2015 Final Report

Lake Quinault Aquatic Invasive Species Survey

By or on behalf of the
Quinault Indian Nation

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2015 Final Report

Lake Quinault Aquatic Invasive Species Survey

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Abstract

Corbicula fluminea (Asian clam) clams were collected in Lake Quinault in 2015. No other aquatic invasive species (AIS) were collected in Lake Quinault during WBS sampling efforts in 2015. Live adult *Corbicula* clams were collected in 10 locations in Lake Quinault. All of the living clams were collected in Lake Quinault near the mouth of Ziegler Creek. Several empty shells were also collected in a shoreline area near Canoe Creek.

Biological sampling for AIS bivalves, gastropods, crayfish, and submerged macrophytes included sampling existing submerged man-made and natural surfaces, shoreline surveys, Portland Samplers for AIS settlement on artificial settlement surfaces, and plankton sampling for bivalve larvae. A total of 705 locations or samples were collected in Lake Quinault by WBS in 2015 using hand pat-downs (n= 73), a surface scraper (n=85), grab sampler (n=117), thatch rake and/or sight tube (n= 280), shoreline surveys (n= 15), artificial settlement substrates (n= 8), crayfish traps (n= 29), and plankton samples (n= 98). Submerged surfaces included logs, dock floats, tree stumps, macrophytes, rocks, gravel, and sand. Sampling targeted several taxa including but not limited to, *Potamopyrgus antipodarum* (New Zealand mudsnails), *Corbicula fluminea* (Asian clams), *Procambarus clarkii* (Red swamp crayfish), *Dreissena polymorpha* (zebra mussels), *D. rostriformis bugensis* (quagga mussels), *Myriophyllum spicatum* (Eurasian water milfoil), *M. heterophyllum* (Variable leaf milfoil), *M. aquaticum* (Parrotfeather), and *Egeria densa* (Brazilian elodea). More than 75,000-L of Lake Quinault were filtered through a plankton net, and over 460 slides were microscopically analyzed during plankton analysis using cross-polarized light microscopy at the Western Biological Services laboratory.

The habitat conditions in Lake Quinault in the August to September period were ideal for the collection and identification of mollusk, crustacean, and macrophyte specimens (e.g., macrophytes with seeds, mussels are spawning, NZMS are brooding young, etc.). AIS sampling occurred during three sampling trips during August and September. The greatest sampling effort occurred during the third sampling trip.

Native and other non-target invertebrates and macrophytes were opportunistically collected by WBS during AIS sampling efforts in 2015. Four different types of bivalves, one species of crayfish, and five types of gastropods were collected from Lake Quinault in 2015. Members from 11 plant families were collected including at least five species of *Potamogeton, Elodea canadensis, Juncus, Eleocharis, Sparganium, Fontinalis, Chara, Nitella, Equisetum, Polygonum, Ranunculus,* and *Isoetes.* Many of the invertebrates and macrophytes collected were native or were from groups with lots of natives and a few invasive, e.g., Physidae and *Potamogeton.* Many specimens were not identified to the species level because the collected specimens often lacked the necessary morphological characters such as flowers and seeds.

Introduction

Aquatic invasive species (AIS) represent one of the most significant habitat alterations for fish, wildlife, and plants, and are rapidly becoming a dominant component of aquatic ecosystems within the Pacific Northwest (Sanderson, Barnas & Rub 2009). AIS of concern to the Quinault Indian Nation (QIN) include, but are not limited to, *Potamopyrgus antipodarum* (New Zealand mudsnails), *Corbicula fluminea* (Asian clams), *Procambarus clarkii* (Red swamp crayfish), *Dreissena polymorpha* (zebra mussels), *D. rostriformis bugensis* (quagga mussels), *Myriophyllum spicatum* (Eurasian water milfoil), *M. heterophyllum* (Variable leaf milfoil), *M. aquaticum* (Parrotfeather), and *Egeria densa* (Brazilian elodea).

P. antipodarum, referred to hereafter as NZMS, are considered to be a high invasive threat to freshwater habitats, and are a priority species of concern in the State of Washington (Washington State Invasive Species Council 2013). NZMS are small snails that possess an operculum, which allows them to seal themselves inside their shells and resist desiccation as well as many fishes' digestive systems (Haynes, Taylor & Varley 1985). NZMS can become the dominant macroinvertebrate species, reaching densities over 500,000 individuals per square meter, e.g., Snake River near Hagerman Idaho (Richards, Cazier & Lester 2001), and they can dominate carbon and nitrogen fluxes (Hall, Tank & Dybdahl 2003). While the dramatic predicted impacts of NZMS have yet to manifest in the West, there is continued concern about the spread of this species.

NZMS are spreading in western water bodies. NZMS can be easily spread to new water bodies by recreational and research equipment (e.g., boats, boots, and angling equipment), aquaculture contamination as well as natural vectors such as downstream movement with water currents (Haynes et al. 1985; Richards and Lester 2003). NZMS were first detected in the Western United States in the mid-1980s in the Snake River, and since then have rapidly expanded their distribution to 10 Western States and one province (Richards et al. 2001). NZMS have established populations in several Washington water bodies proximate to the Quinault system including but not limited to: Chehalis River, WA; Capitol Lake, WA; Thornton Creek, WA; Lake Washington, WA; and several creeks near Lake Washington such as Sears Creek, Valley Creek, Kelsey Creek, and May Creek (Bersine, Brenneis, Draheim, Wargo, Zamon, Litton, Hinton, Sytsma, Cordell & Chapman 2008; Benson 2013; Richards and Lester 2003). The risk posed to Lake Quinault and its associated tributaries by the proximity of these AIS infestations is significant, and increases the likelihood of the successful transport and introduction of these species into the Quinault system where the QIN is located.

D. polymorpha and *D. rostriformis bugensis* mussels, referred to hereafter as ZQM, are invasive freshwater mussels that cause extensive economic and ecological impacts in areas outside their native range (Dermott and Kerec 1997; Mann, Radtke, Huppert, Hamilton, Hanna, Duffield & Netusil 2010; Ricciardi, Neves & Rasmussen 1998). ZQM attach to hard submerged surfaces

such as rock and concrete using byssal threads and this biofouling can create operational problems for hydroelectric, irrigation, and fish facilities (Boelman, Neilson, Dardeau & Cross 1997; Claudi and Mackie 1994; Jenner, Whitehouse, Taylor & Khalanski 1998; Neitzel, Johnson, Page, Young & Daling 1984). ZQM can form large dense populations and through their collective filter feeding and deposition of feces and pseudofeces, they change the manner energy moves in an ecosystem, as well as increasing water clarity, light penetration, and the growth of rooted macrophytes (Bastviken, Caraco & Cole 1998; Botts, Patterson & Schloesser 1996; Burlakova 1995; Caraco, Cole & Strayer 2006; Effler and Siegfried 1998; Effler, Matthews, Brooks-Matthews, Perkins, Siegfried & Hassett 2004; Fahnenstiel, Lang, Nalepa & Johengen 1995; Horvath, Martin & Lamberti 1999; Strayer 2008).

ZQM larvae develop in the water column, and are referred to as veligers. ZQM larvae swim in the water column during larval development (Raven 1958). The planktonic larvae will likely be spatially and temporally clumped in the water column because ZQM spawning is generally synchronized, and ciliated larvae cannot swim horizontally towards specific objects (Boelman et al. 1997; Mackie and Schloesser 1996; Marsden 1992; Nichols 1996; Ram, Fong & Garton 1996; Sprung 1993). Planktonic bivalve larvae may be concentrated by water current and wind conditions, e.g., eddies (Kraft, Garton, Johnson & Hieb 1996). In lakes and reservoirs with low mixing, veligers tend to be concentrated above the thermocline (Boelman et al. 1997; Gallager, Davis, Alatalo & Girard 1996; Mackie and Schloesser 1996; Sprung 1993). In a mixed system, the ZQM larvae, if present, are expected to be mixed throughout the water column, ranging from near the surface to depths greater than 122-m (400-ft) (Sprung 1993).

Bivalve larvae actively settle out of the water column onto to a variety of submerged substrates including macrophytes, rocks, gravel, sand, woody debris and submerged man-made objects where they undergo metamorphosis and become juveniles (Ackerman, Sim, Nichols & Claudi 1994; Roe and MacIsaac 1997; Sprung 1993). ZQM juveniles are generally found in the Midwest of North America between August and September (Thorp, Alexander, Greenwood, Casper, Kessler, Black, Fang, Westin & Lewis 1994). Both juvenile and adult ZQM translocate year-round to preferred substrates and areas such as the sides of hard surfaces (Claudi and Mackie 1994). ZQM adults are found year-round in epilimnion, littoral and profundal areas (Roe and MacIsaac 1997).

In 2007, ZQM were found to have established populations west of the Rocky Mountains, and have since continued to spread to other western water bodies, e.g., Lake Powell, Utah and Lake Winnipeg, Canada. ZQM larvae have been detected in multiple water bodies in Colorado and Utah (Benson 2012). Watercraft with attached, hitchhiking mussels are repeatedly detected being trailered into Washington, Oregon, Idaho, and the surrounding states at watercraft inspection stations (Phillips 2013).

C. fluminea is a freshwater bivalve that was introduced to the Columbia River Basin in the 1930s (Burch 1944), and is causing macrofouling problems in hydroelectric facilities on the main stem Columbia River, e.g., *Corbicula* were removed from cooling condenser tubes during main unit overhauls every five years (Athearn and Darland 2007; Kovalchuk 2007). *Corbicula* adults do not attach to hard surfaces using byssal threads, but these clams do accumulate in collection channels, fishways, under diffuser gratings, behind and lodged in valves, screens and on separator bars (Kovalchuk 2007). In addition to the macrofouling problems associated with blockage, *Corbicula* can cause physical injury to fish during passage (Kovalchuk 2007). *C. fluminea* is often the dominant bivalve in freshwater systems (McMahon 2015), and there are potential ecological impacts, e.g., competition with native bivalves. *C. fluminea* are hermaphroditic and new populations can develop from a single adult clam (Cummings and Graf 2010).

Invasive macrophytes such as *M. spicatum*, *M. heterophyllum*, *M. aquaticum*, and *E. densa* can have a detrimental impact to both the QIN's and Washington's natural environment and recreational use of water resources. Invasive macrophytes can form dense monospecific beds that degrade habitat for waterfowl, fish and other wildlife (e.g., altering water quality), and crowd out native plant species. Dense macrophyte beds also interfere with recreational activities such as swimming, boating, fishing, and water skiing. *M. spicatum* populations that are proximate to the Lake Quinault system include, but are not limited to, Duck Lake, Conner Creek, Lake Sutherland, Mason Lake, Island Lake, and Long Lake (EDDMaps 2015). Known proximate *M. aquaticum* populations occur in the Chehalis River and the Grayland Ditch (EDDMaps 2015). Known *E. densa* populations that are proximate to the Lake Quinault system include Duck Lake, Chehalis River, Leland Lake, and Lake Limerick (EDDMaps 2015).

The milfoils are capable of hybridizing, and the known distribution of hybrids between the introduced *M. spicatum* and the native *M. sibiricum* (northern watermilfoil) are growing in northern USA. This hybrid species is a serious concern because it appears that the hybrid exhibits more aggressive growth and is more tolerant to herbicides compared to the already invasive *M. spicatum* (LaRue et al. 2012). Milfoil hybrids are difficult to identify using morphology alone, and genetic testing is required.

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

The QIN recognized the need to develop management measures for the aforementioned AIS in the region and specifically Lake Quinault. In its request for bids, QIN identified the aforementioned AIS as priority species of concern for the Project area. The QIN request for bids specifically mentioned survey locations where AIS are likely to be introduced and established in the Quinault system such as boat launches, in shoreline areas near parks, and areas most likely to contain target species based on public use and hydraulic conditions. Western Biological Services LLC was contracted to develop the AIS survey plan (i.e., Quality Assurance Project Plan or QAPP); implement the plan including the field collection of data, laboratory analysis of samples; and writing the technical report describing the plan, the sampling methodology, results, and recommendations based on the findings. This document represents the final report for this project.

Objectives

The primary goals of the project are to develop and implement an AIS survey in Lake Quinault focusing on the early detection of invasive freshwater bivalves, gastropods, crayfish, and submerged macrophytes; generating baseline data for aquatic species composition and preliminary spatial distribution in Lake Quinault; and collecting voucher specimen collections. Project goals also include agency and general public outreach and education, and preventing the transfer of organisms and/or genetic material between water bodies and samples. Early detection monitoring of AIS and generating baseline data for species composition will inform the planning and management efforts as deemed appropriate by QIN and other stakeholders such as Ecology, WDFW, U.S. National Park Service, and the U.S. Forest Service. Outreach and education will increase awareness of AIS issues, increase the effectiveness of early detection efforts, and augment support for management efforts.

Specific project objectives are:

- Develop a detailed survey plan for monitoring the presence/ non-detect of AIS in Lake Quinault targeting, but not limited to, NZMS, ZQM, *C. fluminea*, *P. clarkia*, *M. spicatum*, *M. aquaticum*, *E. densa*, and other AIS,

- Implement the survey plan with field collection of biological samples in Lake Quinault (i.e., infaunal, epifaunal, and planktonic samples) during three sampling trips during the August to September period in 2015,

    - Monitor for presence of AIS and other aquatic organisms on naturally occurring and man-made submerged surfaces including, but not limited to, rocks, macrophytes, sand, gravel, dock floats, fish pens, pilings, submerged trees, and large natural debris,

    - Monitor for the presence of AIS and other aquatics in shoreline areas,

    - Monitor for the presence of AIS bivalve larvae in the water column,
- Analyze the samples in the laboratory to identify specimens and create composition lists,
- Generate distribution maps based on early detection collection data,
- Provide voucher specimens of collected specimens,
- Generate a final report detailing the survey plan, field and laboratory methods, results, and recommendations based on the findings.
- Prevent the unintentional transfer of organisms within and between water bodies and samples through field and laboratory equipment decontamination.

Methods

Project location

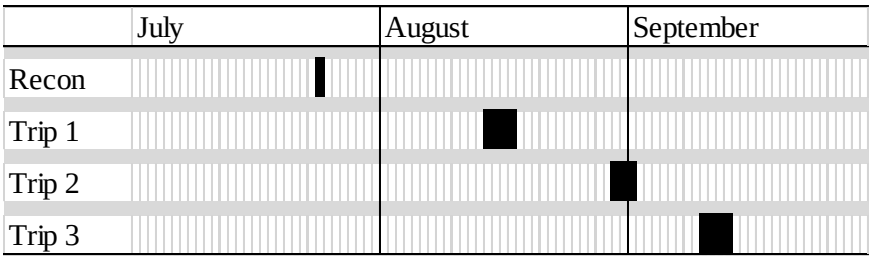
The project location is Lake Quinault. Lake Quinault is located in the Olympic Peninsula in Washington (Figure 1). The Upper Quinault River is the primary inflow into Lake Quinault, and the Quinault River is the primary outflow.

Figure 1: Lake Quinault in the Pacific Northwest.

Schedule

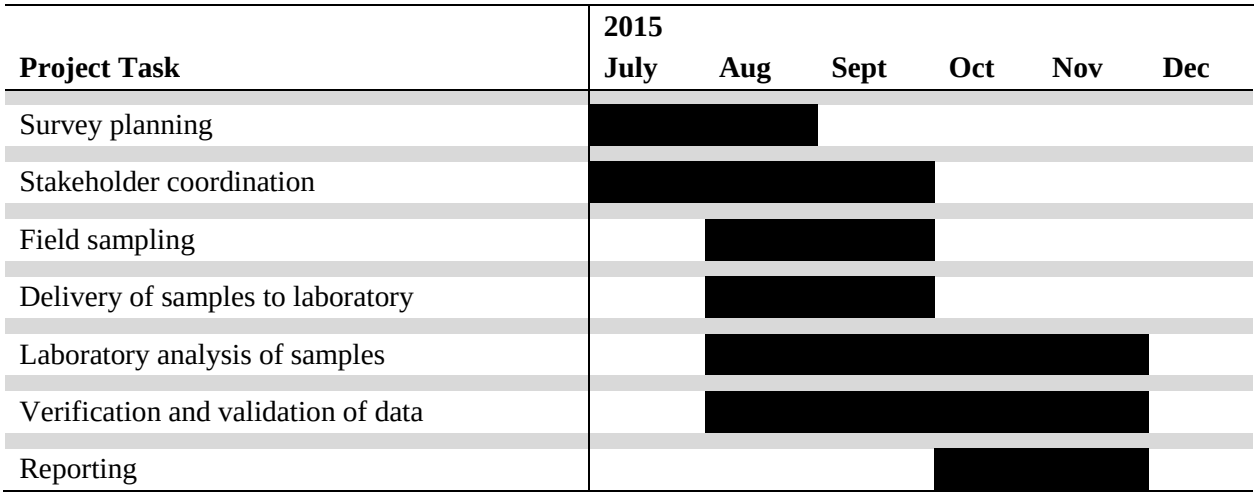
Sampling was done in Lake Quinault during three sampling trips conducted during the August to September period in 2015 (Table 1). Sampling occurred during multiple sampling events to account for temporal variability in phenology and inter-annual variations in habitat conditions. A reconnaissance trip was conducted in late July to observe the Project area, and to discuss logistics of field sampling and the survey plan with QIN personnel. Based on water temperatures from the USGS station #12039500 located in the Quinault River at Quinault Lake (47º27'28" 123º53'17"), the habitat conditions in the August to September period were expected to be ideal for the collection and identification of mollusk, crustacean, and macrophyte specimens (e.g., macrophytes possess reproductive parts, mussels are spawning, NZMS are brooding young, etc.). Additionally, the summer period is the peak recreational period, and this represented the time most likely for AIS introduction. Each sampling trip consisted of at least three days on the water.

Table 1: Field sampling schedule for AIS survey in Lake Quinault during 2015.



The annual work schedule included survey planning, stakeholder coordination, field sampling, delivery of samples to the laboratory, laboratory analysis of samples, data management, and final reporting. The annual work schedule is identified in Table 2.

Table 2: Annual work schedule for AIS survey in Lake Quinault during 2015.



Standard protocols

WBS developed the survey plan and it is described in the Quality Assurance Project Plan (QAPP) (Wells 2015a). The QAPP detailed the types of data to be collected to satisfy the AIS monitoring project in Lake Quinault, described the methods for data analysis, and detailed the decontamination guidelines required to prevent the cross contamination of samples and the inadvertent spread of invasive species.

Standard Field Collection Protocols (SFPs) were developed for WBS field crews working in Lake Quinault for the collection of AIS bivalves, gastropods, crayfish, and submerged macrophytes (Appendix A). WBS developed these SFPs based on previous sampling experiences, acceptance criteria identified in the QAPP, a reconnaissance trip to Lake Quinault in July, and through consultations with Quinault Division of Natural Resources personnel (Armstrong 2015; Fielding 2015; Martorano 2015).

The SFPs developed and used by WBS were designed for the early detection, i.e., presence/ non-detect of AIS in Lake Quinault. To the author's knowledge, there have been no prior reports of AIS bivalves, gastropods, and crayfish in Lake Quinault. The target population, if present, was assumed to be rare and spatially clumped.

Labeling

The information listed below was recorded for each sample on the waterproof field datasheets (Appendix B). Each sample container was pre-assigned a unique number, e.g., LQ3 4456, and this number was recorded on the sample container using a waterproof sticker covered with clear tape. This number was used to track the sample during field collection and transport to the laboratory, receiving, analysis and long-term storage. While in the field, the sample information was recorded on the field datasheet, including the unique sample container number. In general, a single labeled sample container was used for all taxa collected at one sample location, and may represent sampling via multiple methods, e.g., hand pat-downs and surface scraper to sample old fish pens. More than one sample container were used at a single sample location, if needed, depending on the abundance and type of specimens collected. Specimens of concern, if noted in the field, were placed into their own sample container.

- Sample container number, e.g., LQ3 4456
- Date of collection
- Water body name
- Sample location (GPS coordinates)
- Monitoring methods employed, e.g., hand pat-down
- Type of substrate inspected, e.g., sand, floating structure.
- Location description, e.g., near outlet
- Number and length of tows (if applicable)
- Type of tow, e.g., vertical/ oblique or trawling (if applicable)
- Trawling time and speed (if applicable)
- Name and affiliation of person collecting sample
- Preservative and concentration used, e.g., 70% ethanol

All data was recorded on datasheets developed for this purpose.

Sample collection

Refer to Appendix A, Field Collection Protocols for AIS Monitoring (Wells 2015b), and QAPP (Wells 2015a) for complete details on inspecting existing submerged surfaces, using a sediment grab sampler, sampling macrophytes with a thatch plant rake, Portland Sampler deployment and inspections, and conducting shoreline surveys for AIS.

Field collection was focused on adult and juvenile life forms with the exception of plankton sampling targeting introduced bivalve larvae. A targeted sampling design was employed to increase the likelihood of AIS collection, and involved a multi-pronged approach sampling many small quadrats distributed throughout Lake Quinault in areas likely to contain AIS. Different sampling techniques were used to target different types of potential habitat, species, and life stage. Biological collections involved sampling existing submerged natural and man-made surfaces, sampling exposed shoreline areas, deploying and monitoring artificial settlement substrates (i.e., Portland Samplers) (Figure 2A), deploying Frabill and pillow style crayfish traps, and sampling the plankton in the water column. Existing submerged natural and man-made surfaces were inspected for AIS and other species using various methods including: hand-pat downs; surface scraper (Figure 2B); grab sampler (Figure 2C); and a thatch plant rake (Figure 2D). Submerged surfaces included logs, dock floats, rope and mooring chains, fish pens, breakwaters, macrophytes, woody debris, rocks, gravel, sand, and mud.

Figure 2: AIS sampling equipment including A) Portland Sampler, B) surface scraper, C) grab sampler, and thatch plant rake.

AIS and other specimens collected, with the exception of plankton, were retained in sample containers with lake water, held on ice, and transported to WBS laboratory for identification, photomicroscopy, and preparation of voucher specimens. Native unionid mussels were collected in Lake Quinault, and these animals, when alive, were photographed and then returned to the water body. Field notes were made, when relevant, e.g., live/ dead status upon collection.

Sampling locations

Because early detection monitoring is inherently difficult, sample collection efforts were focused in locations that were likely to be at high risk for AIS introduction and establishment, and where different target AIS species may be most abundant.

The sampling locations were determined during the Survey Planning, and WBS worked with the QIN CO and other relevant stakeholders to identify the final sampling locations. These sampling locations represented areas that were estimated to be at high risk for AIS introduction and/ or establishment, such as, but not limited to, areas near boat launches, high use areas, eddies, and littoral areas less than 10-m water depth with sand-silt-gravel substrate. In the field, the sampling locations identified in the QAPP served as guidelines, identified and navigated to using GPS waypoints. The exact location of sample collection, however, was modified while in the field based on data collected on-the-water using sonar, a sight tube, and the expertise of WBS staff

(e.g., identifying locations of eddies based on the accumulation of bubbles on water surface, visible currents, rafting macrophytes, etc.) in order to best target mollusk, crustacean, and submerged macrophyte populations.

Hand pat-downs

Accessible submerged surfaces (i.e., within arm's reach) were visually and tactilely inspected for AIS and other mollusk, crustacean, and macrophyte specimens. Sampling locations included the undersides and sides of submerged rocks and logs, dock floats, log breakwaters, and old fish pens. The fingers and palm of the hand were used to explore underwater surfaces, and questionable objects were removed for visual inspection. Multiple areas of each surface were inspected to sample a representative portion. A total of 73 locations were sampled in Lake Quinault using hand pat-downs during the period between August and September 2015 (Figure 3).

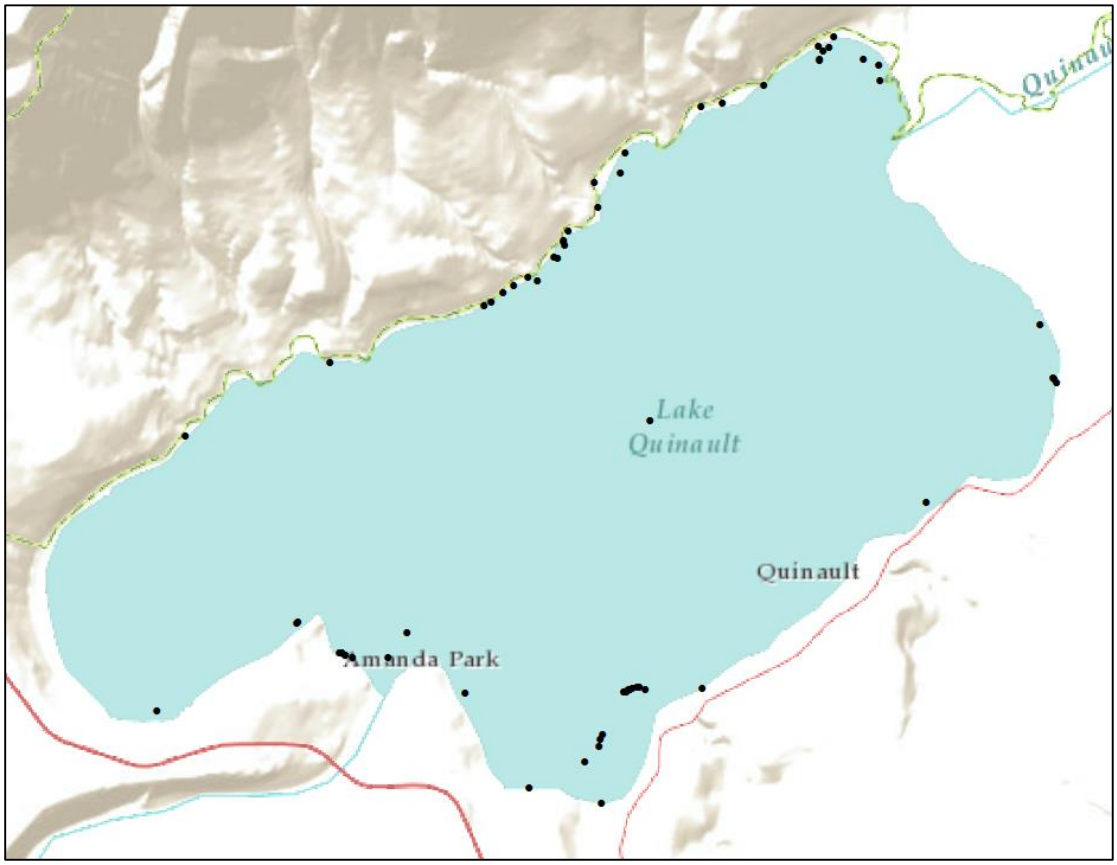


Figure 3: Hand pat-down sampling locations in Lake Quinault in 2015.

Surface scraper

A surface scraper was used to sample for gastropods, bivalves, and macrophytes attached to submerged pilings, fish pens, the undersides of dock floats, and submerged trees. The surface scraper was lowered into the water as deep as the attached pole allowed (approximately 3-m), and the metal rim of the mesh box was brought into contact with the substrate surface and then quickly pulled up, keeping the metal rim in contact with the surface to be sampled. The removed material was collected in the mesh and visually inspected at the water surface. This scraping process was repeated at multiple locations per structure in order to sample a representative portion. A total of 85 locations were sampled with the surface scraper during 2015 AIS sampling, and the sampling locations are identified in Figure 4.

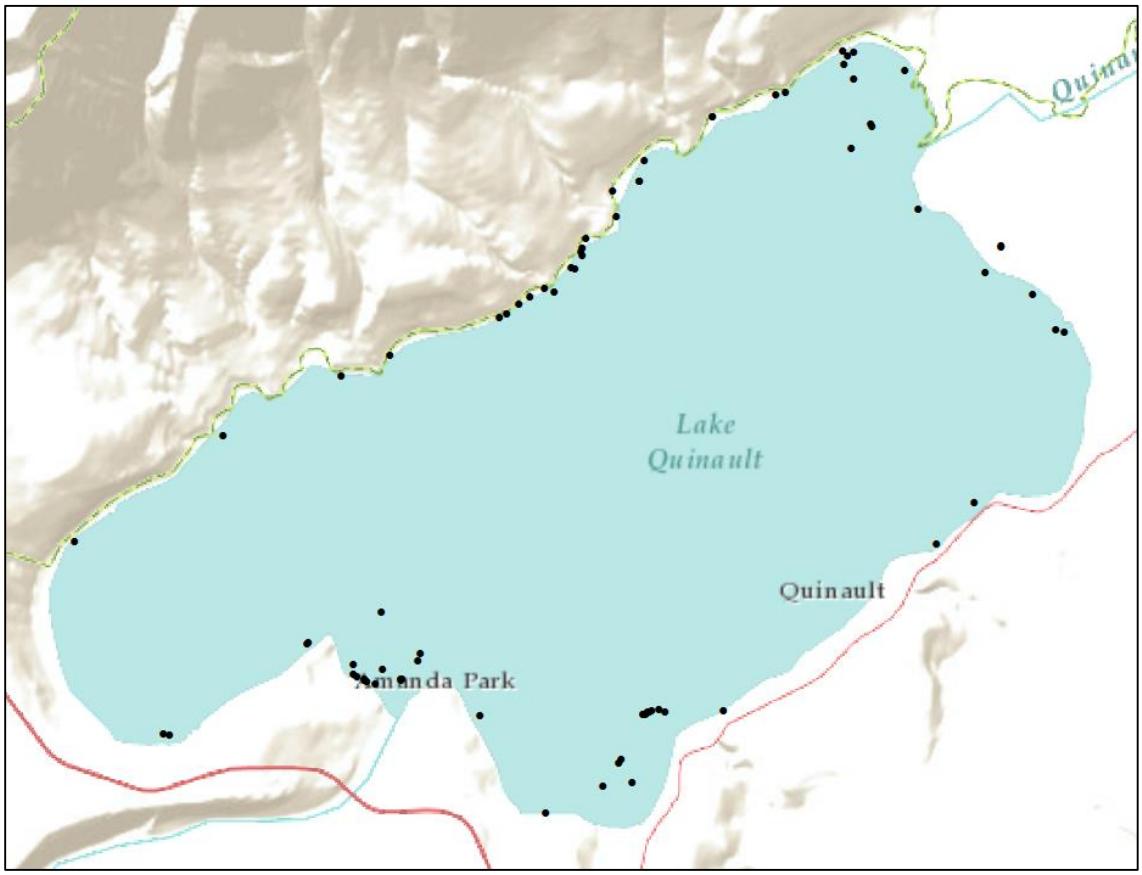


Figure 4: Surface scraper locations in Lake Quinault in 2015.

Grab sampler

A sediment grab sampler, i.e., Petite Ponar, was used to sample gravel, sand, mud, and small rock in water depths between 1- and 65-m for the presence of bivalves, gastropods, and macrophytes. The grab sampler was lowered into position on the benthos, the dredge was deployed using a spring-released pin, and then the sampler was manually retrieved. Grab sampler contents were inspected by dumping into a 700-µm mesh sieve and rinsing in lake. A total of 117 locations were sampled with the grab sampler, and the sampling locations are identified in Figure 5.

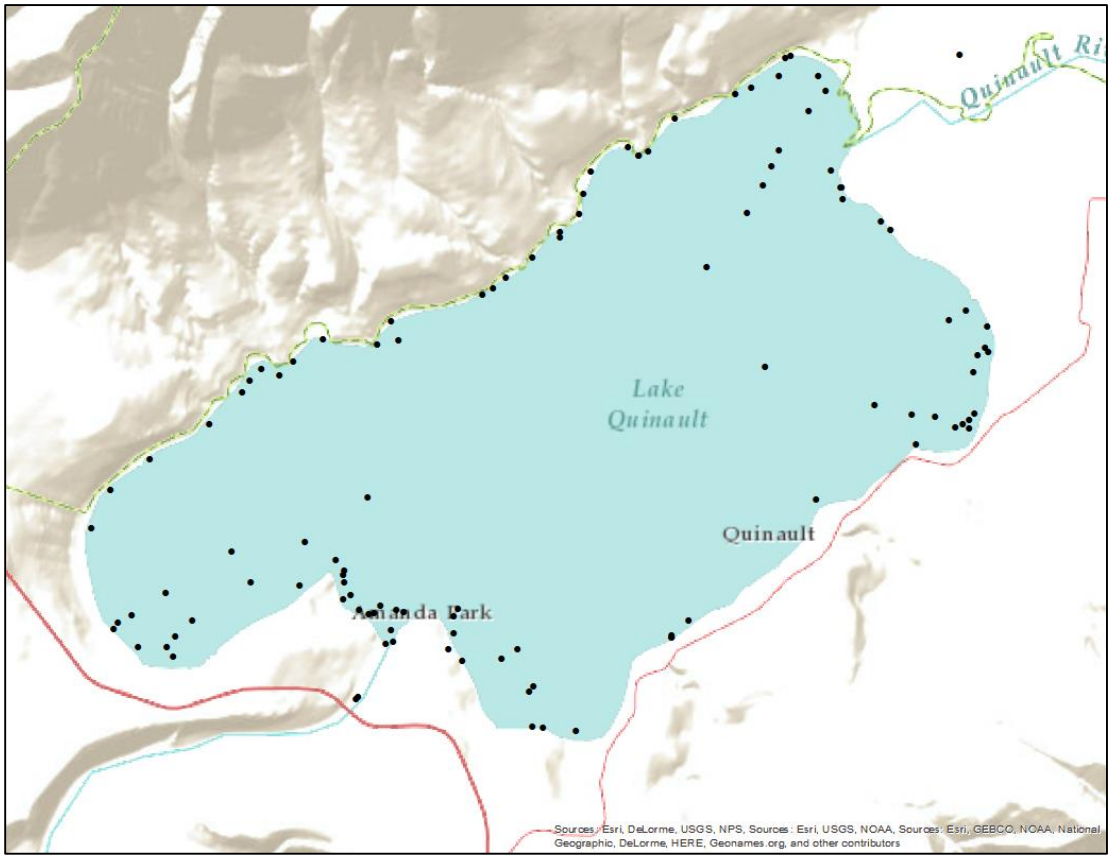


Figure 5: Grab sampler locations in Lake Quinault in 2015.

Plant rake

Macrophytes and associated fauna (e.g., gastropods) were sampled using a thatch plant rake attached to a rope and through visual observation using a sight tube. The thatch rake was thrown over visible macrophytes, and allowed to sink to the lake sediment. The plant beds were sampled by dragging the thatch rake across the lake bottom and through the bed. The retrieved macrophytes were deposited into a white-colored 5-gallon bucket half filled with lake water, and vigorously shaken to remove invertebrates and clean plants of periphyton and sediment. The cleaned macrophytes were removed from the bucket and visually inspected for attached bivalves, gastropods, and other fauna, and then macrophytes were placed into labeled sealable plastic bags filled with lake water, and placed into a cooler on ice. The contents of the rinse bucket were poured through the 700-µm sieve and the sieve was visually inspected for invertebrates. The sight tube reduced the glare on the water's surface, thereby increasing the effectiveness of visual surveys made at the surface. The sight tube was used to visually scan sampled plant beds and/or littoral areas to locate flora and fauna to sample as well as to confirm that all the taxa present in a sampled area were collected. The thatch rake and sight tube were used to sample a total of 280 locations, and the sampling locations are identified in Figure 6.

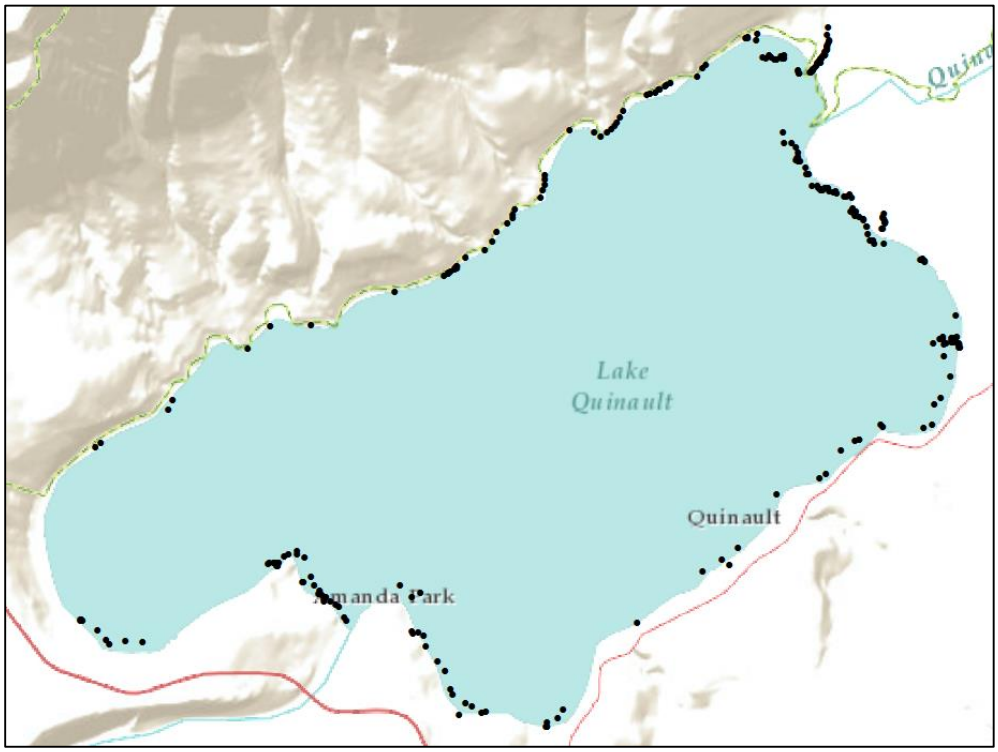


Figure 6: Thatch rake sampling locations in Lake Quinault in 2015.

Shoreline walks

Shoreline surveys were conducted near access points and other exposed shoreline areas where debris accumulated. Starting 30-m on either side of an access point or other shoreline area, WBS personnel waded toward the access point following a zig-zag pattern perpendicular to the shoreline. The width of the zig-zag was determined by the depth of the water. Personnel stopped after every other step to pull out and investigate loose rocks, cobble, woody debris and /or macrophytes for gastropods, and other species. When removing rocks for inspection, the rock was quickly lifted vertically and the area under the rock was inspected for crayfish. If suitable habitat was not adjacent the access point, a nearby area where gravel, sand, silt and rocks accumulated was sampled. Each shoreline area was inspected for a minimum of thirty minutes. A total of 15 shoreline areas were inspected during sampling in August and September, and these sampling locations are identified in Figure 7.

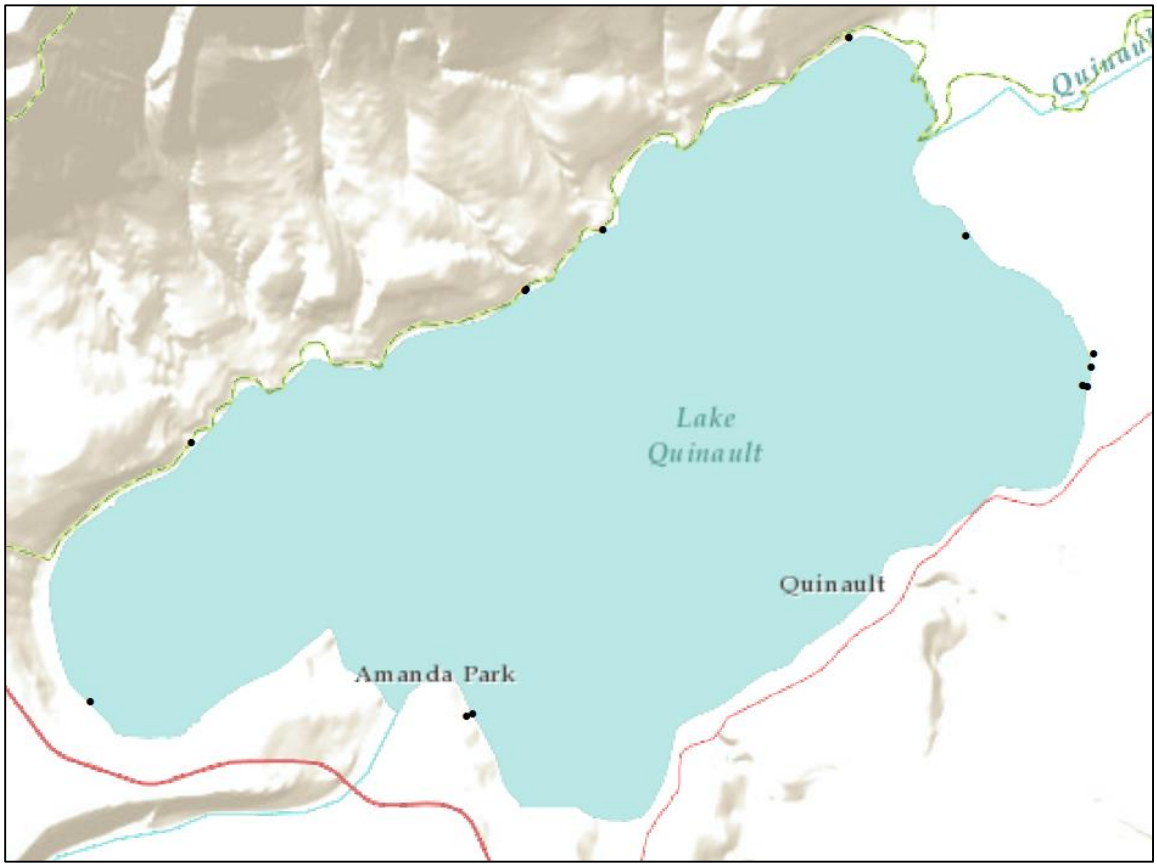


Figure 7: Shoreline survey locations in Lake Quinault in 2015.

Portland sampler

A total of eight Portland Samplers were deployed from secure surface structures to monitor for gastropod, bivalve, and crayfish colonization in deeper waters. Portland Samplers consisted of multiple PVC and ABS plastic pipe sections oriented horizontally and secured at different water depths along a rope weighted with a concrete anchor at the terminal end. The terminal end of the substrate rested near the lake sediment. Several types of artificial materials were incorporated into the design of each sampler to maximize the potential for settlement. This design increased surface area for settlement throughout the water column as well as near the benthos.

Efforts were focused on deploying Portland Samplers in areas where the planktonic or entrained organisms would be most likely to encounter them (e.g., near the outlet), and in areas most likely to encounter AIS introduced into Lake Quinault via trailered watercraft (e.g., near boat launches, and high public use areas). Portland Samplers were tied-off in locations that would not interfere with boat navigation and other recreational activities, and were intended to not be visible to the general public to minimize theft. The Portland Sampler locations are identified in Figure 8, and are associated with the outlet, hatchery operations area, and near July Creek. Most of the Portland Samplers (n= 5) were deployed from large logs and uprooted trees embedded into lake sediment and/ or rooted tree stumps, and three Samplers were deployed from old fish pens and a hatchery breakwater.

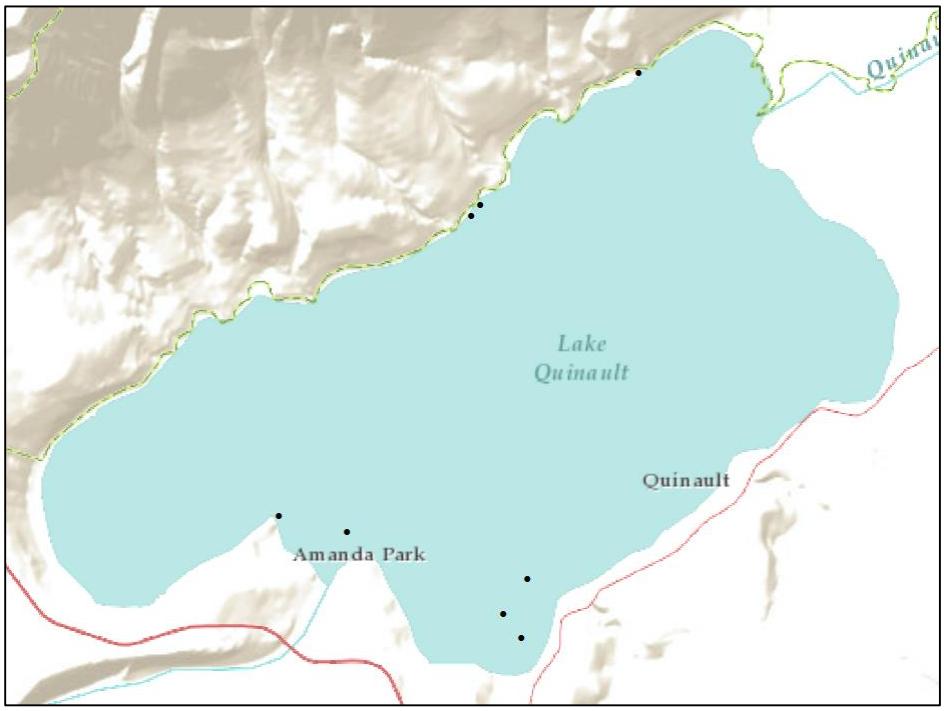


Figure 8: Portland Sampler locations in Lake Quinault in 2015.

Crayfish traps

Crayfish were sampled using modified Frabill and pillow style crayfish traps based on protocols outlined by Larson and Tait (2011). A total of 29 traps were baited with fresh salmon fillets (skin on), and baited traps were deployed for a minimum period of 24-hrs in areas with hard substrate, areas with macrophytes, near confluences with tributaries, and along shoreline areas with evidence of crayfish activity (e.g., carapaces, observations of live crayfish with sight tube). The minnow trap sampling locations are identified in Figure 9.

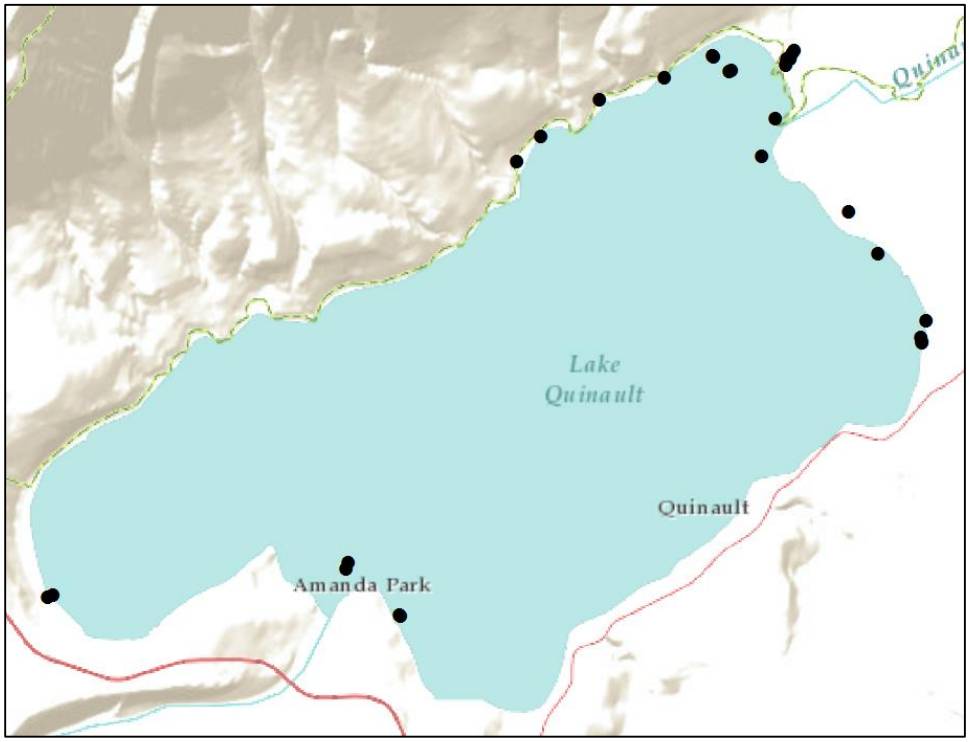


Figure 9: Crayfish trapping locations in Lake Quinault in 2015.

Plankton

Refer to Appendix A, Field Collection Protocols for AIS Monitoring (Wells 2015b), for complete details on plankton sampling for invasive bivalve larvae. Plankton sampling was done at locations throughout Lake Quinault including open water areas and near the outlet. Plankton sampling locations are identified in Figure 10.

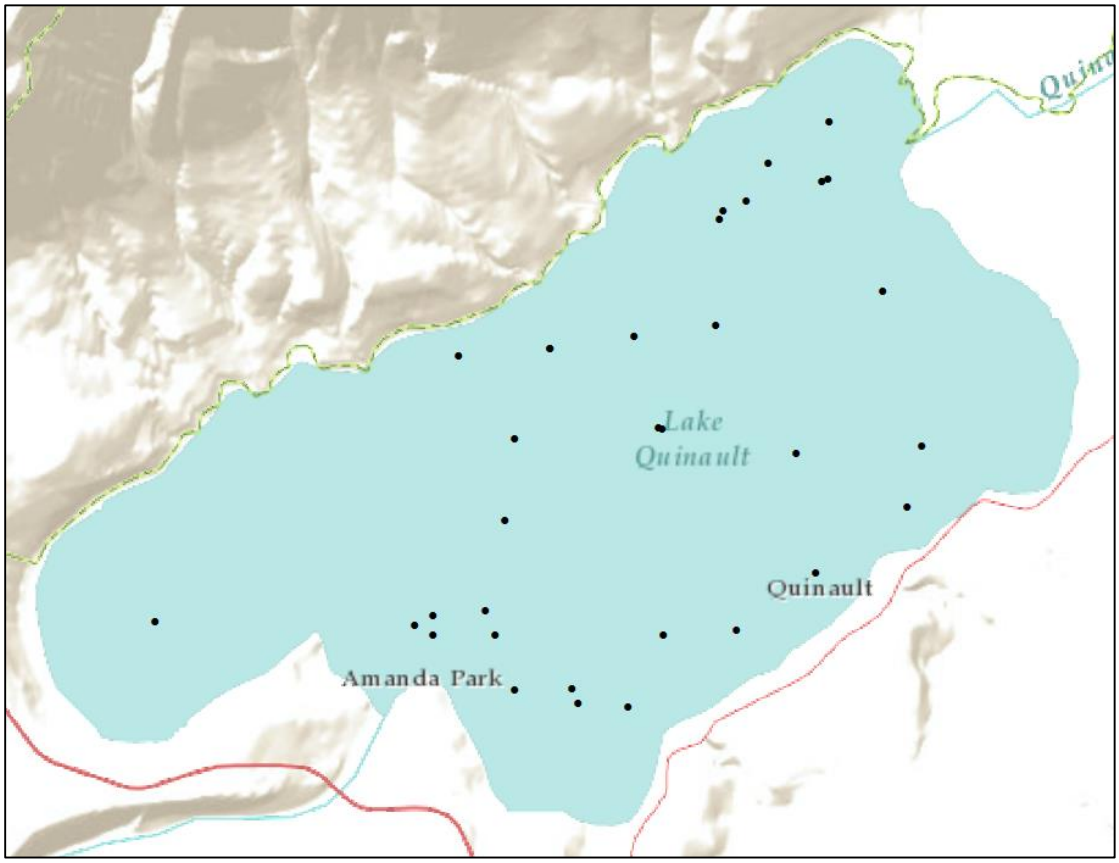


Figure 10: Plankton sampling locations in Lake Quinault in 2015.

Plankton samples were collected using a combination of vertical tows and trawling at 44 sampling locations throughout the August and September period. Plankton sampling locations represented areas where 1) plankton was likely to be concentrated (e.g., eddies and in front of outlet near Amanda Park), 2) near access points, and 3) longitudinally distributed throughout Lake Quinault. Sampling was done from a boat. Plankton samples were collected using a simple, conical plankton net with a 63-µm mesh. Vertical/ oblique tows collected a depth-integrated sample throughout the water column at a discrete spatial location. Trawling sampled a specific strata of the water column over a larger horizontal distance. Plankton samples were stored in 125-mL polyethylene, leak proof containers, and preserved with regular ethanol to a final concentration of 70%. Ethanol was pre-buffered using Tris (2 drops per 125-mL sample) in order to maintain the pH of preserved plankton samples at a pH above 7.5.

Water quality

Water temperature, dissolved oxygen, and pH were measured at the mid-lake white buoy using a Hydrolab Quanta™ water quality multi-probe. Water quality was measured at 1-m depth increments from the surface to within 1-m of the sediment or the end of the sonde cord, e.g., 25-m. The sonde was held at 1-m for at least 5 minutes or until readings stabilized, and then for at least 1 minute at each subsequent depth. Values were recorded in field datasheets. The measurement at 1-m was repeated as a field precision check. Accuracy of the temperature probe was assured through factory calibration. The accuracy of the dissolved oxygen and pH probes was assured by calibration at the start of each sampling day according to the protocols identified in Appendix A, Field Collection Protocols for AIS Monitoring (Wells 2015b).

Laboratory analysis

Bivalves, gastropods, crayfish, and submerged macrophytes were identified in the laboratory to the lowest possible taxonomic level using external anatomy and visible light microscopy. A Leica S8 APO Stereozoom 1.0 – 8.0X stereo-microscope with 10X eyepieces and fitted with a digital camera on a trinocular tube was used for adult and juvenile specimen identification and to document key morphology using photomicrographs. Specimen identification relied on readily available anatomical features such as gastropod operculum type and nucleus position, shell sculpture and shape, spire length, and adult size; bivalve hinge structures, beak position, and shell shape; and macrophyte seed anatomy. Internal tissue dissections were not completed for specimen identification, and this affected the resolution of certain taxonomic groups, e.g., gastropods in the Hydrobiidae family are morphologically diverse, and further identification requires soft tissue dissection using compound microscopy.

Bivalve identifications were verified by Steve Wells and by using Thorp and Rogers (2011). Gastropod identifications were verified by Steve Wells and by using Frest and Johannes (1999), Harrold and Guralnick (2010), Lysne (2009), Perez, Clark & Lydeard (2004), and Thorp and Rogers (2011). Crayfish identifications were verified by Steve Wells using Fetzner, Jr. (2006), Larson and Olden (2011), Larson and Tait (2011), Olden (2009) and Pearl, McCreary & Adams (2011).

Macrophyte identifications were verified by Steve Wells, and by using Crow and Hellquist (2000, 2006), Hamel and Parsons (2001), and Brayshaw (2001). The identification of *Myriophyllum* specimens, if collected, would be based on molecular analyses conducted at Grand Valley State University or another comparable laboratory. A maximum of 50 *Myriophyllum* specimens could have been submitted for genetic analyses if determined necessary. For example, hybrid milfoils can be intermixed within macrophyte beds of one or more *Myriophyllum* species, and multiple specimens per bed may need to be analyzed.

After identification in the laboratory, voucher specimens were prepared and preserved. Voucher specimens were selected to be representative of the collected group. Invertebrates were preserved by covering with 95% regular ethanol buffered with tris in labeled sample containers. Voucher plant specimens were prepared in a plant press using wax paper and cardboard. Preserved specimens were stored at WBS until the project completion upon which time they were relinquished to the QIN CO.

Plankton samples were analyzed at the WBS laboratory for the presence of ZQM larvae using cross-polarized microscopy. Light microscopy is an established methodology that has been demonstrated to be the most reliable and accurate method for detecting ZQM larvae in low densities with interfering plankton matrices such as phyto- and zooplankton (Frischer et al. 2011).The primary objective of plankton sample analysis was early detection of bivalve larvae, i.e., presence/ non-detect data of rare event. Microscopy efforts were focused on analyzing large amounts of samples with rapid turnaround time. Suspect specimens were inspected to determine if they were bivalve larvae.

Plankton samples were prepared for analysis using gravitational settlement. Samples were gravitationally settled for 6 hours in Imhoff settling cones. The settled particulate was then selectively removed into 50-mL centrifuge tubes by opening a valve located at the bottom of the cone. The particulate was then covered with regular ethanol to maintain preservative concentration of 70%, and the sample was allowed to gravitationally settle in the centrifuge tubes for another 4 hours prior to analysis. The pelleted particulate was the concentrated sample.

Compound light microscopes were used to analyze the concentrated plankton sample. At a minimum, 20% of the concentrated sample was microscopically analyzed in two-mL Sedgewick-Rafter cells under 40X to 200X total magnification using a Leica DM750 compound light microscope fitted with 10X lens pieces and 4X, 10X, and 20X plan achromatic objectives with a polarization kit, mechanical stage with Y- and X-axis travel knobs, sub stage condenser, and a trinocular-mounted digital microscope color camera equipped with digital micrometer. The concentrated sample was diluted in the counting chamber using regular ethanol as needed to achieve a matrix density permissible to the visual inspection of all specimens. Photomicrographs were taken to document suspect specimens. Cameras were calibrated using a stage micrometer, and shell measurements were made with a digital micrometer to assist in identification. Microscopic analysis was non-destructive, and the supernatant and concentrated sample were retained in the original sample container and stored at WBS in 70% regular ethanol buffered with Tris.

Blind matrix spike samples (BMS) were used to evaluate the accuracy of microscopic analysis. BMS samples were prepared by adding a known amount of ZQM larvae to 50-mL of sample with a similar matrix of sediment and algae. At least one BMS was analyzed in every batch of 15 samples. The criterion for acceptance of bivalve larvae quality control samples was BMS

detection. Failure to detect larvae in a BMS required reanalysis of all samples in the batch with a new BMS. The analyst was allowed to repeat analysis once per sample batch. If after reanalysis, data still exceeded control limits (i.e., non-detect of larvae in BMS), the sample results were flagged, and modifications were made to procedures (e.g., re-training analyst, increasing subsample volume, and increasing the dilution factor) and recorded with data results.

Positive results for the presence of ZQM larvae, 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. Sample splits would be sent to these laboratories if requested. Molecular tests would be performed if requested by QIN or deemed appropriate by WBS. Cross-validated positive results for ZQM would result in the immediate notification of Caroline Martorano (QIN), Ecology, and WDFW. In addition, should ZQM be detected, the Columbia River Basin Interagency Invasive Species Response Plan: Zebra Mussels and Other *Dreissena* Species notification actions would be followed (Heimowitz and Phillips 2011).

Equipment decontamination

WBS field survey personnel adhered to the decontamination protocols outlined by the Standard Operating Procedures for Areas of Moderate Concern developed by Ecology (Ecology 2012) as applicable to this project except when WBS's internal decontamination protocols exceeded those of Ecology, e.g., protocols to prevent sample cross-contamination between sampled locations.

Deck brushes, long-handle bristle brushes, and fresh water were first used to physically scrub all surfaces of sampling equipment. Sampling equipment was then soaked in a 2% quaternary ammonium solution (Quat), e.g., Virkon Aquatic® or Sparquat 256®, for ten minutes. Quat cleaners are broad spectrum disinfectants and virucides widely used in aquaculture and janitorial services. Equipment was then soaked in solutions of 5% bleach for 15 minutes. Bleach denatures protein (Prince & Andrus 1992). Lastly, plankton sampling equipment was soaked in solutions of 5% acetic acid (i.e., white vinegar) to dissolve the calcite in the shells of bivalve larvae. The ideal soak time in acetic acid solution was 24 hours and the minimum soak time was 6 hours. Equipment was thoroughly rinsed with fresh water after chemical soaks. For large equipment that could not be soaked in large plastic tubs, the chemical soaks were repeatedly applied using spray bottles, alternating between spraying and scrubbing with brushes.

Prior to launching in Lake Quinault and upon the completion of sampling, boat bilge, hull, through-hull fittings, anchor, anchor lines, bow line, and propulsion system were decontaminated using a combination of scrubbing, and both Quat and oxidizing chemicals. Surfaces were scrubbed with a brush to loosen and remove debris and then sprayed with Quat. This procedure was immediately repeated. The engine cooling water was flushed by lowering the engine unit into bucket containing fresh water and running the engine for 2 minutes. Bleach was added to the bucket of water to make a 5 to 7% bleach solution, and this solution was applied to the boat hull

and through-hull fittings using a spray bottle and bucket. The remaining bleach solution was then poured into boat, rinsing down the sides, seats, and floor, and the bleach solution was allowed to sit in the bilge for a minimum of 30 minutes. Bleach and acetic acid are corrosive and equipment was thoroughly rinsed with tap water following decontamination.

Field equipment decontamination was done on level paved surfaces at least 122-m (400-ft) from open water. Several areas with ample space to conduct equipment decontamination were identified during the reconnaissance trip. The 2% Quat, 5% bleach, and 5% acetic acid solutions were retained in plastic carboys and disposed of into WBS drains connected to a sewage treatment facility. Anthropocentric waste materials from the job site were collected, bagged in plastic trash bags and disposed of in a dumpster that went to a landfill.

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

Data management

All field data were recorded on waterproof field datasheets and these datasheets were digitally scanned upon return to the laboratory. Scanned data were stored on a WBS hard drive as well as external flash drives. All sample containers were assigned a unique sample container number. All data were entered into a Microsoft Excel® spreadsheet that was be stored on WBS computers.

Results

AIS detections in Lake Quinault

Corbicula fluminea (Asian clam) adults were collected in Lake Quinault in 2015. No other AIS were collected in Lake Quinault during WBS sampling efforts in 2015.

Live adult *Corbicula* clams were collected in 10 locations in Lake Quinault concentrated near the confluence of Ziegler Creek during the first and third sampling trips (Figure 11). Shells of dead *Corbicula* were collected in two additional locations within this Ziegler Creek area as well as the Canoe Creek area of Lake Quinault, and these shell locations are included in Figure 11. Live clams were collected in 10 locations using several methods including the plant rake (n= 5), grab sampler (n= 3), and shoreline walks (n= 2) (one location in Ziegler Creek area yielded both live clams and shells) (Table 3). The water depth where live clams were collected was 1.3-m ± 1.2-m (mean ± 1SD, n= 9), and depth ranged from 0.1- to 3.6-m. *C. fluminea* specimens were in six sample containers from the first sampling trip (QL 27, QL 92, QL 93, LQ 224, LQ 43, and QL 62), and in six sample containers from the third sampling trip (LQ3 5517, LQ3 5524, LQ3 5525, LQ3 5516, LQ3 5552, and LQ3 5551) (Table 3).

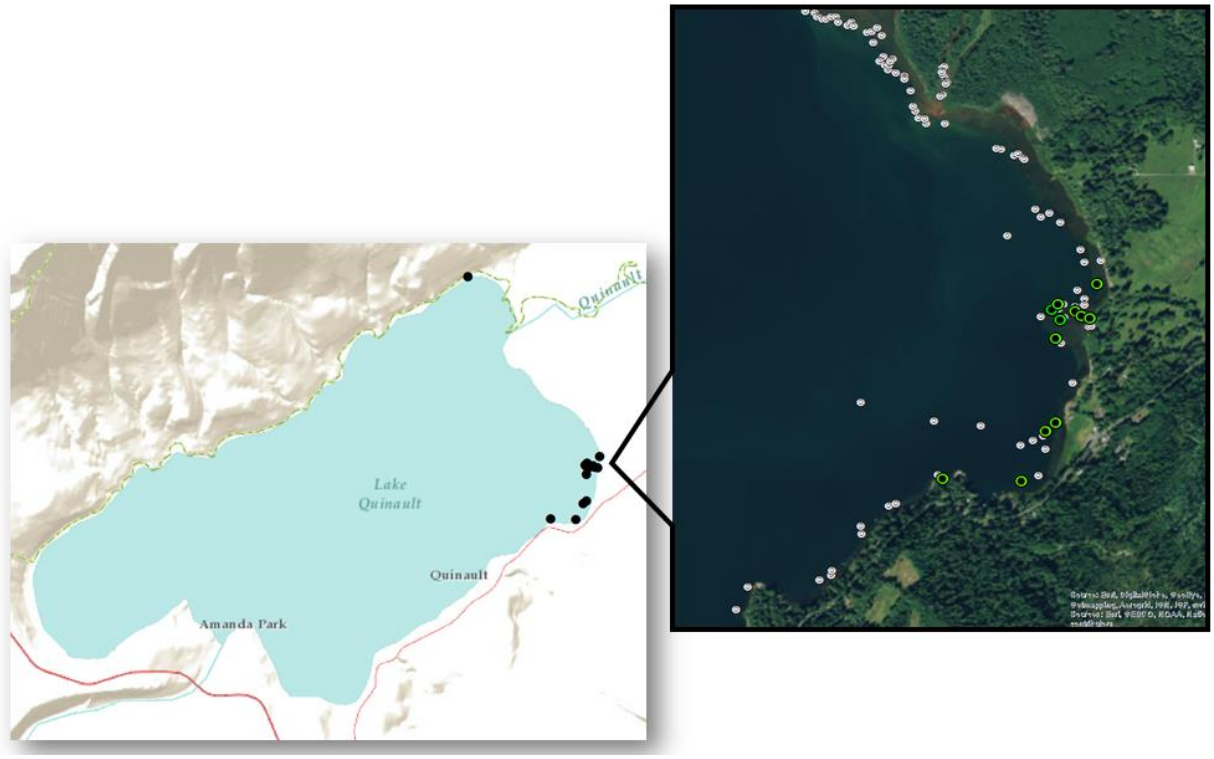


Figure 11: *Corbicula fluminea* locations in Lake Quinault (black circle in map on left, and green circle in map on right) in relation to sampling locations (white circle).

Table 3: Sampling details regarding *Corbicula fluminea* collections in Lake Quinault in 2015.

Date	Latitude	Longitude	Depth (m)	Method	Container
08/16/15	47.4739833	-123.8385833	0.3	Dredge	QL 27
08/16/15	47.47545	-123.83385	3.6	Dredge	QL 92
08/16/15	47.4783167	-123.8334	1.4	Dredge	LQ 224
08/16/15	47.4739	-123.8349667	2.8	Rake	LQ 43
08/16/15	47.4974333	-123.8504667	0.1	Shoreline	QL 93*
08/17/15	47.48	-123.8315167	0.1	Shoreline	QL 62
09/11/15	47.4757167	-123.8334		Rake	LQ3 5517
09/11/15	47.47915	-123.8325		Rake	LQ3 5524
09/11/15	47.4792167	-123.8335667	0.5	Rake	LQ3 5525*
09/11/15	47.4793667	-123.8333	1.0	Rake	LQ3 5516
09/11/15	47.4788833	-123.8331833	1.2	Tube	*
09/14/15	47.4790167	-123.8322333	0.8	Rake	LQ3 5552
09/14/15	47.478938	-123.831829	0.1	shoreline	LQ3 5551

*Only shells collected. Clams dead upon collection.

AIS sampling efforts

A total of 705 locations or samples were collected in Lake Quinault by WBS in 2015 using hand pat-downs (n= 73), a surface scraper (n=85), grab sampler (n=117), thatch rake and/or sight tube (n= 280), shoreline surveys (15), artificial settlement substrates (n= 8), crayfish traps (n= 29), and plankton samples (n= 98). A total of 607 discrete physical locations or quadrats were sampled looking for adult and juvenile AIS in 2015, and these sampling activities involved hand pat-downs, scraper, grab sampler, rake, shoreline surveys, artificial settlement substrates, and crayfish traps. The overall sampling effort was greatest during the third sampling trip, especially via the plant rake, hand pat-downs, and plankton sampling methods (Figure 12). The plankton sampling effort presented in Figure 12 represents the total number of plankton samples collected.

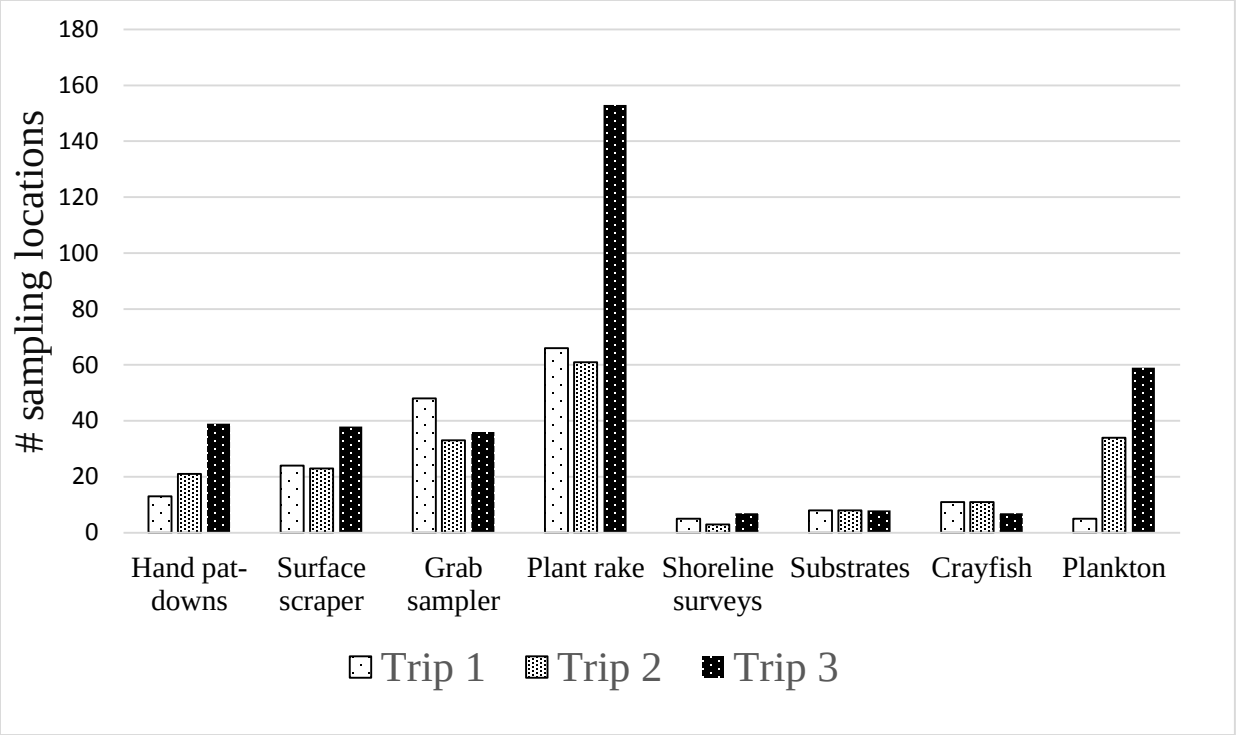


Figure 12: Number of locations sampled by method and trip.

A total of 98 plankton samples (125-mL total sample volume) were collected and analyzed by WBS during the project period (Table 4). More than 75,000-L of reservoir water were filtered through 63-µm plankton nets during sampling that occurred during the August and September period. Plankton sampling effort increased during the project period, and plankton sampling effort was greatest during the third sampling trip (46,579-L of water filtered, 59 samples) as compared to the second trip (25,164-L and 34 samples) and first trip (3,336-L and 5 samples) (Table 4 and Figure 12).

Table 4: Summary of WBS plankton field sampling in 2015. The number of plankton samples collected (#) and the volume of Lake Quinault water sampled with the plankton net (vol fil (L)) are shown.

Trip 1		**Trip 2**		**Trip 3**		**Total**	
#	vol fil (L)	#	vol fil (L)	#	vol fil (L)	#	vol fil (L)
5	**3,336**	**34**	**25,164**	**59**	**46,579**	**98**	**75,079**

The calculated volume of water filtered in Table 4 assumed 80% net filtering efficiency. Therefore, the calculated volume of water sampled (V_m = л * r^2 net aperture * length tow) was reduced by 20% to account for potential net clogging. Net clogging, however, did not appear to be a problem during Lake Quinault plankton sampling based on the lack of visible pressure waves preceding the net during retrieval, and the turbidity and productivity of the samples.

AIS bivalve larvae were not detected in the plankton samples collected from Lake Quinault in 2015. A total of 465 slides were microscopically analyzed while looking for the presence of AIS bivalve larvae in Lake Quinault plankton samples (Table 5). Approximately, 41% (average) of the concentrated sample was microscopically analyzed at WBS (Figure 13; Appendix C). The concentrated sample represented the pelleted particulate in the plankton sample that was isolated from the reservoir water and ethanol using gravitational settlement. *Corbicula* straight-hinge juveniles were not detected, in Lake Quinault plankton samples. A single ostracod was detected in plankton samples collected from all sampling locations in Lake Quinault (Table 5; Appendix C). The detected ostracod was not identified beyond the Class Ostracoda.

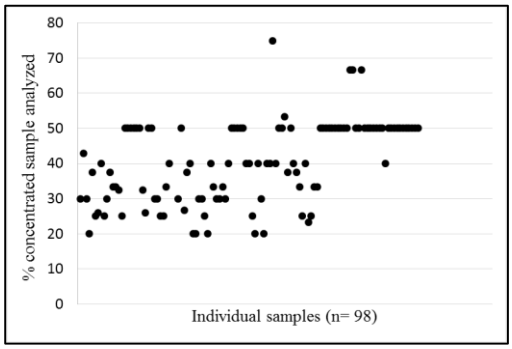


Figure 13: Percent of concentrated plankton sample analyzed in WBS laboratory in 2015.

Table 5: Summary of microscopic analysis of plankton samples done at WBS in 2015. AIS bivalve larvae such as ZQM and *C. fluminea* were not detected in plankton samples. Samples were gravitationally concentrated in settling cones, and the pelleted particulate, or Conc. sample vol (mL), was subsampled and analyzed in 2-mL Sedgewick-Rafter counting cells. The concentrated sample was diluted in counting cells as needed using ethanol. The total number of counting cells analyzed as well as the amount of the concentrated sample, or pelleted particulate that was analyzed in the plankton sample is shown. The number of *Dreissena* bivalve larvae detected in samples is indicated (# ZQM). The number of *Corbicula* spp. straight-hinge juveniles is shown as well as the number of ostracods

Sampling trip	Conc. sample vol (mL)	Total # SR cells analyzed	mL of conc sample analyzed	# ZQM	# *Corbicula*	# Ostracods
Trip 1 (8/15-8/17)	50	30	15	0	0	0
Trip 2 (8/28-9/1)	270	190	89	0	0	1
Trip 3 (9/10-9/14)	260	245	118	0	0	0
Total	**580**	**465**	**222**	**0**	**0**	**1**

A total of ten BMS were submitted with the Lake Quinault plankton samples in 2015. The spiked ZQM larvae were detected in all of the BMS during routine sample analysis. Details of the microscopic analysis of each plankton sample including the blind matrix spike samples are in Appendix C.

Species composition

Native and other non-target invertebrates and macrophytes were opportunistically collected by WBS during AIS sampling efforts in 2015. Four different types of bivalves, one species of crayfish, and five types of gastropods were collected from Lake Quinault in 2015 (Table 6). Members from 11 plant families were collected including at least five species of *Potamogeton*, *Elodea canadensis*, *Juncus*, *Eleocharis*, *Sparganium*, *Fontinalis*, *Chara*, *Nitella*, *Equisetum*, *Polygonum*, *Ranunculus*, and *Isoetes* (Table 7). Many of the invertebrates and macrophytes collected were native or were from groups with lots of natives and a few invasive, e.g., Physidae and *Potamogeton.* Many specimens were not identified to the species level because the collected specimens often lacked the necessary morphological characters such as reproductive parts. In other cases further identification would have required microscopic dissection of soft tissue parts, e.g., *Fluminicola*, and these efforts were outside of the project primary scope of work. Uncertain identifications are in parentheses and indicated that there was supporting evidence for determination but considerable uncertainty remained, e.g., *Anodonta*.

Table 6: Composition lists of mollusks and crayfish collected in Lake Quinault in 2015. AIS are indicated in bold.

Type	Family	Genus	Species
Bivalve	**Corbiculidae**	***Corbicula***	***fluminea***
Bivalve	Sphaeriidae	*Spaerium*	
Bivalve	Sphaeriidae	*Pisidium*	
Bivalve	Unionidae	*Anodonta*	(*kennerlyi*)
Crayfish	Astacidae	*Pacifastacus*	*leniusculus*
Gastropod	Semisulcospiridae	*Juga*	*plicifera*
Gastropod	Hydrobiidae	*Fluminicola*	
Gastropod	Planorbidae	*Gyraulus*	
Gastropod	Physidae		
Gastropod	Ancylidae	*Ferrissia*	

Table 7: Composition of macrophytes collected in Lake Quinault in 2015.

Type	Family	Genus	Species
Submerged plant	Potamogetonaceae	*Potamogeton*	*amplifolius*
Submerged plant	Potamogetonaceae	*Potamogeton*	(*foliosus*/ *pusillus)*
Submerged plant	Potamogetonaceae	*Potamogeton*	*foliosus*
Submerged plant	Potamogetonaceae	*Potamogeton*	*robbinsii*
Submerged plant	Potamogetonaceae	*Potamogeton*	*natans*
Submerged plant	Potamogetonaceae	*Potamogeton*	*perfoliatus* var. *richardsonii*
Submerged plant	Hydrocharitaceae	*Elodea*	
Submerged plant	Hydrocharitaceae	*Elodea*	*canadensis*
Shoreline plant	Juncaceae/Cyperaceae	*Juncus/Eleocharis*	
Shoreline plant	Juncaceae	*Juncus*	
Shoreline plant	Cyperaceae	*Eleocharis*	
Shoreline plant	Typhaceae	*Sparganium*	
Bryophyte	Fontinalaceae	*Fontinalis*	
Plant-like algae	Characeae	*Chara*	
Plant-like algae	Characeae	*Nitella*	
Shoreline plant	Equisetaceae	*Equisetum*	
Shoreline plant	Polygonaceae	*Polygonum*	
Submerged plant	Ranunculaceae	*Ranunculus*	
Submerged plant	Isoetaceae	*Isoetes*	

Preliminary species distribution

The data generated by WBS during AIS early detection monitoring can be used as point collection data to make preliminary distribution maps for species and habitat, and these maps can inform subsequent sampling and experiments. For example, the distribution of the native bivalves, *Anodonta* and Sphaeriidae, in relation to the introduced *Corbicula* are shown in Figure 14 with the locations of all sampling efforts. *Anodonta* were the most abundant bivalve in Lake Quinault, and *Anodonta* were distributed throughout the littoral area in water depths ranging from 0.1-m to 19.9-m (3.7-m ± 5.1-m, mean ± 1 SD, n= 16). *Anodonta* mussels were found in deeper water compared to *Corbicula*, although as Figure 14 shows, *Anodonta* were also found in more shallow areas alongside *Corbicula*. Live *Corbicula* were collected in water depths ranging from 0.1-m to 3.6-m, with the average depth being 1.4-m ± 1.3-m (mean ± 1 SD, n= 7).

Other data generated during this project can also be mapped with species distribution to better inform management decisions. For example, the presence of large woody debris, sand, and rocks are shown in relation to the distribution of *Corbicula* and Sphaeriidae clams in Figure 15.

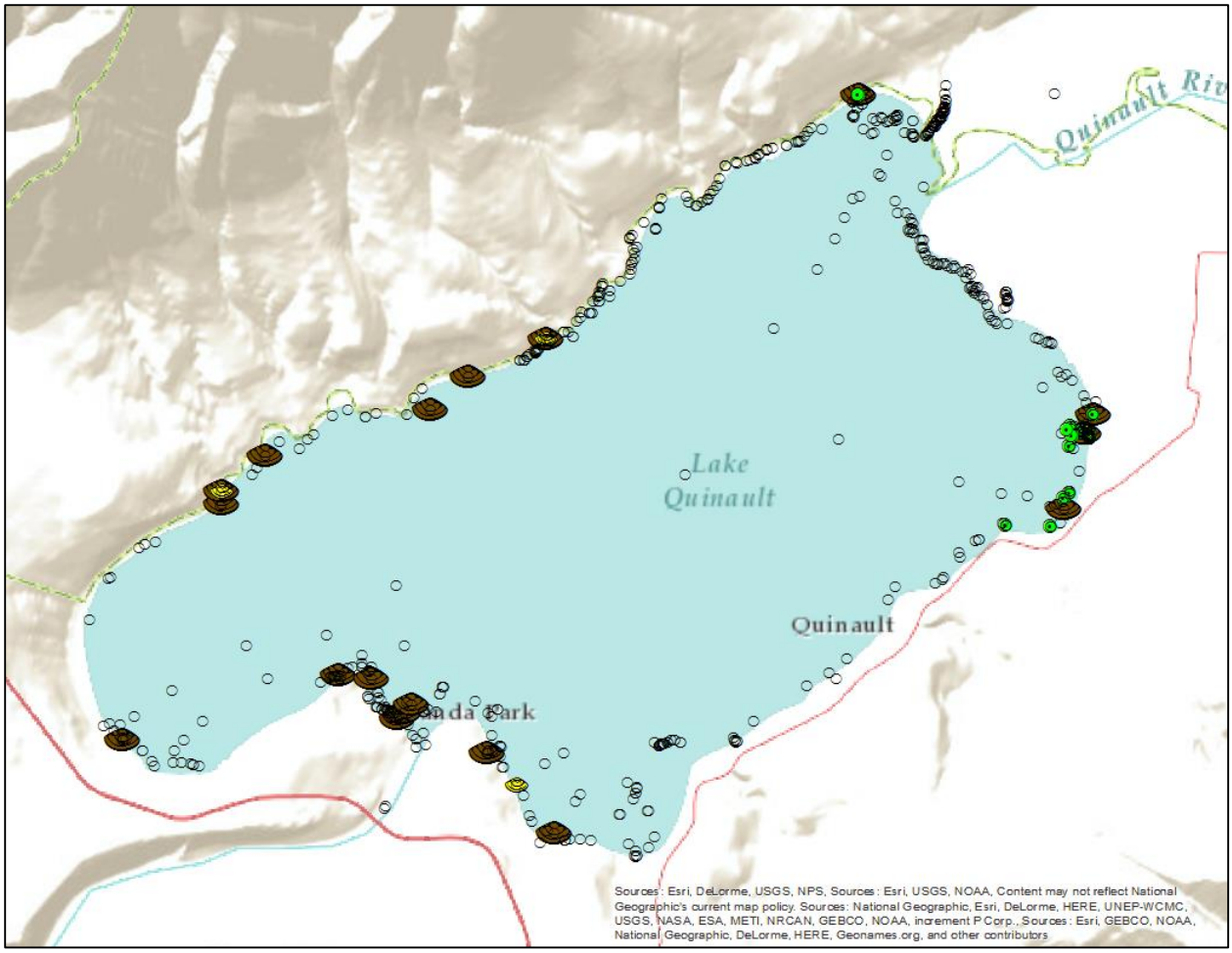


Figure 14: Distribution of bivalves in Lake Quinault. *Anodonta* are large and brown. Sphaeriidae clams are smaller and yellow. *Corbicula fluminea* locations of shells and live clams are indicated with green circles. Sampling locations are empty circles.

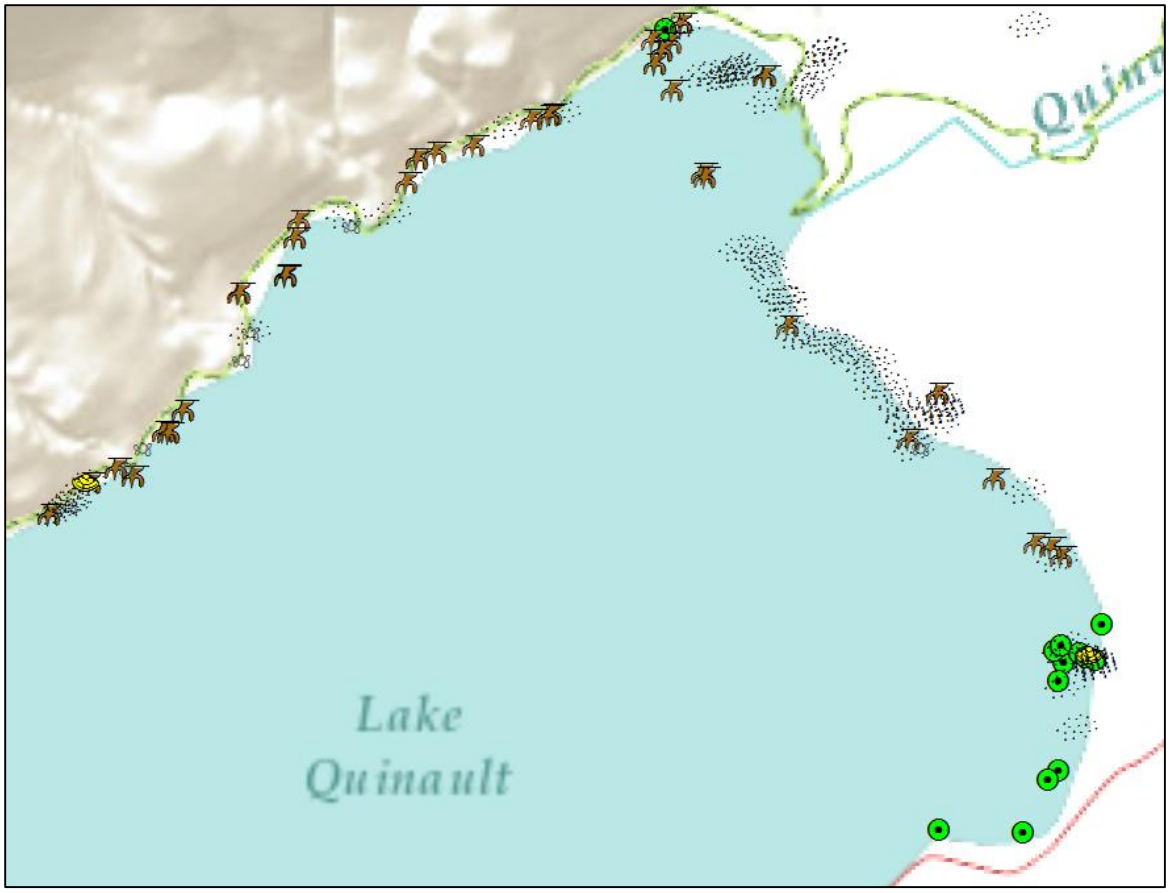


Figure 15: *Corbicula* and Sphaeriidae locations in relation to large woody debris, sand, and rocks.

Water Quality

Water quality was collected at the white buoy located mid-lake during the second and third sampling trips. Sonde malfunctions precluded measurements during the first sampling trip. The pH probe failed calibration during the second sampling trip, and the pH data from the second sampling trip were not used. The pH probe was repaired, calibrated, and used during the third trip.

Biological sampling for AIS was conducted when epilimnion water temperatures were above 16ºC (Figure 17). Lake Quinault was stratified during the sampling period and the entire sampled water column was suitable habitat for the AIS species of concern (Figure 17).

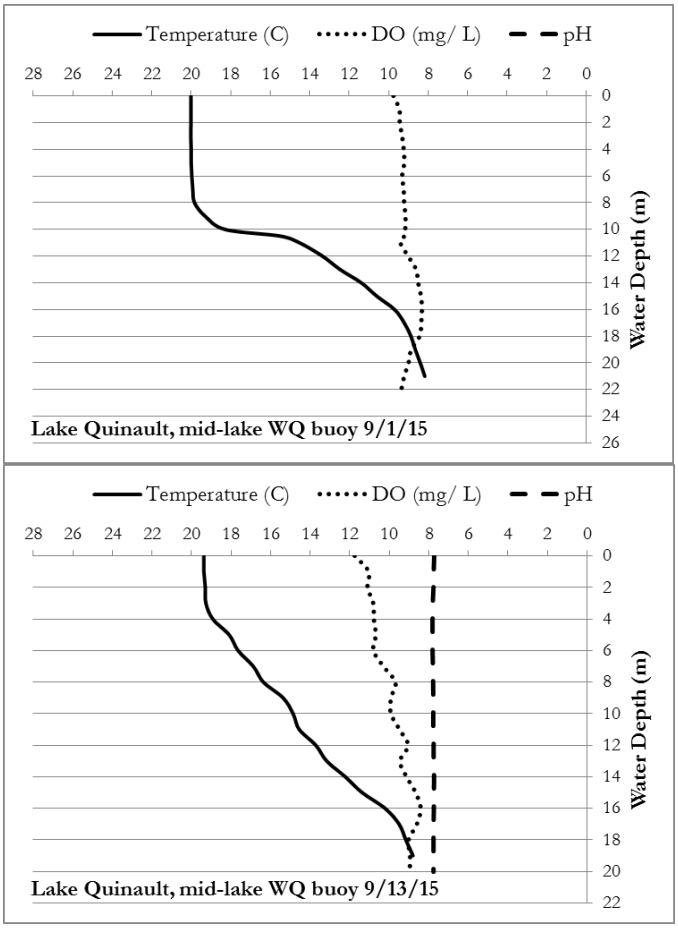


Figure 16: Profiles for water temperature (C), dissolved oxygen (mg/L), and pH in Lake Quinault.

Discussion

The monitoring efforts conducted by WBS in 2015 under this project provided valuable early detection monitoring data for AIS in Lake Quinault as well as providing preliminary composition

and distribution data for bivalves, gastropods, crayfish, and macrophytes. AIS monitoring for adult and juvenile life forms was focused in areas where introduction was most likely to occur (e.g., near popular recreational areas and access points), where establishment was most likely (e.g., macrophyte beds), and where dispersal and settlement of new recruits was most likely to occur (e.g., hydrodynamics, bathymetry). Plankton monitoring targeted areas where bivalve larvae were most likely to be concentrated or be entrained such as in front of the outlet.

There is a high likelihood of false negative results, i.e., failing to detect them when present, with early detection monitoring for AIS bivalves, gastropods, macrophytes, and other rare and spatially clumped discrete organisms. Many AIS such as NZMS exhibit spatial and temporal patchiness, and the sheer size of potential habitat makes collection difficult. Unknown factors such as the life stage at which a species is introduced can further complicate collection efforts. Additionally, the target organisms are small, and the interfering matrix found in biological samples complicates the detection.

The collection and identification of AIS by WBS followed a strategy identified in the QAPP to maximize the likelihood of collecting a rare species and accurately identifying specimens. WBS employed a multi-pronged approach to sample different habitats using multiple methods including sampling for adult and juvenile life forms on existing submerged natural and man-made substrates, inspecting exposed and wade-able shoreline areas, Portland Sampler deployment and inspection, and plankton sampling for bivalve larvae. Each field sampling technique employed was optimized to enhance the likelihood of early detection of AIS bivalves, gastropods, crayfish, and submerged macrophytes. SFPs were developed, and calibrated equipment ensured adequate data collection.

Efforts were focused on sampling a large number of small quadrats distributed throughout the different types of available habitat. The distribution of quadrats, however, was not random but stratified to concentrate efforts in areas thought to be at higher risk for AIS introduction and/ or establishment. These priority areas were identified in the QAPP using input from QIN Department of Natural Resources, a reconnaissance trip in July 2015, aerial photography, and public water quality data. Field sampling occurred in these priority areas as well as other appropriate locations identified in the course of sampling.

The timing of sampling was chosen to maximize the likelihood of collecting and identifying several AIS taxa of concern at the earliest point following introduction and/ or establishment. Both animal and plant AIS were targeted. Morphology based identification often requires sexually mature adults, flowers, and seeds. The optimal time to sample ZQM larvae in North America is during the peak of spawning, which occurs on average, when water temperatures are between 16^{o} and 19^{o}C (McMahon 2015). The sampling period was adjusted according to phenology when feasible. Based on the water temperatures from the USGS station in the outlet river and data provided by QIN Department of Natural Resources, the timing of sampling was

focused during the late summer period. In 2015, WBS sampled Lake Quinault three times in the August to September period when the epilimnion water temperatures were between 16º and 21ºC (Figure 17). Additionally, a higher sampling frequency increased the likelihood of collecting small populations exhibiting spatial and temporal patchiness in the environment.

The materials and design of the Portland Samplers were chosen to increase the likelihood of AIS colonization. Several types of materials were incorporated into the design of each Portland Sampler (concrete, pvc, abs plastics, and steel), and these materials were readily colonized by both ZQM and NZMS (Ackerman, Cottrell, Ethier, Allen and Spelt 1996; Kilgour and Mackie 1993). ZQM and NZMS prefer to colonize protected areas, and holes were drilled into the Portland Sampler pipe sections to provide ample water flow through the protected area inside the pipe section. Multiple pvc and abs plastic pipe sections were secured at different water depths along a rope or cable that was woven through long strips of plastic mesh. This design increased surface area for AIS settlement throughout the water column.

Portland Samplers are effective but provided limited surface area, and the inspection of additional habitat for AIS increased the likelihood of detection. A total of 570 natural and man-made surfaces within Lake Quinault were sampled with hand pat-downs, scrapings, grab samples, thatch rake, and shoreline inspections. Underwater surfaces in the upper 3-m were scraped at 85 locations using the surface scraper. A total of 117 benthic locations were sampled with the grab sampler. One hundred locations were sampled with the plant rake and another 180 locations were visually inspected using the sight tube. These efforts significantly increased the surface area of natural and artificial habitat inspected for AIS.

Corbicula fluminea (Asian clams) were the only AIS found in Lake Quinault during the AIS survey in 2015. Live adult *C. fluminea* were found in ten locations concentrated in Lake Quinault near the mouth of Ziegler Creek. Dead *Corbicula* shells were also found at two additional sample locations within the Ziegler Creek area, as well as one location in the Canoe Creek area of Lake Quinault. All the locations where *Corbicula* were found are identified in Figure 11. *C. fluminea* straight-hinge juveniles were not detected in the 98 plankton samples collected in Lake Quinault in 2015. *C. fluminea* adult clams brood their young inside the adult clam, and release the juveniles from the parent's brood pouch, and these juveniles crawl on benthos using a foot. *Corbicula* straight-hinge juveniles are often entrained in higher water flows, and can be common in plankton samples collected in lotic systems.

It is difficult to determine the spatial distribution and density of the *Corbicula* population within Lake Quinault using the early detection data generated under this project; however, the 2015 data suggests that the distribution of *Corbicula* in Lake Quinault is not widespread. It appears that the *Corbicula* population in Lake Quinault is mainly distributed within the Ziegler Creek area. There were numerous quadrats sampled in Lake Quinault that lacked adult *Corbicula* (Figure 11), and this is especially informative within the Ziegler Creek area where live *Corbicula* were found.

Additionally, the lack of *Corbicula* straight-hinge juveniles in the plankton samples supports the notion that the *Corbicula* population is not yet widespread within Lake Quinault. The collection, however, of numerous dead adult clam shells in the Canoe Creek area of Lake Quinault, many of which were two valves still joined by the ligament, suggests that the distribution of live *Corbicula* in Lake Quinault is not resolved, and is likely more broadly distributed than these data indicate.

It appears that the *Corbicula* population has been established in Lake Quinault for several years based on the size distribution of a limited number of the collected adult clams. Most measured clams were between 20- and 30-mm along the antereo-posterior axis, but several specimens had shell lengths of 35-mm. It is unlikely that all of these older individuals collected in 2015 were introduced into Lake Quinault as adult clams, and therefore, it can be assumed *Corbicula* has been established in Lake Quinault for at least several years. An evaluation of the *Corbicula* population structure would help elucidate the estimated time period *Corbicula* have been established in Lake Quinault.

C. fluminea is a freshwater bivalve that was introduced to the Columbia River Basin in the 1930s (Burch 1944), and is causing macrofouling problems in hydroelectric facilities on the main stem Columbia River, e.g., *Corbicula* were removed from cooling condenser tubes during main unit overhauls every five years (Athearn and Darland 2007; Kovalchuk 2007). *Corbicula* adults do not attach to hard surfaces using byssal threads (young clams can attach to surfaces using byssal threads for anchoring, but this ability is lost in adults). *Corbicula* clams do accumulate in collection channels, fishways, under diffuser gratings, behind and lodged in valves, screens and on separator bars (Kovalchuk 2007). In addition to the macrofouling problems associated with blockage, *Corbicula* can cause physical injury to fish during passage (Kovalchuk 2007) as well as other potential ecological impacts, e.g., competition with native bivalves.

Corbicula are becoming the dominant bivalve in North America (McMahon 2015), and these clams are likely to receive more attention as our understanding of their distribution and interactions with native communities continues to develop. Lake Quinault presents an opportunity to study ecological interactions between the several native bivalves found in Lake Quinault and the introduced *Corbicula* clams. For example, based on the preliminary distribution data generated in this project, it appears that *Anodonta* is distributed throughout Lake Quinault including the area where *Corbicula* is found (Figure 14).

There are improvements that could be made upon the WBS effort in 2015. The abundance of large woody debris accessible at water surface, e.g., log jammed into sediment at an oblique angle, provided numerous relatively secure surface structures to deploy Portland Samplers. Although the number of Portland Samplers deployed met the project objectives, there were abundant opportunities to deploy more Portland Samplers in Lake Quinault, i.e., increase the sample size. Portland Samplers are effective because they sample a variety of artificial materials

at water depths that are typically inaccessible. More permanent tamper-resistant artificial settlement substrates could have been deployed by securing eyebolts into large woody debris and swaging on steel cable or chain. A different type of artificial settlement substrate (e.g., screened box placed onto sediment attached to a retrieval line) could be developed and deployed to augment sampling targeting the distribution of live *Corbicula fluminea* juveniles and young adults (McMahon 2015). The idea of the screened box is to provide a contained, surface-retrievable, and protected area where naturally settled particulate would collect, presumably including entrained straight-hinge juveniles of *Corbicula*.

WBS addressed the bias associated with sample collection. False-positive results during sample collection are caused by cross-contamination of field sampling equipment. Multiple sets of gear and decontamination procedures were used to minimize these sources of bias. False-negative results during sample collection are caused by inadequate sample size, inappropriate location and frequency of sampling, and poor sample handling. WBS sampling utilized several methods to target multiple taxa, life stages, and types of habitat during multiple sampling events throughout the peak spawning and boater recreational period. Samples were immediately placed on ice or preserved in solutions of 70% regular ethanol that was pre-buffered with Tris to maintain sample pH and specimen integrity.

The sampling effort by WBS during 2015 was adequate. More than 75,000-L of reservoir water were filtered through 63-µm plankton nets in order to collect a total of 98 plankton samples from 44 locations sampled in August and September for the early detection of AIS bivalve larvae. Efforts targeting juvenile and adult AIS sampled submerged surfaces including wood, plastic, metal, concrete, wood, rock, sand, gravel, rubber, mud, and submerged macrophytes. A total of 705 locations or samples were collected in Lake Quinault by WBS in 2015 using hand pat-downs (n= 73), a surface scraper (n=85), grab sampler (n=117), thatch rake and/or sight tube (n= 280), shoreline surveys (15), artificial settlement substrates (n= 8), crayfish traps (n= 29), and plankton samples (n= 98). The target number

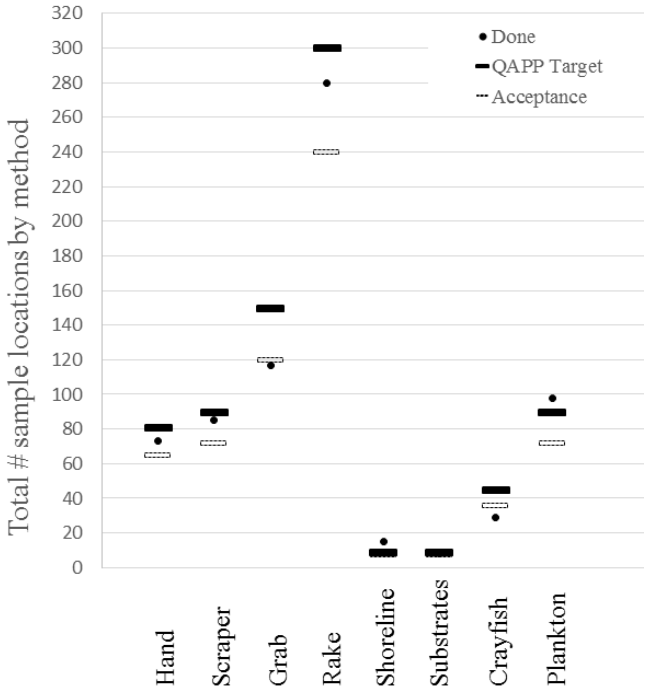


Figure 17: Sampling effort, or the number of locations sampled using each (•) in relation to sampling objectives (▬) and the completeness criteria (□) identified in the QAPP (Wells 2015b).

of sample locations to be sampled was identified in the QAPP, and the acceptable level of data completeness was 80%. WBS sampling in 2015 met or exceeded the minimum number of sample locations for all sampling methods except the grab sampler and crayfish trapping (Figure 18). A total of 117 grab samples were collected, and this was close enough to the 80% completeness threshold of 120 for actual acceptance. Crayfish trapping by WBS in 2015 did not meet acceptance criteria as identified in the QAPP for completeness. In part, the reduced crayfish trapping effort was a result of collecting very large numbers of native crayfish with no evidence of other taxa present.

It was difficult to quantify the level of sampling effort, and this can be especially true for plankton sampling. The amount of reservoir water filtered is the preferred matrix to evaluate the plankton sampling effort as compared to the total number of plankton samples collected, e.g., highly turbid and productive waters can increase the number of samples collected without a concomitant increase in the amount of water filtered. It is difficult to measure the amount of water filtered through a net, and in order to be conservative, the calculated volume of water filtered through the plankton net during sampling in 2015 was reduced by 20% to account for potential net clogging.

Light microscopy was used to detect bivalve larvae in plankton samples, and this is an established methodology that has been demonstrated to be the most reliable and accurate method for detecting bivalve larvae in low densities with interfering plankton matrices such as phyto- and zooplankton (Frischer et al. 2011). Plankton samples were concentrated to preferentially target the portion of plankton most likely to contain ZQM larvae. A minimum of 20% of the concentrated sample was analyzed, but on average, 41% of the concentrated sample was analyzed during this project. The subsample was diluted in the counting chambers using regular ethanol to create workable matrices so that all specimens and associated morphology were visually observed under the microscope; the 222-mL of concentrated plankton sample that was analyzed in 2015 required the analysis of 465 microscope slides (2-mL Sedgewick-Rafter cells). Microscopy was done using compound light microscopes using closed counting chambers that provided for total magnifications up to 200X with a resolution of 0.4 numerical aperture.

WBS addressed the bias associated with plankton analysis to produce reliable data that managers would be willing to use to guide actions. Incorrect and ambiguous results confuse policymakers and managers, complicate other agency efforts, and compromise trust in the scientific community. Bias associated with bivalve larval detection during light microscopy analysis includes false-positive and false-negative results. The sources of these biases and the corrective measures were identified in the QAPP. Misidentification was addressed using appropriate equipment, laboratory control samples and other identification tools (e.g., image database), and increasing subsample volume to locate additional specimens. Contamination was addressed by field and laboratory decontamination procedures. Analyst error, matrix effects, and low

abundances of target specimens cause false-negative results with light microscopy. Analyst error was 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 were addressed by increasing aliquot dilution, thus reducing confounding matrix. Low abundance of larvae was addressed by sample handling procedures in the laboratory, sample concentration, and increasing the subsample volume.

WBS laboratory plankton analysis was acceptable in 2015. The WBS laboratory met its acceptance criteria used for plankton quality control samples. Ten BMS were submitted with routine analysis, and the spiked ZQM larvae were detected in all of these BMS during routine analysis. The percent recovery of spiked larvae in the BMS during routine analysis in 2015 averaged 57% (Figure 19), which involved analyzing approximately 28% of the concentrated sample. This suggests that the percent recovery would have been higher if more of the concentrated sample had been analyzed. ZQM larvae are small, discrete organisms that are not uniformly distributed within a sample matrix. Additionally, the interfering matrix in typical plankton samples complicates the detection of bivalve larvae. It is time consuming and expensive to analyze the entire plankton sample in a manner that is likely to detect all present larvae. It is also difficult to compare plankton analysis via light microscopy to the analysis of other water quality parameters (e.g., the use of atomic absorption spectroscopy to measure dissolved calcium concentration) that typically require BMS recovery within 10% of the actual concentration for acceptance. The recovery rates in Figure 19 demonstrated that the WBS laboratory was able to reliably detect bivalve larvae at low densities in real plankton samples; however, these recovery rates also indicated the inherent problems with detecting discrete organisms at low densities, and reinforced 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), and developing and implementing a regional laboratory certification process.

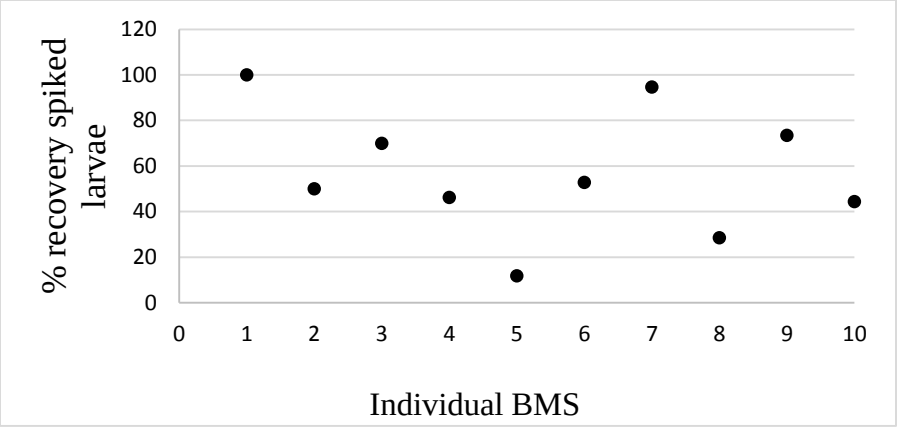


Figure 18: Percent recovery of spiked larvae in the blind matrix spiked samples. All spiked samples were detected during routine plankton analysis, and percent recovery represents larvae abundance in the portion of the concentrated sample that was subsampled.

The risks posed to Lake Quinault by AIS are real. One AIS was detected in Lake Quinault during 2015 sampling (*Corbicula fluminea*), as well as many native and non-invasive species of bivalves, gastropods, crayfish, and macrophytes. Non-detect is different than absent, and despite sampling over 700 locations within Lake Quinault, there are still areas that need better coverage. Early detection will provide the greatest amount of time to organize and mount rapid response efforts.

Next Steps/ Recommendations

- Communicate the new *Corbicula fluminea* detection data with shareholders, scientists, and the general public, e.g., posting data on the Nonindigenous Aquatic Species website <nas.er.usgs.gov>.

- Develop outreach and education materials to communicate with lake users and local residents the risks posed to Lake Quinault by AIS, and actions they can take to help. Based on the interactions with five of the residents along the north shore, the local residents seemed invested in Lake Quinault, and many have been coming here their entire lives. Advisory signs should be posted at the boat launches warning boaters of the risks posed to Lake Quinault, and how to safely their clean gear.

- Determine the distribution of *Corbicula* in Lake Quinault to better inform management decisions. Management options for controlling a widespread bivalve population are different compared to the options when dealing with a population contained in a limited area. Screened boxes deployed on the lake bottom and tethered to surface structures could be a relatively inexpensive, repeatable, and effective way to sample for *Corbicula* juveniles and young adults throughout Lake Quinault.

- Analyze the early detection data produced in this report to explore relationships between the collected taxa, sediment types, and water depth. These analyses may improve the effectiveness of subsequent sampling efforts and contribute to development of ecological studies.

- Continue AIS early detection monitoring in Lake Quinault targeting multiple taxa and types of habitat with several methodologies. Efforts should be focused on sampling a large number of locations to increase the likelihood of collection, filling spatial gaps from 2015 efforts, and expanding search efforts for *Myriophyllum* and other macrophytes.

- Continue employing the multi-pronged AIS sampling strategy using several sampling methodologies. Each sampling method was effective at collecting specimens, and each method was better suited to a particular habitat or taxa.

- Continue recording the amount of water filtered through the plankton net to quantify the plankton sampling effort. The total number of plankton samples does not adequately capture the quality of the sampling effort.

- Continue the use of blind matrix spiked samples during plankton sample analysis to check the accuracy of plankton analysis via light microscopy.

- Continue proper field and laboratory decontamination to prevent cross-contamination of samples and the unintentional transfer of organisms between water bodies.

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Appendix A. **Field Collection Protocols for AIS Monitoring**

2015

Steve Wells

Western Biological Services LLC

SAFETY

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, and some have been serious, e.g., trailer tire blow-out on HWY; rolling truck after swerving onto shoulder.
- You are 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 vehicles.

Safety Protocols

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

Collecting biological and water quality samples from a boat

- Death and/or bodily harm can occur when working on boats in large rivers, reservoirs, and lakes.
- There is a history of accidents, 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 boat while conducting official duties only.
- You are required to possess an Oregon Boater Education card to operate boat. You should have the card with you on the boat.

Safety Protocols

1. **A personal floatation device (PFD)** is worn anytime a person is in boat.
2. Carry cell phone and quarters.
3. Bring a fire extinguisher, throw ring/seat cushion, and sound device (horn or whistle) onto boat. When boating on the Columbia River, you must also bring flares. Do NOT use flares on other inland water bodies (wildfire risk).
4. Valid Oregon Boater Education card.
5. Bring drinking water, sun protection, snacks, and non-slip shoes.
6. Complete/update a field and float plan prior to launch. The Float plan is left on dashboard in parked vehicle while you are on water (fold along dotted line to hide information on bottom half of sheet).
7. Lift with your legs and avoid straining muscles. Use cranes, pulleys, and multiple people as needed.
8. Verbally communicate to other people when shifting position within boat, starting engine, throwing plant rake, etc.

9. Secure lightweight equipment before moving.
10. Distribute weight appropriately Port-Starboard and Stern-Bow.
11. Maintain a clean and clear working area.
12. Be safe!!

GENERAL STRATEGY

Prioritization of activities by species per sampling trip.

Task	Time
1. Hand pat-downs (27 sample locations)	2:45
2. Surface scraper (30 sample locations)	2:30
3. Grab sampler (50 sample locations)	8:15
4. Thatch plant rake & opportunistic hand grabs (100 sample locations)	12:00
5. Shoreline walks (3 sample locations)	1:30
6. Portland Samplers (9 sample locations)	1:30
7. Minnow traps (15 sample locations)	3:00
8. Plankton (15 sample locations)	2:30
9. Water quality (1 sample location)	1:00
Total SAMPLING time on water	**35 hrs.**

- Total time on water will vary by weather, presence and abundance of organisms, and other unforeseen issues. Each trip will have three days available for on-water work, and work is expected to occur during sunlight hours.
- Gastropods, bivalves, crayfish, and submerged macrophytes are the primary targets.
- Sampling locations are targeted to maximize the likelihood of collection.
- Generalized sample locations are pre-identified using GPS coordinates. Navigate to approximate coordinates and use sonar, sight tube, and expert judgement to determine specific sampling location in this area. Record the GPS coordinates for sampling locations while in field.
- More sampling locations and bigger samples are desired if possible. Compositing is okay.
- Collect a minimum of 30 125-mL plankton samples per trip, or a total of ninety 125-mL samples during project period.
- Macrophytes are sampled at a minimum of 100 sample sites within littoral zone (i.e., periphery of Lake Quinault). In the field, GPS coordinates will be used to navigate to the pre-identified sampling location, and then the actual location of macrophyte sampling will be adjusted according to *in-situ* data from sonar, sight tube, and the presence of visible beds in order to target individual macrophytes and beds. Macrophyte sampling at a particular site will be repeated if *in-situ* data (e.g., sight tube) suggests that present species were not collected.

- Be careful of submerged logs with thatch rake. If it gets stuck reverse position to remove.
- Voucher specimens are needed for identification and documentation. ID occurs in laboratory. Sort quickly in field, and err on side of collecting numerous specimens with similar appearance.
- Ethanol is pre-buffered using Tris and plankton samples should require no additional buffering.
- Deploy crayfish traps at start of sampling trip, and collect the last day. Preferably use fresh salmon, but other fish are acceptable. Bait must be either frozen or fresh. Target is total 15 traps deployed.
- Sample containers are pre-labeled with number, e.g., FY15-5555. If missing sample container number, assign one using following: FY15- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY15-062513a.
- **On field datasheet, record the sampler container number**, e.g. FY15-5555, as well as any other pertinent information.
- Write legibly. Use pencil preferably for field datasheet and sharpie for sample containers.

GASTROPODS, BIVALVES, & CRAYFISH

Objective: early detection (presence/ non-detect) and preliminary spatial distribution of adult and juvenile *Potamopyrgus antipodarum* (New Zealand mud snail), *Radix auricularia* (Big-eared radix), *Dreissena* mussels (zebra and quagga mussels), *Corbicula fluminea* (Asian clam), *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 old fish pens, logs, docks, 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
- (9) substrates (pvc, ABS, concrete anchor, rope)
- Knife
- Datasheet (Appendix C)
- 1 gallon zip lock bags
- thatch rake on rope
- 5-gallon bucket (white color)
- GPS unit
- 12V battery
- (1) sharpie pen, (2) pencils
- cooler with ice
- (8) 250-mL sample containers
- digital camera
- Ethanol

- Metal sieve
- Petite Ponar sediment dredge
- Minnow traps, clips and rope
- Salmon or other fish 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, logs, fish pens, 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. Gently 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 sealable plastic 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 smooth solid surfaces such as concrete walls, bridge abutments and cutwaters, logs, channel markers, pilings, underwater floats, and breakwaters.
2. Carefully position boat near structure to sample (e.g., log) and maintain position either using the motor or using water current and wind to position boat against structure.
3. When using the surface scraper on vertical surfaces, e.g., piling, 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 bucket in contact with the submerged surface and quickly pull up, keeping the metal rim in contact with the surface to be sampled. The scraped material will be collected in the mesh bucket, and material is inspected for the presence of specimens at water surface. Inspection is visual and tactile and is done in the field. After inspecting collected material, the mesh bucket is rinsed in waterbody and readied for next sampling.
4. Repeat step #3 at multiple locations per structure in order to sample a representative portion.
5. **Record GPS location and sampling activities on datasheets and record waypoint on chart plotter.**

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 at water surface from which the substrate can be suspended at depths of ideally 25-ft, e.g., vertically oriented log in sediment, 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, and record waypoint on chart plotter. On datasheet, include both GPS coordinates and a general site description with enough detail to guide a stranger to the general deployment location without GPS.

Inspection

1. When checking an existing substrate, remove it slowly from the water to avoid specimen loss. Inspection for adult and juvenile gastropods, bivalves, and other AIS is both tactile and visual. You are looking for a visible (naked eye) specimens. Small specimens and juveniles (<1-mm) 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 AIS 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**. Record waypoint. Note if previously deployed substrate is gone. Replace missing substrates with new substrate.
4. Retain suspect specimens in sealable plastic sample container with small amount of lake water. Record the sample container number with the GPS location on field datasheet. Place

specimen in cooler on ice. Different taxa collected at one site may be combined into one sample container when appropriate, e.g., no risk of predation such as crayfish with bivalves.

5. Redeploy substrate after inspection.

Petite Ponar® Grab Sampler

1. Deploy the sediment dredge in areas of gravel, small cobble, sand and mud in any water depths. 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. Releasing tension on the rope should trigger the pin release. In most cases, you can feel the dredge deploy.
2. Quickly retrieve dredge and dump contents into sieve and rinse in lake. Inspect sieve for bivalves, gastropods, and other AIS.
3. **Record GPS location and sampling activities at each site on datasheet and record waypoint**.
4. Retain suspect specimens in sealable plastic sample container with small amount of lake water. Record the sample container number with the GPS location on field datasheet. Place specimen in cooler on ice. Different taxa collected at one site may be combined into one sample container when appropriate, e.g., no risk of predation such as crayfish with bivalves.

Shoreline walks

1. Walk in a zig-zag pattern parallel to shoreline in wade-able depths near boat launches and other areas where debris (e.g., driftwood and senescing macrophytes) is collecting on shore, rocky areas such as spits and jetties, and areas that contain 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 bivalves, gastropods, and other AIS. Look for specimens partly buried in sediment as well as on top of sediment.
2. **Record GPS location and sampling activities at each site on datasheet, and record waypoints.**
3. Retain suspect specimens in sealable plastic sample container with small amount of lake water. Record the sample container number with the GPS location on field datasheet. Place specimen in cooler on ice. Different taxa collected at one site may be combined into one sample container when appropriate, e.g., no risk of predation such as crayfish with bivalves.

Crayfish traps

- Deploy minnow traps as soon as you arrive at a water body if you are not camping nearby. Retrieve these traps before departing. When camping near the water body, trap overnight.
- Bait traps using fresh fish, preferably salmon or trout. Canned sardines or herring can be used in emergencies. Secure trap together with clips or zip ties. Use dark-colored rope (e.g., dark green Paracord) to minimize visibility.
- Deploy traps throughout water body. Focus efforts in shallow littoral areas with hard substrate and protected areas, preferably rock, as well as areas with macrophytes. Attach rope to stake in ground or tie to rock or other surface structure. Chose trapping locations that are inconspicuous to avoid tampering, theft, etc.
- Retrieve and inspect traps. **Record sampling activities on field datasheet**.
- Retain suspect specimens in sealable plastic sample container with small amount of lake water. Record the sample container number with the GPS location on field datasheet. Place specimen in cooler on ice. Different taxa collected at one site may be combined into one sample container when appropriate, e.g., no risk of predation such as crayfish with bivalves.

Plant shake-down

1. Place collected macrophytes into a 5-gallon white-colored bucket with lake water. Vigorously shake the macrophytes in bucket and water to detach debris, periphyton, and invertebrates. Look for crayfish, bivalves and gastropods on macrophytes when placing plants into bucket, and again when placing rinsed macrophytes into sealable plastic containers with lake water. Allow bucket and water to sit in sunlight while sorting plants.
2. Inspect the sides of bucket for attached gastropods.
3. Pour liquid and debris out of bucket through sieve. Visually inspect collected debris for gastropods and bivalves. Discard debris in lake.
4. **Record activities on datasheets and record waypoint**. Record GPS location, sample container number, and sampling activities at each site on datasheet.
5. Retain suspect specimens in sealable plastic sample container with small amount of lake water. Record the sample container number with the GPS location on field datasheet. Place specimen in cooler on ice. Different taxa collected at one site may be combined into one sample container when appropriate, e.g., no risk of predation such as crayfish with bivalves.

6. Place sample container with suspect specimens in cooler on ice.

Sample Preservation:

- Bivalve and gastropod suspect specimens are kept alive, and transported to WBS held on ice. Bivalves and gastropods 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 if sampling multiple water bodies.**
- Sample containers with living organisms (e.g., mollusks and plants) are stored in the refrigerator upon returning to the WBS laboratory.

MACROPHYTES

Objective: sampling to create species composition lists and conduct early detection monitoring for invasive plants, e.g., *Myriophyllum*.

Site Locations:

- Submerged rooted plant beds visible from surface and/or sight tube.
- 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
- Down scan sonar
- GPS unit and (4) AA batteries
- bathymetric maps
- macrophyte identification books
- pencils and permanent marker
- Sight tube

Sample Collection:

Thatch 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 macrophyte bed.
3. **Record sampling activities on field datasheet and record waypoint**.
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: FY15- collection date (MMDDYYYY) and if multiple numbers needed for same collection date, add "a", "b", etc. as needed to end, e.g. FY15-062513a.

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 WBS. 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 if not retaining plants for laboratory identification**.**

9. Use the sight tube to look for species that were not collected at the site. Repeat rake toss as necessary.

Macrophyte hand grabs

In shallow water, wade into water and manually retrieve the macrophytes.

Repeat steps #3-7 for thatch rake on rope detailed above.

Opportunistic macrophyte hand grabs

Opportunistically grab macrophytes that are floating at water surface in the course of other activities and moving between sites.

Repeat steps #5-7 for plant rake on rope detailed above.

Sample Preservation:

- Plants are placed in sealable plastic 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)

Submerged

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

Floating

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

Emergent

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

ZQM VELIGERS

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

Site Locations:

- A minimum of 15 individual sites, but more if time permits. A site is a particular location separated from other sites by at least 61-m (200-ft).
- Collect, at a minimum, a combination of 8 vertical tows and 7 trawling events (i.e., 30 125-mL samples per trip).

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):

- (30) 125-mL sample containers
- GPS unit w/ batteries
- (2) 64-µm mesh plankton net with cod-end
- (2) rope wheels w/ 31-m (100-ft) rope ea.
- veliger datasheet (Appendix B)
- (2) pencils and (1) sharpie pen
- (1) net anchor
- (10-L) regular ethanol
- watch or clock
- cooler with ice

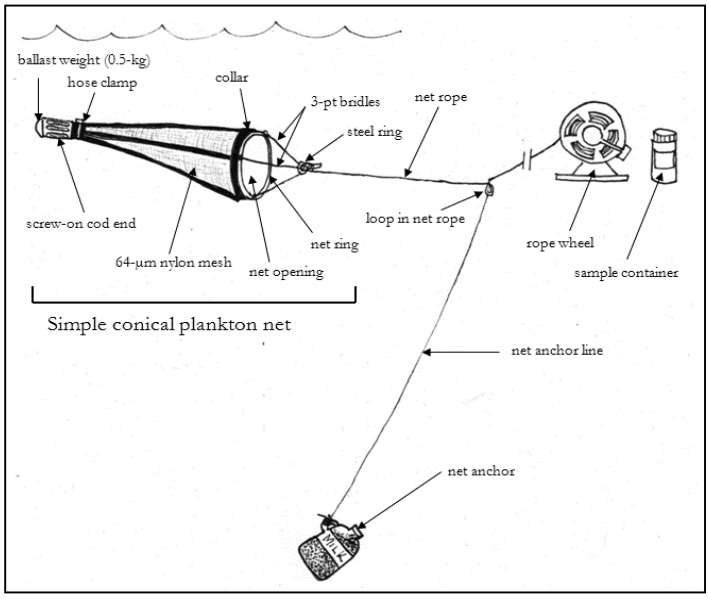

Figure 19: Plankton net set-up.

Sample Collection:

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

Vertical/Oblique Plankton Tow

1. Secure the cod-end piece, check the hose clamp and check that the rope is securely attached to plankton net at steel ring. Attach net anchor (milk jug of gravel) to a second 2- or 3-m section of rope that is tied to a loop placed in the net rope approximately one meter in front of net opening.
2. Lower the net 30-m (100-ft) below water surface, or to 1-m above the sediment, whichever is deeper. Keep the net off the lake bottom. Record GPS location on datasheet (Appendix B).

3. Keep net at this depth for five seconds and then manually retrieve using a hand-over-hand technique at a rate of 0.5-m/s (1.5-ft/s). Slow and steady retrieval is the key to collecting a good plankton tow.
4. Rinse the net by raising the net so that the cod end of the net is at the water surface. Rinse organisms into the cod end of the net by lowering the net back into the water, keeping the opening above the water surface. Then quickly pull net straight up; this action will move collected plankton into the cod-end piece. Repeat this procedure several times to ensure that all the organisms inside the net are in the cod end.
5. **Condense the sample as much as possible before pouring into sample container**. Condense the sample by swirling the cod-end piece while still attached to net. Then carefully unscrew the cod-end piece without spilling collected water and plankton. You may need to use tweezers, spatula, or your finger to gently clear the mesh netting in the cod-end piece to allow the water to filter through. The cod-end piece, once separated from net, should also be swirled to further condense sample. After pouring sample into sample container, dip the cod end into water body to add small amount of water in order to rinse out remaining plankton into sample container.
6. Record the length of each tow on the datasheet (Appendix B) 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 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.

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
- 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 <u>small amount</u> 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 <u>off</u> 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.
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.

<u>pH</u>

Calibrated **<u>at every water body</u>** 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 <u>small amount</u> of "used" pH 7 standard. Discard standard.
3. Turn <u>off</u> 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.

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°, 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) 5% bleach solution
- bottle of household bleach (appx. 6% NaOCl)
- (2) large plastic tubs with lids (> 10 gallons)
- spray bottle 2% Virkon Aquatic
- (> 4 gallon) 2% Virkon Aquatic
- large brush
- spray bottle of tap water
- (> 13 gallons) fresh water
- spray bottle 5% bleach solution
- spray bottle of 2% Virkon Aquatic
- 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 <u>not</u> 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, 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.

13. Stop engine after flushing cooling lines. Raise lower engine unit out of tub, and slide tub of water clear of engine area.

14. Add approximately 2.5-L (0.7-gallons) of household bleach to the tub containing approximately 49-L (13-gallons) of tap water. This is a 5% solution of bleach, and this solution is caustic and appropriate safety equipment should be worn.

15. Soak the plankton net, cod-end, plankton rope, net anchor, dredge, sieve, plant rake, and surface scraper in the plastic tub containing 5% solution of bleach for 15 minutes.

16. Remove equipment from bleach solution, and thoroughly rinse with tap water.

17. The plankton net and the cod-end are then soaked in 5% acetic acid solution for a minimum of 6-hrs. The preferred soak time in acetic acid is 24-hour.

18. Plug the bilge drain. Transfer the 49-L (13 gallons) of 5% bleach solution into the boat, washing down seats, flooring, and other surfaces. Use the scrub brush to wash down the insides of the boat using the 5% bleach solution. Focus efforts on rinsing all surfaces and forcing all debris into boat bilge area. Let bleach solution soak in boat bilge for 15 minutes.

19. Pull bilge plug on impervious surfaces to drain bilge when at least 61-m (200-ft) from open water.

Appendix B: Field datasheet

Lake Quinault AIS Survey 2015

#	Date	Time	Depth	Latitude	Longitude	WP	Collect?	Container#	Notes
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									

If found, please contact Steve Wells, Western Biological Services LLC, 503-713-3579, zqmsww@gmail.com

Appendix B: Field datasheets

WATERBODY: _____ **Page___of____for Water body and sampling date**

Date: ___ / ___ / ____ **Collector(s)**: _____

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

Vertical/ Oblique Tows

Tow #	Latitude	Longitude	Depth (m)	Net efficiency (e.g. 100%)	Sample Container # (e.g. FY15-145)	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	Sample Container #	Notes
1										
2										
3										
4										
5										
6										
7										
8										

Appendix B: Field datasheets

Secchi	Down depth (m)	Up depth (m)	average	Water column depth (m):
1				Latitude:
2				Longitude:

Water Quality Information					
	Temp (C)	SpC (us/cm)	DO (mg/ L)	pH	Other/ Notes
Surface					
1 m					
2 m					
3 m					
4 m					
5 m					
6 m					
7 m					
8 m					
9 m					
10 m					
11 m					
12 m					
13 m					
14 m					
15 m					
16 m					
17 m					
18 m					
19 m					
20 m					
21 m					
22 m					
23 m					
2 m repeat					

Comments:

Appendix C

Sample Analysis Tracking Form **Point of contact:** Steve Wells **Western Biological Services (WBS)**
Zebra/ Quagga Mussel Early Detection Monitoring **PAGE** 1 **of** 10

Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
10/7/15	SWW	DM750	LQ3 5816a	4	2	4:1	3	1.5	0	0	0	
10/7/15	SWW	DM750	LQ3 5816b	4	2	4:1	1	0.5	20*	0	0	*Blind matrix spike sample
				8			**4**	**2.0**	**0**	**0**	**0**	
10/7/15	SWW	DM750	LQ3 5802	6	2	4:1	9	4.5	0	0	0	
				6			**9**	**4.5**	**0**	**0**	**0**	
10/8/15	SWW	DM750	LQ2 5800	5	2	4:1	4	2.0	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
10/8/15	SWW	DM750	LQ3 6022	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
10/21/15	SWW	DM750	LQ3 5526	4	2	4:1	4	2	0	0	0	
10/21/15	SWW	DM750	LQ3 5526	5	2	4:1	2	1	13*	0	0	*Blind matrix spike sample
				9			**6**	**3**	**0**	**0**	**0**	
10/22/15	SWW	DM750	LQ3 6002	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
10/22/15	SWW	DM750	LQ3 6024	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
10/22/15	SWW	DM750	LQ3 6009	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/3/15	SWW	DM750	LQ3 5531	5	2	6:1	3	1	0	0	0	
				5			**3**	**1**	**0**	**0**	**0**	
11/3/15	SWW	DM750	LQ3 5530	4	2	6:1	3	1	0	0	0	
				4			**3**	**1**	**0**	**0**	**0**	
11/3/15	SWW	DM750	LQ3 5956	3	2	6:1	3	1	0	0	0	
				3			**3**	**1**	**0**	**0**	**0**	

Sample Analysis Tracking Form **Point of contact: Steve Wells, WBS**

Zebra/ Quagga Mussel Early Detection Monitoring **Page PAGE 2 of 10**

Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/3/15	SWW	DM750	LQ3 5955a	4	2	6:1	3	1	0	0	0	
11/3/15	SWW	DM750	LQ3 5955b	3	2	6:1	3	1	0	0	0	
				4			**3**	**1**	**0**	**0**	**0**	
11/4/15	SWW	DM750	342	4	2	6:1	3	1	0	0	0	
				4			**3**	**1**	**0**	**0**	**0**	
11/4/15	SWW	DM750	305	5	2	6:1	4	1.3	0	0	0	
				5			**4**	**1.3**	**0**	**0**	**0**	
11/4/15	SWW	DM750	341	4	2	6:1	4	1.3	0	0	0	
				4			**4**	**1.3**	**0**	**0**	**0**	
11/4/15	SWW	DM750	310a	4	2	6:1	4	1.3	0	0	0	
11/4/15	SWW	DM750	310b	5	2	6:1	4	1.3	0	0	0	
				9			**8**	**2.6**	**0**	**0**	**0**	
11/5/15	SWW	DM750	LQ3 5534	5	2	6:1	3	1	0	0	0	
				5			**3**	**1**	**0**	**0**	**0**	
11/5/15	SWW	DM750	LQ3 5951	4	2	6:1	3	1	0	0	0	
				4			**3**	**1**	**0**	**0**	**0**	
11/5/15	SWW	DM750	LQ2 303b	10	2	6:1	6	2	0	0	0	
11/7/15	SWW	DM750	LQ2 303c	7.5	2	4:1	6	3	0	0	0	
11/15/15	SWW	DM750	LQ2 303a	8	2	6:1	6	2	0	0	0	
				25.5			**18**	**7**	**0**	**0**	**0**	
11/5/15	SWW	DM750	LQ3 5950	3	2	6:1	3	1	0	0	0	
				3			**3**	**1**	**0**	**0**	**0**	
11/5/15	SWW	DM750	LQ3 5953	3	2	6:1	2	0.7	0	0	0	
				3			**2**	**0.7**	**0**	**0**	**0**	

Appendix B: Field datasheets

Sample Analysis Tracking Form **Point of contact: Steve Wells, WBS**

Zebra/ Quagga Mussel Early Detection Monitoring **Page PAGE 3 of 10**

Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/7/15	SWW	DM750	LQ2 307	5	2	4:1	3	1.5	0	0	0	
				5	**2**	**4:1**	**3**	**1.5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ2 300a	5	2	6:1	3	1	0	0	0	
11/7/15	SWW	DM750	LQ2 300b	5	2	6:1	3	1	14*	0	0	*Blind matrix spike sample
11/7/15	SWW	DM750	LQ2 300c	5	2	6:1	3	1	0	0	0	
				15			**9**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ2 302	10	2	4:1	6	3	0	0	0	
				10			**6**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	313	7	2	4:1	6	3	0	0	0	
				7			**6**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ 300a	5	2	4:1	3	1.5	0	0	1	
11/7/15	SWW	DM750	LQ 300b	5	2	4:1	3	1.5	0	0	0	
				10			**6**	**3**	**0**	**0**	**1**	
11/7/15	SWW	DM750	306	7.5	2	4:1	6	3	0	0	0	
				7.5			**6**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ3 5533	5	2	4:1	3	1.5	0	0	0	
				5			**3**	**1.5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ2 301	10	2	4:1	6	3	0	0	0	
				10			**6**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	315	8	2	4:1	6	3	0	0	0	
				8			**6**	**3**	**0**	**0**	**0**	

Sample Analysis Tracking Form **Point of contact: Steve Wells, WBS**

Appendix B: Field datasheets

Zebra/ Quagga Mussel Early Detection Monitoring **Page PAGE 4 of 10**

Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/7/15	SWW	DM750	LQ 304a	10	2	4:1	6	3	0	0	0	
	SWW	DM750	LQ 304b	10	2	4:1	4	2	0	0	0	
				20			**10**	**5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ2 308a	5	2	4:1	3	1.5	0	0	0	
11/7/15	SWW	DM750	LQ2 308b	6	2	4:1	4	2	0	0	0	
				11			**7**	**3.5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	311	5	2	4:1	3	1.5	0	0	0	
				5			**3**	**1.5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ3 5952	7.5	2	4:1	6	3	0	0	0	
				7.5			**6**	**3**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ 306a	8	2	4:1	6	3	0	0	0	
11/8/15	SWW	DM750	LQ 306b	10	2	4:1	5	2.5	0	0	0	
11/8/15	SWW	DM750	LQ 306c	9	2	4:1	4	2	12*	0	0	*Blind matrix spike sample
				27			**15**	**7.5**	**0**	**0**	**0**	
11/8/15	SWW	DM750	LQ2 309	5	2	4:1	3	1.5	0	0	0	
				5			**3**	**1.5**	**0**	**0**	**0**	
11/8/15	SWW	DM750	LQ2 304	7.5	2	4:1	5	2.5	0	0	0	
				7.5			**5**	**2.5**	**0**	**0**	**0**	
11/8/15	SWW	DM750	LQ2 101	7.5	2	4:1	6	3	0	0	0	
				7.5			**6**	**3**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/8/15	SWW	DM750	LQ2 102	6	2	4:1	1	0.5	0	0	0	
11/10/15	SWW	DM750	LQ2 102	5.5	2	4:1	2	1	0	0	0	
				6			**3**	**1.5**	**0**	**0**	**0**	
11/10/15	SWW	DM750	LQ2 103	5	2	4:1	3	1.5	0	0	0	
				5			**3**	**1.5**	**0**	**0**	**0**	
11/10/15	SWW	DM750	LQ2 106	8	2	4:1	6	3	0	0	0	
				8			**6**	**3**	**0**	**0**	**0**	
11/11/15	SWW	DM750	308	8	2	4:1	4	2	0	0	0	
				8			**4**	**2**	**0**	**0**	**0**	
11/1/15	SWW	DM750	LQ2 105	7.5	2	4:1	4	2	0	0	0	
				7.5			**4**	**2**	**0**	**0**	**0**	
11/11/15	SWW	DM750	312	5	2	4:1	3	1.5	0	0	0	
				5			**3**	**1.5**	**0**	**0**	**0**	
11/7/15	SWW	DM750	LQ 305d	6	2	4:1	4	2	0	0	0	
11/11/15	SWW	DM750	LQ 305a	8	2	4:1	4	2	2*	0	0	
11/11/15	SWW	DM750	LQ 305b	8	2	4:1	4	2	0	0	0	
11/11/15	SWW	DM750	LQ 305c	8	2	4:1	4	2	0	0	0	
				30			**16**	**8**	**0**	**0**	**0**	
11/14/15	SWW	DM750	307a	4	2	4:1	4	2	0	0	0	
11/14/15	SWW	DM750	307b	4	2	4:1	4	2	0	0	0	
				8			**8**	**4**	**0**	**0**	**0**	
11/14/15	SWW	DM750	LQ2 104	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/14/15	SWW	DM750	316	6	2	4:1	4	2	0	0	0	
				6			**4**	**2**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/14/15	SWW	DM750	309a	3	2	4:1	3	1.5	0	0	0	
11/14/15	SWW	DM750	309b	3	2	4:1	3	1.5	0	0	0	
11/14/15	SWW	DM750	309c	3	2	4:1	2	1	19*	0	0	*Blind matrix spike sample
				9			**8**	**4**	**0**	**0**	**0**	
11/14/15	SWW	DM750	314a	4	2	4:1	3	1.5	18*	0	0	
11/14/15	SWW	DM750	314b	4	2	4:1	4	2	0	0	0	
11/14/15	SWW	DM750	314c	4	2	4:1	4	2	0	0	0	
				12			**11**	**5.5**	**0**	**0**	**0**	
11/14/15	SWW	DM750	LQ2 107	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/14/15	SWW	DM750	344	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/14/15	SWW	DM750	343	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/14/15	SWW	DM750	340	6	2	4:1	4	2	0	0	0	
				6			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6025	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 5801	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6028	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/15/15	SWW	DM750	LQ3 6012	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6018	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 5529	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6007	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 5528	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6026	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6011	3	2	4:1	4	2	0	0	0	
				3			**4**	**2**	**0**	**0**	**0**	
11/17/15	SWW	DM750	LQ3 6017	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 5805	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6013	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 5803	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/15/15	SWW	DM750	LQ3 6019	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/15/15	SWW	DM750	LQ3 5807a	4	2	4:1	3	1.5	0	0	0	
11/15/15	SWW	DM750	LQ3 5807b	3	2	4:1	3	1.5	0	0	0	
				7			6	3	0	0	0	
11/15/15	SWW	DM750	LQ3 5806a	4	2	4:1	3	1.5	8*	0	0	*Blind matrix spike sample
11/15/15	SWW	DM750	LQ3 5806b	7.5	2	4:1	8	4	0	0	0	
				11.5			11	5.5	0	0	0	
11/17/15	SWW	DM750	LQ2 5809	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/17/15	SWW	DM750	LQ3 6006	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/17/15	SWW	DM750	LQ3 6000	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/17/15	SWW	DM750	LQ3 6023	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/17/15	SWW	DM750	LQ3 6027	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/17/15	SWW	DM750	LQ3 6003	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/18/15	SWW	DM750	LQ3 6020	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/18/15	SWW	DM750	LQ3 5535	5	2	4:1	4	2	0	0	0	
				5			4	2	0	0	0	
11/18/15	SWW	DM750	LQ3 6029	4	2	4:1	4	2	0	0	0	
				4			4	2	0	0	0	
11/18/15	SWW	DM750	LQ3 6050	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/18/15	SWW	DM750	LQ3 6021	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/21/15	SWW	DM750	LQ3 6014	3	2	4:1	4	2	0	0	0	
				3			**4**	**2**	**0**	**0**	**0**	
11/21/15	SWW	DM750	LQ3 5527a	4	2	4:1	4	2	0	0	0	
11/21/15	SWW	DM750	LQ3 5527b	4	2	4:1	4	2	0	0	0	
				8			**8**	**4**	**0**	**0**	**0**	
11/21/15	SWW	DM750	LQ3 6010	3	2	4:1	4	2	0	0	0	
				3			**4**	**2**	**0**	**0**	**0**	
11/21/15	SWW	DM750	LQ3 6015	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/21/15	SWW	DM750	LQ3 6004	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6005	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 5808	5	2	4:1	4	2	0	0	0	
				5			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6001	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6016	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ2 5954	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6052	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	

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Date Analysis	Analyst initials	Scope #	Sample tracking number	Conc. sample vol (mL)	Cell vol (mL)	Dilution factor total: solute	# slides done	mL of conc sample analyzed	# ZQM	# Corb	# ostracod	Notes
11/22/15	SWW	DM750	LQ3 5804a	4	2	4:1	4	2	0	0	0	
11/22/15	SWW	DM750	LQ3 5804b	4	2	4:1	3	1.5	25*	0	0	*Blind matrix spike sample
				8			**7**	**3.5**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6051	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6008	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 6053	4	2	4:1	4	2	0	0	0	
				4			**4**	**2**	**0**	**0**	**0**	
11/22/15	SWW	DM750	LQ3 5532a	5	2	4:1	4	2	0	0	0	
11/22/15	SWW	DM750	LQ3 5532b	5	2	4:1	3	1.5	4*	0	0	*Blind matrix spike sample
				10			**7**	**3.5**	**0**	**0**	**0**	