# Zebra and Quagga Mussel and Other Aquatic Invasive Species Early-Detection Monitoring in Eastern Oregon

**2012 Final Report**

S. Wells and M. Sytsma

Center for Lakes and Reservoirs at Portland State University

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## **Abstract**

Five Oregon water bodies located east of the Cascade Mountain Range (Lake Billy Chinook, Prineville Reservoir, Ochoco Reservoir, East Lake and Paulina Lake) were each sampled twice during the summer of 2012 for the early detection of zebra (*Dreissena polymorpha*) and quagga (*D. rostriformis bugensis*) mussels and other aquatic invasive species. Zebra and quagga mussels were not detected. Two non-native snails were detected as well as one non-native submersed aquatic plant. Big-eared radix (*Radix auricularia*) snails were detected in both East Lake and Lake Billy Chinook, and to the authors' knowledge these are new detections. New Zealand mud snails (*Potamopyrgus antipodarum*) and curlyleaf pondweed (*Potamogeton crispus*) were detected in Lake Billy Chinook.

## **Introduction**

Aquatic invasive species (AIS) are spreading rapidly through the western United States. These nonnative species degrade habitat for native species, interfere with recreation, and alter water quality. Many AIS are transported and introduced on trailered watercraft and on recreational gear. Some AIS are of particular concern because of their impacts, including *Dreissena* spp. (zebra and quagga mussel), *Hydrilla verticillata* (water thyme/ hydrilla), *Trapa natans* (water chestnut), *Myriophyllum* spp. (Eurasian watermilfoil and parrotfeather), *Egeria densa* (Brazilian elodea), *Potamopyrgus antipodarum* (New Zealand mud snail), and *Orconectes rusticus* (rusty crayfish).

*Dreissena* spp. mussels are invasive epifaunal freshwater mussels that cause extensive economic and ecological impacts in areas they are not native. These mussels have established populations west of the Rocky Mountains in California and Nevada, and larvae have been detected in multiple water bodies in Colorado and Utah. Watercraft with attached, hitchhiking mussels are repeatedly detected being trailered into Oregon or surrounding states. The risk posed to Oregon water bodies by the proximity of these infestations is significant.

Invasive aquatic plants or 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. *Hydrilla* is present in a tributary to the Snake River in Idaho. Other invasive macrophytes such as *Myriophyllum spicatum* (Eurasian watermilfoil) have established populations in Oregon water bodies.

*Potamopyrgus antipodarum* is an invasive 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. Once established, these snails may out-compete native invertebrate grazers that provide important food resources for fish. *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.

Prevention and containment efforts for AIS are dependent on efficient early detection and 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) was contracted by the Oregon Department of Agriculture and the Bureau of Land Management to conduct early detection monitoring efforts in high-risk Oregon water bodies located east of the Cascade Mountain Range during the summer of 2012 for the presence/ non-detect of *Dreissena* spp. mussels and other AIS.

## **Methods**

Standard Field Protocols (SFPs) were developed by PSU for the collection of both larval and adult life stages for *Dreissena* spp. mussels, as well as the collection of other AIS invertebrates and macrophytes and water quality (Appendix A). The objective of these field collection efforts was early detection, i.e. presence/ non-detect. Therefore, a targeted sampling design was employed to increase the likelihood of collection, i.e. sampling was conducted in areas most likely to contain target species using methods that emphasized large sample size and qualitative data. SFPs included equipment decontamination protocols to prevent the unintentional transport of organisms between water bodies as well as field datasheets, multi-probe calibration procedures and a pre-launch checklist.

The Oregon water bodies located east of the Cascade Mountain Range that were targeted for early detection monitoring were identified from a monitoring priority assessment for the Columbia River Basin (Wells et al. 2009) that identified 45 water bodies in Oregon that are at high risk for introduction or establishment of *Dreissena* spp. mussels. 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 2012. Monitoring was focused on water bodies with a high to medium likelihood of *Dreissena* spp. mussel introduction and/or establishment according to Wells et al. (2009). These water bodies received large amounts of boater recreational use and/ or exhibited dissolved calcium concentrations and pH values conducive for mussel survival and growth. Sampling types and locations are shown in Figures 1- 4. Lake Billy Chinook, Prineville Reservoir, Ochoco Reservoir, East Lake and Paulina Lake were sampled in the period of August 21- 23, 2012 and September 12-14, 2012.



**Figure 1: PSU early detection AIS sampling locations on East Lake, OR and Paulina Lake, OR during 2012.**

#### **Planktotrophic bivalve larvae**

*Dreissena* spp. larvae, which develop as they float in the water column and hereafter are referred to as veligers, were sampled with plankton samples collected using a 63-µm mesh simple, conical plankton net according to the SFPs (Appendix A) and based on previous SFPs jointly developed by PSU and the US Bureau of Reclamation maintained at [www.musselmonitoring.com.](http://www.musselmonitoring.com/) Plankton samples were collected at a minimum of 15 locations within each water body using a boat in near shore and in the open water areas and focused on areas near boat launches, marinas, dams, outflows downstream and downwind position 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 collect plankton throughout the water column at discrete spatial locations. Trawling collects plankton at discrete water depths across a large horizontal spatial area. The plankton collected from several tows or trawling events were composited into the sample container, labeled and preserved using regular ethanol to reach a final solution of 70% ethanol. Sampling effort was similar in all the water bodies (Table 1). Preserved plankton samples were buffered with a small amount of sodium bicarbonate  $\leq 1$  gram NaHCO<sub>3</sub> per 500-mL sample) to maintain the pH above 6.8.



**Figure 2: PSU early detection AIS sampling locations on Lake Billy Chinook, OR during 2012.**



**Figure 3: PSU early detection AIS sampling locations on Ochoco Reservoir, OR during 2012.**

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**Figure 4: PSU early detection AIS sampling locations on Prineville Reservoir, OR during 2012.**





Plankton samples were analyzed at the Veliger Identification Laboratory at PSU for the presence of planktotrophic bivalve veligers using cross-polarized microscopy. Large zooplankton were removed using 500-µm filters, and plankton samples were concentrated using gravity and centrifugation. The settled particulate of the plankton sample was placed in multiple centrifuge tubes and covered with ethanol. Twenty percent of the settled particulate, or concentrated sample, was microscopically analyzed in one-, two- or three-mL Sedgewick-Rafter cells using Leica DME and DM750 compound microscopes fitted with polarizing filters. The concentrated sample was diluted in the counting chamber using 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 DFC290 and 295 cameras for documenting suspect specimens. Shell measurements were made with digital micrometer to assist in identification. Microscopic analysis was non-destructive and large zooplankton, supernatant and concentrated sample were retained in original sample container and stored at PSU in 70% ethanol.

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 50-mL of previously analyzed, *Dreissena*-free sample with a similar matrix of sediment and algae. One spiked matrix sample was analyzed in every batch of samples. Failure to detect the veligers in the spiked sample required reanalysis of all samples in the batch. 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.

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 would be performed if deemed appropriate by PSU or requested and funded by ODA or BLM. Cross-validated positive results for *Dreissena* spp. mussels would result in the immediate notification of appropriate agency staff in ODA and BLM.

## **Adult bivalves and snails**

Adult *Dreissena* spp. mussels as well as other non-native bivalves and snails were sampled opportunistically using multiple methods including artificial settlement substrates, tactile and visual inspections of existing submersed surfaces and shoreline areas, a surface scraper, a sediment grab sampler and a thatch rake on a rope. Suspect specimens, snails and non-native mussels were retained and placed in sampler containers with lake water and transported to the laboratory for identification held on ice. GPS location and preliminary species identifications were noted on field datasheets. Native mussels, e.g. *Anodonta* spp., if collected, were photographed and released. Identifications were verified by Robyn Draheim and Steve Wells at PSU and by using Harrold and Guralnick (2010), Lysne (2009), Nedeau et al. (2009), Perez et al. (2004) and Thorp and Rogers (2011).

Artificial settlement substrates, which are often called "Portland Samplers", are used for early detection and monitoring of newly-settled juvenile and adult *Dreissena* mussels as wells as *P. antipodarum* that colonize substrate surfaces. For example, in 2002, a settlement substrate in Garrison Lake, Oregon facilitated the first detection of *P. antipodarum* in that water body. An artificial settlement substrate consists of multiple pvc and abs pipe sections oriented horizontally and secured at different water depths along a rope that is woven through strips of plastic mesh and weighted

with a small concrete anchor at end, near or on the reservoir/ lake bottom. Settlement substrates are tied to a secure surface structure and suspended in the water throughout the year. Settlement substrates deployed in the summer of 2011 were used when available, and new substrates were deployed during the first sampling trip during 2012, and replaced if needed during the second trip. Inspections 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 bivalves and adult snails 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 opportunistically sample submerged portions of hard, smooth surfaces including concrete walls, bridge abutments, pilings, 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<sup>TM</sup> grab sampler to sample sand, silt, gravel, and small rock substrate for the presence of bivalves and snails. 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 for the presence of bivalves and snails.

Submersed macrophytes were collected to sample for attached bivalves and snails. *P. antipodarum* were first detected in Lake Billy Chinook by sampling *Elodea canadensis* beds. Submersed macrophytes 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<sup>2</sup>. Macrophyte sampling occurred opportunistically at locations with plant beds visible from water surface, in areas near marinas and boat launches, and in shallow littoral areas. The collected macrophytes were visually inspected for bivalves and snails and then shaken in 5-gal buckets of water to detach invertebrates. Bucket water was poured through a sieve and the sieve and bucket were inspected for bivalves and snails.

## **Crayfish**

Crayfish were sampled using modified Gee minnow traps based on protocols outlined by Larson and Tait (2011). Three to five minnow traps were baited with wet cat food and deployed from late evening to early morning in shallow areas (< 2-m depth) with hard substrate. Crayfish sampling was done opportunistically at water bodies that were proximate to where field survey crews were camping. Captured crayfish were identified in the field, preserved in regular ethanol and transported to the laboratory in sample containers for verification. Trap GPS location, general site description and the preliminary identifications were recorded on field datasheets. Identifications were verified by Steve Wells at PSU using Fetzner, Jr. (2006), Larson and Olden (2011), Larson and Tait (2011), Olden (2009) and Pearl et al. (2011).

#### **Submersed macrophytes**

Submersed macrophytes were opportunistically sampled with a thatch rake on a rope for the early detection of AIS. Submersed macrophytes were collected from a boat by throwing a thatch rake

attached to a rope, allowing to sink and then dragging for approximately 1- to 2-m along the sediment, which sampled an area of approximately 0.3-m<sup>2</sup>. Macrophyte sampling occurred opportunistically at locations with plant beds visible from water surface, in areas near marinas and boat launches, and in shallow littoral areas. GPS location, sample depth, and preliminary species identifications were noted on field datasheets. Voucher specimens were placed in labeled plastic bags with water and placed on ice for verification in the laboratory. Field species identifications were verified by Steve Wells at PSU using Crow and Hellquist (2000, 2006), Hamel and Parsons (2001), and Brayshaw (2001). Selected species were pressed and stored at PSU.

#### **Water quality data collection**

Water temperature, dissolved oxygen, pH and specific conductivity were measured near the deepest location of each water body using either a Eureka Manta<sup>TM</sup> or Hydrolab Quanta<sup>TM</sup> water quality multi-probe. Accuracy of specific conductivity and pH sensors was assured by calibration at the start of each sampling day using NIST certified 500-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 2-m measurements after profiles were completed.

Water transparency was measured using a Secchi disk. The depth of disappearance on the way down, and the depth of reappearance on the way up of a 20-cm diameter Secchi disk were recorded from the sunny side of the boat by two separate observers. Values were recorded in field datasheets as well as time of day and general weather.

#### **Field and laboratory equipment decontamination**

Field and laboratory equipment were decontaminated using physical scrubbing and both acid and bleach solutions to prevent the transfer of larvae and genetic material between samples and the unintentional transfer of organisms to new water bodies. Physical scrubbing with a stiff bristle brush or one's fingers removed organisms and other debris. Acid solutions (5% acetic acid and 4% hydrochloric acid) were used to dissolve the shells of veligers, which are composed of calcium carbonate. Genetic material was denatured using a solution of 5% household bleach (50 mL bleach in 1 L of water). Additionally, multiple sets of field equipment were used to allow complete air drying after decontamination and before future use.

Sampling gear such as plankton nets, cod-end pieces, net rope and net anchors were soaked in 5% bleach solution for at least 15 minutes, thoroughly rinsed with tap water and then soaked in 5% acetic acid solution for at least 8 hours. Anchors, thatch rake, sediment dredge, rope, sieve, surface scraper and secchi disk were scrubbed with a bristle brush to remove debris, soaked in 5% bleach solution for at least 15 minutes and then thoroughly rinsed with tap water. Engine cooling water was decontaminated by lowering engine lower unit into a bucket containing fresh tap water and running the engine for approximately five minutes to thoroughly flush cooling system. The boat hull, through-hull fittings and trailer were scrubbed with the bristle brush to remove mud, plants and other visible contaminants and then sprayed with 5% bleach solution. The 5% bleach water used for soaking equipment was then poured into boat and used with the brush to scrub down seats, flooring and inside of boat. Bleach water was held in boat bilge for at least 30 minutes and then bilge drain plug was pulled when trailered boat was on pavement at least 200-ft from open water.

Laboratory equipment and surfaces were decontaminated using both acid and bleach solutions to prevent the transfer of larvae and genetic material between samples. Laboratory decontamination for genetic material in microscopy laboratories was necessary because multiple analytical methods may be used with the same plankton sample to confirm identification made via light microscopy. Equipment such as counting chambers, pipettes, filters, and settling cones were soaked in a solution of 5% household bleach for at least 10 minutes, rinsed with fresh water and soaked in acid solution for a minimum of 8 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 and plastic trays, 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.

#### **Online database**

*Dreissena* spp. mussel early detection monitoring locations were entered into a database and presented in an online interactive map maintained by PSU. The online interactive map visually displays monitoring locations and allows data queries to show site-specific information such as the status of a particular monitoring event. The link to the online interactive map is [<http://mussels.geos.pdx.edu/>](http://mussels.geos.pdx.edu/).

## **Results**

Sampling was done when water temperatures were ideal for *Dreissena* spp. mussel spawning (Figures 5 and 6). Water temperatures between 16 and 19˚ C are considered optimal for *Dreissena* mussel spawning (Claxton and Mackie 1998; Garton and Haag 1993; McMahon 1996; Nichols 1996). Sampling was targeted at areas likely to contain *Dreissena* spp. mussels and other AIS, and sampling effort was distributed throughout the water body. Sampling was conducted when epilimnion water temperatures were between 16° and 22°C. Most of the water bodies were thermally stratified during sampling. The dissolved oxygen concentrations at Ochoco Reservoir may have been limiting to *Dreissena* spp. populations in water depths greater than 12 m during the summer period.

Specific conductance was fairly uniform throughout depth within a water body. The specific conductance measured during the August and September sampling trips during 2012 was  $334 \pm$ 1.515  $\mu$ S/ cm (mean  $\pm$  1SD; n = 16) and 331  $\pm$  2.109  $\mu$ S/ cm (mean  $\pm$  1SD; n = 21), respectively for East Lake,  $201 \pm 4.651 \mu\text{S/m}$  (mean  $\pm 1$ SD; n = 21) and  $127 \pm 22.87 \mu\text{S/m}$  (mean  $\pm 1$ SD; n  $=$  21), respectively for Lake Billy Chinook, 216  $\pm$  3.043  $\mu$ S/ cm (mean  $\pm$  1SD; n = 21) and 215  $\pm$  $3.273 \text{ uS/m}$  (mean  $\pm$  1SD; n = 21), respectively for Ochoco Reservoir, 602  $\pm$  7.202  $\mu$ S/ cm (mean  $\pm$  1SD; n = 21) and 596  $\pm$  6.939  $\mu$ S/ cm (mean  $\pm$  1SD; n = 21), respectively for Paulina Lake and  $122 \pm 30.10 \,\mu\text{s/m}$  (mean  $\pm 1$ SD; n = 21) and  $203 \pm 3.868 \,\mu\text{s/m}$  (mean  $\pm 1$ SD; n = 21), respectively for Prineville Reservoir. Secchi transparency ranged from 1.2 m in Prineville Reservoir to 7.2 m in East Lake (Table 2).

#### **Planktotrophic bivalve larvae**

A total of 47 plankton samples (500-mL to 1-L total sample volume) were collected and analyzed by PSU (Table 1). More than 1.8 million liters of lake/ reservoir water were filtered through 64-µm

plankton nets during sampling that occurred during August and September. A net efficiency of 60% was used to calculate the volume of water filtered, which allowed for 40% clogging of net mesh. Sampling effort was similar among the water bodies (Table 2). More plankton samples were collected during the September sampling trip compared to the August trip, but the relative sampling effort was similar, i.e. the amount of water filtered.



**Figure 5: Profiles of pH, dissolved oxygen (mg/ L) and water temperature (C) in East Lake Lake, Billy Chinook, and Ochoco Reservoir.**

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**Figure 6: Profiles of pH, dissolved oxygen (mg/ L) and water temperature (C) in Paulina Lake and Prineville Reservoir.**



**Table 2: Secchi disk readings, i.e. water transparency, during August and September, 2012.**

Planktotrophic veligers e.g. *Dreissena* spp., were not detected in the plankton samples from East Lake, Lake Billy Chinook, Ochoco Reservoir, Paulina Lake and Prineville Reservoir during field sampling in 2012 (Table 3). Twenty percent, at minimum, of the concentrated sample of each plankton sample was microscopically analyzed at PSU. The concentrated sample represented the pelleted particulate in the plankton sample that was isolated from the lake water and ethanol using gravitational settlement and centrifugation. *Corbicula* spp. straight-hinge juveniles were detected in low densities in East Lake, Paulina Lake and Prineville Reservoir. Ostracods were detected in all water bodies sampled. Ostracods detected in plankton samples were not identified beyond the Class Ostracoda. Twelve blind matrix spike samples were submitted with the plankton samples, and the spiked *Dreissena* spp. veligers were detected in all spiked samples. Details of the microscopic analysis including the blind matrix spike samples are in Appendix B.

**Table 3: Summary of microscopic analysis of plankton samples at PSU for the early detection of zebra and quagga mussels planktotrophic larvae, veligers. Dreissena spp. veligers were not detected in samples. Samples were analyzed in 2- and 3 mL Sedgewick-Rafter counting cells, and the concentrated sample was diluted in counting cells as needed using ethanol. The total number of counting cells analyzed as well as the amount of the concentrated sample, or pelleted particulate that was analyzed in the plankton sample is shown. The number of planktotrophic bivalve veligers detected in samples is indicated (# ZQM). The number of Corbicula spp. straight-hinge juveniles is shown as well as the number of ostracods. Individual specimens in a cell were not counted beyond 20 and were thereafter indicated with a +.** 



#### **Adult bivalves and snails**

Adult *Dreissena* spp. mussels were not detected in any of the sampled water bodies. Several *Radix auricularia* (big-eared Radix) were collected from both East Lake and Lake Billy Chinook (Table 4). To the authors' knowledge, *Radix auricularia* has not been previously detected in either East Lake or Lake Billy Chinook. *Radix auricularia* have been found in other Oregon water bodies including Carmen Pond, Hemlock Lake, Ash Pond, Toketee Lake and Lost River. *Potamopyrgus antipodarum* (New Zealand mud snails) were collected from Lake Billy Chinook, but PSU previously collected these snails in Lake Billy Chinook in 2011. All other bivalve and gastropod species collected were native (e.g. *Stagnicola elodes*) or unidentified species that belong to taxonomic groups that have many native species and few AIS.



**Table 4: Adult and juvenile gastropods and bivalves collected during August and September, 2012.**

#### **Crayfish**

Crayfish were collected in Lake Billy Chinook and Ochoco Reservoir during 2012 field sampling (Table 5). All crayfish collected were native species, *Pacifasticus leniusculus* (signal crayfish).

**Table 5: Crayfish collected during August and September, 2012.**



#### **Submersed macrophytes**

The only non-native submersed macrophyte collected during August and September, 2012 was *Potamogeton crispus* (curly leaf pondweed) in Lake Billy Chinook (Table 6). *P. crispus* was previously found in Lake Billy Chinook. Several plants were not identified to species level due to the lack of morphological features, e.g. seeds. There were several *Myriophyllyum* spp. specimens that could not be identified to species level based solely on morphology, but none of these specimens were thought to be *M. spicatum*.





## **Discussion**

The monitoring efforts conducted by PSU in 2012 under this project provided valuable early detection data for zebra and quagga mussels and other AIS in five Oregon water bodies located east of the Cascade Mountain Range. More than 1.8 million liters of lake/reservoir water were filtered through 64-µm plankton nets in order to collect a total of 47 plankton samples (500-mL to 1-L total sample volume). The calculated quantity of the water body filtered with the plankton net during sampling was conservative. A 60% net efficiency was used to calculate the volume filtered. Efforts were focused on reducing the net clogging by reducing the length of the individual tows but collecting a greater number of tows. It is likely that net efficiency was higher than 60%, and more than 1.8 million liters of lake/reservoir water were filtered through the plankton net during *Dreissena* veliger sampling. The total plankton sample volume ranged from 500-mL to 1-L, and sampling effort was best described by the volume of water filtered compared to total number of samples collected.

The detection of spiked *Dreissena* spp. veligers in all twelve of the blind matrix spike samples indicated that the microscopic analysis conducted at PSU met quality control and quality assurance objectives.

It is likely that *R. auricularia* is more widely distributed in Oregon water bodies than currently known. The other known populations of *R. auricularia* in Oregon are not proximate to East Lake and Lake Billy Chinook, and these snails lack an operculum, or trap door, which helps them to resist desiccation during overland transport. *R. auricularia* is widely distributed in North America (Harrold and Guralnick 2010), but previously reported populations in Oregon were limited to the Umpqua River drainage in Douglas County and the Lost River in Klamath County. Other sampling efforts may have overlooked this species.

The population of *P. antipodarum* located in the Crooked River arm of Lake Billy Chinook is well established and offers insight to their habitat preferences and sampling techniques. *P. antipodarum* in Lake Billy Chinook were found in the highest densities for two consecutive years in water depths greater than 2-m by sampling submersed macrophytes with a plant rake and inspecting plants for snails. Inspecting shallow water areas surrounding these plant beds yielded low densities of snails. Inspecting submersed macrophytes in deeper areas as well as shallow water areas will likely improve early detection efforts for *P. antipodarum.*

The risks posed by zebra and quagga mussels and other AIS are significant and there is a likelihood of false negative results with early detection monitoring for zebra and quagga mussels and other rare and spatially clumped discrete organisms. The mussels exhibit spatial and temporal patchiness, and the sheer size of potential habitat makes collection difficult. Additionally, the interfering matrix in typical plankton samples complicates the detection of veligers under microscopes. *Dreissena* spp. mussels and other priority AIS, such as *Hydrilla verticillata,* were not detected during these sampling efforts, but non-detection is different than absent. If they are present in Oregon water bodies, their early detection will provide the greatest amount of time to organize and mount rapid response efforts.

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## Appendix A

## **Field Collection Protocols**

 $\quad$  for Aquatic Invasive Species

#### 2012

Steve Wells, Rich Miller and Vanessa Howard

#### **Contents**



## **ZQM Veligers**

**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 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 100'.
- Cluster one third of site locations near dam and spread out other sites according to horizontal and vertical distribution comments below.

#### Horizontal distribution

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

Reservoirs- near dams and outflows, open water areas, downwind positions, near shore areas such as marinas and boat launches, and other areas of eddies.

Vertical distribution, i.e. depth

River- entire water column, but focus efforts from 15 m to surface. Reservoir-entire water column, but focus efforts from 15-20 m to surface, or 1 m from bottom.

#### **Equipment List (for sampling one water body):**

- 
- (40) 250-mL sample containers (1) sharpie pen
- (2) 63-um mesh plankton net with cod-end (14-L) regular ethanol
- (2) rope wheels w/ 100' rope ea. watch or clock
- veliger datasheet (Appendix A) cooler with ice
- 
- (4) AA batteries knife
- (3) substrates (pvc, abs, concrete anchor, rope) (2) pencils
- (20) 500-mL sample containers **(a)** (15) 500-mL graduated cylinders
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	-
- GPS unit **surface scraper** 
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	-

#### **Sample Collection:**

- Combination of <u>at least</u> 10 vertical/ oblique tows and 5 trawling events per water body. When trawling, record site location at the start of trawling as well as the end. Trawling is done at lowest engine speed to avoid net clogging.
- Keep net and line clear of boat engine prop. Avoid snagging net on sharp objects.
- Keep net off bottom.
- Attach milk jug filled with gravel to net line approximately 1 m in front of net opening.
- Composite samples of sites within similar area of water body, e.g. dam. Fill sample container 30% full.
- Condense plankton in net and cod-end as much as possible prior to pouring into sample container.
- Do not preserve samples on boat. Keep samples in cooler on ice while on boat.

Vertical/ Oblique Plankton Tow

- 1. Secure the cod-end piece and check that the line is securely attached to plankton net. Attach additional weight (milk jug of gravel) to a second 3 m section of line that is tied to a loop placed in the net line approximately one meter in front of net opening.
- 2. Lower the net 15-20 m (50-67 ft) below water surface, or to 1 m above the sediment, whichever is deeper. Keep the net off the lake bottom. Record GPS location and the depth the net is lowered 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, may also be swirled gently 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. 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, and water will be forced to the surface prior to the net emerging from the water. 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 (0.25 m)  $d =$  depth to which the net is lowered (30 m)

#### Trawling

- 1. Secure the cod-end piece and check that the line is securely attached to plankton net. Attach additional weight (milk jug of gravel) to a second 3 m section of line that is tied to a loop placed in the net line approximately one meter in front of net opening.
- 2. Lower the net 10 m (33 ft) below surface of water. Keep net off lake bottom. Record GPS position (start), and start time of trawling on datasheet. Maintain net at this depth for at least 5 minutes while driving the boat in transect. Keep net and line clear of engine prop.
- 3. 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 GPS location (end), stop time of trawling, and an estimate of average boat speed.
- 4. Follow steps #4 through #5 used for vertical/oblique tows regarding condensing and collecting plankton from the net.

5. Lower the net to 5 m (16 ft) depth for next trawling event. Alternate depths of trawling between 5-10 m in order to capture different layers of water column near the thermocline.

#### **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. Add a small amount of sodium bicarbonate (< 1 g NaHCO<sub>3</sub> per 500-mL sample) to sample container to buffer the pH. Shake closed sample container to mix contents. Preserved samples are stored at temperatures equal to or less than room temperature.

#### **ZQM Adult and Juveniles**

**Objective:** early detection (presence/ non-detect) of rare adult mussels attached to hard surfaces.

#### **Site Locations**:

- Existing submerged hard surfaces including docks, pilings, channel markers, floating bathrooms, buoys, bridge abutments, seawalls, rocks, and logs.
- Artificial settlement substrates deployed from dam buoy line, speed limit buoys, floating bathrooms, docks, channel markers and other surface structures.
- Shoreline areas for dead shells.
- Focus efforts on the bottom and sides of objects; in protected and shaded areas such as nooks, crannies and junction of two different surfaces.
- Periphyton may obscure attached bivalves.
- Adult mussels are also sampled using methods covered in Invertebrates sampling section.

#### Horizontal distribution

River- in areas where the water currents and/ or wind patterns are likely to concentrate the planktonic larvae, e.g. near dam and outflow; main stem; near marinas, boat launches, and rocky substrate areas like rip rap; behind islands or downstream of obstructions that cause eddies, and along shore in areas of eddies.

Reservoirs- in areas where the water currents and/ or wind patterns are likely to concentrate the planktonic larvae, e.g. near dam and outflow; open water areas; near shore areas such as marinas, boat launches, and areas of eddies.

#### Vertical distribution

Entire water column but focus efforts 7.6 m (25 ft) to surface.

#### **Equipment List (for sampling one water body):**

- surface scraper GPS unit
- (3) substrates (pvc, abs, concrete anchor, rope) (4) AA batteries
- 
- datasheet (Appendix B) (2) pencils
- 
- 
- 
- 
- knife (1) sharpie pen
	-
- 1 gallon zip lock bags **a** waterproof paper labels
	- cooler with ice  $(2)$  250-mL sample containers

#### **Sample Collection:**

#### Existing submerged surfaces

- 1. Locate suitable existing surfaces to inspect. Surface scraper works well on vertical concrete walls, bridge abutments and cutwaters, channel markers, pilings, underwater booms, and breakwaters. Accessible surfaces (i.e. within arm's reach) are good candidates for visual and tactile inspections. These relatively easy-to-access surfaces include the undersides and sides of docks, vessel hulls, buoys, and the underside and sides of rocks found in shoreline areas.
- 2. Carefully position boat near structure to sample (e.g. channel marker) and maintain position either using the motor, securing boat to structure with bow line, or using current and wind to position boat against structure.

WARNING: Be careful not to pin arms between the boat and 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 inspected for the presence of bivalves 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).

6. When performing visual and tactile inspections on structures, carefully pat surface with the palm of your hand. Do not run your hand along surfaces because of sharp objects. Remove hard protruding objects for visual inspection.

7. Retain suspect specimens in 250-mL sample container. Record date, water body and waypoint number on waterproof paper label with a pencil, and insert into container with specimen. Place specimen in cooler on ice.

Artificial settlement substrates

- 1. Make sure substrate is complete and includes pvc (white) and abs (black) pipe sections suspended along a rope. 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 15 ft, e.g. dam buoy line.
- 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 7.6 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 on datasheet (Appendix B).
- 7. When checking the substrate, remove it slowly from the water to avoid specimen loss. Inspection for adults and juveniles *Dreissena* spp. mussels is both tactile and visual. You are looking for a bivalve attached to a hard surface. Small juveniles may feel gritty to the touch. A hand lens (10X magnification) may be used.
- 8. 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.
- 8. Retain suspect specimens in 250-mL sample container. Record date, water body and waypoint number on waterproof paper label with a pencil, and insert into container with specimen. Place specimen in cooler on ice.
- 9. Redeploy substrate after inspection.

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#### **Sample Preservation:**

Suspect specimens and waterproof paper labels are retained in 250-mL containers and placed in cooler on ice while in boat. Specimens should be submerged in 95% to 100% ethanol on shore. Preserved specimens can be kept at room temperature out of direct sunlight.

#### **Invertebrates**

**Objective:** opportunistic sampling for early detection and determining the distribution of *Dreissena* spp. (zebra/ quagga mussels), *Corbicula fluminea* (Asian clam), *Potamopyrgus antipodarum* (New Zealand mud snail), *Orconectes rusticus* (rusty crayfish), and *Procambarus clarki* (red swamp crayfish).

#### **Site locations:**

- macrophyte beds in large rivers and reservoirs
- shoreline areas including gravel, sand, mud, cobble, and woody debris.
- on the sides and bottoms of rocks, woody debris, buoys, artificial settlement substrates, and other submerged surfaces

#### **Equipment List (for sampling one water body):**

- thatch rake on rope GPS unit
- 5-gallon white-colored bucket (4) AA batteries
- 
- datasheet (Appendix B) (2) pencils
- 1 gallon zip lock bags <br>• digital camera
- 
- 
- 
- minnow traps rope
- trap stakes and clips **regular ethanol**
- sediment dredge
- 
- 
- surface scraper (1) sharpie pen
	-
	-
- cooler and ice  $\bullet$  (2) 500-mL sample containers
- waterproof paper labels (4) 250-mL sample containers
- metal sieve **cat food or other bait** 
	-
	-

#### **Sample Collection:**

#### Plant rake

- 1. Shake down macrophytes in white-colored 5-gallon bucket containing lake water. Look for crayfish, bivalves and snails on plants. Allow bucket 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. Discard debris in lake.
- 4. Record GPS location and sampling activities at each site on datasheet (Appendix B).
- 5. Retain suspect specimens in 250-mL sample container with lake water. Record the water body and way point number on waterproof paper label using pencil and insert into sample container. Place in cooler on ice.

#### Sediment dredge

- 1. Deploy the sediment dredge in areas of sand-mud in water depths of 20 ft or less as determined by plant rake sampling, proximate shoreline areas, sight viewer, and/ or randomly. Dump dredge contents into metal sieve and rinse in lake. Inspect for bivalves and snails.
- 2. Record GPS location and sampling activities at each site on datasheet (Appendix B).
- 3. Retain suspect specimens in 250-mL sample container with lake water. Record the date, water body and way point number on waterproof paper label using pencil and insert into sample container. Place in cooler on ice.

#### Submerged surfaces

- 1. Inspect for snails when sampling submerged surfaces for adult *Dreissena* spp. mussels including artificial settlement substrates, existing substrates within arms-reach, e.g. buoys, and the debris collected using the surface scraper.
- 2. Record GPS location and sampling activities at each site on datasheet (Appendix B).
- 3. Retain suspect specimens in 250-mL sample container with lake water. Record the date, water body and way point number on waterproof paper label using pencil and insert into sample container. Place in cooler on ice.

#### Shoreline walks

- 1. Walk in a zig-zag pattern parallel to shoreline in wade-able depths near boat launches and other areas for approximately 50 linear feet. Stop every other step to pull out loose rocks, cobble and woody debris and/ or aquatic plants to inspect for snails. Look for bivalve shells on top of sediment. Record GPS location. Retain suspect specimens.
- 2. Record GPS location and sampling activities at each site on datasheet (Appendix B).
- 3. Retain suspect specimens in 250-mL sample container with lake water. Record the date, water body and way point number on waterproof paper label using pencil and insert into sample container. Place in cooler on ice.

#### Crayfish traps

- 1. Deploy minnow traps at reservoirs and rivers when camping near the water. Bait traps using cat food or other bait and secure trap together with clips. Attach rope to traps and place in shallow littoral areas with hard substrate, preferably rock, from late evening through early morning, or as time permits. Attach rope to stake in ground. Chose trapping locations that are inconspicuous to avoid tampering, theft, etc.
- 2. Record GPS locations and sampling activities at each site on datasheet (Appendix B).
- 3. Retrieve and inspect traps. Photograph collected specimens, including the ventral and dorsal side of crayfish. Retain voucher specimens for crayfish in 250-mL or 500-mL sample containers.

#### **Sample Preservation:**

Suspect specimens are retained in sample containers with lake water and placed in cooler on ice while on the boat. Snails are placed in sample containers with water and held on ice for verification in the laboratory. Bivalves and crayfish are preserved. Decant lake water and add 95% to 100% regular ethanol to cover specimens when on shore. Preserved specimens can be stored at room temperature out of direct sunlight.

#### **Macrophytes**

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

#### **Site Locations**:

- 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.
- Near boat ramps, marinas, and in bays and inlets.
- The geographic spread of water body, i.e. across the entire water body.
- Random locations during veliger sampling.

#### **Equipment List (for sampling one water body):**

- thatch rake on rope **GPS** unit
- waterproof paper labels (4) AA batteries
- 
- datasheet (Appendix C) (2) pencils
- 
- bathymetric maps  $\bullet$  cooler and ice
- 
- 
- 5-gallon white-colored bucket macrophyte identification books
	-
- 1 gallon zip lock bags **depth sounder or secchi disk depth sounder or secchi disk** 
	-

#### **Sample Collection:**

- 1. Position boat near plant bed or area to sample. Anchor boat only when necessary, e.g. windy conditions.
- 2. Record GPS location and depth. If no depth sounder available, depth can be determined using secchi disk.
- 3. Throw rake into or beyond the plant bed. 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.
- 4. Remove plants from rake and deposit into white-colored 5-gallon bucket half filled with lake water. Vigorously shake plants in water to remove invertebrates.
- 5. Sort plants.

At each site, select a representative specimen for each species; this specimen should have as many portions of the plant (submersed leaves, floating leaves, inflorescences, seeds/fruit, rhizomes, roots, etc) represented as possible. Place the specimens representing all species present at one site into a zip lock bag with enough lake water to cover plants. Record the date, water body, and waypoint number on the waterproof paper label using a pencil.. Place label into zip lock bag with plants and water. On datasheet, record the preliminary identifications for the specimens for that particular site. . Place bags of plants in cooler on ice. Do not freeze.

- 6. If you suspect you've found one of the high priority EDRR species listed below, retain several specimens in a separate zip lock bag for verification at PSU. These should be placed in a zip lock bag filled with lake water. Record the date, water body, way point number(s), and field id on waterproof paper label using pencil and insert into zip lock bag with plants and water. Place bags of plants in cooler on ice. Do not freeze.
- 7. Discard the other plants back into water body.
- 8. Use the view finder to look for species that were not collected at the site. Repeat rake toss if necessary.

#### **Sample Preservation:**

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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. Refrigerate plants upon returning to laboratory prior to confirming identifications and pressing voucher specimens. Any samples of *Myriophyllum* (milfoil) that do not cleanly key out to a species should be sent out for molecular tests. Voucher specimens are pressed once identifications are confirmed.

#### **High Priority EDRR Species**

#### **Submerged**

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

#### Floating

- 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*)\*

\* Record the presence of yellow flag iris, but do not collect specimens unless unusual flowers/foliage is noted

#### **Water Quality/ Multi-Probe Unit**

**Objective:** obtain relatively accurate data for water temperature, specific conductance, pH and dissolved oxygen along depth profiles, as well as collecting other metadata.

#### **Site locations:**

Dam, within main channel, or at deepest open water site.

#### **Equipment List (for sampling one water body):**

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

#### **Multi-probe unit calibration:**

#### **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. Rinse calibration cup, lid, and probes 3X with DI water. Discard water.
- 2. Rinse 2X with small amount of "used" conductivity standard. Discard standard.
- 3. Turn off unit circulator for conductivity calibration. Hold unit upside down (probes facing up).
- 4. Add enough "new" conductivity standard to cover probes, and equilibrate for several minutes.
- 5. Record the temperature of the standard and initial conductivity reading on calibration sheet (Appendix E).
- 6. Calibrate unit to appropriate value for conductivity standard (e.g.  $100 \mu S/cm$ ).
- 7. Record the second conductivity reading as well as the time for values to stabilize ( $\pm 0.01 \,\mu\text{s/m}$ ). Retain standard in container marked "used" for rinsate in subsequent calibrations.
- 8. Acceptable range= 7% from reference (e.g. 93 to 107 µS/ cm for 100 µS/ cm conductivity standard).
- 9. Repeat steps #1-7 if calibration fails.

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#### pH

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

- 1. Rinse cup, lid, and probes 3X with DI water. Discard water.
- 2. Rinse 2X 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. Record the temperature of standard, and initial pH 7 reading on calibration sheet (Appendix E).
- 6. Determine the temperature-corrected pH value using the Table of pH Calibration Standards (Appendix F).
- 7. Calibrate unit to calculated temperature-corrected pH value for pH 7 standard (e.g. 7.02 @ 20°C).
- 8. 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.
- 9. Acceptable range= 0.2 units from reference (e.g. 6.82 to 7.22 for pH 7  $\omega$  20 °C).
- 10. Repeat steps #1-8 with "new" standard if calibration fails.
- 11. Calibrate unit for pH 10 standard by repeating steps #1-10 using pH 10 standard.

#### Dissolved oxygen

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

- 1. Shake closed 1-L wide-mouth sample container containing approximately 800-mL of tap water for one minute.
- 2. Attach the slotted-probe cover to unit, and turn on the unit 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. Use equations to convert from different units or calculate from altitude.
- 7. Record the temperature and initial dissolved oxygen reading (mg/ L) on calibration sheet (Appendix E).
- 8. Calibrate unit to calculated temperature-corrected value determined from DO Saturation Values Table (Appendix F), and by entering calculated barometric pressure in mmHg.
- 9. Record the second DO reading and time to stabilize  $(\pm 0.01 \text{ mg/L})$ .
- 10. Acceptable range= 0.2 mg/ L from reference.
- 11. Repeat steps #1-9 if calibration fails.

Trouble-shooting Multi-probe Calibration

- $\bullet$  Bad standard  $\rightarrow$  rinse with additional DI water and use "new" standard.
- Incorrect units or values  $\rightarrow$  check units and values entered.
- $\bullet$  Low battery  $\rightarrow$  check unit voltage, charge unit or replace batteries.
- Faulty sensor $\rightarrow$  check sensors for obvious contamination and maintenance needs:
	- o SpC sensor cleaned with cotton swab and ethanol, rinse with tap water.
	- o pH sensor cleaned with cotton swab and ethanol, rinse with tap water.
	- o 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:**

- 1. Anchor boat or tie-off to structure such as buoy 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 water 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.
- 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  $\pm 0.01$ <sup>o</sup>, depth  $\pm 0.1$  m, DO  $\pm 0.01$  mg/L, and pH  $\pm 0.01$ ).
- 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  $0.2 \text{ mg/L}$ .
- 12. Remove slotted probe cover, and attach probe storage cup with <sup>1</sup>/4" tap water. Do not use DI water. If no tap water is available use lake water.
- 13. Record GPS location.

#### **Sample preservation:**

Not applicable.

#### **Decontamination**

**Objective:** remove and/ or kill any plant and animals on gear and boat.

#### **Site locations:**

launch ramp parking lot at least 200 ft from open water.

#### **Equipment List (for sampling one water body):**

- $(> 4 \text{ gallon})$  5% acetic acid large brush
- $\bullet$  (> 4 gallon) 5% bleach solution  $\bullet$  towels
- bottle of household bleach (appx. 6% NaOCl) (13 gallons) fresh water
- (2) large plastic tubs with lids  $(> 10 \text{ gallons})$  spray bottle 5% bleach solution
- 
- 
- -

#### **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. Spray plankton net, cod-end, plankton net rope, anchor, anchor rope and bow lines with 5% bleach solution and let sit.

3. Position an empty plastic tub under engine lower unit so that prop is inside tub.

4. Add fresh water to tub until water surface reaches the bottom of the cavitation plates (approximately 13 gallons).

5. Put boat engine in neutral and start boat engine. Run engine for approximately two minutes. Do not allow water level to fall below cavitation plates. A suggestion is to collect the discharged cooling water in a container, and return liquid to the tub. Stop engine if cooling water temperature becomes hot.

6. Stop engine. Raise lower engine unit out of tub. Spray external engine casing with 5% bleach solution and wipe down with towel.

7. Add approximately 2.5-L (0.7 gallons) of household bleach to tub containing 49-L (13 gallons) of tap water, and mix with hand. Wear appropriate safety equipment. This is a 5% solution of bleach and is caustic. 8. Place plankton net, cod-end, net rope, milk jug of gravel, secchi disk, thatch rake and rope, sediment dredge and surface scraper basket into tub with 5% bleach solution and soak for at least 15 minutes.

9. Spray boat hull and trailer with 5% bleach solution and scrub with brush. Remove plants, mud and debris.

10. Remove items from the tub containing 5% bleach solution, and thoroughly rinse with fresh tap water.

11. Place plankton net, cod-end and plankton net rope in 5% acetic acid and soak for a minimum of 8 hours.

12. Plug the bilge drain. Pour the 49-L of 5% bleach solution into the boat, washing down seats, flooring, and other surfaces. Use the scrub brush to remove plants, animals, mud and debris. Allow solution to soak in boat bilge for 30 minutes and then pull bilge drain plug when trailered boat is located on pavement at least 200-ft from open water.

## **Appendix A: ZQM Veliger Datasheet**

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#### **Appendix B: Adult bivalves, NZMS, crayfish, and other Invertebrates Datasheet**



## **Appendix C: Opportunistic Macrophyte Datasheet**



Zebra/ Quagga Mussels and Other Aquatic Invasive Species Early Detection Monitoring in Eastern Oregon



Zebra/ Quagga Mussels and Other Aquatic Invasive Species Early Detection Monitoring in Eastern Oregon

**Appendix D: Water Quality Datasheet Secchi Down depth (m) Up depth (m) average Water column depth (m): 1 Latitude: 2 Longitude: Water Quality Information** Temp (C)  $\left|\int \text{SpC (us/cm)}\right|$   $\left|\text{DO (mg/L)}\right|$   $\left|\int \text{pH}\right|$   $\left|\int \text{Other/ Notes}\right|$ **Surface 1 m 2 m 3 m 4 m 5 m 6 m 7 m 8 m 9 m 10 m 11 m 12 m 13 m 14 m 15 m 16 m 17 m 18 m 19 m 20 m 2 m repeat**

**Appendix E: Calibration Datasheets** 



## **Appendix F: Calibration Tables**



## **DO Saturation Values Oxygen Content of Air-Saturated Freshwater at 760 mm Hg**

## **Table of pH Calibration Standards**



#### **Appendix G: Pre-Launch Checklist**



#### **Appendix B**

**Sample Analysis Tracking Form Laboratory:** PSU **Point of contact:** Steve Wells **Zebra/ Quagga Mussel Early Detection Monitoring PAGE\_\_**1**\_\_\_ of \_**7\_**\_\_\_\_\_ Date Analysis Analys t initials Scope # Sample tracking number SR cell vol Dilution factor (total:sample) Total # SR cells analyze d mL of conc. sample analyze d # ZQ M # Cor b # ostrco d Notes** 9/29/12 TR DME FY12-5498 9/12/12 3 4:1 2 1.5 0 1 0 Paulina Lake, OR 9/29/12 TR DME FY12-5498 9/12/12 3 2:1 1 1.5 0 0 2 9/29/12 TR DME FY12-5498 9/12/12 3 3:1 2 2 0 0 2 10/1/12 TR DME FY12-5498  $9/12/12$  3 11:1 11 3 0 0 4 **16 8 0 1 8** 10/1/12 IR DM75 0 FY12-5507  $9/12/12$  3 3:1 5 5 0 0 0 East Lake, OR 10/1/12 IR DM75 0 FY12-5507  $9/12/12$  3 2:1 20 30 0 0 0 **25 35 0 0 0** 10/8/12 LC DM75 <sup>0</sup> FY12-5488 9/12/12 <sup>3</sup> 7:1 <sup>21</sup> <sup>9</sup> <sup>0</sup> <sup>0</sup> <sup>15</sup> Paulina Lake, OR 10/9/12 LC DM75  $0 \qquad FY12-5488 \quad 9/12/12 \quad 3 \qquad 7:1$  21 9 0 0 12 **42 18 0 0 27** 10/9/12 LC DM75 <sup>0</sup> FY12-5585 9/12/12 <sup>3</sup> 7:1 <sup>33</sup> 14.1 <sup>0</sup> <sup>0</sup> <sup>13</sup> East Lake, OR 10/9/12 LC DM75  $0 \qquad FY12-5585 \frac{9}{12/12} \qquad 3 \qquad 4:1$  3 2.3 0 0 2 **36 16.4 0 0 15** 10/10/12 PR DME FY12-5490 9/14/12 3 5:1 13 7.8 0 0 1 Lake Billy Chinook, OR 10/11/12 IR DME FY12-5490 9/14/12 3 5:1 15 9 0 3 10/11/12 IR DM75  $0 \qquad FY12-5490 \frac{9}{14/12} \qquad 3 \qquad 6:1$  16 8 0 0 7 **44 24.8 0 0 11**















![](_page_47_Picture_607.jpeg)

**Sample Analysis Tracking Form Laboratory:** <u>PSU</u>

Point of contact: <u>Steve Wells</u>

![](_page_47_Picture_608.jpeg)

![](_page_48_Picture_482.jpeg)

![](_page_48_Picture_483.jpeg)