L., K. A. Barnas and A. Michelle Wargo Rub, 2009. Nonindigenous species of the Pacific Northwest: an overlooked risk to endangered salmon? BioScience **59:** 245-256.

Sprung, M., 1993. The Other Life: An Account of Present Knowledge of the Larval Phase of *Dreissena polymorpha*. *In* Zebra mussels: biology, impact and control. Edited by T.F. Nalepa and D.W. Schloesser. Lewis Press, Boca Raton, Fla. Pg. 39-52.

Strayer, D.L., 2008. Twenty years of zebra mussels: lessons from the mollusk that made headlines. *Frontiers of Ecological Environment*. **7**(3):135-141.

Thorp, J.H., J.E. Alexander, K.S. Greenwood, A.F. Casper, R.K. Kessler, A.R. Black, W. Fang, A.F. Westin and B. Lewis, 1994. Predicting Success of Riverine populations of Zebra mussels (*Dreissena polymorpha*)- Early Colonization and Microhabitat Distribution in the Ohio River. Proceedings from the Fourth International Zebra Mussel Conference.

Thorp, J.H. and D.C. Rogers, 2011. Freshwater Invertebrates of North America. Academic Press, Elsevier, San Diego, CA, 274 pg.

USDA, NRCS, 2014. The PLANTS Database (http://plants.usda.gov, 25 February 2014). National Plant Data Team, Greensboro NC.

USGS, 2014. *Orconectes rusticus*. USGS Nonindigenous Aquatic Species Database, Gainsville, FL. http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=214 Revision Date: 1/30/2008.

Wells, S., T.D. Counihan, A. Puls, M. Sytsma and B. Adair, 2010. Prioritizing zebra and quagga mussel monitoring in the Columbia River Basin. Prepared for Bonneville Power Administration, 82 pg.

Wilson, K.A., J.J. Magnuson, D.M. Lodge, A.M. Hill, T.K. Kratz, W.L. Perry and T.V. Willis, 2004. A long-term rusty crayfish (*Orconectes rusticus)* invasion: dispersal patterns and community changes in a north temperate lake. Canadian Journal of Fisheries and Aquatic Science. **61**:2255-2266.

Winterbourn, M., 1970. The New Zealand Species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia*. **10**(2):283-321.

Appendix A

Field Collection Protocols: *Dreissena* mussels and other AIS

2014

Steve Wells

SAFETY

Driving state-owned vehicle while towing boat

- Death and/or bodily harm can occur because of vehicle accidents, and towing boat while carrying heavy loads increases risks of sudden loss of control.
- There is a history of vehicle accidents at the CLR, and some have been serious, e.g., trailer tire blow-out on HWY; rolling truck after swerving onto shoulder.
- You are authorized to drive state-owned vehicle as employee of PSU traveling on official business only. You must carry your valid driver's license when operating vehicle, and have a good driving record.
- Alcohol is prohibited in state-owned vehicles.
- State rigs can be brought to your residence for particular situations (e.g., early departure from residence saves the State money), but this requires prior ESM Dept approval.

Safety Protocols

1. **Maximum speed limit when towing is 55 MPH.** Speed is reduced as necessary depending on weather, road conditions, etc.
2. Seatbelts are worn at all times when vehicle is in motion.
3. Courteous and defensive driving. When in doubt, err on the side of caution.
4. Inspect both truck and trailer tire pressure and tread before each trip. Maintain tire pressure of trucks at psi labeled on inside of truck door. Maintain the trailer tire psi at 10 psi less than maximum psi labeled on trailer tire wall, e.g., 70 psi on tire labeled "Max 80 psi".
5. Trailer lights inspected before leaving launch. Maintain all trailer lighting.
6. Grease trailer axle prior to leaving boat storage each trip.
7. Bring spare trailer tire and lug wrench for changing tire. If you get a flat tire, slowly move off the road to the right shoulder. Get as far off road as possible, and avoid areas with reduced visibility from behind, e.g., below hill crest or after bends in road.

Collecting biological and water quality samples from a boat

- Death and/or bodily harm can occur when working on boats in large rivers, reservoirs, and lakes.
- There is a history of accidents at the CLR, e.g., struck by cod-end piece that was flying out of boat when underway at high boat speed, straining muscles in back, dehydration, etc.
- You are authorized to operate a state-owned boat as an employee of PSU while conducting official duties only.
- You are required to possess an Oregon Boater Education card to operate boat. You should have the card with you on the boat.

Safety Protocols

1. **A personal floatation device (PFD)** is worn anytime a person is in boat.
2. Operate boat with a minimum of two people present. Buddy system.
3. Bring a fire extinguisher, throw ring/seat cushion, and sound device (horn or whistle) onto boat. When boating on the Columbia River, you must also bring flares. Do NOT use flares on other Oregon water bodies (wildfire risk).
4. Valid Oregon Boater Education card.
5. Bring drinking water, sun protection, snacks, and non-slip shoes.
6. Complete/update a field and float plan prior to launch. The Float plan is left on dashboard in parked vehicle while you are on water (fold along dotted line to hide information on bottom half of sheet).
7. Lift with your legs and avoid straining muscles. Use cranes, pulleys, and multiple people as needed.
8. Verbally communicate to each other when shifting position within boat, starting engine, throwing plant rake, etc.
9. Secure lightweight equipment before moving.
10. Distribute weight appropriately Port-Starboard and Stern-Bow.
11. Maintain a clean and clear working area.
12. Be safe!!

GENERAL STRATEGY

Prioritization of activities by species.

Task	Time
1. Zebra/quagga mussel veliger (plankton net)	2:45
2. Zebra/quagga mussel adult (scraper, Portland Sampler, hand pat-down, Petite Ponar®, plant shake-down, shoreline walks)	2:05
3. Multi-probe water quality measurements (Quanta® or Eureka®)	0:45
4. Macrophytes (plant rake on rope, opportunistic hand grabs)	0:45
5. Snails & *Corbicula* (see #2)	
6. Crayfish (minnow traps, scraper, opportunistic hand grabs)	0:15
Total SAMPLING time on water*	**6:40 hrs**

*Additional time will be required for boating to different locations on water body.

- Total time on water will vary by water body size and boat speed.
- At least 15 plankton sites; 8 vertical and 7 trawling events. More sampling locations and bigger samples are desired if possible.

Water body	# trips	Loop	Target # 500-mL samples each trip
Upper Klamath Lake	1x	OSMB	5 - 8
Howard Prairie Lake	1x	OSMB	5 – 8
Emigrant Lake	1x	OSMB	5 – 8
Lake Billy Chinook	1x	OSMB	5 – 8
Prineville Reservoir	1x	OSMB	5 – 8
Lake Owyhee	3x	Eastern	9 - 11
Snake River, Farewell Bend	3x	Eastern	9 - 11
Columbia River, Boardman	3x	Eastern	9 - 11

- Zebra/quagga mussels are the primary target.
- Other top priorities include *Corbicula* clams (everywhere but Columbia nr Boardman), snails (everywhere), crayfish (especially Howard Prairie, Emigrant and Snake River), flowering rush and hydrilla (Columbia and Snake), and *Myriophyllum* (everywhere). See Vanessa's photograph ID guide.
- Voucher specimens are needed for identification and documentation. ID occurs in laboratory. Sort quickly in field, and err on side of collecting numerous specimens with similar appearance.
- Ethanol is pre-buffered using Tris and plankton samples should require no additional buffering.
- Deploy crayfish traps at start of sampling if not camping nearby. If camping, set out overnight. Preferably use sardines or herring. Target is total 9 traps deployed in three locations.
- Sample containers are pre-labeled with number, e.g., FY14-5555. If missing sample container number, assign one using following: FY14- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY14-062513a.
- **On field datasheet, record the sampler container number**, e.g. FY14-5555, as well as 1) vertical tow length, 2) time starting and stopping for trawling, 3) trawling boat speed, 4) latitude and longitudes, 5) water body name, 6) sampling date, 7) collectors, and any other pertinent information.
- Write legibly. Use pencil preferably for field datasheet and sharpie for sample containers.

ZEBRA/ QUAGGA MUSSEL VELIGERS (2 HOURS AND 45 MINUTES)

Objective: early detection (presence/non-detect) of rare planktonic specimens that have clumped spatial distribution, i.e., sampling at multiple locations within water body targeted where plankton likely collects.

Site Locations:

- A minimum of 15 individual sites, but more if time permits. A site is a particular location separated from other sites by at least 61-m (200-ft).
- Collect, at a minimum, a combination of 8 vertical tows and 7 trawling events (i.e., approximately five to eleven 500-mL samples or 750- to 1,650-mL of actual plankton).
- Cluster one quarter of site locations near dam and spread out other sites according to horizontal and vertical distribution comments below.

Horizontal distribution

River- main stem, near dam, near marinas and boat launches, behind islands or downstream of large obstructions that cause eddies, in downwind bays, and along shore in areas of eddies and downwind positions.

Reservoirs- near dam and outflows, open water areas, downwind positions (e.g., in a particular bay), near shore areas such as marinas and boat launches, and other areas of eddies.

Vertical distribution, i.e. depth

River and non-stratified reservoir- entire water column for vertical tows, and 10-m (33-ft) depth for trawling.

Stratified reservoirs- just above thermocline to surface for vertical tows, and just above the thermocline for trawling.

Equipment List (for sampling one water body):

- (15) 500-mL sample containers
- GPS unit w/ (4) AA batteries
- (2) 64-µm mesh plankton net with cod-end
- (2) rope wheels w/ 31-m (100-ft) rope ea.
- veliger datasheet (Appendix A)
- (2) pencils and (1) sharpie pen
- (1) net anchor
- (10-L) regular ethanol
- watch or clock
- cooler with ice

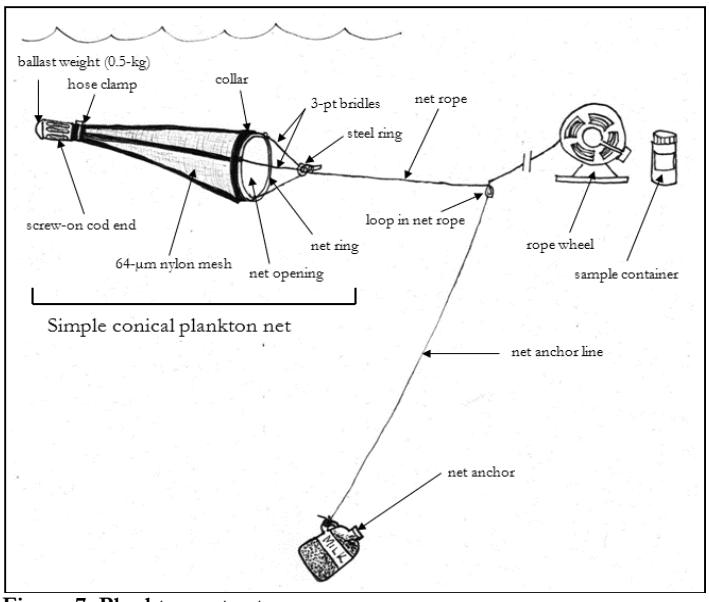

Figure 7: Plankton net set-up.

Sample Collection:

- Combination of <u>at least</u> **8 vertical**/oblique tows and **7 trawling** events per water body. When trawling, record time at the start of trawling as well as the end. Trawling is done at lowest engine speed to avoid net clogging.
- Keep net and rope clear of boat engine prop. Avoid snagging net on sharp objects.
- Keep net off lake bottom.
- Attach milk jug filled with gravel to net rope approximately 1 m in front of net opening (Figure 1).
- Composite samples from sites in similar area of water body, e.g., dam.
- Condense plankton in net and cod-end as much as possible prior to pouring into sample container.
- Fill sample container 30% full of concentrated plankton + lake water.
- Keep samples in cooler on ice until preserved on-shore.

Vertical/Oblique Plankton Tow- (1 hour)

1. Secure the cod-end piece, check the hose clamp and check that the rope is securely attached to plankton net at steel ring. Attach net anchor (milk jug of gravel) to a second 2- or 3-m section of rope that is tied to a loop placed in the net rope approximately one meter in front of net opening.
2. Lower the net 30-m (100-ft) below water surface, or to 1-m above the sediment, whichever is deeper. Keep the net off the lake bottom. Record GPS location on datasheet (Appendix A).
3. Keep net at this depth for five seconds and then manually retrieve using a hand-over-hand technique at a rate of 0.5-m/s (1.5-ft/s). Slow and steady retrieval is the key to collecting a good plankton tow.
4. Rinse the net by raising the net so that the cod end of the net is at the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the opening above the water surface. Then quickly pull net straight up; this action will move collected plankton into the cod-end piece. Repeat this procedure several times to ensure that all the organisms inside the net are in the cod end.

5. **Condense the sample as much as possible before pouring into sample container**. Condense the sample by swirling the cod-end piece while still attached to net. Then carefully unscrew the cod-end piece without spilling collected water and plankton. You may need to use tweezers, spatula, or your finger to gently clear the mesh netting in the cod-end piece to allow the water to filter through. The cod-end piece, once separated from net, should also be swirled to further condense sample. After pouring sample into sample container, dip the cod end into water body to add small amount of water in order to rinse out remaining plankton into sample container.
6. Record the length of each tow on the datasheet (Appendix A) as well as the sample container number, and the latitude and longitude coordinates.
7. The volume of water sampled is determined using the formula below, assuming a net filtering efficiency of 100% (i.e., no clogging). If clogging occurs, a pressure wave develops reducing effectiveness of sampling; water will be forced to the surface prior to the net emerging from the water if a pressure wave forms. If clogging occurs, first try reducing the depth of the tow. If it still occurs, estimate the net filtering efficiency and record with data.

Maximum volume of filtered water, V_m is

$$V_m = \pi * r^2 * d$$

where r = radius of the net opening (0.15 m)
d = depth to which the net is lowered (30 m)

$1\ m^3 = 1{,}000$-L

Trawling (1 hour and 45 minutes)

1. Secure the cod-end piece, check the hose clamp and check that the rope is securely attached to plankton net at steel ring.
2. Attach net anchor (milk jug of gravel) to a second 2- or 3-m section of rope that is tied to a loop placed in the net rope approximately one meter in front of net opening. **A net anchor is required for trawling**.
3. In a vertically mixed water body, lower the net 10-m (33-ft) below surface of water. In a stratified water body, lower the net to just above the thermocline. Keep net off lake bottom. Record start time of trawling and GPS position on datasheet. Maintain net at this depth for 1- to 30-minutes, depending on net filtering efficiency, while driving the boat at lowest boat speed, e.g., 1 MPH. Use the hand-held GPS device to estimate boat speed.
4. Keep net and rope clear of engine prop.
5. Stop engine or idle, and manually retrieve net using a hand-over-hand technique at a rate of 0.5-m/s (1.5-ft/s). Record stop time of trawling (end), GPS location, and an estimate of average boat speed.
6. Follow steps #4 through #5 used for vertical/oblique tows regarding condensing and collecting plankton from the net.

Sample Preservation:

- Keep samples in cooler on ice while on boat.
- Samples are preserved in solutions of 70% regular ethanol on shore. Add 350-mL of 95% regular ethanol to 150-mL of plankton in a 500-mL sampler container to achieve a 70% solution of regular ethanol.
- Gently shake closed sample container to mix contents.
- Preserved samples are stored at temperatures equal to or less than room temperature.
- Place all sample containers from the same water body into a large plastic trash bag and tie off.
- Ethanol is pre-buffered with tris(hydroxymethyl)aminomethane, and the target pH for preserved samples is between 7.64 and 9.00. A pH below 6.8 will result in shell dissolution and birefringence.

ZEBRA/ QUAGGA MUSSEL ADULTS, SNAILS, CLAMS (2 HOURS)

Objective: early detection (presence/non-detect) of epifaunal adult *Dreissena* mussels attached to hard surfaces as well as opportunistic sampling for early detection and distribution of *Corbicula fluminea* (Asian clam), *Potamopyrgus antipodarum* (New Zealand mud snail), *Radix auricularia* (Big-eared radix), *Orconectes rusticus* (rusty crayfish), *Procambarus clarki* (red swamp crayfish) and others.

Site Locations:

- Existing submerged hard surfaces including docks, pilings, channel markers, floating bathrooms, buoys, bridge abutments, seawalls, rocks, and logs.
- Artificial settlement substrates (deploy from dam buoy line, speed limit buoys, floating bathrooms, docks, channel markers and other surface structures).
- Shoreline areas including gravel, sand, mud, cobble and woody debris, especially in downwind, downstream, or other positions where shells and other debris are collected.
- Focus on the bottom and sides of submerged objects; in protected and shaded areas such as nooks, crannies and junction of two different surfaces.
- Periphyton may obscure attached bivalves and other specimens.

Horizontal distribution

- Areas where the water currents and/or wind patterns are likely to concentrate the planktonic larvae, as well as dead adult shells, e.g., near dam or outflow, particular bays, eddies, etc.
- High boater use areas and points of entry, e.g., near marinas and launches
- Main stem, open water areas and near-shore areas.

Vertical distribution

- Lake bottom to exposed shoreline areas in well mixed water bodies, e.g., reservoirs along Columbia River.
- Thermocline to surface in stratified lakes and reservoirs.
- The default minimum water depth for Portland Sampler deployment is 8-m (25-ft).

Equipment List (for sampling one water body):

- surface scraper
- (3) substrates (pvc, abs, concrete anchor, rope)
- knife
- datasheet (Appendix B)
- 1 gallon zip lock bags
- thatch rake on rope
- 5-gallon bucket (white color)
- Metal sieve
- Petite Ponar sediment dredge
- GPS unit
- (4) AA batteries
- (1) sharpie pen, (2) pencils
- cooler with ice
- (8) 250-mL sample containers
- digital camera
- Ethanol
- Minnow traps, clips and rope
- Sardines, herring or other bait

Sample Collection:

Hand pat-down- (20 minutes)

1. Locate suitable existing submerged surfaces to inspect. Accessible surfaces (i.e., within arm's reach) are good candidates for visual and tactile inspections and include the undersides and sides of dock floats, floating bathrooms, buoys and mooring chains, and the underside and sides of rocks found in shoreline areas.
2. Carefully pat surface with the palm of your hand and fingers. Do not run your hand along surfaces because of sharp objects. Remove hard protruding objects for visual inspection.
3. **Record efforts on field datasheets** (Appendix B) even if nothing is detected.
4. Retain suspect specimens in 250-mL sample container or zip lock bag with small amount of lake water. Record the sample container number on field datasheet (Appendix B). Place specimen in cooler on ice.

Surface scraper- (15 minutes)

1. Locate suitable submerged structures to inspect. Surface scraper works well on vertical concrete walls, bridge abutments and cutwaters, channel markers, pilings, underwater booms, and breakwaters.
2. Carefully position boat near structure to sample (e.g., channel marker) and maintain position either using the motor or using water current and wind to position boat against structure.
3. When using the surface scraper, lower it into the water as deep as the pole will allow. Using both hands on the pole, bring the metal rim of the mesh box in contact with the substrate surface and quickly pull up, keeping the metal rim in contact with the surface to be sampled. The sessile communities collected in the mesh are visually inspected for the presence of bivalves while in the field.
4. Repeat step #3 at multiple locations per structure in order to sample a representative portion.
5. **Record GPS location and sampling activities on datasheet** (Appendix B).

WARNING: Be careful not to pin arms between the boat and structure.

Portland Samplers- (5 minutes)

Deployment

1. Make sure substrate is complete and includes pvc (white) and abs (black) pipe sections suspended along a rope with pvc mesh. A concrete anchor or other heavy object should be attached to the bottom of the substrate rope.
2. Locate a suitable deployment location that provides a secure structure from which the substrate can be suspended at depths of at least 25-ft, e.g., dam buoy line, floating bathroom, channel marker, buoys, etc. Shallower deployment locations are acceptable, but are not preferred.
3. Position boat to provide safe access. **Minimize the visibility of the deployment to other lake users to avoid tampering, theft, etc.**
4. Determine the depth of the deployment location. If you are making the substrate in the field, cut the rope to an appropriate length. The substrate at the end of the rope should be as deep in the water as reasonably possible. The substrate does not need to touch lake bottom, although this is acceptable. The default depth to suspend settlement substrates is 8-m (25-ft), i.e., pre-made substrates.

5. Tie the loose end of the rope to the structure. Lower substrate into water.
6. Record GPS location and activities on datasheet (Appendix B). Include both GPS coordinates and a general site description with enough detail to guide a stranger to deployment location.

Inspection

1. When checking an existing substrate, remove it slowly from the water to avoid specimen loss. Inspection for adult and juvenile *Dreissena* mussels is both tactile and visual. You are looking for a visible (naked eye) bivalve attached to a hard surface. Small juveniles may feel gritty to the touch. A hand lens (10X magnification) may be used.
2. A biofilm will develop after immersion in natural waters. Do not remove biofilms because mussel settlement is greater on surfaces with biofilms compared to surfaces lacking biofilms. Freshwater sponges, however, should be removed.
3. **Record activities on field datasheet regardless if anything found** (Appendix B). Note if previously deployed substrate is gone. Replace missing substrates with new substrate.
4. Retain suspect specimens in 250-mL sample container or zip lock bag with small amount of lake water. Record the sample container number and GPS location on field datasheet. Place specimen in cooler on ice.
5. Redeploy substrate after inspection.

Petite Ponar® Grab Sampler- (40 minutes)

1. Deploy the sediment dredge in areas of gravel, small cobble, sand and mud in water depths up to 6-m (20-ft). Engage the spring-pin into dredge and carefully lower the dredge keeping tension on the rope. Lower dredge until it settles in or on bottom, and then quickly jerk the rope up to trigger the dredge. You can feel the dredge deploy.
2. Quickly retrieve dredge and dump contents into metal sieve and rinse in lake. Inspect sieve for bivalves and snails.
3. **Record GPS location and sampling activities at each site on datasheet** (Appendix B).
4. Retain suspect specimens in 250-mL sample container or zip lock bag with lake water. Record the sample container number in the datasheet. Place in cooler on ice.

Shoreline walks- (30 minutes)

1. Walk in a zig-zag pattern parallel to shoreline in wade-able depths near boat launches and other areas that contain shells, cobble, gravel, and sand. Sample multiple areas if time permits. Stop every other step to pull out loose rocks, cobble and woody debris and/or aquatic plants to inspect for mussels and snails. Look for bivalve shells partly buried in sand as well as dead shells on top of sediment.
2. **Record GPS location and sampling activities at each site on datasheet** (Appendix B).

3. Retain suspect specimens in 250-mL sample container or zip lock bag with small amount of lake water. Record the sample container number in the datasheet. Place in cooler on ice.

Crayfish traps- (15 minutes)

1. Deploy minnow traps at reservoirs and rivers as soon as you arrive at a water body if you are not camping nearby. Retrieve these traps before departing. When camping near the water body, trap overnight.
2. Bait traps using canned sardines or herring. Wet cat food can be used in emergencies. Secure trap together with clips or zip ties. Use dark-colored rope (e.g., dark green Paracord) to minimize visibility.
3. Deploy a total of nine traps per water body (three traps in a location and sample at least three locations per water body). Deploy traps in shallow littoral areas with hard substrate, preferably rock, as well as areas with macrophytes. Attach rope to stake in ground or tie to rock. Chose trapping locations that are inconspicuous to avoid tampering, theft, etc.
4. Retrieve and inspect traps. **Record sampling activities on field datasheet** (Appendix B).
5. Retain several voucher specimens for crayfish in 250-mL or 500-mL sample containers and preserve with regular ethanol; cover specimens with ethanol. On the field datasheet, record the sample container number.

Plant shake-down- (15 minutes)

1. Place collected macrophytes into a 5-gallon white-colored bucket with lake water. Vigorously shake the macrophytes in bucket and water to detach invertebrates. Look for crayfish, bivalves and snails on plants when placing plants into bucket, and again when removing plants and sorting for macrophyte collection. Allow bucket and water to sit in sunlight while sorting plants.
2. Inspect the sides of bucket for small attached snails.
3. Pour liquid and debris out of bucket through metal sieve. Visually inspect collected debris for snails and bivalves. Discard debris in lake.
4. **Record activities on datasheets** (Appendix B). Record GPS location, sample container number, and sampling activities at each site on datasheet.
5. Retain suspect specimens in 250-mL sample container or zip lock bag with small amount of lake water.
6. Place sample container with suspect specimens in cooler on ice.

Sample Preservation:

- Bivalve and snail suspect specimens are kept alive, and transported to PSU held on ice. Bivalves and snails are retained in sample containers or bags with lake water and placed in cooler on ice. Identification will be done in the laboratory.
- **Place all sampler containers and bags collected from the same water body into a large plastic trash bag and tie off.**

- Crayfish are preserved with regular ethanol in 250- or 500-mL sample containers. Decant lake water and add 95% regular ethanol to cover specimens when on shore. Preserved specimens can be stored at room temperature out of direct sunlight.
- Sample containers with living organisms (e.g., mollusks and plants) are stored in the refrigerator upon returning to the PSU laboratory.

MACROPHYTES (45 MINUTES)

Objective: opportunistic sampling to create species composition lists and conduct early detection monitoring for invasive plants, e.g., *Hydrilla verticillata*, *Trapa natans*, *Myriophyllum*, and *Butomus* (flowering rush).

Site Locations:

- Submerged rooted plant beds visible from surface and/or sight viewer.
- Areas known or suspected to contain plants from previous efforts or surveys.
- Areas with extensive littoral zones, especially areas with shallow slopes based on bathymetric maps or depth sounder.
- In downwind positions, sheltered bays and near islands.
- Near boat ramps, marinas, in bays and inlets and stream inlets.
- Shallow shoreline areas with visible submerged plants.
- Emergent plant beds along shoreline.

Equipment List (for sampling one water body):

- thatch rake on rope
- cooler and ice
- datasheet (Appendix C)
- 1 gallon zip lock bags
- GPS unit and (4) AA batteries
- bathymetric maps
- macrophyte identification books
- pencils and permanent marker

Sample Collection:

Plant rake on rope- (25 minutes)

1. Position boat near plant bed or area to sample in deeper water areas. Anchor boat only when necessary, e.g., windy conditions.
2. Throw rake side-arm style while firmly holding onto the free end of rope with your other hand. Allow rake to sink to bottom, and then slowly retrieve rake by dragging rake across lake bottom and through plant bed.
3. **Record sampling activities on field datasheet** (Appendix C).
4. Deposit plants into white-colored 5-gallon bucket half filled with lake water. Vigorously shake plants in water to remove invertebrates.
5. Continue sampling an area until all apparent species have been collected.

   At each site, select numerous representative specimens for each species; these specimens should have as many portions of the plant (submersed leaves, floating leaves, inflorescences, seeds/fruit, rhizomes, roots, etc.) represented as possible. When in doubt, collect additional specimens. Place the specimens representing all species present at one site into zip lock bags with enough lake water to cover plants. Place bags of plants in cooler on ice. Do not freeze.
6. On datasheet, record the sample container number. If a sample container is lacking a sample container number, assign one using following: FY14- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY14-062513a.

7. If you suspect you've found one of the high priority EDRR species listed below, retain extra specimens in a separate zip lock bag for verification at PSU. These should be placed in a zip lock bag filled with lake water. On datasheet, record the sample container number. Place bags of plants in cooler on ice. Do not freeze.
8. **In Oregon,** discard the other plants back into water body**.**

   In Washington, retain all plant material in plastic garbage bags and dispose of in dumpsters**.**
9. Use the view finder to look for species that were not collected at the site. Repeat rake toss as necessary.

Plant hand grabs- (15 minutes)

1. In shallow water, wade into water and manually retrieve the plants. Focus 10 minutes of these efforts on submerged plants in shoreline areas lacking emergent plants and the other 5 minutes sampling emergent plants.
2. Repeat steps #3-7 for plant rake on rope detailed above.

Opportunistic plant hand grabs- (5 minutes)

1. Opportunistically grab plants that are floating at water surface in the course of other activities and moving between sites.
2. Repeat steps #5-7 for plant rake on rope detailed above.

Sample Preservation:

- Plants are placed in zip lock bags filled with lake water, and placed in cooler on ice. Keep bagged plants on ice while in field. Do not freeze.
- Place all sample containers collected from the same water body into a large plastic trash bag and tie off.
- Refrigerate plants upon returning to laboratory.
- Any samples of *Myriophyllum* (milfoil) that do not cleanly key out to a species should be sent out for molecular tests. Fresh specimens are required.
- Voucher specimens are pressed once identifications are confirmed.

High Priority EDRR Species (see laminated field guides for preliminary identification)

<u>Submerged</u>

- hydrilla (*Hydrilla verticillata*)
- South American Waterweed (*Egeria densa*)
- milfoil *(Myriophyllum* species)
- fanwort (*Cabomba caroliniana*)

<u>Floating</u>

- parrots feather (*Myriophyllum aquaticum*)
- yellow floating heart (*Nymphoides peltata*)
- water primrose (*Ludwigia* species)
- *Limnobium laevigatum* (West Indian spongeplant)
- *Hydrocharis morsus-ranae* (European frogs-bit)

- European water chestnut (*Trapa natans*)

Emergent

- flowering rush (*Butomus umbellatus*)
- common reed (*Phragmites australis* ssp. *australis*)
- yellow flag iris (*Iris pseudacorus*)

MULTI-PROBE UNIT/ WATER QUALITY (45 MINUTES ON WATER)

Objective: to determine presence of and location of thermocline, and to obtain relatively accurate data for water temperature, specific conductance, pH, and dissolved oxygen along depth profiles, as well as collecting other metadata.

Site locations:

- At deepest open water site.
- Anchor or tie-off (e.g., tie to channel marker) to maintain boat position during multi-probe deployment.

Equipment List (for sampling one water body):

- multi-probe unit sensors
- multi-probe interface
- multi-probe charger
- multi-probe cable
- probe storage cup
- probe slotted cover
- calibration cup and lid
- tap water
- DI water
- datasheet (Appendix D, E, and F)
- barometric gauge
- secchi disk
- GPS unit
- (4) AA batteries
- (2) pencils
- DC/ AC power inverter and charge cable (Eureka)
- (3) C batteries (Quanta)
- conductivity standard
- pH 7 standard
- pH 10 standard
- pH reference electrolyte
- KCl salt pellets
- pH and DO tables and equations for mmHg
- 1-L wide-mouth container with lid

Multi-probe unit calibration:

NOTE: Specific sequence and buttons listed below are for Hydrolab Quanta (e.g., ↵↑). The basic steps, however, apply to all multi-probe units.

Conductivity

Calibrated in laboratory at both start and end of field trip, and in-field on 4th consecutive field day and every 4th day thereafter, or upon reason to suspect reported values or as required by specific project protocols.

1. Take off storage cup. Put on calibration cup.
2. Rinse calibration cup, rubber lid, and probes 5X with DI water. Discard water.
3. Rinse 3X with small amount of "used" conductivity standard (including rubber lid). Discard standard.
4. Hold O|| for approximately 5-seconds to turn unit on. It should be on "Screen".
5. Turn off unit circulator for conductivity calibration. If circulator is on (i.e., spinning), hit Esc to turn off.
6. Hold unit upside down (probes facing up).

7. Add enough "new" conductivity standard to cover probes, and equilibrate for several minutes. Do not cover with rubber lid.
8. From "Screen", record the temperature of the standard and initial conductivity reading on calibration sheet (Appendix E).
9. ↑↓ 2x to "Calib".
10. ↵
11. →← to "SpC".
12. ↵
13. Enter value for the conductivity standard in correct units, (e.g., 0.100 mS/cm at 25C), using ↑↓ and →←.
14. ↵
15. Esc to "Screen".
16. Record the second conductivity reading as well as the time for values to stabilize (±0.01 µS/cm). Retain standard in container marked "used" for rinsate in subsequent calibrations.
17. Acceptable range= 7% from reference (e.g., 93 to 107 µS/cm for 100 µS/cm conductivity standard).
18. Repeat steps #2-16 if calibration fails.

pH

Calibrated **at every water body** as well as in laboratory at both start and end of field trip, and as required by specific project protocols.

1. Rinse cup, rubber lid, and probes 5X with DI water. Discard water.
2. Rinse 3X with small amount of "used" pH 7 standard. Discard standard.
3. Turn off unit circulator for pH calibration.
4. Add enough "used" pH 7 standard to cover probes, and equilibrate for several minutes.
5. From "Screen", record the temperature of standard, and initial pH 7 reading on calibration sheet (Appendix E).
6. ↑↓ 2x to "Calib".
7. ↵
8. →← to "pH".
9. ↵
10. Enter the temperature-corrected pH value for pH 7 standard (e.g., 7.02 @ 20°C) using ↑↓ and →←. Determine the temperature-corrected pH value using the Table of pH Calibration Standards (Appendix E).
11. ↵
12. Esc to "Screen".

13. Record the second pH 7 reading and the time for values to stabilize (±0.01 pH units). Retain standard in container marked "used" for subsequent calibrations.
14. Acceptable range= 0.2 units from reference (e.g., 6.82 to 7.22 for pH 7 @ 20°C).
15. Repeat steps #1-13 with "new" standard if calibration fails. See Trouble-shooting section below.
16. Calibrate unit for pH 10 standard by repeating steps #1-13 using pH 10 standard.

Dissolved oxygen

Calibrated **at every water body**, and in laboratory at both start and end of field trip, and as required by specific project protocols.

1. Add 620-mL of tap water (NOT distilled water) to wide-mouth container. Close lid and shake for at least one full minute to saturate water with oxygen.
2. Attach the slotted-probe cover to unit, and hit Esc to turn on the unit circulator. It may be stuck. Wait 20-seconds and then gently turn the circulator with your finger to start circulator.
3. Open 1-L container and place on level surface.
4. Lower probes into jar of water until slotted-probe cover rests on bottom. All probes must be submerged.
5. Allow unit to equilibrate for several minutes.
6. Determine the barometric pressure in mmHg. Calculate the barometric pressure in inches using gauge (e.g., 30.2-in). Multiple this by 25.4 to convert to mmHg (see Appendix E). Round to whole number (e.g., 767).
7. From "Screen", record the temperature and initial dissolved oxygen reading (mg/L) on calibration sheet (Appendix E).
8. ←↑ 2x to "Calib".
9. ↵
10. ↓→ to "DO".
11. ↵
12. Enter temperature-corrected dissolved oxygen value using ←↑ and ↓→. Determine the temperature-corrected value determined from DO Saturation Values Table (Appendix E), and by entering calculated barometric pressure in mmHg. The assumption is that agitated water is 100% saturated with oxygen at given altitude.
13. ↵
14. Enter barometric pressure in mmHg (e.g., 767) using ←↑ and ↓→.
15. ↵
16. Esc to "Screen".
17. Record the second DO reading and time to stabilize (±0.01 mg/L).
18. Acceptable range= 0.2 mg/L from reference.

19. Repeat steps #1-17 if calibration fails.

Trouble-shooting Multi-probe Calibration

- Bad standard→ rinse with additional DI water and use "new" standard.
- Incorrect units or values→ check units and values entered.
- Low battery→ check unit voltage, charge unit or replace batteries.
- Faulty sensor→check sensors for obvious contamination and maintenance needs:
    - SpC sensor cleaned with cotton swab and ethanol, rinse with tap water.
    - pH sensor cleaned with cotton swab and ethanol, rinse with tap water.
    - Replace pH reference solution and clean reference junction:
        - Gently pull off reference sleeve and discard liquid.
        - Add two KCl salt pellets into sleeve and refill sleeve with reference electrolyte.
        - Hold unit with probes facing down, and push reference sleeve back onto mount until sleeve covers first O-ring.
        - Turn unit over so probes face up, and push sleeve completely into mount. This may take some force. Reference electrolyte should push out of junction.
        - Rinse with tap water.

Sample collection:

Multi-probe- (45 minutes)

1. Anchor boat or tie-off to structure such as boom line in front of dam. Record GPS location on datasheet (Appendix D)
2. Attach the slotted probe cover to multi-probe unit sensor. Immerse probes in lake and turn unit on. Allow unit to equilibrate.
3. Deploy secchi disk on sunny side of boat. Do not use polarized sunglasses or view finder.
4. Record the depth the disk disappears on datasheet (Appendix D).
5. Slowly raise disk until it reappears, and record this depth.
6. Repeat secchi measurement with the other field operator.
7. Determine anchor site depth using a depth sounder or by lowering secchi disk to bottom.
8. Record multi-probe readings at 1-m depth intervals. Start at surface and move down. Keep the unit at least 1-m off the lake bottom.
9. Allow unit to stabilize at each depth (temperature $\pm 0.01^{o}$, depth ± 0.1 m, DO ± 0.01 mg/L, and pH ± 0.01).
10. Record values on datasheet.
11. Continue to obtain profile. Raise unit to 2-m depth and record values a second time. Compare first and second measurements to assess instrument drift. Repeat profile if outside acceptable range (SpC 7%, pH 0.2 units, and DO 0.2 mg/L).
12. Remove slotted probe cover, and attach probe storage cup with ¼-inch tap water. Do not use DI water. If no tap water is available use lake water or pH standard.

Sample preservation:

- Keep probes moist during storage by attaching the probe storage cup containing 1/4-inch of tap water.
- Do NOT use distilled water for probe storage.
- If no tap water is available, use pH 4 or pH 7 standards, or lake water.

DECONTAMINATION (40 MINUTES)

Objective: remove and/or kill any plant and animals on gear and boat using a combination of physical scrubbing, and chemical processes to prevent cross-contamination of samples, and/or the accidental introduction of nonnative species.

Site locations:

- Launch ramp parking lot at least 61-m (200-ft) from open water.

Equipment List (for sampling one water body):

- (> 4 gallon) 5% acetic acid
- (> 4 gallon) 5% bleach solution
- bottle of household bleach (appx. 6% NaOCl)
- (2) large plastic tubs with lids (> 10 gallons)
- spray bottle 2% Virkon Aquatic
- (> 4 gallon) 2% Virkon Aquatic
- large brush
- spray bottle of tap water
- (> 13 gallons) fresh water
- spray bottle 5% bleach solution
- spray bottle of Formula 409 (or Virkon)
- 1 large tub (> 10 gallon) no lid

Procedures:

1. Rinse multi-probe unit sensors with ample fresh tap water. Replace water in probe storage cup with fresh tap water. Do not use DI water.
2. On the boat launch, remove the bilge drain to drain lake water out of boat.
3. Manually remove any visible contaminants on equipment, e.g., macrophytes, and dispose in upland trash container.
4. Move the boat to a level, paved area at least 61-m (200-ft) from open water.
5. Soak the plankton net, cod-end, plankton rope, net anchor, dredge, sieve, plant rake, and surface scraper in a plastic tub containing 2% solution of Virkon Aquatic for 20 minutes. Virkon Aquatic re-used for 1-week.
6. Using the spray bottle of 2% Virkon Aquatic or undiluted Formula 409 Disinfectant, thoroughly spray down the exterior boat hull, interior hull, bench seats, flooring, through-hull fittings, and exterior of lower engine unit. Let soak for 10 minutes.
7. Repeat step #6, and soak for another 10 minutes.
8. Using a bristle brush, scrub the boat hull, benches, flooring and engine exterior casing.
9. Rinse boat surfaces with spray bottle containing tap water.
10. Position an empty plastic tube under engine lower unit so that prop is inside tub, and cavitation plates are lower than the rim of the plastic tub.
11. Add tap water to tub until water surface reaches at least the bottom of the cavitation plates (approximately 13-gallons).
12. Put the engine in neutral, and start the boat engine. Run engine for approximately two minutes. Do not return cooling water discharged from engine into the tub. Do not allow water level in tub to fall below cavitation plates. Keep extra tap water at hand when running engine to maintain water level. Stop engine if cooling water discharge becomes hot, and replace the water.
13. Stop engine after flushing cooling lines. Raise lower engine unit out of tub, and slide tub of water clear of engine area.
14. Add approximately 2.5-L (0.7-gallons) of household bleach to the tub containing approximately 49-L (13-gallons) of tap water. This is a 5% solution of bleach, and this solution is caustic and appropriate safety equipment should be worn.
15. Soak the plankton net, cod-end, plankton rope, net anchor, dredge, sieve, plant rake, and surface scraper in the plastic tub containing 5% solution of bleach for 15 minutes.

16. Remove equipment from bleach solution, and thoroughly rinse with tap water.
17. The plankton net and the cod-end are then soaked in 5% acetic acid solution for a minimum of 6-hrs. The preferred soak time in acetic acid is 24-hour.
18. Plug the bilge drain. Transfer the 49-L (13 gallons) of 5% bleach solution into the boat, washing down seats, flooring, and other surfaces. Use the scrub brush to wash down the insides of the boat using the 5% bleach solution. Focus efforts on rinsing all surfaces and forcing all debris into boat bilge area. Let bleach solution soak in boat bilge for 15 minutes.
19. Pull bilge plug on impervious surfaces to drain bilge when at least 61-m (200-ft) from open water.

Appendix B

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
12/16/2014	SWW	DME	FY14-8173	66	2	0.2000	45	18	27	0	0	6	Prineville Reservoir, 8/5/14
12/16/2014	SWW	DME	FY14-8173	7.5	2	0.2000	5	2	27	20*	0	0	*Blind matrix spike sample
				73.5			**50**	**20**	**27**	**0**	**0**	**6**	
1/10/2015	TDR	DME	FY14-8145	23	3	0.1000	20	6	26	0	0	2	Prineville Reservoir, 8/5/14
				23			**20**	**6**	**26**	**0**	**0**	**2**	
1/5/2015	RP	DM750	FY14-8175	2	2	0.1111	9	2	100	0	0	2	Lake Billy Chinook, 8/4/14
1/5/2015	RP	DM750	FY14-8175	13	2	0.0714	29	4.1	32	0	0	3	
				15			**38**	**6.1**	**66**	**0**	**0**	**5**	
1/11/2015	TDR	DME	FY14-8142	2	3	0.1250	3	1.1	56	0	0	2	Lake Billy Chinook, 8/4/14
1/11/2015	TDR	DME	FY14-8142	8	3	0.1000	7	2.1	26	0	0	6	
1/11/2015	TDR	DME	FY14-8142	4	3	0.1000	3	0.9	23	5*	0	0	*Blind matrix spike sample
				14			**13**	**4.1**	**105**	**0**	**0**	**8**	
1/9/2015	LC	DM750II	FY14-8144	40	2	0.1111	39	8.7	22	0	0	1	Lake Billy Chinook, 8/4/14
1/9/2015	LC	DM750II	FY14-8144	10	2	0.1111	10	2.2	22	14*	0	1	*Blind matrix spike sample
1/13/2015	TDR	DME	FY14-8144	22	3	0.1000	18	5.4	25	0	0	0	
1/13/2015	TDR	DME	FY14-8144	10	3	0.1000	7	2.1	21	15*	0	0	*Blind matrix spike sample
				82			**74**	**18.4**	**22**	**0**	**0**	**2**	

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
2/3/2015	SWW	DME	FY14-8239	35.5	2	0.1250	29	7.3	20	0	0	2	Emigrant Lake, 8/8/14
2/3/2015	SWW	DME	FY14-8239	5	2	0.1250	4	1.0	20	12*	0	0	*Blind matrix spike sample
				40.5			**33**	**8.3**	**20**	**0**	**0**	**2**	
2/9/2015	SWW	DME	FY14-15006	15	2	0.2000	26	10.4	69	0	0	35	Howard Prairie Lake, 8/7/14
2/9/2015	SWW	DME	FY14-15006	5	2	0.2000	4	1.6	32	16*	0	0	
				20			**30**	**12**	**51**	**0**	**0**	**35**	
2/12/2015	SWW	DME	FY14-8092	7.5	2	0.1667	5	1.7	22	8*	0	1	Upper Klamath Lake, 8/6/14
2/12/2015	SWW	DME	FY14-8092	30.5	2	0.1667	23	7.7	25	0	0	0	*Blind matrix spike sample
				38			**28**	**9.3**	**24**	**0**	**0**	**1**	
2/13/2015	SWW	DME	FY14-8102	20.5	2	0.1429	20	5.7	28	0	0	76	Howard Prairie Lake, 8/7/14
				20.5			**20**	**5.7**	**28**	**0**	**0**	**76**	
12/10/2014	LC	DM750II	FY14-8154	25.5	2	0.1111	26	5.8	23	0	0	2	Prineville Reservoir, 8/5/14
12/11/2014	LC	DM750II	FY14-8154	120.5	2	0.1111	122	27.1	22	0	0	14	
12/11/2014	LC	DM750II	FY14-8154	7.5	2	0.1111	8	1.8	24	17*	0	1	*Blind matrix spike sample
				153.5			**156**	**34.7**	**23**	**0**	**0**	**17**	
12/31/2014	LC	DM750II	FY14-8153	20	2	0.1111	20	4.4	22	0	0	27	Lake Billy Chinook, 8/4/14
				20			**20**	**4.4**	**22**	**0**	**0**	**27**	
1/2/2015	LC	DM750II	FY14-8176	12	2	0.1111	12	2.7	22	0	0	2	Lake Billy Chinook, 8/4/14
				12			**12**	**2.7**	**22**	**0**	**0**	**2**	

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
1/6/2015	LC	DM750II	FY14-8174	4	2	0.1111	4	0.9	22	0	0	0	Lake Billy Chinook, 8/4/14
1/7/2015	LC	DM750II	FY14-8174	17	1	0.1111	37	4.1	24	0	0	3	
				21			**41**	**5**	**23**	**0**	**0**	**3**	
1/7/2015	LC	DM750II	FY14-8146	12.5	2	0.1111	12	2.7	21	0	0	5	Lake Billy Chinook, 8/4/14
1/8/2015	LC	DM750II	FY14-8146	26.5	2	0.1111	27	6.0	23	0	0	11	
				39			**39**	**8.7**	**44**	**0**	**0**	**16**	
1/8/2015	LC	DM750II	FY14-8177	26	2	0.1111	26	5.8	22	0	0	3	Lake Billy Chinook, 8/4/14
				26			**26**	**5.8**	**22**	**0**	**0**	**3**	
1/9/2015	LC	DM750II	FY14-8136	24	2	0.1111	24	5.3	22	0	0	1	Lake Billy Chinook, 8/4/14
1/9/2015	LC	DM750II	FY14-8136	10	2	0.1111	10	2.2	22	15*	0	0	*Blind matrix spike sample
				34			**34**	**7.6**	**22**	**0**	**0**	**1**	
2/7/2015	LC	DM750II	FY14-8245	24	2	0.0667	40	5.3	22	0	0	13	Emigrant Lake, 8/8/14
2/9/2015	LC	DM750II	FY14-8245	41.5	2	0.0667	68	9.1	22	0	0	25	
2/10/2015	LC	DM750II	FY14-8245	24	2	0.0667	39	5.2	22	0	0	20	
				89.5			**147**	**19.6**	**22**	**0**	**0**	**58**	
2/10/2015	LC	DM750II	FY14-8242	55	2	0.1111	57	12.7	23	0	0	12	Emigrant Lake, 8/8/14
2/11/2015	LC	DM750II	FY14-8242	62.5	2	0.1111	63	14.0	22	0	0	5	
2/11/2015	LC	DM750II	FY14-8242	5	2	0.1111	5	1.1	22	9*	0	2	*Blind matrix spike sample
2/11/2015	SWW	DME	FY14-8242	36.5	2	0.0909	40	7.3	20	0	0	12	
2/12/2014	SWW	DME	FY14-8242	32	2	0.1667	25	8.3	26	0	0	24	
				191			**190**	**43.4**	**23**	**0**	**0**	**55**	

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells **PAGE** 4 **of** 6

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
2/17/2015	SAC	DM1000	FY14-8082	14	3	0.1000	14	4.2	30	0	0	30	Howard Prairie Lake, 8/7/14
				14			**14**	**4.2**	**30**	**0**	**0**	**30**	
2/16/2015	SWW	DME	FY14-8081	4	2	0.2000	3	1.2	30	0	0	1	Upper Klamath Lake, 8/6/14
2/16/2015	SWW	DME	FY14-8081	32.5	2	0.1429	31	8.9	27	0	0	7	
				36.5			**34**	**10.1**	**29**	**0**	**0**	**8**	
2/16/2015	SWW	DME	FY14-8098	23	2	0.1429	25	7.1	31	0	0	20	Prineville Reservoir, 8/5/14
				23			**25**	**7.1**	**31**	**0**	**0**	**20**	
2/16/2015	SWW	DME	FY14-8093	30	2	0.1667	28	9.3	31	0	0	1	Upper Klamath Lake, 8/6/14
2/16/2015	SWW	DME	FY14-8093	3	2	0.1667	2	0.7	22	38*	0	0	*Blind matrix spike sample
				33			**30**	**10**	**27**	**0**	**0**	**1**	
2/17/2014	SWW	DME	FY14-8084	5	2	0.0909	3	0.5	11	0	0	0	Upper Klamath Lake, 8/6/14
2/17/2014	SWW	DME	FY14-8084	32.5	2	0.1667	28	9.3	29	0	0	10	
				37.5			**31**	**9.9**	**20**	**0**	**0**	**10**	
2/15/2015	MS	DM750	FY14-8241	27.5	3	0.0588	47	8.3	30	0	0	0	Emigrant Lake, 8/8/14
2/17/2014	SWW	DME	FY14-8241	16	2	0.0909	18	3.3	20	0	0	7	
				43.5			**65**	**11.6**	**25**	**0**	**0**	**7**	
2/17/2015	SWW	DME	FY14-15003	20	2	0.1667	20	6.7	33	0	0	55	Howard Prairie Lake, 8/7/14
				20			**20**	**6.7**	**33**	**0**	**0**	**55**	
2/17/2015	SWW	DME	FY14-8094	30	2	0.1667	24	8	27	0	0	7	Upper Klamath Lake, 8/6/14
				30			**24**	**8**	**27**	**0**	**0**	**7**	

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells **PAGE** 5 **of** 6

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
2/17/2015	SWW	DME	FY14-8101	27	2	0.1667	19	6.3	23	0	0	3	Upper Klamath Lake, 8/6/14
				27			**19**	**6.3**	**23**	**0**	**0**	**3**	
2/18/2015	SWW	DME	FY14-8080	5	2	0.1429	4	1.1	23	22*	0	0	Upper Klamath Lake, 8/6/14
2/18/2015	SWW	DME	FY14-8080	34.5	2	0.1429	31	8.9	26	0	0	0	*Blind matrix spike sample
				39.5			**35**	**10**	**24**	**0**	**0**	**0**	
2/18/2015	SWW	DME	FY14-8091	22	2	0.1667	21	7	32	0	0	55	Prineville Reservoir, 8/5/14
				22			**21**	**7**	**32**	**0**	**0**	**55**	
2/18/2015	LC	DM750II	FY14-8090	26	2	0.1111	26	5.8	22	0	0	0	Upper Klamath Lake, 8/6/14
				26			**26**	**5.8**	**22**	**0**	**0**	**0**	
2/18/2015	LC	DM750II	FY14-8000	3	2	0.1111	3	0.7	22	0	0	0	Applegate Reservoir, 9/11/14
				3			**3**	**0.7**	**22**	**0**	**0**	**0**	(ODFW collection)
2/18/2015	LC	DM750II	FY14-8185	3	2	0.1111	3	0.7	22	0	0	0	Applegate Reservoir, 9/11/14
				3			**3**	**0.7**	**22**	**0**	**0**	**0**	(ODFW collection)
1/6/2015	MS	DM750	FY14-8178	14	3	0.1250	35	13.1	94	0	0	0	Prineville Reservoir, 8/5/14
1/7/2015	MS	DM750	FY14-8178	11	3	0.1250	29	10.9	99	0	0	4	
				25			**64**	**24**	**96**	**0**	**0**	**4**	
12/31/2014	LC	DM750II	FY14-8009	3	2	0.1111	3	0.7	22	0	0	5	Applegate Reservoir, 9/11/14
				3			**3**	**0.7**	**22**	**0**	**0**	**5**	(ODFW collection)

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Portland State University **Point of contact:** Steve Wells

Date Analysis	Analyst initials	Scope #	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
12/31/2014	LC	DM750II	FY14-8158	12.5	2	0.1111	14	3.1	25	0	0	0	Applegate Reservoir, 9/11/14
				12.5			**14**	**3.1**	**25**	**0**	**0**	**0**	(ODFW collection)
11/10/2015	TDR	DME	FY14-8328	3	3	1.0000	1	3	100	0	0	4	Applegate Reservoir, 9/11/14
				3			**1**	**3**	**100**	**0**	**0**	**4**	(ODFW collection)
11/10/2015	TDR	DME	FY14-8276	9	3	0.1250	7	2.6	29	0	0	0	Applegate Reservoir, 9/11/14
				9			**7**	**2.6**	**29**	**0**	**0**	**0**	(ODFW collection)