Zebra and Quagga Mussel Monitoring in the Columbia River Basin by the U.S. Army Corps of Engineers and Portland State University in 2017

2017 Final Report

Submitted by:

M. D. Sytsma and R. Miller
Portland State University

and

S. Wells
Aquaticus LLC

To

Pacific States Marine Fisheries Commission, Bonneville Power Administration and U.S. Army Corps of Engineers

December 2017

Table of Contents

List of Acronyms

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

Abstract

Neither zebra nor quagga mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*, respectively) were detected by the U.S. Army Corps of Engineers (USACE) and Portland State University (PSU) during their early detection sampling in the Columbia River Basin during 2017. Nine USACE Projects in the Northwestern Division and PSU conducted sampling for planktonic larvae and adult mussels during the May to October period. A total of 121 plankton samples were collected and over 3.4 million liters of river/lake water were filtered through 64-µm mesh nets during plankton sample collection. The greatest sampling effort occurred in the Columbia River and Snake River, but sampling also occurred in the Kootenai River, Pend Oreille River, Lake Pend Oreille, Long Tom River, Middle Fork Willamette River, and Lake Oswego. Sampling was focused during the July to September period coinciding with water temperatures favorable for mussel spawning. Non-native invertebrates were opportunistically collected during *Dreissena* sampling in 2017. *Corbicula fluminea* (Asian clam) straight-hinge juveniles were collected in the Columbia River and Snake River plankton samples. None of these collections represent new detections.

Introduction

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. These mussels have established several populations west of the Rocky Mountains, e.g., San Justo Reservoir, CA, Lake Powell, UT, and Lake Mead, NV, and planktonic larvae, or veligers, have been detected in multiple water bodies west of the Mississippi River. *Dreissena* larvae were detected in Tiber Reservoir, MT in 2016, and this water body is proximate to the CRB headwaters. Watercraft with attached, hitchhiking mussels were trailered into the Columbia River Basin (CRB) on multiple occasions in the period between 2009 and 2017 as well as in previous years (Phillips 2017). The risk posed to water bodies in the CRB by the proximity of these infestations is significant.

Monitoring and early detection of *Dreissena* mussels 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.

The U.S. Army Corps of Engineers (USACE) in the Northwestern Division has collaborated with the Center for Lakes and Reservoirs at Portland State University (PSU) for more than eleven years regarding early detection monitoring for *Dreissena* mussels. USACE biologists have regularly monitored artificial substrates, Portland samplers, at several CRB projects since as early as 2003. PSU partnered with USACE in 2005 regarding veliger sampling at the Bonneville, John Day, Ice Harbor and Lower Granite Projects. USACE partnered with PSU in 2010, 2011, 2012, 2013, 2014, 2015, and 2016 to augment early detection monitoring efforts throughout the CRB for *Dreissena* mussels.

PSU was contracted by the Pacific States Marine Fisheries Commission and Bonneville Power Administration to coordinate early detection monitoring efforts with USACE in the Columbia River Basin during 2017 for the presence/non-detect of *Dreissena* mussels. The primary project objectives were to conduct early detection monitoring in the CRB for all mussel life stages at similar spatial and temporal scopes as done in 2016, and to communicate to a large audience in a timely and effective manner, the results of the monitoring activities. The work reported here was expanded upon using funding from the Corps or Engineers provided under the Water Resource Reform Development Act. Results of that expanded work was reported by Turner et al. (2017)

Project Objectives

- Provide equipment and training to USACE biologists to conduct early detection monitoring.
- Coordinate sample collection by USACE biologists and PSU staff at a level commensurate with efforts in previous years.
- Analyze plankton samples collected by USACE and PSU for the presence of *Dreissena* larvae and provide identification of adult specimens.
- PSU sample collection at high priority CRB water bodies not monitored by USACE or others.
- Data management including providing results to USACE biologists and posting data on-line for managers and general public.
- Reporting

Methods

Both USACE and PSU staff conducted early detection monitoring at high-priority water bodies throughout the CRB. At USACE Projects in the CRB, USACE staff opportunistically inspected submersed and exposed hard surfaces for the presence of settled juveniles and adults, and collected plankton samples for veligers. PSU provided sampling equipment and training to USACE biologists, and coordinated the sampling and data management. PSU contracted Aquaticus LLC (Aquaticus) for the laboratory analysis of the plankton samples collected by USACE and PSU biologists. At priority water bodies in the CRB that were not sampled by USACE, PSU staff collected plankton samples for the presence of veligers as well as opportunistically sampling hard surfaces and shoreline areas for the presence of settled juveniles and adults.

Coordination of Sampling

High priority water bodies were identified from a prioritization project conducted by US Geological Survey and PSU (Wells et al. 2010), and emphasis was placed on sampling water bodies or river reaches that were identified as high to medium risk for *Dreissena* mussel establishment and/or introduction. The water body list was further narrowed by identifying anticipated spatial and temporal gaps in early detection monitoring efforts occurring in CRB

during 2017. USACE biologists and PSU finalized the target water body sampling list, and jointly identified the proposed sampling schedule for 2017.

PSU coordinated the sampling done by USACE biologists and PSU staff in the CRB during 2017. PSU surveyed USACE biologists that participated in the 2016 monitoring efforts to solicit concerns and comments, and these comments were incorporated into the 2017 sampling design. PSU established points of contact for each participating USACE Project and identified USACE equipment and training needs. PSU also obtained shipping addresses and arranged the shipment of plankton samples to laboratory for analysis.

Equipment and Training

USACE biologists and PSU staff were equipped and trained to conduct early detection monitoring for *Dreissena* mussels. Equipment and training was provided as needed in 2017, e.g., replacement nets and cod-end pieces. In addition to replacing worn-out equipment, new gear was provided to USACE biologists to provide plankton nets dedicated to a particular water body in order to reduce concerns regarding equipment decontamination and the cross-contamination of samples. PSU staff provided information as needed regarding updates to sample collection protocols, and offered to provide in-person training/joint sampling at the beginning of the field season. A training video that was previously developed by PSU was posted on You-Tube to visually demonstrate the principles of veliger sample collection that were described in the written standard operating procedures. The video is available at http://www.youtube.com/watch?v=v4Qpzi2p0PE&feature=youtube.

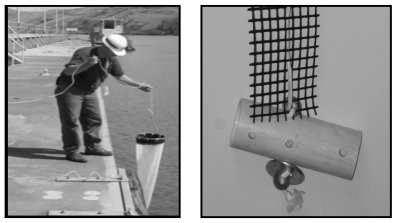


Figure 1: Simple plankton net and artificial settlement substrate.

Sampling equipment included a simple conical plankton net (64-micron mesh, 30-cm diameter opening, 90-cm long, screw-on cod-end piece with mesh and weight) (Figure 1), 100-ft marked nylon rope and rope wheel, artificial settlement substrates (e.g., Portland sampler) (Figure 1), labeled sample collection bottles (500-mL polypropylene screw cap, wide mouth), collection/shipping protocols, field datasheets, regular pre-buffered ethanol, zip-lock bags, permanent markers, and pencils. For each sampling event, PSU provided USACE biologists with labeled sample containers 70% full of regular ethanol pre-buffered with Tris (8 drops of 4-M Tris per 500-mL ethanol), return postage (e.g., FedEx label to print), packing tape, and the packaging materials for Class 3 Flammable Liquids such as “ORM-D” and “49 CFR 173.4” stickers.

USACE Sample Collection

USACE biologists conducted veliger sampling at nine Projects located throughout the Columbia River Basin during the anticipated spawning period for *Dreissena* mussels in 2017 (Table 1). Efforts were focused on sampling during the peak spawning period for *Dreissena* mussels according to water temperature (e.g., July to September) as well as capturing the entire potential spawning period (May to October) (Adrian et al. 1994; Claxton and Mackie 1998; Garton and Haag 1993; McMahon 1996; Nichols 1996; Roe and MacIsaac 1997).

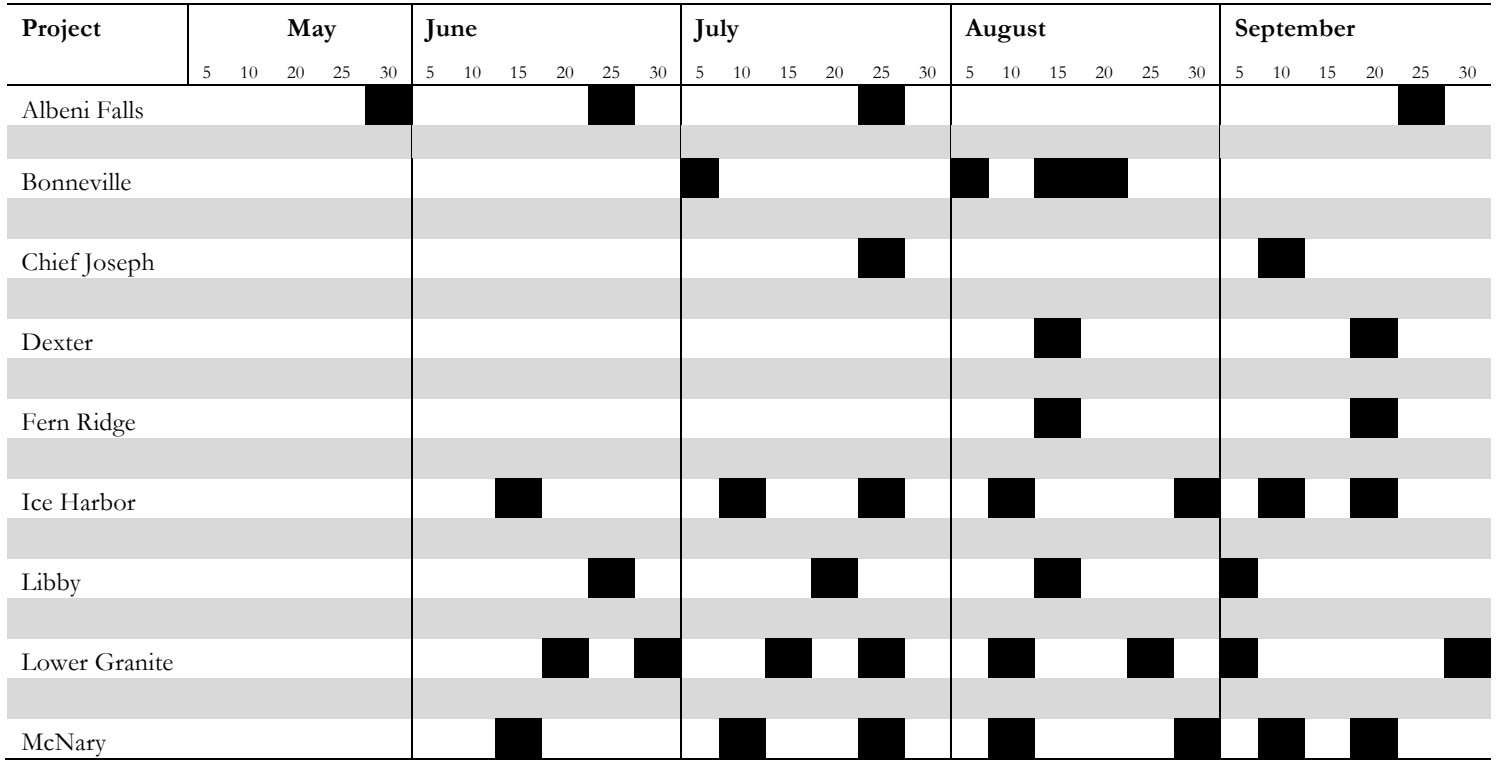
USACE biologists sampled for *Dreissena* veligers, which develop as they float in the water column, by collecting plankton samples using a 64-µm mesh simple, conical plankton net according to the protocols provided by PSU (Appendix A). PSU developed these protocols based

on previous experiences with USACE biologists. In most cases, USACE biologists collected plankton samples from multiple locations within each water body using a boat in near shore and in the open water areas. Sampling was focused on areas near boat launches, marinas, dams, outflows, downwind positions, and in other areas plankton collected, e.g., eddy, to increase the likelihood of collecting veligers. 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 across a large horizontal spatial area.

The plankton collected from several tows or trawling events was composited into a labeled sample container, and preserved using pre-buffered regular ethanol to reach a final solution of 70% ethanol. 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). Although the ethanol used to preserve plankton samples was pre-buffered using Tris, USACE biologists could add a small amount of Tris or sodium bicarbonate if necessary to maintain the pH above 7.0.

USACE biologists opportunistically inspected artificial settlement substrates and other submersed and exposed hard surfaces, both man-made and natural, for the presence of settled *Dreissena* juveniles and adults. Field collection protocols for adult mussel sampling were provided by PSU (Appendix A). Artificial settlement substrates, or Portland Samplers, were provided upon request, and these were tied to a secure surface structure and suspended in the water to provide hard surface area for *Dreissena* colonization throughout the year. Portland Samplers consist of multiple PVC and ABS pipe sections oriented horizontally and secured at different water depths along a rope weighted with a small concrete anchor at end. Other materials and designs for artificial settlement substrates were also used depending on USACE biologists' preferences, e.g., concrete blocks, steel bars, etc. USACE biologists have deployed artificial settlement substrates from a variety of surface structures including USACE docks and boat houses, in adult fish ladders, and public access launch docks.

Table 1: USACE field sampling schedule for *Dreissena* mussels in 2017.



PSU Sample Collection

PSU staff focused *Dreissena* mussel sampling for all shelled life stages in two areas of the Columbia River (Lake Bonneville/ Bonneville Reservoir and Lake Celilo/ The Dalles Reservoir) (Table 2). The Columbia River is a high priority water body in the CRB, and sampling was conducted at multiple sample locations in the late September and early October period (Table 2). PSU also sampled Lake Oswego, Oregon for *Dreissena* mussels. PSU collected plankton samples for veligers as well as opportunistically sampling for settled juvenile and adult mussels by inspecting shoreline areas according to protocols that were developed for PSU field crews (Appendix B). These protocols were developed by PSU for the collection of both larval and adult life stages for *Dreissena* mussels. 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 to increase the likelihood of collection, i.e., sampling was conducted in areas most likely to contain target *Dreissena* life stage using methods that emphasized rapid inspection of large surface areas and qualitative data. Other AIS that were opportunistically collected during *Dreissena* sampling were identified and voucher specimens were retained. The protocols included equipment decontamination protocols to prevent the unintentional transport of organisms between water bodies and the cross contamination of samples for both traditional microscopy and genetic analyses.

Table 2: PSU field sampling schedule for *Dreissena* mussels in 2017.

Water body	September						October					
	5	10	15	20	25	30	5	10	15	20	25	30
Columbia River, Cascade Locks					■		■					
Columbia River, Hood River							■					
Columbia River, Mayer State Park							■					
Columbia River, Rooster Rock							■					
Columbia River, Starvation Creek					■							
Columbia River, The Dalles							■					
Columbia River, Celilo Park							■					
Columbia River, Deschutes River							■					
Lake Oswego, OR							■					

Plankton samples were collected using a 64-µm mesh simple, conical plankton net at several locations within each water body using a boat in near shore and in the open water areas or using 64-µm mesh filters and buckets of water collected near boat launches. 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 using conical plankton nets. Oblique/vertical tows collected plankton throughout the water column at discrete spatial locations. Trawling collected plankton at discrete water depths, e.g., near and above thermocline, across a large horizontal spatial area. The plankton collected from plankton nets was deposited into sample containers, labeled and preserved using pre-buffered regular ethanol to reach a final solution of 70% ethanol. Ethanol was pre-buffered using

Tris (8 drops per 500-mL sample), and additional Tris was added upon receipt into the laboratory if the measured pH was less than 7.0.

Adult *Dreissena* mussels were opportunistically sampled using tactile and visual inspections of existing submersed and exposed surfaces in shoreline areas. Natural and other man-made surfaces in shoreline areas near boat launches including the undersides of launch docks and the sides of rocks, logs, and macrophytes in wadable water depths were inspected for the presence of attached adult and juvenile *Dreissena* mussels.

Suspect AIS specimens were retained in labeled sample containers with lake water, held on ice, and transported to the laboratory for identification. Collection data was recorded on field datasheets. Bivalve identifications were verified by Steve Wells at Aquaticus by using Thorp and Rogers (2011). Following identification, invertebrate voucher specimens were placed in 95% regular ethanol buffered with Tris and stored. Unionid mussels such as *Anodonta* spp. and *Gonidea angulata*, if collected, were photographed and released; unionid mussels dead upon collection were retained.

Sample Analysis

Plankton samples were analyzed at Aquaticus for the presence of *Dreissena* veligers using cross-polarized microscopy. Upon receiving the plankton samples in the 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 laboratory, total sample volume, and the collecting agency were recorded. The laboratory notified PSU staff upon receiving plankton samples via email with the following subject line, "ZQM Monitoring: plankton samples received", and the email contained information regarding the status of the samples and the sample container number(s), e.g., 77011. Plankton samples were analyzed within 14 business days of receipt into the laboratory. Additional alcohol 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 an appropriate pH electrode in the laboratory, and additional tris(hydroxymethyl)aminomethane (Tris) was added as needed to maintain sample pH above 7.0.

Plankton samples were prepared for analysis using gravitational settlement and filtration. Large zooplankton were removed using 750-µm filters. Plankton samples were gravitationally concentrated, and the settled particulate of the plankton sample was placed into multiple centrifuge tubes and covered with alcohol. Twenty percent, at minimum, of the settled particulate, or concentrated sample, was microscopically analyzed in three-mL Sedgewick-Rafter cells using Leica DM750 compound light microscopes fitted with polarizing filters. The concentrated sample was diluted in the counting chamber using alcohol as needed to achieve a matrix density permissible to the visual inspection of all specimens. Microscopes are fitted with high-resolution, color digital Leica EC3 cameras for documenting suspect specimens. Shell measurements were made with digital micrometers to assist in identification.

Other freshwater bivalves such as the introduced *Corbicula fluminea*, are sometimes found in the plankton. Each bivalve specimen encountered during microscopic analysis for the presence of *Dreissena* veligers was identified and documented with photomicrographs, 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 Aquaticus in 70% alcohol buffered with Tris. Following analysis, the contents of the Sedgewick-Rafter cell, were decanted and rinsed with clean alcohol 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* veligers to one of the centrifuge tubes containing a subset of the sample. For example, a typical plankton sample was divided into six 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., 072917_119450. 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 alcohol. 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 sample tracking number, water body name and date of collection. The centrifuge tube contents were thoroughly mixed, placed with the other 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 20 samples, at minimum. 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 planktonic 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 Timothy Dykstra, USACE, 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 viricides used in aquaculture and janitorial services. Genetic material was denatured using a solution of 10% household bleach (100-mL bleach in 1-L of water) (Prince and Andrus 1992). Acid solutions (5% acetic 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 B. 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, and boat anchors 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 10% 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 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 bleach solution for 15 minutes, and then drained on impervious surfaces at least 61-m (200-ft) from open water or retained and disposed of into a sewer system. 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 settling 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, filters, and settling cones) was soaked in a solution of 10% household bleach for at least 10 minutes, rinsed with fresh water and soaked in acid solution (i.e., 5% acetic 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 results were summarized and communicated to USACE through email throughout and at the end of the season. At the completion of the sampling and plankton analysis, USACE provided monitoring data collected by USACE biologists to PSMFC in a spreadsheet format for inclusion in the following online map: http://crbans.psmfc.org/monitoring.html. PSU provided monitoring data collected by PSU staff to PSMFC in a spreadsheet format for inclusion in the aforementioned online map.

Results

USACE Sample Collection

A total of 107 plankton samples (e.g., 500-mL total sample volume) were collected by USACE biologists throughout the Columbia River Basin in 2017 (Table 3). More than 3.2 million liters of river/reservoir water were filtered through 64-µm plankton nets during sampling that occurred during the May to September period (Table 3). The sampling effort was greatest during August, and sampling effort peaked in the July to September period (Table 3). The greatest *Dreissena* veliger sampling effort by USACE occurred in the Snake River (45 samples, 2,079,987-L of river filtered) and the Columbia River (36 samples, 1,179,058-L river filtered) (Table 3). The sampling effort was greatest in the Walla Walla District (number of samples and volume filtered) (Figure 2).

Table 3: USACE *Dreissena* veliger sampling effort at each Project during 2017.

Water body	Project	May		June		July		August		September		Total	
		#	vol fil (L)	#	vol fil (L)	#	vol fil (L)	#	vol fil (L)	#	vol fil (L)	#	vol fil (L)
Columbia River	Bonneville	0	0	0	0	3	73,059	9	138,292	0	0	12	211,351
	Chief Joseph	0	0	0	0	2	8,506	0	0	1	6,578	3	15,084
	McNary	0	0	3	138,957	6	279,587	6	259,861	6	274,218	21	952,623
	Water body Total	0	0	3	138,957	11	361,152	15	398,153	7	280,796	36	1,179,058
Kootenai River	Libby	0	0	3	6,160	3	6,160	3	6,552	3	7,567	12	26,439
	Water body Total	0	0	3	6,160	3	6,160	3	6,552	3	7,567	12	26,439
Long Tom River	Fern Ridge	0	0	0	0	0	0	1	357	1	357	2	714
	Water body Total	0	0	0	0	0	0	1	357	1	357	2	714
MF Willamette River	Dexter	0	0	0	0	0	0	1	462	1	462	2	924
	Water body Total	0	0	0	0	0	0	1	462	1	462	2	924
Pend Oreille River	Albeni Falls	4	5,320	2	2,401	2	2,352	0	0	2	2,352	10	12,425
	Water body Total	4	5,320	2	2,401	2	2,352	0	0	2	2,352	10	12,425
Snake River	Ice Harbor	0	0	3	140,364	6	283,934	6	285,089	6	280,021	21	989,408
	Lower Granite	0	0	6	281,722	6	252,952	6	277,907	6	277,998	24	1,090,579
	Water body Total	0	0	9	422,086	12	536,886	12	562,996	12	558,019	45	2,079,987
Total USACE effort		**4**	**5,320**	**17**	**569,604**	**28**	**906,550**	**32**	**968,520**	**26**	**849,553**	**107**	**3,299,547**

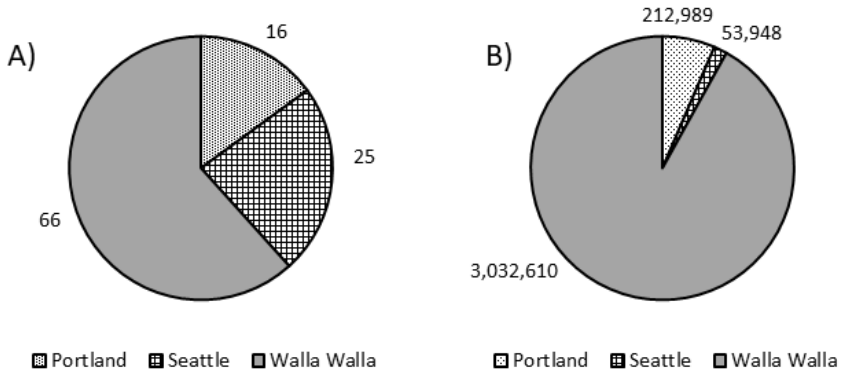


Figure 2: USACE sampling effort by District regarding A) number of veliger samples collected, and B) volume of reservoir water sampled (liters) with plankton net.

No adult or juvenile *Dreissena* mussels were detected by USACE biologists during visual and tactile inspections of existing submerged and exposed surfaces and artificial settlement substrates during 2017.

PSU Sample Collection

A total of 14 plankton samples (e.g., 500-mL total sample volume) were collected by PSU in three reaches or areas within the Columbia River Basin in 2017 (Table 4). More than 48,000 liters of river/reservoir water were filtered through 64-µm plankton nets and filters during sampling that occurred during the September to October period (Table 4). The *Dreissena* veliger sampling effort was greatest in the Columbia River within Lake Bonneville/ Bonneville Reservoir in terms of the number of plankton samples collected and the amount of river/reservoir water filtered (Table 4). The PSU sampling effort was the greatest in October (12 plankton samples and 34,090-L water filtered) (Table 4).

No adult or juvenile *Dreissena* mussels were detected by PSU during 2017.

Table 4: PSU Dreissena veliger sampling effort in the CRB during 2017.

Water body	Reach/ Area	Site	September		October		Total	
			#	vol fil (L)	#	vol fil (L)	#	vol fil (L)
Columbia River	Lake Bonneville/ Bonneville Res.	Cascade Locks	1	4,256	2	15,967	3	20,223
		Hood River	0	0	1	210	1	210
		Mayer SP	0	0	4	16,814	4	16,814
		Rooster Rock	0	0	1	210	1	210
		Starvation Creek	1	10,647	0	0	1	10,647
		The Dalles	0	0	1	210	1	210
	Water body Total		2	14,903	9	33,411	11	48,314
Columbia River	Lake Celilo/ The Dalles Res.	Celilo Park	0	0	1	210	1	210
		Mouth Deschutes River	0	0	1	210	1	210
	Water body Total		0	0	2	420	2	420
Lake Oswego	Lake Oswego	Mid lake	0	0	1	259	1	259
	Water body Total		0	0	1	259	1	259
Total PSU effort			**2**	**14,903**	**12**	**34,090**	**14**	**48,993**

Sample Analysis

Aquaticus microscopically analyzed a total of 121 plankton samples that were collected by both USACE and PSU during 2017. Approximately 77% of the concentrated sample of each plankton sample was microscopically analyzed in Sedgewick-Rafter counting slides (a minimum of 20%) (Table 5, Appendix C and D). The concentrated sample represented the pelleted particulate in the plankton sample that was isolated from the reservoir/river water and ethanol using gravitational settlement. A total of 1,019 Sedgewick-Rafter counting chambers (3-mL slides) were analyzed under compound light microscopes using total magnifications ranging from 40X to 200X (Table 5). A total of 505-mL of concentrated sample was analyzed in these counting chambers (Table 5).

Dreissena mussel veligers were not detected in these plankton samples. *Corbicula fluminea* straight-hinge juveniles were detected in seven of the eleven sample locations in the Columbia River as well as both sampling locations in the Snake River (Table 5, Appendix C and D). *Corbicula* is a non-native bivalve with established populations in the Columbia River Basin. Larvae of the native unionid mussel, *Gonidea angulata* (western ridged mussel) were detected in 10 USACE samples collected from the Snake River near Ice Harbor and Lower Granite Dams and the Columbia River above McNary (collected on 6/15/17, 6/20/17, 6/30/17, 7/13/17 & 7/27/17) (Table 5, Appendix C). Larvae of another native unionid mussel, *Anodonta* spp. (floater mussel), were detected in seven USACE samples collected from the Columbia River above Bonneville Dam (collected on 7/5/17, 8/1/17 & 8/22/17) (Table 5, Appendix C). Ostracods were detected throughout the sampling period, and in all of the sampling locations except the Columbia River near the mouth of the Deschutes River and the Willamette River above Dexter Dam (Table 5, Appendix C and D). Ostracods detected in plankton samples were not identified beyond the Class Ostracoda.

A total of 12 BMS were submitted with these samples. The spiked *Dreissena* veligers were detected in all of the BMS during routine analysis in 2017. The criterion for acceptance is the detection of spiked veligers. 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 for USACE-collected samples in Appendix C, and PSU-collected samples in Appendix D.

Analytical results for the plankton samples were provided within 14-business days of laboratory receipt for 88% (n = 107) of the plankton samples collected during this project (Appendix C and D). Eleven samples exceeded the turn-around time by seven days due to laboratory shut-down for Hurricane Irma. Three other samples exceeded the turn-around time by three days.

During routine analysis, five *Dreissena* veligers that were the result of field contamination were detected in sample, FY2017-223 (Table 5, Appendix D). Upon detecting veligers, steps were immediately taken in the laboratory to address potential sources of bias, specifically false positives due to contamination. The sample was split into several uniquely labeled capped centrifuge tubes during sample preparation prior to analysis. Upon veliger detection in the first centrifuge tube, new sets of laboratory equipment (e.g., Sedgewick-Rafter counting cells, cover slips, pipette, and preparation tray) were used to analyze each of the remaining centrifuge tubes. Veligers were detected in all of the sample subsets using multiple sets of equipment, thus indicating a low likelihood of contamination due to laboratory equipment. PSU was contacted to inquire about potential blind field spikes (e.g., spiking veligers into whole sample in field), and the labeled field sampling gear that was used to collect the positive sample was identified to address potential bias due to contamination during field collection. The plankton net that was used to collect sample, FY2017-223 had been last used to collect zebra mussel veligers from an infested reservoir in 2015. The contaminated net was labeled and stored dry for over a year prior to use in 2017, thus the organisms were dead and there was no risk of inadvertent inoculation. No other plankton samples were collected with the contaminated net. The contaminated plankton net was rinsed with water, the rinsate was collected and microscopically analyzed, and additional veligers were detected in the rinsate.

PSU personnel thoroughly reviewed their field and laboratory decontamination protocols in response to using *Dreissena*-contaminated equipment for the collection of an early detection sample. PSU utilized separate sets of equipment dedicated for either sampling water bodies for the early detection of *Dreissena* mussels (i.e., expecting many zeros and rare, low abundance of *Dreissena* mussels) or sampling water bodies infested with *Dreissena* mussels (i.e., expecting many veligers in samples). The separate sets of equipment were used for different purposes, and physically stored in separate areas of PSU. The decontamination protocols for the set of equipment used to collect early detection samples always involved full equipment decontamination after each use according to Appendix B. The decontamination procedures for equipment used to sample *Dreissena*-infested water bodies was not consistent and varied by project objectives. For example, a set of hoses and 64-µm filters used to sample veliger density at discrete water depths were cleaned after each field sampling event using 5% acetic acid to dissolve the veliger shell. Veligers were counted under the microscope, therefore no efforts were made to denature genetic material contaminating the hose and filters. The contaminated net used to collect FY2017-223 had been used to collect veligers for laboratory control stock purposes, and was not cleaned after use. The inadvertent use of inappropriate equipment for early detection

purposes, highlighted a failure of the labeling and storage system for separating the two sets of field equipment. In order to avoid future mishaps, PSU modified their decontamination procedures to include full decontamination of all equipment following each use according to Appendix B and/or equipment removal from inventory.

Table 5: Summary of Aquaticus microscopic analysis of plankton samples collected by USACE and PSU in 2017. Samples were analyzed in 3-mL Sedgewick-Rafter counting cells diluted with regular alcohol. Total amount of concentrated sample, or pelleted particulate, is shown (Con sample). Total number of counting cells analyzed (# slide), amount of the concentrated sample that was analyzed (mL conc sample), and the average percent subsampled ($\bar{X}$ %) are shown. Number of specimens detected in samples is shown for *Dreissena* veligers (# ZQM), *Corbicula* spp. juveniles (*Corbicula*), ostracods (Ost), *Anodonta* (Ano) and *Gonidea angulata* (Go) unionid larvae, and snails (Gas). *Dreissena* veligers were not detected in samples. *During routine analysis, *Dreissena* veligers were detected in sample #FY2017-223, however, these veligers were the result of field contamination from a plankton net previously used to collect zebra mussel veligers from an infested reservoir. The contaminated net was labeled and stored dry for over a year prior to use in 2017, and no other samples were collected with the contaminated net.

Water body	Project/Area	Agency	Con sample	# slide	mL conc. sample	$\bar{X}$ %	ZQM	*Corbicula*	Ost	Ano	Go	Gas
Columbia Rv	Bonn.	Corps	94	118	59	65	0	427	152	11	0	346
	Chief J.	Corps	19	33	17	91	0	20	15	0	0	0
	McNary	Corps	104	124	60	58	0	1	220	0	3	5
	Cascade L.	PSU	32	41	21	68	0	29	25	0	0	125
	Hood River	PSU	3	6	3	100	0	0	8	0	0	15
	Mayer SP	PSU	46	63	32	72	0	43	109	0	0	137
	Rooster R.	PSU	2	4	2	100	0	0	1	0	0	0
	Starvation C	PSU	26	32	16	63	0	8	23	0	0	27
	The Dalles	PSU	3	6	3	100	0	2	11	0	0	5
	Celilo Park	PSU	1	2	1	100	0	0	4	0	0	0
	Deschutes R	PSU	2	4	2	100	0	0	0	0	0	0
	Water body Totals		332	433	216	83	0	530	568	11	3	660
Kootenai Rv	Libby	Corps	41	63	32	79	0	0	16	0	0	0
	Water body Totals		41	63	32	79	0	0	16	0	0	0
Pend Oreille Rv	A. Falls	Corps	44	59	30	67	0	0	15	0	0	0
	Water body Totals		44	59	30	67	0	0	15	0	0	0
Lake Oswego	Mid lake	PSU	7	14	7	100	5*	0	5	0	0	0
	Water body Totals		7	14	7	100	0	0	5	0	0	0
Long Tom Rv	Fern Ridge	Corps	14	20	10	74	0	0	3	0	0	0
	Water body Totals		14	20	10	74	0	0	3	0	0	0
Willamette Rv	Dexter	Corps	7	11	6	79	0	0	0	0	0	0
	Water body Totals		7	11	6	79	0	0	0	0	0	0
Snake River	Ice H.	Corps	225	243	119	55	0	10	240	0	4	0
	L. Granite	Corps	150	176	85	58	0	45	473	0	26	4
	Water body Totals		375	419	204	57	0	55	713	0	30	4
Project Totals			**820**	**1,019**	**505**	**77**	**0**	**585**	**1,320**	**11**	**33**	**664**

Discussion

The monitoring efforts conducted by USACE and PSU in 2017 under this project provided valuable early detection data for zebra and quagga mussels throughout the Columbia River Basin. 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 Oregon, Washington, Idaho and Montana and involved dozens of USACE and PSU personnel. The targeted water bodies/river reaches received large amounts of boater recreational use and/or exhibited dissolved calcium concentrations and pH values conducive for mussel survival and growth. USACE and PSU *Dreissena* sampling, targeting both veligers and adult life stages, covered a large geographical area including the major river systems and reservoirs/lakes in the Columbia River Basin (Figures 3 and 4).

Sampling was focused during the period of expected peak mussel spawning based on water temperature (July – September), but sampling occurred from May to October to account for the uncertainty associated with predicting mussel spawning and water temperatures (Tables 1 and 2). Water temperatures between 16 and 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).

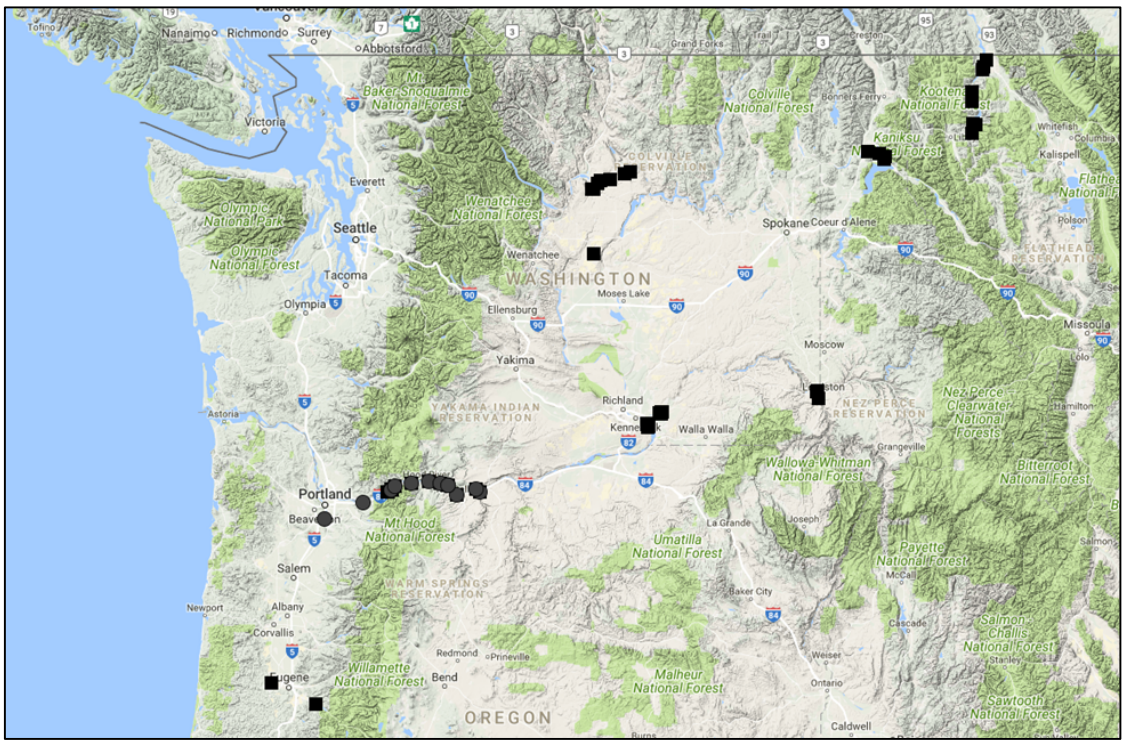


Figure 3: *Dreissena* mussel veliger sampling locations throughout the Columbia River Basin in 2017. USACE veliger sampling locations are indicated by black triangles (■), and PSU veliger sampling locations are indicated by grey circles (●).

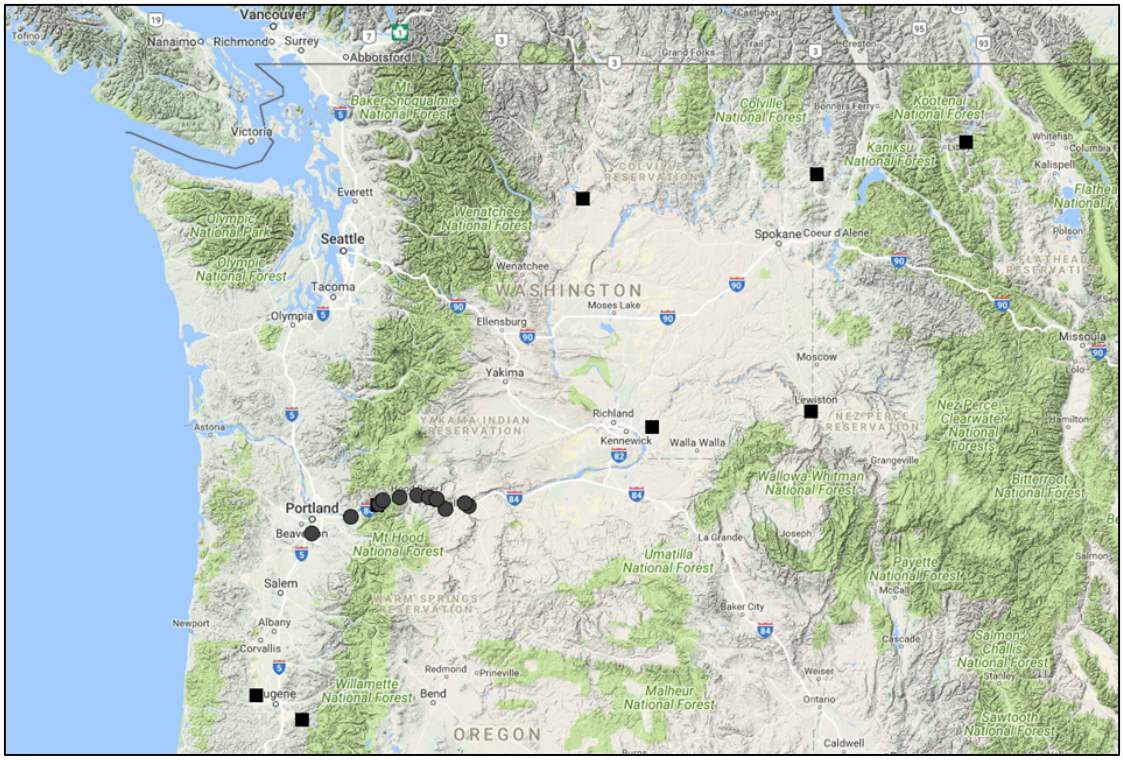


Figure 4: *Dreissena* sampling locations for adult and juvenile mussels throughout the Columbia River Basin in 2017. USACE sampling locations are indicated with black triangles (■), and PSU sampling locations are indicated with grey circles (●).

The USACE and PSU early detection sampling effort in 2017 was comparable to the level of effort done in previous years (2016 – 2011) regarding both spatial and temporal coverage. Nine USACE Projects were involved throughout the CRB, and a similar number of USACE projects were involved with sampling in previous years (Table 6). USACE and PSU biologists sampled during the May to October period in 2017, and similar to other years, sampling in 2017 was focused during the July to September period (Table 6). The greatest sampling effort in 2017 occurred in the Columbia and Snake Rivers as in previous years (Table 6).

The quality of veliger sampling in 2017 was similar to the efforts done in previous years. The total number of plankton samples collected and analyzed in 2017 (n = 121), was less than some previous years (Table 6); however, the total volume of river/lake water filtered through the nets during plankton sample collection in 2017 (3.4 million liters) was comparable to previous years including 2016 (2.9 million liters), 2015 (8.6 million liters), 2014 (4.6 million liters), 2013 (2.1 million liters), 2012 (9.1 million liters), and 2011 (1.6 million liters) (Table 6).

Table 6: Comparison of sampling efforts between 2017 and previous years (2016 – 2011).

Parameter	2017	2016	2015	2014	2013	2012	2011
# USACE Projects	9	11	11	11	11	9	10
# plankton samples	111	165	159	251	170	132	156
Total vol. filtered (millions L)	3.4	2.9	8.6	4.6	2.1	9.1	1.6
USACE vol. filtered (L)	3,299,547	2,521,195	4,666,831	3,614,570	941,106	4,385,800	432,927
PSU vol. filtered (L)	48,993	418,564	3,972,449	1,032,676	1,117,470	4,722,540	1,168,865
Net efficiency (trawling vol.)	70%	70%	70%	75%	70%	80%	80%
Sampling period	May-Oct	May-Oct	May-Oct	May-Nov	May-Nov	June-Oct	
Peak sampling period	July-Sept	June-Sept	June-Aug	June-Sept	July-Sept	July-Sept	
Focus water bodies	Columbia & Snake Rivers	Columbia, Snake, & Lake Owyhee	Columbia, Snake, & Lake Owyhee	Columbia Snake, & Lake Owyhee	Snake, Columbia, & Lake Owyhee	Columbia Snake, & Lake Owyhee	Columbia Snake, & Lake Owyhee

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. If the open spaces in the 64-µm mesh net become clogged, however, then the water can be pushed in front of the net reducing the actual amount of water passing through the net and being filtered. It is difficult to accurately measure the volume of water filtered through a plankton net. Sampling efforts were focused on preventing net clogging by reducing the length of the individual tows, collecting a greater number of smaller tows, and by reducing net tow speed. In order to be conservative with volume estimates, a degree of net clogging was assumed to occur with sample collection. Therefore, the calculated volume filtered was reduced by the assumed net efficiency (i.e., 70%). The assumed net efficiency was 70% but the actual net efficiency was likely variable depending on the turbidity and productivity of the water body, and the distance the net was towed. The assumed net filtering efficiency per sample in 2017 was 70%, and this was comparable to previous years (Table 6).

The opportunistic inspection of exposed and submerged natural and man-made hard objects in shoreline and other areas allowed for the rapid inspection of surfaces, thereby increasing the total surface area sampled for invertebrate colonization and increasing the likelihood of early detection.

The level of effort expended on veliger laboratory analyses in 2017 was comparable to previous years. In 2017, a greater proportion of each plankton sample was analyzed (Table 7). Fewer total samples, however, were collected in 2017 as compared to previous years resulting in similar numbers of slides being analyzed (Table 7). In 2017, approximately 77% of the collected concentrated sample was analyzed under the microscope. In 2016, 2015, 2014, and 2013 the amount of the concentrated sample that was analyzed averaged 42%, 38%, 40%, and 40%, respectively (Table 7). In 2017 laboratory analyses, a total of 1,019 microscope slides were analyzed, whereas in 2016, 2015, 2014, and 2013, the total number of slides analyzed were 1,746, 2,616, 7,535, and 1,762 slides, respectively (Table 7).

Table 7: Comparison of laboratory effort between 2017 and 2016, 2015, 2014, 2013, and 2012.

Parameter	2017	2016	2015	2014	2013	2012
% done	77%	42%	38%	40%	40%	-
# slides	1,019	1,746	2,616	7,535	1,762	2,681
mL sample done	505	658	769	1,765	431	1,354
# *Corbicula*	585	12,092	17,567	15,019	297	4,568
# ostracods	1,320	2,743	5,393	7,206	5,689	2,308
# *Anodonta*	11	8	5	8	1	-
# *Gonidea*	33	2	30	172	7	-
# snails	664	1,564	3,801	89	-	-

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 USACE biologists have been involved with these collaborative efforts for several years. The use of trained USACE biologists and dedicated PSU field crews provided for quality samples, and the use of trained laboratory analysts provided for rapid and quality sample analysis, which is invaluable when looking for an incipient population at presumed low densities. USACE biologists were in direct communication with PSU staff throughout the 2017 spring, summer, and fall periods. Travel costs associated with field collection were reduced, PSU staff had more time available for coordination. Both USACE biologists and PSU field crews provided valuable outreach and education within their respective agencies and to the general public.

The effectiveness of the USACE and PSU early detection monitoring efforts are reflected in the opportunistic detections of other species during *Dreissena* sampling efforts in 2017. Both USACE and PSU detected non-native species during sampling including

Corbicula fluminea. These non-native clams were previously detected in these water bodies. The repeated detection of *C. fluminea* as well as larvae of the native unionid mussels, *Gonidea angulata* and *Anodonta,* reflects the effectiveness of the field collection and laboratory analysis methodology.

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 contamination of field sampling equipment. Multiple sets of labeled gear and decontamination procedures were used to minimize these sources of bias. The improper use of a plankton net that was contaminated with *Dreissena* veligers to collect a single sample in 2017 (FY2017-223), however, demonstrates the importance of trained personnel, proper labeling and decontamination procedures. The risks of contamination are real, and proper labeling and decontamination procedures are paramount to providing reliable data managers can trust. 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 laboratory, and additional Tris was added as necessary to maintain pH above 7.0.

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 8 and 9. 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 using multiple sets of equipment and 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 8: 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 9: 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)

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. Aquaticus met the acceptance criteria for sample analysis in 2017 and veligers were detected in all 12 of the BMS submitted with USACE and PSU samples during routine analysis. Although veliger detection is the acceptance criterion used for veliger quality control samples by Aquaticus, it is useful to consider the percent recovery of spiked veligers during routine microscopic analysis. The percent recovery of veligers in the BMS in 2017 averaged 42%, and this involved analyzing approximately 69% of the entire concentrated sample in the BMS (Appendix C and D). Figure 5 shows the percent recovery for the BMS analyzed during this project. The mean percent recovery during 2017 was 42% ± 15% (1SD; min= 18%, max= 67%, n = 12). 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 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.

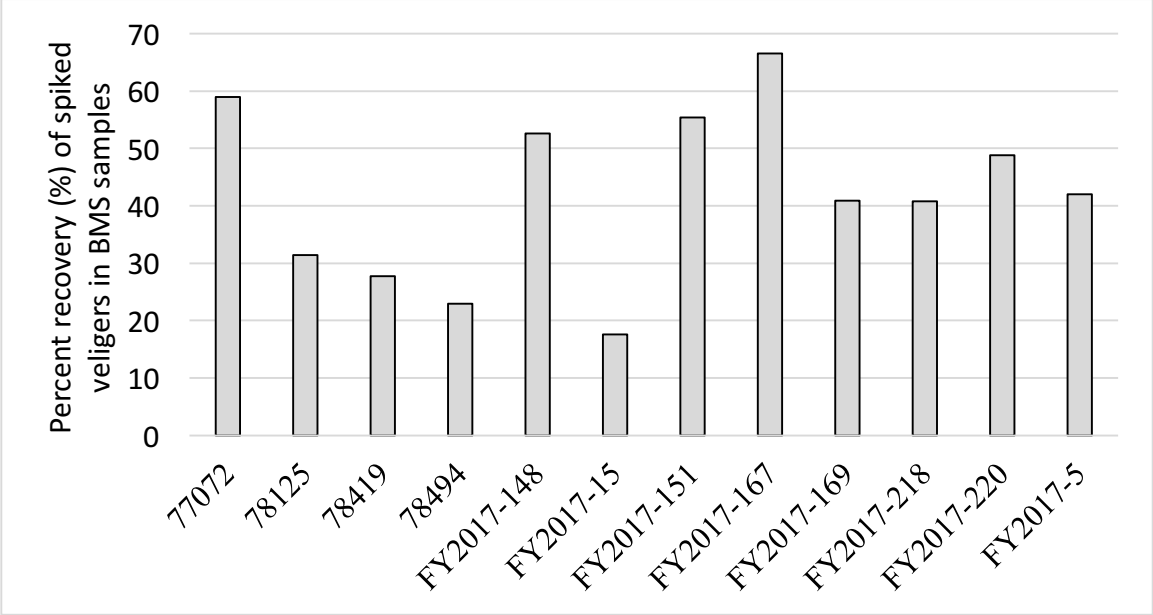


Figure 5: Percent recovery of blind matrix spike samples during 2017. The percent recovery (%) of spiked veligers in the subsample recovered during routine microscopic analysis is shown. On average, 69% of the BMS concentrated sample was subsampled during routine analysis. Veligers were detected in all the BMS samples 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 the Columbia River, and watercraft contaminated with *Dreissena* mussels are continually intercepted at watercraft inspection stations throughout the CRB. If these mussels are present in the CRB, 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 the CRB in 2018.
- Continue coordinating sampling efforts with other federal and state agencies to increase spatial and temporal coverage while reducing unnecessary overlap.

- Continue proper field and laboratory decontamination to prevent contamination of samples and the unintentional transfer of organisms between water bodies. Dedicate plankton nets to each water body sampled to reduce risks of cross-contamination of samples.
- Increase adult mussel surveillance efforts. 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.
- Continue the use of blind matrix spiked samples during plankton sample analysis to check the accuracy of plankton analysis via light microscopy.
- Continue to record the amount of water body filtered through plankton net to quantify the veliger sampling effort, but focus efforts on collecting a greater number of smaller tows to reduce net clogging.
- 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.

References

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.

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.

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.

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.

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.

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

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

Larson, E.R. and C.K. Tait, 2011. Crayfish in the Intermountain Region, A Guide to Sampling and Identification. School of Aquatic and Fisheries Sciences, University of Washington, 21 pg.

LaRue, E.A., M.P. Zuellig, M.D. Netherland, M.A. Heilman and R.A. Thum, 2012. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). *Evolutionary Applications*. **6**:462-471.

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.

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

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

Olden, J., 2009. Brief Guide to Crayfish Identification in the Pacific Northwest. University of Washington.

Pearl, C., B. McCreary and M. Adams, 2011. Invasive crayfish in the Pacific Northwest. US Geological Survey Fact Sheet, 2 pg.

Parsons, Jenifer, Washington Department of Ecology, personal communication, 2015.

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., Pacific States Marine Fisheries Commission, personal communication, 2017.

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

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.

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

Turner, B., C. Gantz, C. de Rivera, A. Strecker, and M. Sytsma. 2017. Zebra and Quagga Mussel Monitoring in the Columbia River Basin. Prepared for Pacific States Marine Fisheries Commission. 8 pg.

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.

Wells, S. and M. Sytsma, 2013. Zebra and Quagga Mussel Monitoring in the Columbia River Basin by the U.S. Army Corps of Engineers and Portland State University in 2012. Prepared for Pacific States Marine Fisheries Commission, Bonneville Power Administration, and U.S. Army Corps of Engineers, 76 pg.

Wells, S. and M. Sytsma, 2014. Early Detection Monitoring Program for Zebra/Quagga Mussels and New Zealand Mudsnails: Boundary Hydroelectric Project (FERC No. 2144). Report prepared for Seattle City Light, 129 pg.

Appendix A

Sample Collection Protocols

For

Zebra and Quagga Mussel Early Detection Monitoring

Rich Miller, Steve Wells and Mark Sytsma

Portland State University

2017

I. SAFETY RECOMMENDATIONS

A. Driving 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 PSU, and some have been serious, e.g., trailer tire blow-out on HWY; rolling truck after swerving onto shoulder.

Safety Protocols

1. The recommended maximum speed limit when towing is 55 MPH. Speed is reduced as necessary depending on weather, road conditions, etc.
2. Seatbelts are worn always 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. Disconnect trailer lights before launching.
6. Grease trailer axle prior to leaving boat storage each trip or as necessary.
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.

B. 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 PSU, 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.

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, as well as other required equipment per your boat and location.

4. Bring drinking water, sun protection, snacks, and non-slip shoes.
5. Complete/update a field and float plan prior to launch. The Field plan is completed prior to the trip, and a copy is left with emergency contact. 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).
6. Lift with your legs and avoid straining muscles. Use cranes, pulleys, and multiple people as needed.
7. Verbally communicate to each other when shifting position within boat, starting engine, etc.
8. Secure lightweight equipment before moving.
9. Distribute weight appropriately Port-Starboard and Stern-Bow.
10. Maintain a clean and clear working area.
11. Be safe!!

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

II. Plankton sampling (planktonic larvae)

A. Sampling Process Design

1. The target organism: planktonic bivalve veligers

The objective of plankton sample collection is early detection monitoring for the shelled, planktonic larvae (veligers) of zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*). *Dreissena* larval development is indirect and occurs in the water column, and this is relatively unique in freshwater bivalves[1]. Planktonic veligers will likely be spatially and temporally clumped in the water column because fertilization is external. Veligers that develop in the water column can be distinguished from other freshwater bivalves using morphological features visible under light microscopy.

Early detection monitoring efforts are focused on the shelled veliger stages found in the plankton that are between 70- and 260-µm (0.07 - 0.26-mm) in size. *Dreissena* bivalve larval development follows a pattern of successional stages and the trochophore stage is the first larval stage developing from the gastrula (Kume and Dan 1968; Raven 1958). Trochophore larvae are damaged during sample preservation, however, and cannot be reliably identified (Nichols and Black 1994). Therefore, veliger monitoring focuses on the straight-hinge or D-shape, umbonal and pediveliger larval stages (Figure 1).

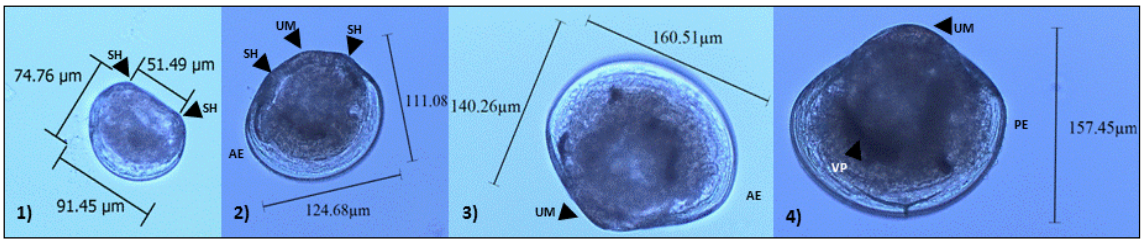


Figure 1: The overall shell shape, general size, and hinge development of the shelled bivalve larval stages found in the plankton that are targeted in early detection efforts including the 1) straight-hinge (D-stage), 2) late-stage straight-hinge/early umbonal (early umbonal), 3) umbonal (umbonal), and 4) late-stage umbonal/pediveliger (pediveliger). The hinge area is marked by the shoulders (SH) and umbo (UM). The velar pigment (VP) is marked. Overall shape includes symmetry of posterior end (PE) and anterior end (AE), umbo height beyond shell margin, and shell outline.

2. Plankton Sample Locations

A minimum of three 500-mL plankton samples should be collected from a boat, if possible, at multiple sites within each water body during a sampling event (Figure 2). A site represents a discrete physical location within a water body, e.g., 100-m intervals

[1] Most other freshwater bivalves either 1) exhibit direct development of larvae without a free-living larval stage, and larvae develop within egg capsules or in a brood-pouch of the adults (e.g., *Corbicula fluminea)*, or 2) exhibit indirect development that involves a parasitic glochidium that attaches to a host, develops in a cyst, and then breaks free from the host and falls to the sediment (e.g., native Unionidae mussels) (Raven 1958). Both glochidia larvae and *Corbicula* straight-hinge juveniles may be found in the water column temporarily but only planktotrophic veligers have a velum and swim in the water column.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

along transect. A water body in this document is a reservoir, run-of-the-river reservoir and/or a river reach. A sampling event is the collection of samples from a water body on a particular date. Collect one plankton tow or trawling event at each site. Plankton samples collected in the same area can be composited together until sample bottle is full. Spread out the sites and increase the number of sites sampled to increase the likelihood of collecting veligers. A boat allows the sampling to be independent of land-accessible structures (e.g., docks).

Sampling is targeted to maximize the likelihood of collection, i.e., not random. In reservoirs, focus sampling near dams, marinas, boat launches, and in areas that are downwind and downstream. Sampling is done in both open water and near shore areas. In rivers, focus sampling in the main stem, downwind and downstream areas, and in near shore areas around boat launches, marinas, and other structures that create eddies.

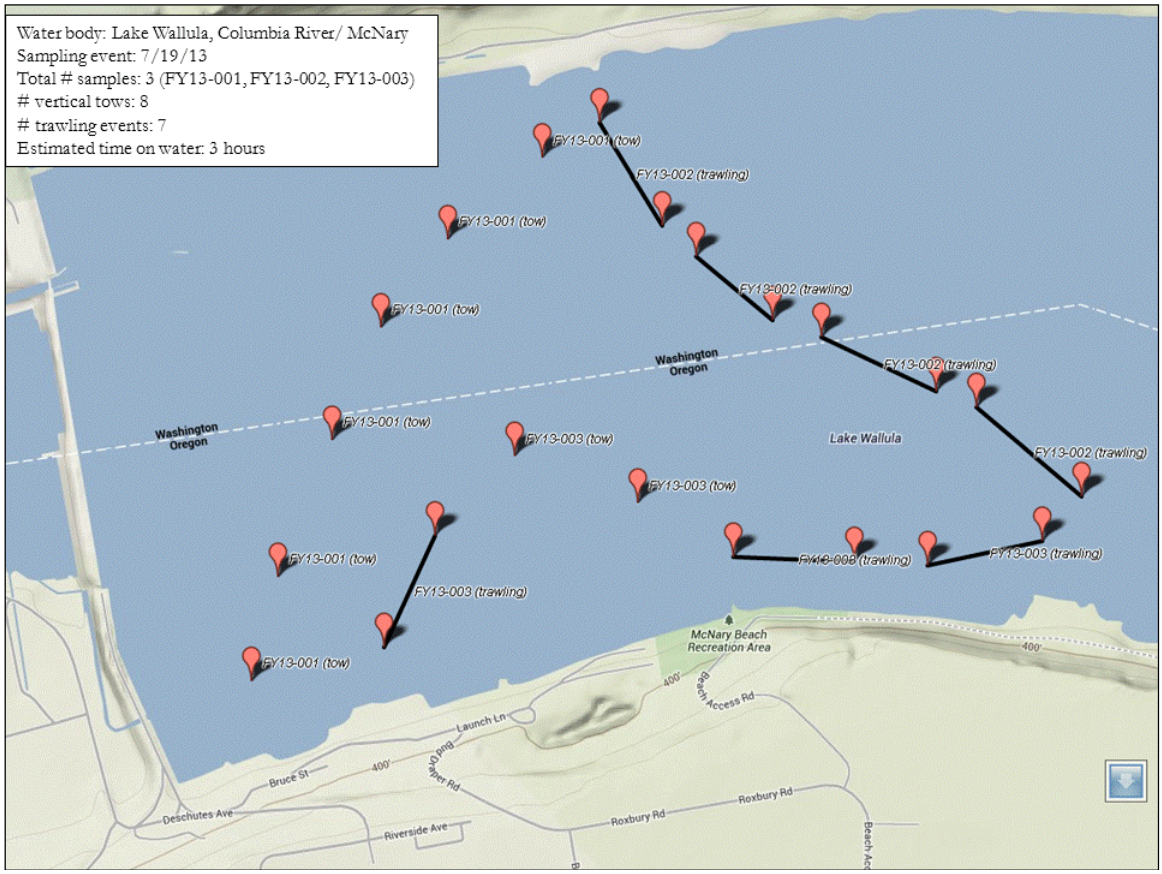


Figure 2: An example of sampling locations at a water body for a given sampling event. The water body is Lake Wallula, Columbia River near McNary Dam, and three 500-mL plankton samples were collected by compositing eight vertical/oblique tows ( ) and seven trawling events ( )that were collected near the launch dock and dam, across the main stem and along shoreline areas.

3. Plankton Sampling Frequency

Veligers can exhibit spatial and temporal patchiness in the water column, and high sampling frequency (weekly or biweekly) increases the likelihood of collecting veligers. Additionally, repeated detection reduces the likelihood of a false positive. The

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

optimal time to sample veligers in North America is when peak spawning is occurring. On the average, optimal time to sample veligers is when water temperatures are between 16^{o} and 19^{o}C (60.8^{o} – 66.2^{o}F). Until site-specific spawning information is known, we suggest that sampling occur a minimum of three times during spawning season, starting once water temperatures are greater than 9^{o}C (48.2^{o}F). Veliger sampling can be performed anytime during the day but preferably not immediately following a storm event. Storm events can increase water turbidity and hence the time required to process the sample.

4. Plankton Sample Collection

A combination of trawling and oblique/vertical plankton tows are recommended for collecting plankton samples. Veligers have been found throughout the water column, ranging from near the surface to depths greater than 122-m (400-ft). The water depth where peak veliger density occurs can vary within and between water bodies, but peak abundances are typically between 3- and 7-m (10- and 23-ft). The mixing in large rivers, e.g., Columbia River, is likely to distribute veligers throughout the water column. Vertical/oblique plankton tows are recommended to collect a depth-integrated sample from the river/lake bottom to water surface. Trawling is recommended to capture a larger horizontal spatial component at a specific depth, and should be targeted in the upper sections of the water column, i.e., 5- and 10-m (16- and 33-ft) depths.

Do not keep plankton tows that contain large amounts of sediment. If the net is dragged across the lake bottom, large amounts of sediment can be captured. Large amounts of sediment interfere with sample analysis, bind up preservative, and may damage sampling equipment. If your sample contains large amounts of sediment, dump the contents of net back into the lake, thoroughly rinse net and cod-end piece in lake, and then repeat the tow. Some sediment (i.e., suspended solids) may be captured in plankton tows, especially in turbid systems, and this small amount of inorganic debris is acceptable.

Plankton tows collected within the same area may be composited into one sample container because the primary objective of sampling is early detection, the veliger spatial data does not indicate the location of adult mussels, and compositing reduces costs. Compositing samples may help reduce the likelihood of false negative results in the field (i.e., failing to collect the veligers when they are present). With sample compositing, spatial information is lost. The location of planktonic veligers, however, is not related to the location of adult mussels.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

B. Plankton Sampling Methods

1. Plankton Equipment

a) Sample Collection

- Plankton net (simple, conical plankton tow net, 64-µm mesh size-*the mesh size is critical*, 0.25-m diameter net opening, removable, weighted cod end) (Figure 3)
- Rope for deploying the net (approximately 31-m/100-ft on rope wheel)
- Sample containers (polyethylene material, 500-mL volume, screw lid)
- Field data sheets, clipboard, labels, permanent marker and pencils
- Net anchor (2.3 kg – 4.5 kg/5 – 10 lbs.) (e.g., 1-gal milk jug filled with sand tied to 3-m section rope)
- Two quick links (*recommended*)
- Stopwatch or timer (*recommended*)
- Global Positioning Satellite unit (GPS) (*recommended*)
- Boat (*recommended*)
- Multiprobe water quality instrument, e.g. Hydrolab® (*optional*)
- Large flathead screwdriver (*optional*)

b) Sample Preservation and Handling

- Regular ethanol (EtOH) OR liquor ≥ 151 proof = 75.5%, e.g., Everclear. Avoid denatured ethanol. Isopropyl alcohol can be used in emergencies.
- Sealable plastic bags (e.g., Ziploc)
- Tris (*optional*)
- pH indicator strips (*optional*)
- Measuring tape or ruler (*optional*)

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

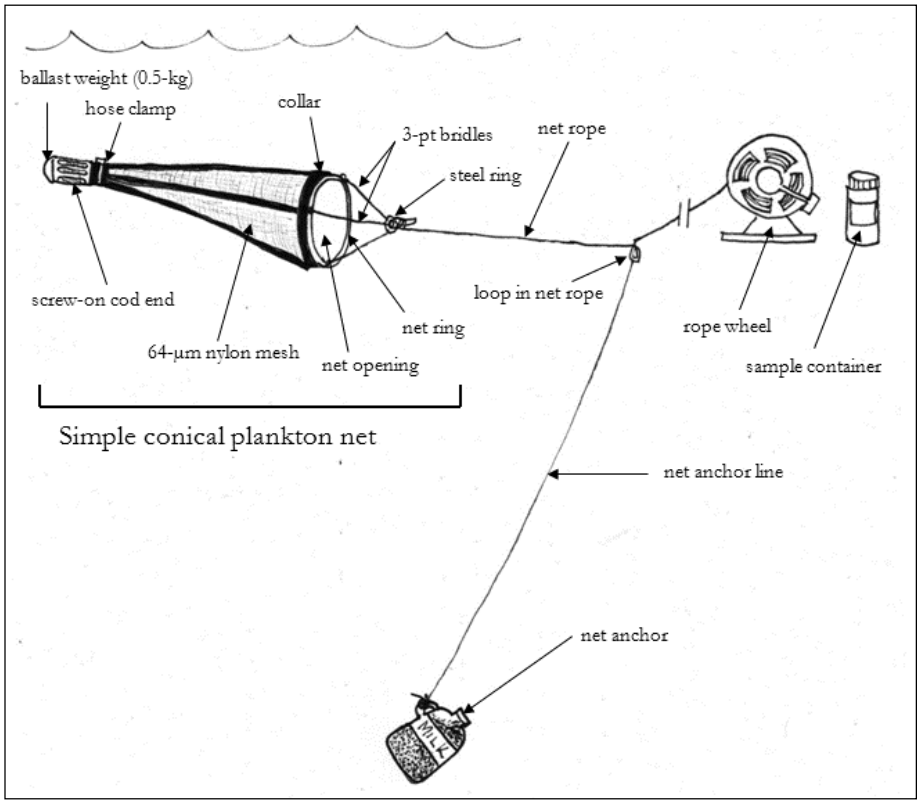


Figure 3: Simple conical plankton net set-up for veliger sampling drawn approximately to scale.

2. Plankton Sample Collection

a) Vertical/oblique tow in mixed, non-stratified water body

1. Screw on the cod end and check that the net rope is securely tied to plankton net at the steel ring. A quick link is convenient way to secure net rope to steel ring. Check that the hose clamp securing the cod end to the net is secure. Use flathead screwdriver to tighten the hose clamp as needed. Secure the net anchor to a second 3-m (10-ft) section of line that is secured to a loop placed in the net rope approximately one meter in front of net opening. A second quick link can be used to attach net anchor to net rope.

2. Lower the net 31-m (100-ft) below water surface, or to 1-m (3-ft) above the sediment, whichever is deeper. **Keep the net off the river/ reservoir bottom**. The net rope will go slightly slack when the net anchor hits the bottom, which should be before the net.

3. If the net anchor hits the bottom, quickly pull-in approximately 2-m of the net rope to keep net opening clear of bottom sediment. Keep net at this depth for five seconds.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

4. 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.**

5. Rinse the net by raising it 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. Repeat this procedure several times to ensure that all the organisms inside the net are in the cod end.

6. **Condense the sample as much as possible before pouring into sample container**. Condense the sample by swirling the cod end while still attached to net. Then carefully unscrew the cod end 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 to allow the water to filter through. The cod end, once separated from net, should also be swirled to further condense sample. After pouring condensed 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.

7. **Record the water depth** the net was lowered, hence the length of the tow, **and the sample container number**, e.g., 77001, in the field datasheet. It is important to record the number and length of tows so that the volume of lake water sampled can be estimated. It is recommended to record the latitude and longitude of the tow.

8. 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 length of the tow. If it still occurs, estimate the net filtering efficiency and multiple the corresponding percent by the maximum volume of water filtered (e.g., 80% filtering efficiency means 0.80 x V_m).

Maximum volume of filtered water, V_m (m^3) 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 (e.g., 30-m)
1-m^3 = 1,000-L

b) Vertical/Oblique Plankton Tow in Stratified Water body

1. Repeat step #1 in II.B.2.a).
2. Lower the net 15-m (49-ft) below water surface, or to 1-m (3-ft) above the sediment, whichever is deeper.
3. Repeat steps #3 through #6 in II.B.2.a).

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

c) Shoreline Toss

1. Remove the net anchor, which is secured to a loop in net rope.
2. Screw on the weighted cod end, check that the hose clamp is secure, and that the net rope is secured to steel ring.
3. Hold the net ring using thumb and forefinger of your throwing hand. Make large loops of the net rope and hold loosely with the same hand holding the net. Grasp the loops of the rope in front of the net opening.
4. Firmly hold the other end of the rope with free hand.
5. Throw the net using a sidearm-style, opening your hand upon release to allow rope to feed out with the net.
6. Allow net to sink into water body. A weighted cod end will aid in pulling the net into the water. If an air bubble gets trapped in the net, retrieve the net and start again.
7. Manually retrieve net using a hand over hand technique at a rate of 0.5-m/s (1.5-ft/s). Keep the net off the sediment to avoid both snagging and collecting debris.
8. Follow steps # 5 - 7 in II.B.2.a).

d) Sampling inside Facilities

1. Open flow valve and purge system for at least two minutes.
2. Estimate the flow using a flow meter valve or calculate the time needed to fill a 5-gal bucket (gal/min).
3. Position the plankton net so that the flow of water passes into the mouth of the net.
4. Record the time that water is entering net so that the volume of water being filtered can be calculated.
5. Follow steps # 6 - 7 in II.B.2.a).

e) Trawling

1. **Attach a net anchor** (4.5 kg/ 10-lbs.) to a loop in the net rope approximately 1-m (3-ft) in front of the net opening to keep the net at a specific depth for given time period. A plastic 1-gal milk jug filled with sand or gravel works well as a net anchor (~8.4 lbs.).
2. Screw on the cod end and check that the hose clamp is secure and that the net rope is secured to steel ring.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

3. Lower the net 5- to 10-m (16- to 33-ft) below surface of water and keep net at this depth. **Record the start time** and the starting location coordinates on field datasheet (Appendix B).
4. Use the boat engines and/or the river current to move the net horizontally through the water for five to 15-minutes. **Trawling should be done at low speeds**, e.g., 1- to 3-MPH. The boat may be driven directly upstream, essentially keeping the boat in the same approximate longitudinal position and allowing river to flow through net. Trawling can also be done transversely to the current. Reduce the trawling time in productive and turbid waters as net may clog.
5. Idle or stop the boat engine and manually retrieve net using a hand-over-hand technique at a rate of 0.5-m/s (1.5-ft/s).
6. **Record the stop time, boat speed** and the coordinates of the stop location on the field datasheet (Appendix B). The trawling time and boat speed are used to estimate the volume of water filtered (i.e., distance = rate x time).
7. Follow steps # 4 - 7 in II.B.2.a).

3. Labeling and Associated Parameters

Record the following information on the field datasheet with a pencil. Permanent marker ink will smear when in contact with ethanol.

- Sample container number (e.g., 77001) (this is printed on sample container label and is unique identifier for each sample)
- Date of collection
- Water body name
- Sample location (GPS if available or detailed description)
- Number and length of tows (vertical/oblique tows)
- Start time, stop time and boat speed (trawling)
- Type of tow (vertical, trawling, etc.)
- Name and agency of person collecting sample
- Preservative and concentration used (e.g., 70% EtOH)

It is recommended to collect the following metadata with plankton samples. Most of these data can be measured using a multi-probe unit (e.g., Hydrolab). Calibrate multi-probe units according to their manuals. Record metadata in field datasheets.

- Water temperature (^{o}C) and depths of reading
- pH
- Specific conductance (µS/cm)
- Dissolved oxygen (mg/L or % saturated)
- Wind speed (two minute average, MPH)
- Secchi depth reading (ft)

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

4. Sample Preservation

Preserve samples using regular ethanol (EtOH) immediately after collection to ensure sample integrity. **Preserve samples in a final solution of 70% EtOH.** Use more preservative with samples that contain greater amounts of plankton, sediment and other debris. Samples that cannot be preserved immediately after collection should be placed on ice until preservative can be added. Do NOT wait more than three hours to preserve samples. EtOH is the preferred preservative. Isopropyl alcohol may also be used. Avoid denatured EtOH because it appears denatured EtOH dissolves the calcite in shells much faster than regular EtOH. Do NOT use Lugol's solution as it may contain acetic acid and dissolve shells.

Sample containers are provided with a quantity of buffered regular ethanol so that a final solution of 70% EtOH will be reached if plankton is added until sample containers are full. Add condensed plankton directly to ethanol until solution reaches the neck of container. **Ethanol is provided that has been buffered** with tris(hydroxymethyl)aminomethane (TRIS), which increases the buffering capacity of the plankton sample and raises the pH. The pH of the preserved sample should be acceptable and require no additional buffering. Samples may need to have additional buffer added, however. **EtOH from the 2015 sampling season was pre-buffered** with TRIS.

To make 70% solution of EtOH and plankton:

1. Make sure sample container is ¼ or less full. If needed, pour some sample into another sample bottle. Tighten cap and thoroughly mix sample prior to pouring into another sample bottle.
2. Allow sample to settle until water level is constant.
3. Mark the level of sample on the outside of sample bottle using a permanent marker.
4. Using a stock solution of 95% to 100% buffered EtOH, add three parts EtOH to one part sample to achieve a final concentration of approximately 70% EtOH. A ruler or measuring tape may be placed alongside the sample container to help estimate the ratios.
5. Mark the level of final solution containing sample and preservative on outside of sample bottle using a permanent marker.
6. The target pH of the preserved sample is between 7.0 and 9.0. The pH of the preserved sample can be measured using the provided pH test strips although the accuracy of the colorimetric strips is questionable. Hand-held pH electrodes and meters are relatively accurate when properly calibrated and approved for use with TRIS. Ethanol is provided already containing the buffer TRIS and the pH of the preserved sample should be in the target range. If the pH is less than 7.0, add a small amount (1-3 drops) of Tris. Secure lid and shake to mix. Measure pH again to ensure pH is above 7.0. Be careful to add Tris in small amounts, and keep Tris away from eyes and skin.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

5. Sample Handling and Storage

Handle samples while in the field after preserving according to following:

1. Tighten sample container lid to prevent leakage.
2. Place sample container into two sealable plastic bags. Sample containers are placed into plastic bags to reduce the likelihood of a leaking sample cross-contamination.
3. Samples preserved in final solution of 70% EtOH and buffered may be stored in a cool, dry place at least 12 months prior to analysis[2]. Avoid placing samples in direct sunlight or freezing conditions. Samples preserved in 70% EtOH may be stored on ice or in the refrigerator although this is not necessary.

PSU will provide return postage, address labels and Class 3 flammable liquid labels for shipping plankton samples to laboratory for analysis. Keep the packaging materials and boxes used to send sample containers with ethanol, and repack for returning plankton samples. No-date postage stickers are provided for those using USPS. FedEx shipping labels will be provided electronically to those using FedEx; FedEx labels will be printed once a shipping date has been identified by Project point of contact.

EtOH is a Class 3 flammable liquid and there are restrictions regarding its transport. EtOH can only be transported on the ground/surface. Do not fly in an airplane with EtOH. Keep preserved samples in a plastic container such as a bin or cooler in the back of the car while in transit. EtOH can be mailed but there are training, certification, labeling and shipping requirements. Ship or mail EtOH preserved samples to PSU laboratory via ground or surface mail using USPS and/ or FedEx according to the protocols below, which allow exemptions for training and certification.

USPS Protocols for mailing EtOH

1. Samples must be in plastic containers with a screw lid. Secure screw lids.
2. Place all containers into a sealable plastic bag (e.g., Zip Lock) and then place this bag into another sealable plastic bag.

[2] The effects of holding time on *Dreissena rostriformis bugensis* (quagga mussel) larval shell birefringence and integrity preserved in solutions of 70% regular ethanol (EtOH) and stored at room temperature and pH 8.5 were determined through repeated non-destructive light microscopy analysis. Repeated measurements were made on three plankton samples collected from Lake Mead, Nevada and preserved in solutions of 70% EtOH to determine the effects of holding time on veliger density. Holding time had no significant effect on veliger density ($F(1.828,3.657) = 6.024$, $p = 0.070$) over a period of 1,310 days in plankton samples preserved in 70% EtOH. These findings suggest that solutions of 70% EtOH stored at room temperature and pH of 8.5 do not adversely affect veliger shell birefringence and shell integrity, and are acceptable means for preserving plankton samples for the early detection of zebra and quagga mussels analyzed via light microscopy.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

3. Place sealed bags and sample containers into box and add cushioning material such as scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.
4. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box either, so long as it is sturdy. Seal box with clear packing tape.
5. Label the outer box with your return shipping address, PSU shipping address, Surface Mail Only stickers, and the no-date poster sticker according to Figure 4.
6. Mail via USPS domestic surface transport as Standard Mail or Parcel Post.

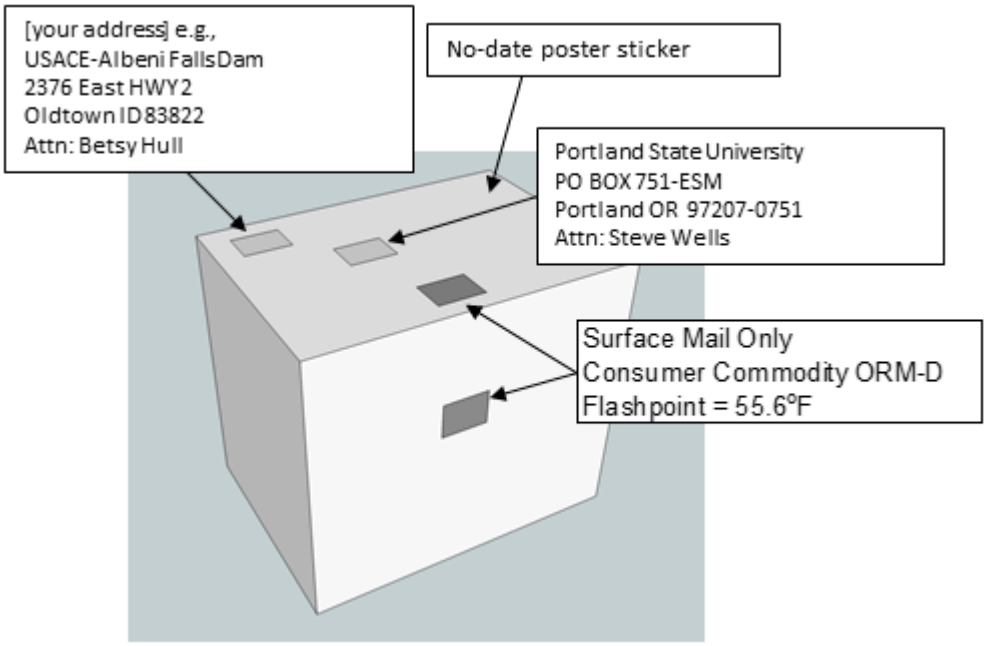


Figure 4: Example of USPS Parcel Post labeling for shipping a ZQM plankton sample to PSU.

FedEx Protocols for mailing EtOH

1. Samples must be in plastic containers with a screw lid. Secure screw lids.
2. Place all containers into a sealable plastic bag (e.g., Zip Lock) and then place this bag into another sealable plastic bag.
3. Place sealed bags and sample containers into a box and add cushioning material such scrap paper. Seal this box with clear packing tape. The box does NOT need to be a specific type of box so long as it is sturdy.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

4. Place this box into another box and add cushioning material as needed. The outer box does NOT need to be a specific type of box so long as it is sturdy. Seal box with clear packing tape.
5. Request a FedEx shipping label/schedule pickup from Rich Miller via email or phone. FedEx label will be emailed to you. Print off the label and tape to outer box.
6. Attach two 49 CFR 173.4 stickers according to Figure 5.
7. Mail via FedEx Ground.

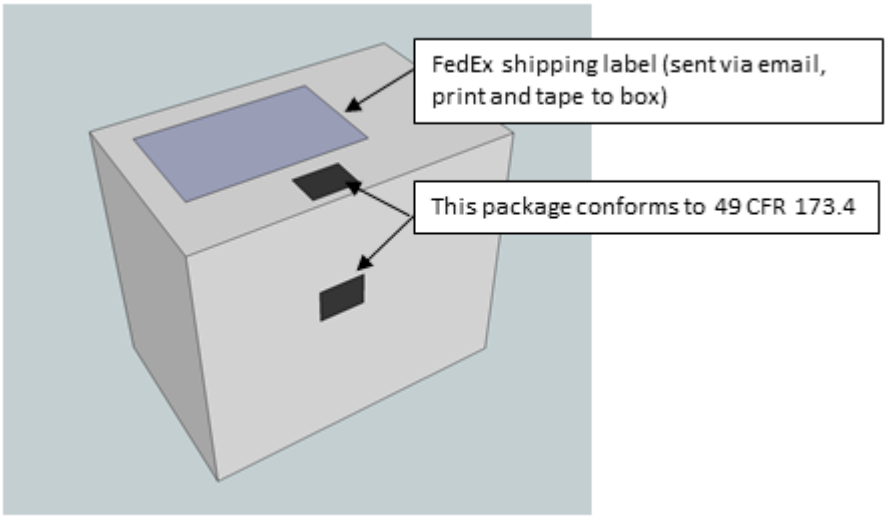


Figure 5: Example of FedEx Ground labeling for shipping a ZQM plankton sample.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

III. Adult sampling (settled, epifaunal adults and juveniles)

A. Sampling Process Design

1. Target organism: settled, epifaunal juvenile and adult mussels

The objective of sampling for adult and juvenile zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*), hereafter referred to as *Dreissena*, is the early detection of bivalves attached to hard submerged surfaces in freshwater environments. Bivalves in freshwater that are firmly attached to a hard surface, i.e., epifaunal, are cause for concern. *Dreissena* mussels are one of the only freshwater mussels capable of adhering to hard surfaces using byssal threads (Figure 6). Another invasive freshwater mussel, *Limnoperna fortunei*, is also capable of adhering to hard surfaces via byssal threads but these mussels have not been detected in the United States. Additionally, brackish water mussels such as *Mytilopsis leucophaeata*, produce byssal threads and firmly attach to hard surfaces, and these on rare occasions, have been found in freshwater areas. Lastly, *Corbicula* spp. juveniles can weakly attach to hard surfaces using threads, but this phenomenon is temporary and associated with only small *Corbicula*. A bivalve firmly attached to hard surfaces in a freshwater habitat constitutes a fouling threat, regardless of the species, and byssal thread formation in freshwater can be used to quickly identify specimens that require greater scrutiny.

Figure 6: An adult quagga mussel with byssal threads.

Dreissena mussels are small, triangular bivalves. Adults are typically 1- to 3-cm (0.4- to 1-inch) in shell length and juveniles range between 250-µm to ~5-mm in size. Shell color varies but they usually have black and white stripes, although some are all dark and other cream colored (Figure 7).

Figure 7: Adult zebra mussels showing size and color variation.

2. Sample Locations

a) Artificial Settlement Substrates

Artificial settlement substrates provide hard surfaces for mussel colonization at specific water depths that can be readily retrieved and inspected from the water surface. An artificial settlement substrate, e.g., Portland Sampler, is shown in Figure 8, but materials and designs may vary. The effectiveness of settlement substrates is proportional to the surface area available for colonization, and multiple substrates may be deployed at each

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

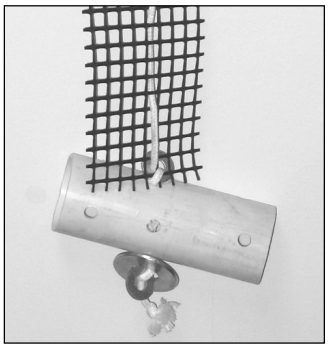


Figure 8: Artificial settlement substrate.

project to increase the likelihood of detecting mussels. Deploy multiple substrates in different areas to further increase the likelihood of collection. Substrates should be deployed in continuous or intermittent water flows less than 1.8-m/s (6-ft/s) in areas that have a means to secure the substrate rope (e.g., hand railing), and provide safe access during inspections. Settlement substrates should be continuously immersed, at depths ranging from 2- to 16-m (5- to 50-ft), preferably at depths greater than 6-m (20-ft). The highest density of settled mussels is typically at a depth of 8-m (25-ft). *Dreissena* settlement is highest at water flows between 0.5- to 0.7-m/s (1.6- to 2.3-ft/s), but mussels tolerate flows ranging from 0.05-cm/s to 1.8-m/s (0.02-in/s to 6.0-ft/s). Deploy settlement substrates in areas where the water currents and/or wind patterns are likely to concentrate the planktonic larvae. Suggested deployment locations for settlement substrates include dam booms, buoys, adult fish ladders, navigation arm, fish raceways, docks, channel markers, and piers.

b) Existing Surfaces

Accessible, existing submerged surfaces such as concrete walls, dock floats, pilings, etc. offer a readily available large surface area for mussel colonization and should be examined for adult and juvenile settlement. Existing submerged surfaces can be actively sampled using tactile and visual inspections (e.g., feeling with hand along underside of dock float). Existing surfaces can also be opportunistically inspected during maintenance and other work activities, e.g., dewatering structure.

3. Sampling Frequency

The frequency of inspections for adults and juveniles is either monthly, as dictated by existing maintenance schedules, or as time allows. Temporal patterns for juvenile settlement usually parallel spawning patterns, and the time period between spawning and settlement is typically 15 to 20 days. Juveniles are generally found in the Midwest between August and September. Substrates should be inspected year round, however, as year round reproduction is possible, and *Dreissena* translocate throughout the year. For example, *Dreissena* juveniles can drift in the water column using a mucous thread that creates drag and entrains the mussel. Adults and juveniles crawl over surfaces using a foot.

4. Sample Collection

Visually and tactilely inspect all submerged surfaces while focusing efforts on the nooks, crannies and other protected areas. The density of settled mussels is greatest on the undersides and sides of objects as well as in nooks and areas of surface irregularity. *Dreissena* colonization on Portland Samplers is greatest on the inside of the pipe sections, especially near the holes and around knots in rope. Settlement is an active process that involves initial settlement, metamorphosis, and translocation. *Dreissena* mussels do not

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

appear to discriminate surfaces on which they initially settle. After completing metamorphosis, however, the mussels translocate to preferred substrates such as the undersides of objects and other protected locations.

5. Data Reporting

Positive results for the presence of *Dreissena* adults and juveniles will be confirmed by PSU according to shell morphology. If a questionable organism is found (e.g., bivalve attached to hard surface), carefully remove the suspect specimen(s) and place into a sealable plastic bag with a small amount of water. Place bagged specimen in refrigerator for short term storage (1 to 5 days). Immediately call or email Rich Miller using contact information below for further instructions on species identification. You may be asked to take a digital photograph, compare to reference shells provided, and/or preserve and mail specimen.

B. Sampling Methods

1. Adult Sampling Equipment

a) Sample Collection

- Portland Sampler (abs and pvc pipe sections suspended along a rope)
- Sealable plastic bag (e.g., Ziplock)
- Field datasheet and pencil
- Field lens (10X total magnification) (*optional*)
- Boat (recommended)

b) Sample Preservation

- Regular ethanol, denatured ethanol, isopropyl alcohol, or cooler with ice.

2. Adult Sample Collection

a) Artificial Settlement Substrate Deployment and Inspection

1. Settlement substrates, Portland Samplers, are available from Portland State University. Upon receiving your settlement substrate please check that your substrate is complete and includes the PVC (white) and abs (black) pipe sections, and rope. A heavy object may be attached to the washer on the bottom of the substrate string to anchor substrate.
2. Locate a suitable deployment location that provides a secure structure from which the substrate can be suspended, e.g., dock.
3. Lower the substrate into the water to determine the depth at that particular site. The substrate at the end of the rope should rest near the sediment or be as deep in

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

the water as possible. The default depth to suspend settlement substrates is 8-m (25-ft). Tie the end of the rope to the structure once the required length of rope is known.

4. When checking the substrate, remove it slowly from the water to avoid loss of mussels. Inspection for adults and juveniles is 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 to assist visual inspections. Efforts are focused on bivalves large enough to be seen with naked eye.
5. A biofilm will develop following submersion 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.
6. Record sampling activities in field datasheet regardless if anything was found.
7. If you think you have a *Dreissena* mussel, either carefully remove suspect specimen or remove the entire substrate, and place into a sealable plastic bag with a small amount of water. Place bagged specimen in refrigerator for short term storage (1 to 5 days). Call or email Steve Wells using contact information below for further instructions on species identification. You may be asked to take a digital photograph, compare to reference shells provided, and/or mail specimen(s) to Portland State University. Portland State University will provide shipping labels or reimburse shipping costs.

b) Existing Submerged Surfaces

1. Locate suitable existing surfaces to inspect. Accessible surfaces (i.e., within arm's reach) in facilities and water body are good candidates for visual and tactile inspections. These relatively easy-to-access surfaces include the walls of adult fish ladders, metal grates/screens, wood and plant material collected on screens, the undersides and sides of docks, vessel hulls, buoys, and the underside and sides of rocks found in shoreline areas.
2. If sampling from a boat, carefully position vessel near structure to sample and maintain position either using the motor or using current and wind to position boat against structure.

WARNING: Be careful not to pin arms between the boat and structure. Beware of conditions on the water. Do not tie boat to structures in water body.

3. 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. Handle questionable organisms according to step #7 in III.B.2.a).

3. Labeling and Associated Parameters

Record the following information on a field datasheet. Use a pencil for datasheet.

Date of inspection/collection
Water body name

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

Sample location (GPS if available or detailed description)
Type of inspection (e.g., artificial substrate)
Inspection results (e.g., non-detect)
Name and agency of person collecting sample
When applicable, preservative and concentration used (e.g., 70% ethanol)

4. Sample Preservation

Preserve adult suspect specimen(s) immediately after collection to ensure sample integrity. Samples that cannot be preserved immediately after collection should be placed on ice until preservative can be added. Samples, placed into a sealable plastic bag with a small amount of water, may be temporarily stored in the refrigerator (1 to 5 days). Do NOT wait more than three hours to preserve or refrigerate samples. Ethanol (ETOH) is the preferred chemical preservative. Denatured ethanol and isopropyl alcohol are acceptable chemical preservatives with adult specimens. When using alcohol as a preservative, use stock that is 70% alcohol and greater, and add enough preservative so that specimen(s) and/or associated substrate are completely submerged. Other chemical preservatives not listed may be acceptable.

5. Sample Handling

Samples preserved using a final solution of 70% ETOH may be stored in a cool, dry place up to three months prior to analysis. Samples that lack chemical preservative must be refrigerated prior to analysis. Refrigeration is a temporary means to preserve biological samples. Avoid placing samples in direct sunlight or freezing conditions. For long term storage, place samples preserved in 70% ETOH.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

IV. Decontamination

- Acetic acid dissolves the calcite in the mollusk shell. Ideal soak time is 24-hrs. The minimum soak time is 8-hrs.
- Bleach kills organisms and degrades cellular macromolecules, i.e., genetic cross-contamination. Soak time in 5-10% bleach solution is 15 minutes. Bleach solutions are unstable, and should be made fresh when using.
- Formula 409 Disinfectant (Alkyl C12-16 dimethylbenzyl ammonium chloride) can be used instead of Virkon Aquatic when not available.
- Decontamination is done on flat pavement or concrete surfaces a minimum of 200-ft from open water.
- Equipment is thoroughly rinsed with tap water after decontamination to reduce corrosion and wear.

Warning: Some of the chemicals used to disinfect equipment are corrosive and can be dangerous to health when working with concentrated stock solutions as well as diluted solutions. Consult MSDS, and wear appropriate personal protective equipment, e.g., gloves, eyewear.

A. Sampling equipment used in same water body

If sampling equipment is used in one water body, then the objective of decontamination is to prevent veliger cross-contamination between sampling events. Early detection monitoring equipment is not cleaned between the sampling sites on the same water body during a given sampling event. The plankton net and cod end are decontaminated using 5% bleach solution (destroys genetic material), and 5% acetic acid (dissolves shell).

a) Equipment

- Large plastic bag
- Large tub with lid
- White vinegar or 5% acetic acid solution (e.g., 2.5-L)
- Bottle of Clorox brand household bleach (approximately 6% NaOCl)
- Spray bottle containing 5% solution of household bleach
- Tap water 5-gal/19-L)
- Spray bottle containing tap water
- Brush

b) Plankton net and cod end

1. Rinse with lake water. Take off cod end and dunk net. Rinse cod end.
2. Physically remove visible debris with fingers or soft brush.
3. Using the spray bottle containing 5-10% solution of bleach, thoroughly spray net, cod end, net anchor, and affiliated rope with fresh 5% Clorox bleach solution (made from bottle 5.25% sodium hypochlorite). Soak for 15 minutes.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

4. Rinse with tap water.

5. Place net and cod end into plastic bag, and add the 5% acetic acid solution to the plastic bag. Cover net and cod end with 5% acetic acid solution. Tie-off bag and place in tub to contain leaks. Soak for a minimum of 8-hrs. The ideal soak time is 24-hrs.

6. Thoroughly rinse with tap water.

B. Sampling equipment used in different water bodies

Field crews can be vectors for the unintentional movement of plants and animals associated with field sampling equipment. Field equipment that is used in multiple water bodies should undergo full decontamination at the field site involving both physical and chemical means to prevent transfer of a variety of taxa within and between systems and samples. An example full decontamination protocol is outlined below.

a) Equipment needed in addition to supplies in IV.A.1.

- 2-3 large plastic tubs with lids (approximately 20-gal./76-L ea.)
- 2% solution of Virkon Aquatic (6-gal./23-L)
- Spray bottle with 2% Virkon Aquatic
- Bristle brush with long handle
- Tap water (20-gal./76-L)

b) Plankton net and cod end

1. Rinse net in lake water. Take off cod end. Dunk net and pull net straight up to flush. Flush out the cod end and use fingers or gentle brush to gently clear mesh.
2. Soak in 2% Virkon Aquatic for 10 minutes.
3. Rinse with tap water.
4. Soak in 5% bleach solution for 15 minutes.
5. Rinse with tap water.
6. Soak in 5% acetic acid solution for 24-hrs. The minimum soak time is 4-hrs.
7. Rinse with tap water.

c) Small sampling equipment, e.g., dredge, rope, minnow traps

1. Rinse in lake water.
2. Manually remove large debris.
3. Scrub all surfaces with a bristle brush. Rinse with tap water.
4. Soak equipment in 2% Virkon Aquatic for 10 minutes.
5. Rinse with tap water.
6. Soak equipment in fresh 5% bleach solution for 15 minutes. The large tub of water used to flush outboard engine cooling lines can be used for equipment bleach soaks.
7. Rinse with tap water.

Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

d) Outboard engines cooling lines

1. Position outboard engine inside large tub. A low sturdy object (e.g., spare tire) can be positioned under the tub to raise tub up to the bottom of engine skeg.
2. Fill tub with tap water so that water level is above engine cooling water intakes (e.g., approximately 18-gal to cover cavitation plates on Honda 30HP outboard).
3. Start engine in neutral, and run engine for approximately two minutes.
4. Do NOT return the cooling water discharge to the tub of water.
5. Keep water level above the cooling water intakes when running engine. Keep a container of tap water handy when engine is running in order to replenish the reservoir of water being used to flush engine cooling system.
6. Turn off engine, and raise engine so that tub of water can be dragged clear.
7. Add store bought bleach (5.25% sodium hypochlorite) to the water in tub to reach a 5% solution of bleach (e.g., add approximately 1-L of store bought bleach to 18-L of tap water to make 5% bleach solution).

e) Boat hull, decking, engine exterior, etc.

1. Manually remove large debris.
2. Spray with 2% Virkon Aquatic.
3. Scrub with bristle brush.
4. Spray with 2% Virkon Aquatic, and let sit for 10 minutes.
5. Rinse with tap water.
6. Plug bilge drain.
7. Use the tub of 5% bleach solution (once done with small equipment soaks) for washing down hull, decking, engine exterior, etc. and scrubbing with brush. Use the 5% bleach solution to rinse debris down into bilge area.
8. Soak bilge area in 5% bleach solution for 30 minutes. Drain bilge on flat pavement or concrete surfaces at least 200-ft. from open water.

V. Contact Information

A. Contact PSU

Rich Miller
Research Assistant
Portland State University
Center for Lakes and Reservoirs
Ph. (office): 503-725-8946
Fax: 503-725-9040
Email: richm@pdx.edu

Physical address:
Portland State University
SRTC, Room 119
Portland OR 97210

USPS shipping:
Portland State University
PO BOX 751-ESM
Portland OR 97207-0751
Attn: Rich Miller

FedEx Shipping:
Portland State University
SRTC, Room 218
Portland OR 97210
Attn: Rich Miller
Tel. 503-725-8946

B. You-Tube Training Video

A video was produced in 2013 to visually demonstrate the principles of veliger sample collection that are described in this document. The video is available at the following link:

< http://www.youtube.com/watch?v=v4Qpzi2p0PE&feature=youtube >

FIELD PLAN

Trip Name__________

Travelers:

Name___________________________________	Home/cell Ph__________________________
Emergency contact_____________________	Ph. Emerg. Contact_______________________
Name___________________________________	Home/cell Ph__________________________
Emergency contact_____________________	Ph. Emerg. Contact_______________________
Name___________________________________	Home/cell Ph__________________________
Emergency contact_____________________	Ph. Emerg. Contact_______________________

ITINERARY

Date	Start FROM	Depart Time	Going TO	Arrive Time	Notes

LODGING

Date	Expected location	Notes

GENERAL NOTES

FLOAT PLAN

DATE LAUNCH_____

TIME LAUNCH_____

LAUNCH LOCATION_____

TRAVELERS:

_____

_____

BOAT_____

IF PROBLEMS, CALL Steve Wells, Portland State University

Monday -Friday (7A to 4P)	**503-725-8946**
All other times	**503-538-7456**

Fold along line. This side facing up on truck dashboard.

EXPECTED DATE RETURN TO VEHICLE_____

EXPECTED TIME RETURN TO VEHICLE_____

ITINERARY SUMMARY

Date	FROM	Depart Time	TO	Arrive Time	Notes

CONTACT INFORMATION OF TRAVELERS

Home Ph			Home Ph		
Cell Ph			Cell Ph		

If found, please call, email or return to Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

USACE Plankton Field Datasheet

WATER BODY: _____ Date:___/ ____/ ____ Collectors:

Weather: Sunny / Pt. Cloudy / Cloudy / Rain / Windy / Calm / Other_____ **Total # samples: _____ Lat. / Long. Datum:_____**

Vertical/ Oblique Tows

Tow #	Latitude	Longitude	Depth (m)	Net efficiency	Sample Container #	Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Trawling

Trawl #	Latitude Start	Longitude Start	Time Start	Latitude End	Longitude End	Time End	Depth (m)	Boat speed (MPH)	Sample Container #	Notes
1										
2										
3										
4										
5										
6										
7										
8										
9										

If found, please call, email or return to Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

WATERBODY: _____ Date___/____/____ Collectors:

Notes

Adult bivalves, snails, crayfish

New artificial substrates deployed	Latitude	Longitude	Site description

Datum:

				Method								**Substrate Surveyed**									**Results**	
WP #	**latitude**	**longitude**	**Site Descrption**	artificial substrate	**hand pat-down**	zig-zag shoreline	**anchor**	other				Portland Sampler	**dock/floats/buoys**	piling/ chnl markr	**bridge abutment**	seawall/ breakwater	**rocks**	woody debris			**Collected (Y/ N)**	Specimen # : AXX e.g. A01 A= animal XX= two digit number for specimen number, i.e. 01-99
e.g.,	45.8725413	-117.2154698	I-5 bridge	x	x		x					x			x	x		x			y	FY15-070113- corbicula and snails

If found, please call, email or return to Portland State University, POB 751-ESM, Portland OR 97207, tel. 503.725.8946, email. richm@pdx.edu

Appendix B

Field Collection Protocols: *Dreissena* mussels and other AIS

2017

Rich Miller, Steve Wells and Mark Sytsma

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

8. **Maximum speed limit when towing is 55 MPH.** Speed is reduced as necessary depending on weather, road conditions, etc.
9. Seatbelts are worn at all times when vehicle is in motion.
10. Courteous and defensive driving. When in doubt, err on the side of caution.
11. 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".
12. Trailer lights inspected before leaving launch. Maintain all trailer lighting.
13. Grease trailer axle prior to leaving boat storage each trip.
14. 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

12. **A personal floatation device (PFD)** is worn anytime a person is in boat.
13. Operate boat with a minimum of two people present. Buddy system.
14. 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).
15. Valid Oregon Boater Education card.
16. Bring drinking water, sun protection, snacks, and non-slip shoes.
17. 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).
18. Lift with your legs and avoid straining muscles. Use cranes, pulleys, and multiple people as needed.
19. Verbally communicate to each other when shifting position within boat, starting engine, throwing plant rake, etc.
20. Secure lightweight equipment before moving.
21. Distribute weight appropriately Port-Starboard and Stern-Bow.
22. Maintain a clean and clear working area.
23. Be safe!!

GENERAL STRATEGY

Prioritization of activities by species.

- Total time on water will vary by water body size and boat speed.
- More sampling locations and bigger samples are desired if possible.
- Zebra/quagga mussels are the primary target.
- 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.
- Sample containers are pre-labeled with number, e.g., 77001. If missing sample container number, assign one using following: FY17- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY17-062513a.
- **On field datasheet, record the sampler container number**, e.g. FY17-5555, 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

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:

- Multiple individual sites as time permits. A site is a particular location separated from other sites by at least 61-m (200-ft).

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

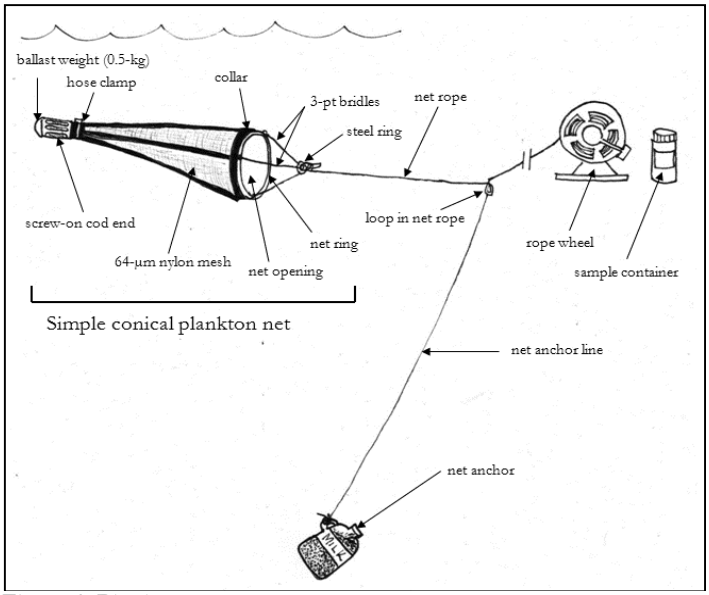


Figure 1: Plankton net set-up.

Sample Collection:

- Combination of **vertical**/oblique tows and **trawling** events in each water body. When trawling, record time and GPS coordinates 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., boat launch.
- 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. 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.
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 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. 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

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

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** 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. Place specimen in cooler on ice.

Surface scraper

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**.

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

Portland Samplers

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**. 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

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**.
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

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**.
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.

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**. 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.**
- 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

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

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**.
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: FY17- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY17-062513a.
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

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

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*)

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*)

MULTI-PROBE UNIT/ WATER QUALITY

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 ○| 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

1. Anchor boat or tie-off to structure such as boom line in front of dam. Record GPS location on datasheet.
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.
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 ±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

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) 10% 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 10% 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 5-L (1.4-gallons) of household bleach to the tub containing approximately 49-L (13-gallons) of tap water. This is a 10% 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 10% 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 10% 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 10% 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 C

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Aquaticus LLC **Point of contact:** Steve Wells

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
6/15/17	6/28/17	78525	14	3	0.14	10	4.3	31	0	0	1	Snake River, ab Ice Harbor; 6/15/17
6/15/17	6/28/17	78525	12	3	0.14	10	4.3	36	0	0	1	0 photos
			26			20	8.6	33	0	0	2	
7/6/17	6/28/17	FY15-2751	5	3	0.17	8	4.0	80	0	0	7	Columbia River, confl; 6/15/17
7/6/17	6/28/17	FY15-2751	5.5	3	0.17	4	2.0	36	0	0	3	*Gonidea angulata*: 2
7/6/17	6/28/17	FY15-2751	4	3	0.17	4	2.0	50	0	0	5	1 photo
			14.5			16	8.0	55	0	0	15	
7/7/17	6/28/17	78496	3	3	0.11	6	2.0	67	0	0	1	Columbia River, McNary confl; 6/15/17
7/7/17	6/28/17	78496	3	3	0.11	3	1.0	33	0	0	2	snail: 1; 0 photos
			6			9	3.0	50	0	0	3	
7/7/17	6/28/17	78494	3	3	0.14	4	1.7	57	0	0	2	Columbia River, McNary Pool; 6/15/17
7/7/17	6/28/17	78494	3	3	0.14	4	1.7	57	132*	0	4	*BMS veligers (132/574)
7/7/17	6/28/17	78494	3	3	0.14	3	1.3	43	0	0	6	0 photos
			9			11	4.7	52	0	0	12	
7/7/17	6/28/17	77069	4.5	3	0.14	4	1.7	38	0	0	0	Snake River, nr Hells Gate Marina; 6/20/17
7/7/17	6/28/17	77069	3	3	0.14	4	1.7	57	0	0	0	0 photos
7/7/17	6/28/17	77069	3	3	0.14	4	1.7	57	0	0	0	
			10.5			12	5.1	51	0	0	0	
7/8/17	6/28/17	77148	4.5	3	0.14	5	2.1	48	0	0	0	Snake River, confl. Clearwater River; 6/20/17
7/8/17	6/28/17	77148	4.5	3	0.14	5	2.1	48	0	0	0	*Gonidea angulata*: 2; lots sediment.
7/8/17	6/28/17	77148	4.5	3	0.14	5	2.1	48	0	0	1	1 photo
			13.5			15	6.4	48	0	0	1	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
7/8/17	6/28/17	FY15-2717	5	3	0.14	5	2.1	43	0	0	1	Snake River, confl. Clearwater River; 6/20/17
7/8/17	6/28/17	FY15-2717	5	3	0.14	5	2.1	43	0	0	4	*Gonidea angulata*: 3; 10 photos
			10			10	4.3	43	0	0	5	
7/9/17	6/28/17	FY15-2719	5	3	0.14	4	1.7	34	0	0	0	Snake River, Ice Harbor; 6/15/17
7/9/17	6/28/17	FY15-2719	4.5	3	0.14	4	1.7	38	0	0	0	
			9.5			8	3.4	36	0	0	0	
7/9/17	6/28/17	78514	3	3	0.14	3	1.3	43	0	0	0	Snake River, ab Ice Harbor; 6/15/17
7/9/17	6/28/17	78514	2	3	0.14	3	1.3	64	0	0	0	
7/9/17	6/28/17	78514	3	3	0.14	3	1.3	43	0	0	0	
			8			9	3.9	50	0	0	0	
7/23/17	7/18/17	77053	4	3	0.17	4	2.0	50	0	0	4	Lake Pend Oreille, Hope; 5/31/17
			4			4	2.0	50	0	0	4	
7/23/17	7/18/17	77086	3.5	3	0.17	3	1.5	43	0	0	2	Lake Pend Oreille, Islands; 5/31/17
			3.5			3	1.5	43	0	0	2	
7/23/17	7/18/17	77070	3.5	3	0.17	4	2.0	57	0	0	0	Lake Pend Oreille, Anderson Pt.; 5/31/17
			3.5			4	2.0	57	0	0	0	
7/23/17	7/18/17	77016	4	3	0.17	4	2.0	50	0	0	0	Lake Pend Oreille; 5/31/17
0			4			4	2.0	50	0	0	0	
8/1/17	7/21/17	FY2017-10	3.5	3	0.17	4	2.0	57	0	0	4	Columbia River, McNary Pool; 7/12/17
			3.5			4	2.0	57	0	0	4	
8/1/17	7/21/17	FY2017-11	4	3	0.17	4	2.0	50	0	0	5	Columbia River, McNary Pool; 7/12/17
			4			4	2.0	50	0	0	5	
8/1/17	7/21/17	FY2017-12	7.5	3	0.17	6	3.0	40	0	0	7	Columbia River, McNary Pool; 7/12/17
			7.5			6	3.0	40	0	0	7	native unionid: 1; 3 photos
8/1/17	7/21/17	FY2017-15	4	3	0.17	4	2.0	50	68*	0	5	Snake River, ab Ice Harbor; *BMS veligers (68/386)
8/1/17	7/21/17	FY2017-15	5.5	3	0.17	4	2.0	36	0	0	2	6/30/17; *Gonidea angulata*: 3; 2 photos
			9.5			8	4.0	43	0	0	7	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
8/1/17	7/21/17	FY2017-3	7.5	3	0.17	6	3.0	40	0	0	2	Snake River, ab Ice Harbor; 6/30/17
			7.5			6	3.0	40	0	0	2	native unionid: 1; 6 photos
8/1/17	7/21/17	FY2017-7	6.5	3	0.17	6	3.0	46	0	0	2	Snake River, confl. Clearwater Rv; 6/30/17
			6.5			6	3.0	46	0	0	2	*Gonidea angulata*: 2; 5 photos
8/1/17	7/21/17	FY2017-9	3.5	3	0.17	3	1.5	43	0	0	5	Snake River, Lower Granite; 7/13/17
			3.5			3	1.5	43	0	0	5	*Gonidea angulata*: 4; 7 photos
8/1/17	7/21/17	FY2017-4	5.5	3	0.17	4	2.0	36	0	0	6	Snake River, Lower Granite; 7/13/17
			5.5			4	2.0	36	0	0	6	*Gonidea angulata*: 7; 8 photos
8/1/17	7/21/17	FY2017-2	4	3	0.17	4	2.0	50	0	0	2	Snake River, Lower Granite; 7/13/17
			4			4	2.0	50	0	0	2	*Gonidea angulata*: 4; 15 photos
8/1/17	7/21/17	FY2017-18	3	3	0.17	3	1.5	50	0	0	2	Snake River, ab Ice Harbor; 7/12/17
			3			3	1.5	50	0	0	2	low sediment
8/1/17	7/21/17	FY2017-8	3.5	3	0.17	3	1.5	43	0	0	2	Snake River, ab Ice Harbor; 7/12/17
			3.5			3	1.5	43	0	0	2	low sediment
8/1/17	7/21/17	FY2017-13	4	3	0.17	4	2.0	50	0	0	0	Snake River, ab Ice Harbor; 7/12/17
			4			4	2.0	50	0	0	0	low sediment
8/22/17	8/9/17	FY2017-53	3.5	3	0.17	5	2.5	71	0	0	2	Koocanusa, Lower; 6/27/17
			3.5			5	2.5	71	0	0	2	
8/22/17	8/9/17	FY2017-41	3.5	3	0.17	4	2.0	57	0	0	0	Koocanusa, Upper; 6/27/17
			3.5			4	2.0	57	0	0	0	
8/22/17	8/9/17	FY2017-50	3.5	3	0.17	4	2.0	57	0	0	0	Koocanusa, Middle; 6/27/17
			3.5			4	2.0	57	0	0	0	
8/22/17	8/9/17	FY2017-36	3.5	3	0.17	4	2.0	57	0	0	0	Koocanusa; 7/20/17
			3.5			4	2.0	57	0	0	0	
8/22/17	8/9/17	FY2017-28	3.5	3	0.17	4	2.0	57	0	0	0	Koocanusa; 7/20/17
			3.5			4	2.0	57	0	0	0	
8/23/17	8/9/17	FY2017-26	3.5	3	0.17	5	2.5	71	0	0	1	Koocanusa; 7/20/17
			3.5			5	2.5	71	0	0	1	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
8/23/17	8/9/17	FY2017-17	4.5	3	0.17	5	2.5	56	0	0	1	Snake River, ab Ice Harbor; 7/26/17
			4.5			5	2.5	56	0	0	1	
8/23/17	8/9/17	FY2017-5	4	3	0.17	4	2.0	50	0	0	10	Snake River, ab Ice Harbor; 7/26/17
8/23/17	8/9/17	FY2017-5	3.5	3	0.17	4	2.0	57	145*	0	14	*BMS veligers (145/345)
			7.5			8	4.0	54	0	0	24	
8/23/17	8/9/17	FY2017-16	4.5	3	0.17	5	2.5	56	0	0	0	
			4.5	3	0.17	5	2.5	56	0	0	0	
8/23/17	8/9/17	FY2017-1	4	3	0.17	5	2.5	63	0	0	5	Snake River, confl Clearwater Rv; 7/26/17
			4			5	2.5	63	0	0	5	
8/23/17	8/9/17	FY2017-130	4.5	3	0.17	5	2.5	56	0	3	0	Snake River, ab confl Clearwater Rv; 7/27/17
8/23/17	8/9/17	FY2017-130	4	3	0.17	5	2.5	63	0	5	0	*Gonidea angulata:* 1; 5 photos
			8.5			10	5.0	59	0	8	0	
8/23/17	8/9/17	FY2017-14	4	3	0.17	5	2.5	63	0	0	3	Columbia River, confl. Snake River; 7/26/17
			4			5	2.5	63	0	0	3	snails e.g. *Valvata;* 3 photos
8/23/17	8/9/17	FY2017-118	4	3	0.17	5	2.5	63	0	0	0	Snake River, bl confl. Clearwater Rv; 7/27/17
			4			5	2.5	63	0	0	0	
8/23/17	8/9/17	FY2017-6	3.5	3	0.17	5	2.5	71	0	0	2	Columbia River, bl confl. Snake River; 7/26/17
8/23/17	8/9/17	FY2017-6	3.5	3	0.17	5	2.5	71	0	0	3	
			7			10	5.0	71	0	0	5	
8/23/17	8/9/17	FY2017-136	4	3	0.17	5	2.5	63	0	0	4	Snake River, bl confl. Clearwater Rv; 7/27/17; 3 photos
			4			5	2.5	63	0	0	4	*G. angulata:* 1, poor specimen, intact valves, no internal
9/4/17	8/22/17	FY2017-109	5	3	0.17	5	2.5	50	0	0	0	Snake River, ab Ice Harbor; 8/9/17
			5			5	2.5	50	0	0	0	
9/4/17	8/22/17	FY2017-115	3.5	3	0.17	4	2.0	57	0	0	0	Snake River, ab Ice Harbor; 8/9/17
9/4/17	8/22/17	FY2017-115	3.5	3	0.17	4	2.0	57	0	0	0	
			7			8	4.0	57	0	0	0	
9/4/17	8/22/17	FY2017-154	3.5	3	0.17	4	2.0	57	0	0	0	Snake River, ab Ice Harbor; 8/9/17
			3.5			4	2.0	57	0	0	0	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
9/4/17	8/22/17	FY2017-151	3.5	3	0.17	4	2.0	57	0	0	13	Columbia River, bl confl. Snake Rv; 8/9/17
9/4/17	8/22/17	FY2017-151	4	3	0.17	4	2.0	50	123*	0	5	*BMS veligers (123/222)
			7.5			8	4.0	54	0	0	18	
9/4/17	8/22/17	FY2017-127	5	3	0.17	5	2.5	50	0	0	10	Columbia River, bl confl. Snake Rv; 8/9/17
			5			5	2.5	50	0	0	10	
9/4/17	8/22/17	FY2017-121	4	3	0.17	4	2.0	50	0	0	17	Columbia River, bl confl. Snake Rv; 8/9/17
9/4/17	8/22/17	FY2017-121	4	3	0.17	4	2.0	50	0	0	19	
			8			8	4.0	50	0	0	36	
9/4/17	8/22/17	FY2017-133	5	3	0.17	5	2.5	50	0	3	5	Snake River, ab confl. Clearwater Rv; 8/11/17
9/4/17	8/22/17	FY2017-133	5	3	0.17	5	2.5	50	0	2	4	4 photos
			10			10	5.0	50	0	5	9	
9/4/17	8/22/17	FY2017-145	3.5	3	0.17	4	2.0	57	0	0	2	Snake River, bl confl. Clearwater Rv; 8/11/17
			3.5			4	2.0	57	0	0	2	
9/4/17	8/22/17	FY2017-124	3.5	3	0.17	4	2.0	57	0	0	4	Snake River, bl confl. Clearwater Rv; 8/11/17
			3.5			4	2.0	57	0	0	4	
9/4/17	8/22/17	77095	5	3	0.17	10	5.0	100	0	0	2	Lake Pend Oreille, Contest Point; 6/24/17
			5			10	5.0	100	0	0	2	
9/5/17	8/22/17	77014	5	3	0.17	6	3.0	60	0	0	0	Lake Pend Oreille, Anderson Point; 6/24/17
			5			6	3.0	60	0	0	0	
9/29/17	9/8/17	77102	4	3	0.17	5	2.5	63	0	0	1	Lake Pend Oreille, Anderson Point; 7/26/17
9/29/17	9/8/17	77102	3	3	0.17	5	2.5	83	0	0	1	
			7			10	5.0	73	0	0	2	
9/29/17	9/8/17	77109	4.5	3	0.17	6	3.0	67	0	0	2	Lake Pend Oreille, Contest Point; 7/26/17
			4.5			6	3.0	67	0	0	2	
9/29/17	9/8/17	FY2017-148	4.5	3	0.17	6	3.0	67	30*	0	4	Snake River, ab Ice Harbor; *BMS veligers (30/57)
9/29/17	9/8/17	FY2017-148	4.5	3	0.17	6	3.0	67	0	0	1	8/28/17; lots sediment
			9			12	6.0	67	0	0	5	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
9/29/17	9/8/17	FY2017-99	4	3	0.17	5	2.5	63	0	0	26	Snake River, ab confl. Columbia; 8/28/17
			4			5	2.5	63	0	0	26	sediment
9/29/17	9/8/17	FY2017-139	4.5	3	0.17	6	3.0	67	0	0	23	Snake River, ab confl. Clearwater Rv; 8/25/17
9/29/17	9/8/17	FY2017-139	5	3	0.17	6	3.0	60	0	6	15	lots of sediment; 4 photos
9/29/17	9/8/17	FY2017-139	5	3	0.17	6	3.0	60	0	3	24	
			14.5			18	9.0	62	0	9	62	
9/30/17	9/8/17	FY2017-142	5.5	3	0.17	6	3.0	55	0	0	8	Snake River, bl confl Clearwater Rv; 8/25/17
			5.5			6	3.0	55	0	0	8	
9/30/17	9/8/17	FY2017-157	4	3	0.17	6	3.0	75	0	1	12	Snake River, bl confl. Clearwater Rv; 8/25/17
			4			6	3.0	75	0	1	12	2 photos
9/30/17	9/8/17	FY2017-116	7.5	3	0.17	8	4.0	53	0	0	21	Snake River, ab confl. Columbia Rv; 8/28/17
			7.5			8	4.0	53	0	0	21	
9/30/17	9/8/17	FY2017-113	3.5	3	0.17	5	2.5	71	0	0	0	Snake River, ab confl. Columbia Rv; 8/28/17
			3.5			5	2.5	71	0	0	0	
9/30/17	9/8/17	FY2017-112	6.5	3	0.17	8	4.0	62	0	0	10	Snake River, ab Ice Harbor; 8/28/17
			6.5			8	4.0	62	0	0	10	
9/30/17	9/8/17	FY2017-111	7.5	3	0.17	8	4.0	53	0	0	1	Snake River, ab Ice Harbor; 8/28/17
			7.5			8	4.0	53	0	0	1	
10/6/17	9/22/17	FY2017-184	4	3	0.17	5	2.5	63	0	0	3	Snake River, ab Ice Harbor; 9/8/17
10/6/17	9/22/17	FY2017-184	4	3	0.17	5	2.5	63	0	0	5	
			8			10	5.0	63	0	0	8	
10/6/17	9/22/17	FY2017-160	4	3	0.17	5	2.5	63	0	5	40	Snake River, ab confl. Clearwater Rv; 9/7/17
10/6/17	9/22/17	FY2017-160	4	3	0.17	5	2.5	63	0	2	50	sediment, snails scarce; 2 photos
			8			10	5.0	63	0	7	90	
10/6/17	9/22/17	FY2017-178	5.5	3	0.17	6	3.0	55	0	0	2	Snake River, ab Ice Harbor; 9/8/17
10/6/17	9/22/17	FY2017-178	4.5	3	0.17	6	3.0	67	0	0	1	
10/6/17	9/22/17	FY2017-178	5	3	0.17	6	3.0	60	0	0	7	
			15			18	9.0	60	0	0	10	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
10/6/17	9/22/17	FY2017-172	4	3	0.17	5	2.5	63	0	0	14	Snake River, ab Ice Harbor; 9/8/17
10/6/17	9/22/17	FY2017-172	4	3	0.17	5	2.5	63	0	0	12	
			8			10	5.0	63	0	0	26	
10/6/17	9/22/17	FY2017-193	3.5	3	0.17	5	2.5	71	0	0	32	Snake River, bl confl. Clearwater Rv; 9/7/17
			3.5			5	2.5	71	0	0	32	sediment
10/6/17	9/22/17	FY2017-190	3.5	3	0.17	5	2.5	71	0	0	26	Snake River, bl confl. Clearwater Rv; 9/7/17
			3.5			5	2.5	71	0	0	26	sediment
10/9/17	9/22/17	FY2017-163	5.5	3	0.17	7	3.5	64	0	0	11	Snake River, ab confl. Columbia Rv; 9/8/17
10/9/17	9/22/17	FY2017-163	4	3	0.17	7	3.5	88	0	1	9	Sediment; 1 photo
			9.5			14	7.0	76	0	1	20	
10/9/17	9/22/17	FY2017-187	4	3	0.17	5	2.5	63	0	0	2	Snake River, ab confl. Columbia Rv; 9/8/17
10/9/17	9/22/17	FY2017-187	5	3	0.17	5	2.5	50	0	2	12	Sediment; 1 photo
			9			10	5.0	56	0	2	14	
10/9/17	9/22/17	FY2017-202	4	3	0.17	5	2.5	63	0	2	14	Snake River, ab confl. Columbia Rv; 9/8/17
			4			5	2.5	63	0	2	14	Sediment; 1 photo
10/15/17	10/2/17	78419	7	3	0.17	9	4.5	64	0	1	0	Columbia River, Bonneville Rv Center; 7/5/17
10/15/17	10/2/17	78419	6	3	0.17	9	4.5	75	0	2	3	*Anodonta*: 1, snails: 1; 4 photos
10/15/17	10/2/17	78419	5	3	0.17	7	3.5	70	133*	0	0	*BMS veligers (133/479)
			18			25	12.5	70	0	3	3	
10/15/17	10/2/17	78416	7	3	0.17	8	4.0	57	0	0	7	Columbia River, Bonneville OR; 7/5/17
10/15/17	10/2/17	78416	5	3	0.17	6	3.0	60	0	0	5	*Anodonta*: 2; 3 photos
			12			14	7.0	59	0	0	12	
10/15/17	10/2/17	78418	10	3	0.17	10	5.0	50	0	2	6	Columbia River, Bonneville WA; 7/5/17
10/15/17	10/2/17	78418	7.5	3	0.17	8	4.0	53	0	1	9	*Anodonta*: 3; 4 photos
			17.5			18	9.0	52	0	3	15	
10/16/17	10/2/17	78125	5	3	0.17	6	3.0	60	0	12	17	Columbia River, Bonneville OR; 8/1/17
10/16/17	10/2/17	78125	4	3	0.17	6	3.0	75	64*	19	13	*BMS veligers (64/204), snails: 27
			9			12	6.0	68	0	31	30	*Anodonta*: 1, snails: 25; 5 photos

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
10/16/17	10/2/17	78415	4	3	0.17	6	3.0	75	0	48	18	Columbia River, Bonneville WA; 8/1/17
			4			6	3.0	75	0	48	18	*Anodonta*: 2, snails: 13; 2 photos
10/16/17	10/2/17	78371	4.5	3	0.17	6	3.0	67	0	37	15	Columbia River, Bonneville mid channel; 8/1/17
			4.5			6	3.0	67	0	37	15	snails: 28; 1 photo
10/16/17	10/2/17	78427	5	3	0.17	6	3.0	60	0	31	7	Columbia River, Bonneville OR; 8/16/17
			5			6	3.0	60	0	31	7	snails: 53; 1 photo
10/16/17	10/2/17	78428	5	3	0.17	6	3.0	60	0	42	4	Columbia River, Bonneville mid channel; 8/16/17
			5			6	3.0	60	0	42	4	snails: 51; 1 photo
10/16/17	10/2/17	78516	5.5	3	0.17	7	3.5	64	0	43	2	Columbia River, Bonneville WA; 8/16/17
			5.5			7	3.5	64	0	43	2	snails: 43; 1 photo
10/16/17	10/2/17	FY2017-30	5	3	0.17	6	3.0	60	0	64	15	Columbia River, Bonneville WA; 8/22/17
			5			6	3.0	60	0	64	15	snails: 36
10/16/17	10/2/17	FY2017-19	4	3	0.17	6	3.0	75	0	33	16	Columbia River, Bonneville OR; 8/22/17
			4			6	3.0	75	0	33	16	*Anodonta*: 1, snails: 22; 1 photo
10/16/17	10/2/17	FY2017-76	4	3	0.17	6	3.0	75	0	92	15	Columbia River, Bonneville mid channel; 8/22/17
			4			6	3.0	75	0	92	15	*Anodonta*: 1, snails: 47; 1 photo
10/24/17	10/12/17	FY2017-166	5	3	0.17	7	3.5	70	0	1	4	Columbia River, bl confl. Snake Rv; 9/22/17
10/24/17	10/12/17	FY2017-166	4	3	0.17	7	3.5	88	0	0	19	1 photo
			9			14	7.0	79	0	1	23	
10/24/17	10/12/17	FY2017-169	5	3	0.17	6	3.0	60	0	0	33	Columbia River, bl confl. Snake Rv; 9/22/17; snails: 1
10/24/17	10/12/17	FY2017-169	4	3	0.17	6	3.0	75	114*	0	31	*BMS veligers (114/279)
			9			12	6.0	68	0	0	64	
10/24/17	10/12/17	FY2017-196	5	3	0.17	6	3.0	60	0	0	6	Columbia River, bl confl. Snake Rv; 9/22/17
10/24/17	10/12/17	FY2017-196	5	3	0.17	6	3.0	60	0	0	9	
			10			12	6.0	60	0	0	15	
10/25/17	10/12/17	FY2017-199	5	3	0.17	6	3.0	60	0	0	12	Snake River, ab Ice Harbor; 9/22/17
			5			6	3.0	60	0	0	12	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
10/25/17	10/12/17	FY2017-175	4	3	0.17	6	3.0	75	0	0	13	Snake River, ab Ice Harbor; 9/22/17
			4			6	3.0	75	0	0	13	
10/25/17	10/12/17	FY2017-181	5	3	0.17	6	3.0	60	0	0	5	Snake River, ab Ice Harbor; 9/22/17
10/25/17	10/12/17	FY2017-181	6	3	0.17	6	3.0	50	0	0	6	
			11			12	6.0	55	0	0	11	
10/25/17	10/12/17	FY2017-167	6	3	0.17	7	3.5	58	0	0	36	Snake River, bl confl. Clearwater Rv; 9/29/17; snails: 1
10/25/17	10/12/17	FY2017-167	5	3	0.17	7	3.5	70	157*	0	33	*BMS veligers (157/236)
			11			14	7.0	64	0	0	69	
10/25/17	10/12/17	FY2017-164	5	3	0.17	6	3.0	60	0	9	34	Snake River, ab confl. Clearwater; 9/29/17
10/25/17	10/12/17	FY2017-164	5.5	3	0.17	7	3.5	64	0	8	37	limpets: 2, snails: 1; 12 photos
			10.5			13	6.5	62	0	17	71	
10/25/17	10/12/17	FY2017-170	4	3	0.17	6	3.0	75	0	3	30	Snake River, bl confl. Clearwater Rv; 9/29/17
10/25/17	10/12/17	FY2017-170	4	3	0.17	6	3.0	75	0	0	37	2 photos
			8			12	6.0	75	0	3	67	
10/30/17	10/19/17	FY13-7043	3.5	3	0.17	5	2.5	71	0	0	2	Fern Ridge Reservoir, #2; 8/16/17
			3.5			5	2.5	71	0	0	2	lots of sodium bicarbonate crystals
10/30/17	10/19/17	FY14-7134	3.5	3	0.17	5	2.5	71	0	0	0	Dexter Reservoir, #1; 8/16/17
			3.5			5	2.5	71	0	0	0	lots of sodium bicarbonate crystals
10/30/17	10/19/17	77030	5	3	0.17	8	4.0	80	0	0	1	Fern Ridge Reservoir, #4; 9/18/17
10/30/17	10/19/17	77030	5	3	0.17	7	3.5	70	0	0	0	lots of sodium bicarbonate crystals
			10			15	7.5	75	0	0	1	
10/30/17	10/19/17	dex_3	3.5	3	0.17	6	3.0	86	0	0	0	Dexter Reservoir, #3; 9/18/17
			3.5			6	3.0	86	0	0	0	lots of sodium bicarbonate crystals
10/30/17	10/20/17	77122	4	3	0.17	5	2.5	63	0	0	0	Lake Pend Oreille; 9/22/17
			4			5	2.5	63	0	0	0	
11/2/17	10/20/17	77120	3.5	3	0.17	7	3.5	100	0	0	3	Lake Pend Oreille; 9/27/17
			3.5			7	3.5	100	0	0	3	

Appendix C: Results of light microscopy analysis of USACE-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued **PAGE_10 of _10_**

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
11/8/17	10/26/17	FY2017-21	3.5	3	0.17	5	2.5	71	0	0	0	Koocanusa, Tobacco Plains; 8/17/17
			3.5			5	2.5	71	0	0	0	
11/8/17	10/26/17	FY2017-33	3.5	3	0.17	7	3.5	100	0	0	4	Koocanusa, Peck Rocky; 8/17/17
			3.5			7	3.5	100	0	0	4	
11/8/17	10/26/17	FY2017-82	3.5	3	0.17	7	3.5	100	0	0	9	Koocanusa, McGillavry; 8/17/17
			3.5			7	3.5	100	0	0	9	
11/8/17	10/26/17	FY2017-32	3	3	0.17	6	3.0	100	0	0	0	Koocanusa, Abayance Bay; 9/5/17
			3			6	3.0	100	0	0	0	
11/8/17	10/26/17	FY2017-73	3	3	0.17	6	3.0	100	0	0	0	Koocanusa, Peck Gulch; 9/5/17
			3			6	3.0	100	0	0	0	
11/8/17	10/26/17	FY2017-34	3	3	0.17	6	3.0	100	0	0	0	Koocanusa, Koocanusa Marina; 9/5/17
			3			6	3.0	100	0	0	0	
12/14/17	12/5/17	77128	4	3	0.17	7	3.5	88	0	0	6	Rufus Woods Reservoir; 7/27/17
12/14/17	12/5/17	77128	4.5	3	0.17	7	3.5	78	0	0	1	sediment
			8.5			14	7.0	83	0	0	7	
12/14/17	12/5/17	77098	4	3	0.17	7	3.5	88	0	0	3	Rufus Woods Reservoir; 7/27/17
			4			7	3.5	88	0	0	3	
12/14/17	12/5/17	77072	3	3	0.17	6	3.0	100	56*	11	4	*BMS veligers (56/95)
12/14/17	12/5/17	77072	3	3	0.17	6	3.0	100	0	9	1	Rufus Woods Reservoir; 9/12/17; 2 photos
			6			12	6.0	100	0	20	5	

Table end

Appendix E: Results of light microscopy analysis of PSU-collected plankton samples for the presence of *Dreissena* veligers in 2017.

Appendix D

Zebra/ Quagga Mussel Early Detection Monitoring **Lab**: Aquaticus LLC **Point of contact:** Steve Wells **PAGE 1 of 2**

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
10/28/17	10/17/17	FY2017-195	2	3	0.17	4	2.0	100	0	0	0	Columbia River, confl. Deschutes Rv; 10/6/17
			2			4	2.0	100	0	0	0	
10/28/17	10/17/17	FY2017-204	1	3	0.17	2	1.0	100	0	0	4	Columbia River, Celilo Park; 10/6/17
			1			2	1.0	100	0	0	4	
10/28/17	10/17/17	FY2017-201	2.5	3	0.17	5	2.5	100	0	1	8	Columbia River, Mayer SP; 10/6/17
			2.5			5	2.5	100	0	1	8	snails: 43; 2 photos
10/28/17	10/17/17	FY2017-210	3	3	0.17	6	3.0	100	0	2	11	Columbia River, The Dalles; 10/6/17
			3			6	3.0	100	0	2	11	snails: 5; 2 photos
10/28/17	10/17/17	FY2017-220	5.5	3	0.17	7	3.5	64	0	6	14	Columbia River, Mayers SP; 10/5/17
10/28/17	10/17/17	FY2017-220	4	3	0.17	7	3.5	88	0	8	9	snails: 24; 3 photos
10/28/17	10/17/17	FY2017-220	4	3	0.17	7	3.5	88	104*	3	20	*BMS veligers (104/213)
			13.5			21	10.5	80	0	17	43	
10/28/17	10/17/17	FY2017-219	4	3	0.17	5	2.5	63	0	3	6	Columbia River, Cascade Locks; 10/5/17
10/28/17	10/17/17	FY2017-219	4	3	0.17	5	2.5	63	0	1	3	snails: 33; 3 photos
			8			10	5.0	63	0	4	9	
10/30/17	10/17/17	FY2017-v140	2	3	0.17	4	2.0	100	0	0	1	Columbia River, Rooster Rock; 10/6/17
			2			4	2.0	100	0	0	1	
10/30/17	10/17/17	FY2017-218	7.5	3	0.17	9	4.5	60	0	2	8	Columbia River, Starvation Creek; 9/26/17
10/30/17	10/17/17	FY2017-218	6	3	0.17	8	4.0	67	0	1	1	snails: 27; 3 photos
10/30/17	10/17/17	FY2017-218	6.5	3	0.17	8	4.0	62	0	3	6	
10/30/2017	10/17/17	FY2017-218	5.5	3	0.17	7	3.5	64	69*	2	8	*BMS veligers (69/169)
			25.5			32	16.0	63	0	8	23	

Appendix E: Results of light microscopy analysis of PSU-collected plankton samples for the presence of *Dreissena* veligers in 2017.

continued **PAGE_2 of_2_**

Date Analysis	Date Rec'd	Sample tracking number	Con vol	SR cell vol	Dil. factor	# SR cells done	mL of conc. sample analyzed	% done	# ZQM	# Corb	# Ost	Notes
10/30/17	10/17/17	FY2017-217	7.5	3	0.17	9	4.5	60	0	6	9	Columbia River, Cascade Locks; 9/26/17
10/30/17	10/17/17	FY2017-217	7.5	3	0.17	9	4.5	60	0	6	1	snails: 65; 6 photos
10/30/17	10/17/17	FY2017-217	5	3	0.17	6	3.0	60	0	10	2	
			20			24	12.0	60	0	22	12	
10/30/17	10/17/17	FY2017-v134b	3.5	3	0.17	7	3.5	100	0	3	4	Columbia River, Cascade Locks; 10/6/17
			3.5			7	3.5	100	0	3	4	snails: 27; 1 photo
10/30/17	10/17/17	FY2017-198	3	3	0.17	6	3.0	100	0	0	8	Columbia River, Hood River; 10/6/17
			3			6	3.0	100	0	0	8	snails: 15
10/30/17	10/17/17	FY2017-221	5	3	0.17	6	3.0	60	0	3	9	Columbia River, Mayer SP; 10/5/17
10/30/17	10/17/17	FY2017-221	7.5	3	0.17	9	4.5	60	0	11	18	snails: 57; 2 photos
10/30/17	10/17/17	FY2017-221	4	3	0.17	5	2.5	63	0	5	9	
			16.5			20	10.0	61	0	19	36	
10/30/17	10/17/17	FY2017-222	7.5	3	0.17	9	4.5	60	0	3	16	Columbia River, Mayer SP; 10/5/17
10/30/17	10/17/17	FY2017-222	6	3	0.17	8	4.0	67	0	3	6	snails: 13; 1 photo
			13.5			17	8.5	63	0	6	22	
11/2/17	10/20/17	FY2017-223	3.5	3	0.17	7	3.5	100	2*	0	2	*2 *Dreissena* veligers (umbonal & late umbonal-early pediveliger stages); veligers from contaminated field net; 12 photos
11/2/17	10/20/17	FY2017-223	3.5	3	0.17	7	3.5	100	3*	0	3	*3 *Dreissena* veligers (two straight-hinge & one late umbonal to early pediveliger stages); veligers from contaminated field net; 20 photos
			7			14	7.0	100	0	0	5	

Table end