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New Zealand Mudsnail Surveys at Lower Columbia River Basin National Fish Hatcheries 2015

FY 2015 Annual Report

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On the cover: *Technician Eric McOmie collecting environmental DNA water sample from fish ladder outflow at Warm Springs National Fish Hatchery, photo by Jennifer Poirier.*

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2015 ANNUAL REPORT

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Abstract

The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum* is a tiny exotic snail species that has invaded brackish and freshwater habitats of at least ten states in the western U.S. including a number of private, state and federal fish hatcheries. The Columbia River Fisheries Program Office (CRFPO) has performed presence/absence surveys for NZMS at lower Columbia River Basin National Fish Hatcheries since 2006. In the fall of 2015, a small pilot study was conducted to test the feasibility of using environmental DNA (eDNA) technology jointly with presence/absence surveys as an NZMS early detection tool. Presence/absence surveys and eDNA sampling were performed concurrently over a two week period. A total of 35 sites were visually surveyed for NZMS at six National Fish Hatcheries. Surveyors observed freshwater snails from six unique families and eleven genera. No NZMS were observed during presence/absence surveys. Four sites were surveyed at Warm Springs National Fish Hatchery using the eDNA technique. For comparison, samples were also taken at a location with known NZMS presence (Burnt Bridge Creek) and a location where NZMS have not been detected (Willard National Fish Hatchery). All eDNA samples from National Fish Hatcheries tested negative for the presence of NZMS. Samples collected at Burnt Bridge Creek tested strongly positive for the presence of NZMS. Environmental DNA sampling was very easy to implement, took considerably less time to perform than visual surveys, and is considered more effective at detecting low density species than traditional sampling methods. Cost of eDNA sampling materials and analysis may be prohibitive for smaller projects, but the investment in prevention is minimal compared to the exorbitant cost of ANS control or eradication measures. There are many potential sources of error when using the eDNA technique. Minimizing the risks of false positives and false negatives and incorporating detection probability into the sample design, can greatly increase the reliability of results. This pilot project demonstrated the ease with which eDNA technology may be integrated into a traditional ANS monitoring program and highlights the utility of the method to accurately detect NZMS in a stream environment. Incorporating annual eDNA sampling into our current monitoring program at National Fish Hatcheries would greatly improve the odds of detecting NZMS should an infestation ever occur.

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Introduction

The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum* is an exotic aquatic snail species that has invaded brackish and freshwater habitats of Australia, Europe, Asia and North America. As its common name implies, this snail is native to New Zealand and may have been introduced globally through contaminated ballast water (Zaranko et al. 1997; Gangloff 1998) or the transport of live fish or eggs for the commercial aquaculture industry (Bowler 1991; Bowler and Frest 1992). In North America, the NZMS was first discovered in the middle Snake River (Idaho) in 1987 (Bowler 1991). Since this time, the NZMS has become established in ten Western states, six Great Lakes states and two Canadian provinces (British Columbia and Ontario) (Benson 2015; Figure 1).

The small size, hardiness and exceptional adaptability of NZMS have likely contributed to the snail's spread within the United States. Adult NZMS range from 3-6 mm in length and have an elongate conical shell with 5-6 whorls coiled in a clock-wise (dextral) direction. Whorls may be smooth or bear a raised keel and shell color varies from grey to light or dark brown. The aperture of the shell has a retractable door or operculum that allows the snail to seal off the shell opening making it relatively impervious to mild pollutants and highly resistant to desiccation (Richards et al. 2004; Schisler et al. 2008). Larger snail can survive up to 24 hours without water and for several weeks on damp surfaces (Cheng and LeClair 2011). The shell wall of NZMS is very hard and difficult for many species of fish to thoroughly digest. In some instances, the snail may pass through the digestive tract of fish unharmed (McCarter 1986; Vinson 2004; Bruce et al. 2009; Oplinger et al. 2009). Fish released from infested aquaculture facilities or rearing in infested rivers may expand the distribution of NZMS by transporting live snails in their stomachs to other locations (Vinson and Baker 2008; Bruce et al. 2009). Within the United States, NZMS populations are comprised almost entirely of self-cloning parthenogenetic females. New Zealand mudsnail become sexually mature at 3mm in length (approximately 3-9 months of age), and may bear offspring up to four times per year. Brood size of an individual female ranges from 20-120 embryos, each of which may mature to produce an average of 230 offspring per year (Alonso and Castro-Díez 2008; Cheng and LeClair 2011). New Zealand mudsnail may inhabit a range of aquatic ecosystems (e.g., estuaries, rivers, lakes, reservoirs), and tolerate a broad range of aquatic conditions (e.g., temperature, salinity, turbidity, water velocity, stream productivity and substrate types) (see ANSTF 2007 and references therein). This flexibility has enabled the snail to successfully colonize and thrive in a wide array of aquatic habitats. New Zealand mudsnail are adept hitchhikers and may be spread through many natural and human-related processes. Within a watershed, snails may be transported on the fur or feathers of terrestrial wildlife, livestock and waterfowl or consumed and dispersed in the excrement of local fish species. Long distance dispersal of NZMS has been

attributed to ballast water discharge, the movement of commercial aquaculture products (i.e., fish, eggs, and ornamental plants), contaminated hatchery transplants or the translocation of recreational watercrafts, boat trailers and personal gear such as boots and waders. Once established in a new habitat, lack of natural predators and/or other population controls in addition to high reproductive potential allow the NZMS to reach extraordinary densities in some locations. Large colonies of NZMS can comprise up to 95 percent of the total invertebrate biomass, and consume up to 75 percent of the food resources in a stream (Hall et al. 2003; Hall et al. 2006). New Zealand mudsnail may outcompete or displace native snails, mussels and aquatic insects which native fish species depend on for food. This disruption to the food chain may ultimately result in reduced growth rates and lower populations of economically important fish species.

In 2002, NZMS were discovered at the first National Fish Hatchery in Hagerman Idaho (Hagerman National Fish Hatchery). Since this time they have been detected at a number of private, state and federal fish hatcheries and aquaculture facilities within the Western United States including: Arizona, California, Colorado, Idaho, Montana, Utah and most recently Washington State. The National Fish Hatchery System produces fish for mitigation, conservation, supplementation, and/or recreational purposes. Aquatic nuisance species such as the New Zealand mudsnail are an issue of concern for federal fisheries managers because fish stocking and transfers of eggs or fish from contaminated hatcheries may introduce or spread aquatic nuisance species to previously uninfested facilities or drainages (ANSTF 2007). Executive Order 13112 (USOFR 1999), was enacted to improve federal response to the growing problem of invasive species. The order prohibits federal agencies (except under certain conditions) from carrying out actions “likely to cause or promote the spread of invasive species”, and directs agencies to implement programs with the goal of preventing the introduction and spread of invasive species in their work. In response, many federal fish hatcheries have developed regional Hazard Analysis and Critical Control Point (HACCP) Plans that are used as a risk assessment and management tool to prevent NZMS invasion, identify pathways of potential introduction or minimize impacts or spread of existing populations. These plans often call for regular visual inspections of hatchery facilities and grounds. Performing annual visual inspections of hatchery water intake and outflow structures may detect NZMS before they become established or are inadvertently spread to new areas.

Traditional aquatic snail survey techniques such as wading, snorkeling, using D-frame dip nets or Hess samplers can be laborious and may not reliably detect the tiny snail when an infestation first occurs or abundance is low. A more sensitive detection tool such as environmental DNA could be employed in addition to presence/absence surveys to increase the chances of early detection. Environmental DNA (eDNA) is genetic material that is shed by an organism in the form of tissue cells, gametes, mucus, urine, feces, etc. This genetic material is released continuously and remains present in an environment until it is diluted, degraded or

dispersed in currents. Fragments of expelled DNA can be captured in an environmental sample (e.g., air, soil, sediment or water) and extracted to confirm the presence of an organism without the need to capture or observe the organism directly. The eDNA technique has a wide range of applications in freshwater ecology and fisheries conservation. It may be used to census all taxa present within a local community (DNA metabarcoding), confirm the presence of a single organism that may be difficult to detect (i.e., rare or threatened), validate classification of species difficult to identify, or track the arrival and/or advancement of invasive species (Díaz-Ferguson 2014; Herder et al. 2014). The application of eDNA as an aquatic nuisance species surveillance tool has gained popularity in recent years due to its increased sensitivity, low environmental impact and potential time and cost savings over traditional field survey techniques. Environmental DNA has been used to detect a wide range of aquatic invasive organisms such as American bullfrog, Asian carp, brook trout, Burmese python, red swamp crayfish, and most recently the New Zealand mudsnail (Rees et al. 2014). By comparing residual DNA within a water sample to the unique genetic markers of a target species, it is possible to detect the presence of an invasive species at the earliest stage of an invasion before densities reach unmanageable levels.

The Columbia River Fisheries Program Office has performed visual surveys for NZMS at lower Columbia River Basin National Fish Hatcheries since 2006 (see Allard and Olhausen 2007a, 2007b; Hogle 2009; Poirier 2012; Poirier 2014). In fall 2015, a small pilot study was conducted at Warm Springs National Fish Hatchery to test the feasibility of using eDNA technology jointly with presence/absence surveys. The goal of the pilot study was to evaluate the suitability of using eDNA as an NZMS early detection tool and to assess the practicality of broadening the scope of sampling to include all six National Fish Hatcheries. This report presents results of NZMS presence/absence surveys and eDNA sampling conducted by U.S. Fish and Wildlife Service CRFPO personnel in 2015.

Methods

Presence/absence surveys

Six lower Columbia River Basin National Fish Hatcheries were surveyed for New Zealand mudsnail including: Carson, Eagle Creek, Little White Salmon, Spring Creek, Warm Springs and Willard National Fish Hatcheries (Figure 2). Presence/absence surveys were conducted over a two week period from 31 August to 9 September, 2015. Site selection focused on areas perceived as likely NZMS introduction points (e.g., headwater springs, water intake and outflow structures), and included locations established during 2011 NZMS surveys. Sample locations were georeferenced using a Trimble handheld global positioning system, and a photograph was taken to document current physical habitat conditions. Baseline habitat characteristics (e.g.,

temperature, maximum water depth, dominant substrate type, dominant aquatic vegetation, percentage aquatic vegetation cover) were also recorded at each sample site. Two field personnel visually inspected up to a 20 meter portion of stream upstream and/or downstream of each survey location for approximately 10 minutes. Surface substrate was manually flipped over at random intervals, aquatic vegetation was sifted through by hand and surfaces of hatchery structures (i.e., pipes, intake/outflow grates, concrete walls, dam boards and log booms) were closely examined (visually and by hand) for NZMS. In water depths greater than 0.6 m, substrate, aquatic vegetation and hatchery structures were visually inspected using an underwater viewing scope or manually swept with a D-frame dip net. If field personnel observed an aquatic snail that could not be identified, a single specimen was collected and placed in a vial with 100% ethanol for preservation. Snail specimen were carefully examined under a dissecting microscope and photographed using an AxioCam ERc 5s microscope camera. Magnified photographs of specimen were sent to Edward Johannes with Deixis Consultants for identity confirmation.

Environmental DNA

Study Area

The eDNA pilot study focused on a single hatchery as well as two control sites in 2015. Warm Springs National Fish Hatchery was selected as the test site for the eDNA pilot study due to its relative close proximity to a number of established snail colonies in the Deschutes River (Figure 2). Warm Springs National Fish Hatchery (NFH) is located at river kilometer (rkm) 16 of the Warm Springs River within the Warm Springs Indian Reservation in north central Oregon. The hatchery is operated by the USFWS in cooperation with the Confederated Tribes of the Warm Springs Reservation of Oregon to produce spring Chinook salmon for tribal and sport harvest opportunities, and promote wild fish conservation. The Warm Springs River is a tributary of the Deschutes River that flows approximately 48 kilometers from its headwaters before joining the Deschutes River at rkm 135. Willard NFH was selected as the negative control for the eDNA pilot study. Past presence/absence surveys have found no freshwater snail in the proximity of the hatchery. Willard NFH currently rears coho salmon for the Yakima Nation Mid-Columbia River coho reintroduction project, and upriver bright fall Chinook for sport and tribal harvest opportunities. The hatchery is located at rkm 6.5 of the Little White Salmon River which flows 31 kilometers from the Cascade Range to its confluence with the Columbia River at rkm 261 (Figure 2). Burnt Bridge Creek was selected as the positive control for the pilot study. Burnt Bridge is a 21 kilometer long urban stream that flows west through the city of Vancouver Washington to its confluence with Vancouver Lake (Figure 2). New Zealand mudsnail were first discovered in the creek in March 2013 by a group of students conducting water quality and macroinvertebrate sampling. Environmental DNA samples were taken at location with known

NZMS presence using GPS coordinates obtained from the USGS Nonindigenous Aquatic Species Database.

eDNA Sample Collection & Filtration

Environmental DNA sampling was conducted over a one week period (31 August to 3 September, 2015), following protocols described in Goldberg and Strickler (2014). Four sites were surveyed at Warm Springs NFH using the eDNA technique including the hatchery intake grate, raceway/fish ladder outflow, pollution abatement pond, and abatement pond discharge channel. A total of three water samples were collected at each site. Samples were taken inside or in the immediate vicinity of hatchery structures, and were balanced spatially along the perimeter or width of structures (i.e., left side, middle, right side). Sterile 0.5L Nalgene bottles were rinsed three times with water from the sample site, submerged until full and placed in a cooler on ice for transport to the CRFPO laboratory. A single field negative water sample was also collected at each eDNA test site and processed in the same manner as field samples to assess the potential for sample contamination associated with handling and transport. Field negatives were collected immediately following the collection of field samples and consisted of filling a sterile 0.5L Nalgene bottle with distilled water and placing it in the cooler on ice alongside field samples. To validate the performance and accuracy of eDNA technology, three water samples and a single field negative were collected at a location with known NZMS presence (Burnt Bridge Creek) and a location where NZMS have not been detected (Willard NFH intake grate). Immediately following the collection of eDNA water samples at all sites, two personnel performed a visual presence/absence survey for NZMS using the methods described above.

Environmental DNA water samples were filtered in the CRFPO laboratory on the same day they were collected. Individual samples were poured into a 250ml disposable filter funnel and strained through a 0.45µm cellulose nitrate membrane using a vacuum flask and hand pump. When a total of 500ml had been filtered, the funnel was removed from the flask and the membrane disk was carefully folded and placed in a sterile 2.0ml vial with 100% ethanol. Sample vials were labeled with a unique site code and stored at room temperature until they were sent to Washington State University eDNA laboratory for analysis.

Quality Assurance

A general concern with eDNA technology is the possibility of obtaining a false positive result due to field or lab contamination. To minimize this risk in the field, care was taken to remain out of the water or downstream of the sample bottle while acquiring water samples to avoid

close contact with field gear. New nitrile gloves were worn between sample collection sites in the field and during sample filtering in the CRFPO lab. Within the lab, equipment in direct contact with water samples (i.e., Nalgene bottles, forceps) were decontaminated between sample sites by soaking in a 50% bleach for a minimum of one minute before rinsing and drying thoroughly. Vacuum flask and other components not in direct contact with water samples (i.e., rubber stopper, silicone tubing, hand-pump) were soaked in a 10% bleach solution and rinsed between sample sites. Lab countertops were sprayed with a 50% bleach solution and wiped down between each sample site. Waders, boots and sampling gear (i.e., nets, viewing scope) were disinfected daily in a 1% solution of Virkon Aquatic for a minimum of 10 minutes. Environmental DNA samples were collected and processed on three separate but consecutive days beginning with Willard NFH (low probability of NZMS) and ending with Burnt Bridge Creek (high probability of NZMS) to further minimize risk of sample contamination.

NZMS assay

The NZMS eDNA assay used in this analysis was designed using published mitochondrial cytochrome *b* sequence data obtained through GenBank (National Center for Biotechnology Information). A target primer-probe set was created using Primer Express software, and tested against all known sequences using primer-BLAST in GenBank to prevent cross amplification with other species. Assay sensitivity and specificity was tested using DNA extracted from a number of NZMS specimen representing six known haplotypes, as well as DNA from six 'non target' snail species commonly found in freshwater streams in Idaho and Montana. The resulting primer-probe set was then validated using eDNA samples obtained from a NZMS dose-response lab experiment and samples collected from a natural river with known NZMS presence (Goldberg et al. 2013).

PCR Amplification

Environmental DNA was extracted from sample membrane discs using the QIAshredder/DNeasy Blood and Tissue DNA extraction kit method (described in Goldberg et al. 2011), and amplified using a real-time quantitative polymerase chain reaction (qPCR) method. All DNA extractions included a negative control (i.e., empty centrifuge tube) that was processed similarly to a real sample to reveal potential cross-contamination during the extraction process. Each PCR plate included an internal positive control (i.e., synthetic non-target sequence) to test for the presence of PCR inhibitors that may lead to a false negative result. Approximately 2.5µL of DNA extract was used in each reaction, and all reactions were run in triplicate to ensure consistent results.

Results

Presence/absence surveys

A total of 35 sites were surveyed for NZMS at six lower Columbia River Basin National Fish Hatcheries (Table 1). Native freshwater snail were present in 19 (54%) of sites sampled in 2015. Surveyors observed freshwater snail from six unique families and eleven genera (Table 2). Snails of genus *Menetus* and *Juga* were the species most commonly observed at National Fish Hatcheries. No NZMS were observed during field surveys or examination of collected snail specimen.

Carson NFH

There were six sites surveyed for NZMS at Carson NFH in 2015 (Figure 3). Water inflow sites included the headwaters of Tyee Springs (hatchery source water), Tyee Springs road crossing culvert and hatchery intake grate. Outflow sample sites included the hatchery abatement pond, earthen pond discharge channel and adult fish ladder/raceway outflow channel. Water temperatures ranged from 7.5˚C at the headwaters to 13.0˚C in the abatement pond. Substrate varied considerably between sites, and submerged aquatic vegetation was dominant in nearly every site covering 25-75% of the area surveyed (Table 3). A total of four unique snail species were observed at Carson NFH, including the button sprite (*Menetus opercularis)*, Pygmy fossaria (*Galba parva*), Rocky mountain duskysnail (*Colligyrus greggi*) and freshwater limpet (*Ferrissia rivularis*) (Table 2). Button sprites were the most commonly observed snail species, prevalent in all but a single sample location.

Eagle Creek NFH

There were five sites surveyed for NZMS at Eagle Creek NFH in 2015 (Figure 4). Water inflow sites included the hatchery water intake grate and microfilter channel. Outflow sites included the adult fish ladder, upper and lower raceway discharge pipes. Water temperatures ranged very little between survey sites (12.5-13.0˚C). Aquatic vegetation was absent at all sites and substrate size was variable between sample locations (Table 3). Surveyors observed the freshwater limpet (*Ferrissia rivularis*) at two separate outflow sites at Eagle Creek NFH (Table 2).

Little White Salmon NFH

There were eight sites surveyed for NZMS at Little White Salmon NFH in 2015 (Figure 5). Water inflow sites included Stairway, Hillside and Baily springs as well as the primary hatchery inflow

grate and microfilter house. Outflow sites included the old pollution abatement pond, hatchery building and raceway outflow/fish ladder entrance. Water temperature ranged from 7.0˚C at the intake grate to 10.0˚C in Hillside and Stairway springs. There was no aquatic vegetation present at any survey site and substrate was predominantly cobble or larger in most locations (Table 3). Three different snail genera were observed at Little White NFH (Table 2). Two different species of juga (i.e., basalt juga and unknown species) were very abundant in Hillside and Stairway springs. Pygmy fossaria were present in the Little White Salmon River near the hatchery building outflow pipe, and the button sprite was observed in the vicinity of the old abatement pond outflow pipe.

Spring Creek NFH

There were six sites surveyed for NZMS at Spring Creek NFH in 2015 (Figure 6). Water inflow sites included four hillside springs located on the north side of Washington State Highway 14. Outflow sites included the adult fish ladder entrance/raceway outflow on the Columbia River and pollution abatement pond channel. Water temperatures varied widely between sample locations ranging from 8.0˚C in the hillside springs to 17.0˚C at the adult ladder outflow (i.e., Columbia River). Most sample sites were devoid of vegetation with the exception of hillside Spring #2 of which less than 25% of the total sample area was covered with floating vegetation. Fine silt and sand were the predominant substrate type in the hillside Springs, while cobble was dominant in the abatement pond and adult ladder outflow (Table 3). A total of five unique freshwater snail species were observed at Spring Creek NFH (Table 2). The button sprite and an unknown species of Juga were numerous in hillside springs. The Artemesian rams-horn (*Vorticifex effusa*), tadpole physa and an unknown species of *Fluminicola* were found at the base of the adult fish ladder, and shells of the button sprite and tadpole physa (*physella gyrina*) were observed in the abatement pond channel.

Willard NFH

There were five sites surveyed for NZMS at Willard NFH in 2015 (Figure 7). Water inflow sites included the hatchery water intake grate, two separate trash racks, and upper debris settling pond. The lower raceway discharge pipe was the only outflow site surveyed in 2015. Water temperature was a consistent 7.5˚C and aquatic vegetation was absent at all sample sites. Sand was the dominant substrate at all intake structures and cobble was the dominant substrate type at the lower pond discharge pipe (Table 3). No freshwater snails were observed at Willard NFH.

Warm Springs NFH

There were five sites surveyed for NZMS at Warm Springs NFH in 2015 (Figure 8). A single hatchery water intake grate and four outflow sites were inspected including the adult holding pond outflow pipe, hatchery raceway/fish ladder entrance, abatement pond discharge channel and eastern perimeter of the pollution abatement pond. Water temperatures were moderate ranging from 14.5˚C at the adult ladder outflow to 17.0˚C within the abatement pond. Submerged aquatic vegetation was present at the intake grate as well as the abatement pond, covering 25% to nearly 100% of the area surveyed. Silt was the dominant substrate type at the intake grate and abatement pond, while boulders and concrete were the predominant substrates in the remaining outflow sites (i.e., adult ladder, adult holding pond discharge and abatement pond discharge pipe) (Table 3). A total of four snail genera were observed at Warm Springs NFH (Table 2). Unknown species of *Juga* and *Fluminicola* were prevalent near the abatement pond outflow pipe, and the big-ear radix (*Radix auricularia*) and rough rams-horn (*Planorbella subcrenata*) were observed clinging to vegetation and structures around the perimeter of the abatement pond.

Burnt Bridge Creek

A single site was surveyed for NZMS at Burnt Bridge Creek in 2015 (Figure 9). Water clarity was very poor so the presence/absence survey was conducted from each bank to avoid reducing visibility further. Water temperature was 17.5˚C, dominant substrate was silt, and submerged aquatic vegetation covered approximately 75% of the total wetted channel (Table 3). Freshwater snails were abundant, but NZMS were not readily apparent along the stream bank. A single snail specimen was collected during the presence/absence survey. This snail was later confirmed to be a New Zealand mudsnail (Appendix A.). Although freshwater snails (other than one NZMS) were not formally documented during the presence/absence survey, a recent macroinvertebrate survey found *Juga sp.*, *Planorbidae*, and a high abundance of *Fluminicola sp.* in the area (Judy Bufford personal communication, November 18, 2015).

Environmental DNA

A total of 24 eDNA water samples were taken at six locations in 2015. Sample acquisition time took approximately one minute or less per sample, and filtration time ranged from 10 minutes (Willard NFH) up to 30 minutes per sample (Burnt Bridge Creek). Environmental DNA samples from Warm Springs and Willard National Fish Hatcheries tested negative for the presence of NZMS. All field negative samples tested negative for NZMS. Three positive control samples taken at Burnt Bridge Creek tested strongly positive for the presence of NZMS.

Discussion

New Zealand mudsnails have been present in the lower Columbia River Basin for nearly two decades, with observations of snails occurring along the Oregon Coast, Columbia River Estuary (including five peripheral bays) (Bersine et al. 2008), coastal lakes, coastal tributaries and in multiple locations along the lower Deschutes River (Benson 2015). The relative close proximity of Warm Springs NFH to snail colonies in the Deschutes River only emphasizes the need for a more comprehensive aquatic nuisance species (ANS) surveillance and monitoring program. Fish hatcheries may be more vulnerable to invasion because NZMS are known to thrive in the stable aquatic conditions of the hatchery environment (i.e., temperature, flow and nutrient load) (Bruce et al. 2009). Many hatcheries are located on rivers that support popular sport fisheries or receive heavy recreational usage where NZMS may be introduced or transported by a multitude of pathways (e.g., boats, trailers, fishing gear, waders), and fish hatchery operations such as fish stocking and the transfer of live fish or eggs are a major vector of spread given the potential for NZMS to pass through the gut of fish alive and intact.

Early detection is the most important, yet most challenging aspect of ANS management (Hulme 2006; Harvey et al. 2009). When an infestation first occurs, population density is usually very low. Detecting species in low abundance may be challenging using traditional sampling methods, particularly if the organism is small, cryptically colored or occurs in a habitat that is difficult to sample effectively (Harvey et al. 2009). Many ANS are not discovered until late in an invasion when the population is abundant and well-established. When NZMS were first observed at Ringold State Hatchery (Ringold, WA), population densities were so prolific many speculate the snails were present in the facility 3-4 years prior to their discovery. Traditional ANS sampling methods are most effective when population abundance is high (Harvey et al. 2009). However, if a species is present in low abundance, detection probability may be improved by increasing sample frequency (i.e., increase total number of surveys), focusing surveys on areas perceived as likely introduction points, or employing a more sensitive detection method such as eDNA (Hulme 2006; Harvey et al. 2009).

The goal of this pilot study was to evaluate the suitability of using eDNA as a NZMS early detection tool. The sampling protocol was very straightforward and could easily be employed at all lower Columbia River National Fish Hatcheries with little additional labor. Sample acquisition and filtration took less time than performing visual surveys and required very little specialized training. Generally, the cost of eDNA is less than other conventional survey methods (e.g., electroshocking, trapping, Hess sampling); however, in this pilot study, the eDNA technique was double the cost of presence/absence surveys. Visual surveys are performed annually over a six day period and only require a viewing scope and pair of waders. Time investment and labor costs are lower using eDNA because sampling can be performed by a

single person, but consumable materials and sample analysis may be cost prohibitive for smaller projects. At the time of writing, eDNA cost an average of 45 dollars per sample, but price of sample analysis continues to decrease as use of the technique increases and DNA sequencing technology improves. The moderate investment in eDNA is minimal compared to the exorbitant cost of control or eradication measures if an infestation is unknowingly overlooked (Pimentel et al. 2005). Environmental DNA has a number of additional benefits that enhance its appeal as an early detection tool. In many instances eDNA has proven to be more effective at detecting aquatic organisms than traditional sampling techniques (Jerde et al. 2010; Thomsen et al. 2011; Biggs et al. 2014; Smart et al. 2015), even at low densities (Dejean et al. 2012; Mahon et al. 2013; Pilliod et al. 2013). Environmental DNA technology is less invasive than many physical sampling techniques (Herder et al. 2014; Tréguier et al. 2014; Thomsen and Willerslev 2015) because there is no need to trap or handle an organism to obtain a positive identification. In addition, most eDNA sampling can be performed from the bank or a boat, eliminating the need to walk in the water which can disturb the aquatic environment or potentially harm benthic invertebrates. Moreover, sampling from the bank lessens the opportunity of spreading ANS or pathogens to other locations (Herder et al. 2014; Tréguier et al. 2014). Environmental DNA is a more practical method for sampling areas that are deep, have poor visibility or dense aquatic vegetation. Nearly half (49%) of the sites surveyed for NZMS at National Fish Hatcheries had water depths greater than one meter. This limited our visibility of bottom substrates and made it difficult to survey some areas adequately. Finally, eDNA can provide more certainty regarding species identity (Herder et al. 2014). Freshwater snail can be difficult to identify and NZMS are often confused with other native and non-native species (Crosier and Molloy 2007). Use of species-specific DNA eliminates potential for misidentification that may lead to false positive or negative outcomes.

There has been considerable work and progress made towards improving and standardizing eDNA collection methods and analysis; however, there is still uncertainty regarding the interpretation of eDNA results specifically pertaining to the potential for false positives and false negatives. False positives (i.e., target DNA is detected in sample, but organism is not present in environment) are generally associated with poor qPCR primer specificity (i.e., primer binds to non-target species DNA) or field/laboratory sample contamination (Herder et al. 2014). The NZMS assay used in this pilot study was thoroughly tested to ensure accurate amplification of target DNA. The primer and probe design was compared to published DNA sequences, screened against tissue samples of six target and six co-occurring snail species and finally tested in dose-response laboratory and field experiments (see Goldberg et al. 2013). Field cross-contamination was eliminated by following proper sampling and decontamination procedures (see Methods). All field negative control samples tested as negative, which validated the success of our efforts. To reduce the chance of contamination in the laboratory environment, Washington State University follows strict sample analysis

protocols (see Goldberg et al. 2013), and includes negative and positive controls throughout the DNA extraction and amplification process to detect contamination should it occur.

False negatives (i.e., target DNA is not present in sample, but organism exists in environment) may occur if DNA amplification is inhibited, eDNA quality is poor or is present in very low abundance. Organic material within a water sample (e.g., sediment particles, tannins) may be co-extracted with target DNA and prohibit its amplification during PCR. Inhibitors can be detected by introducing a positive control (i.e., synthetic DNA sequence) to each PCR plate. If the control sequence is not amplified during PCR, inhibition has occurred and the sample must be purified and run again. Burnt Bridge Creek was very turbid at the time of sampling, yet all three samples tested as strongly positive for the presence of NZMS indicating that no inhibition occurred during PCR amplification. The persistence of DNA in aquatic habitats depends upon a number of environmental factors such as water temperature, flow, UV radiation, water chemistry, bacteria and organic material (Herder et al. 2014; Pilliod et al. 2013; Strickler et al. 2014). DNA may remain in flowing water for a few days up to a few weeks before it becomes degraded, diluted or is swept away in the current (Dejean et al. 2011; Thomsen et al. 2012; Pilliod et al. 2013). In a controlled laboratory experiment, New Zealand mudsnail DNA was detectable in 1.5L of freshwater for three days following snail removal in the lowest density treatment (one snail) and up to 21 days in the highest density treatment (200 snail) (Goldberg et al. 2013). To maximize detection of degraded and/or sparse DNA, all water samples collected during the pilot study were immediately placed on ice and filtered within four hours of collection. Samples were analyzed using quantitative PCR which is highly sensitive at amplifying rare, short or degraded DNA fragments (Wilcox et al. 2013; Herder et al. 2014; Pilliod et al. 2013), and each sample was run for 50 PCR cycles in triplicate. Concentration of eDNA within an environment may depend on the species, its size, population density, time of year, distribution, location and rate of DNA degradation (Ficetola et al. 2008; Jerde et al. 2010; Pilliod et al. 2013; Goldberg et al. 2011). Mesocosm and aquaria experiments have found a significant correlation between eDNA concentration and species abundance (Takahara et al. 2012; Thomsen et al. 2012; Goldberg et al. 2013). This same relationship does not necessarily hold true under natural conditions (Spear et al. 2014; Tréguier et al. 2014; Biggs et al. 2015). It is unclear how few NZMS can be reliably detected by the eDNA technique, and how distance from the population might affect DNA concentration. Several studies indicate DNA may still be detectable up to several kilometers downstream depending on the species of source population (Pilliod et al. 2013; Deiner and Altermatt 2014; Jane et al. 2014). Goldberg et al. (2013) successfully detected low densities of NZMS (11-78 snail/m^2) in Portneuf River (Pocatello, ID), but it is unclear how close NZMS were to sample collection sites. We conduct NZMS surveys in late summer when stream flows are typically at their lowest. This may increase the concentration of DNA in the water column, thereby improving the likelihood of detection (Smart et al. 2015). If NZMS are detected immediately upstream or downstream from the

hatchery, we can be fairly confident they are present within or very near the facility and will persist long enough for a visual verification to be made.

There are many potential sources of error when using the eDNA technique. Understanding the origin of false positives and false negatives and attempting to minimize those risks is an important step toward reducing the uncertainty associated with eDNA results. A positive eDNA outcome is relatively straightforward because presence can (and should) be verified by visual inspection. A negative result is more challenging because it is unclear whether the organism is absent, or was simply not detected by the method (Herder et al. 2014). Some of the uncertainty regarding false negatives can be reduced by incorporating detection probability into the sample design (Schmidt et al. 2013). Most eDNA studies that incorporate detection probability have some prior knowledge of target species distribution and/or the ability to perform a quantitative comparison with traditional survey techniques. In this pilot study we are monitoring a target organism that is not yet present, so traditional statistical models used to estimate species occurrence and/or detection probability are not suitable. In this case Herder et al. (2014) recommends estimating the detection probability for a specific assay as an alternative. This can be achieved by performing eDNA sampling at a number of locations with known NZMS presence and applying this detection probability to test sites at National Fish Hatcheries – as long as environmental conditions are similar. Goldberg et al (2013) reported an estimated NZMS detection probability of 83% in the Portneuf River where densities ranged from 11-144 snail/m^2. Although not directly comparable to our study, these results provide more confidence regarding the strength of eDNA to reliably detect NZMS at National Fish Hatcheries.

This pilot study demonstrated the ease with which eDNA technology can be integrated into a traditional ANS monitoring program and highlights the value of the method to accurately detect NZMS in a stream environment. Incorporating annual eDNA sampling into our current monitoring program at National Fish Hatcheries would greatly improve the odds of detecting NZMS should an infestation ever occur. The ability to reliably detect NZMS early when densities are low may improve success of eradication or prevent the establishment and unintentional spread of snail to neighboring hatchery facilities and/or stocking locations.

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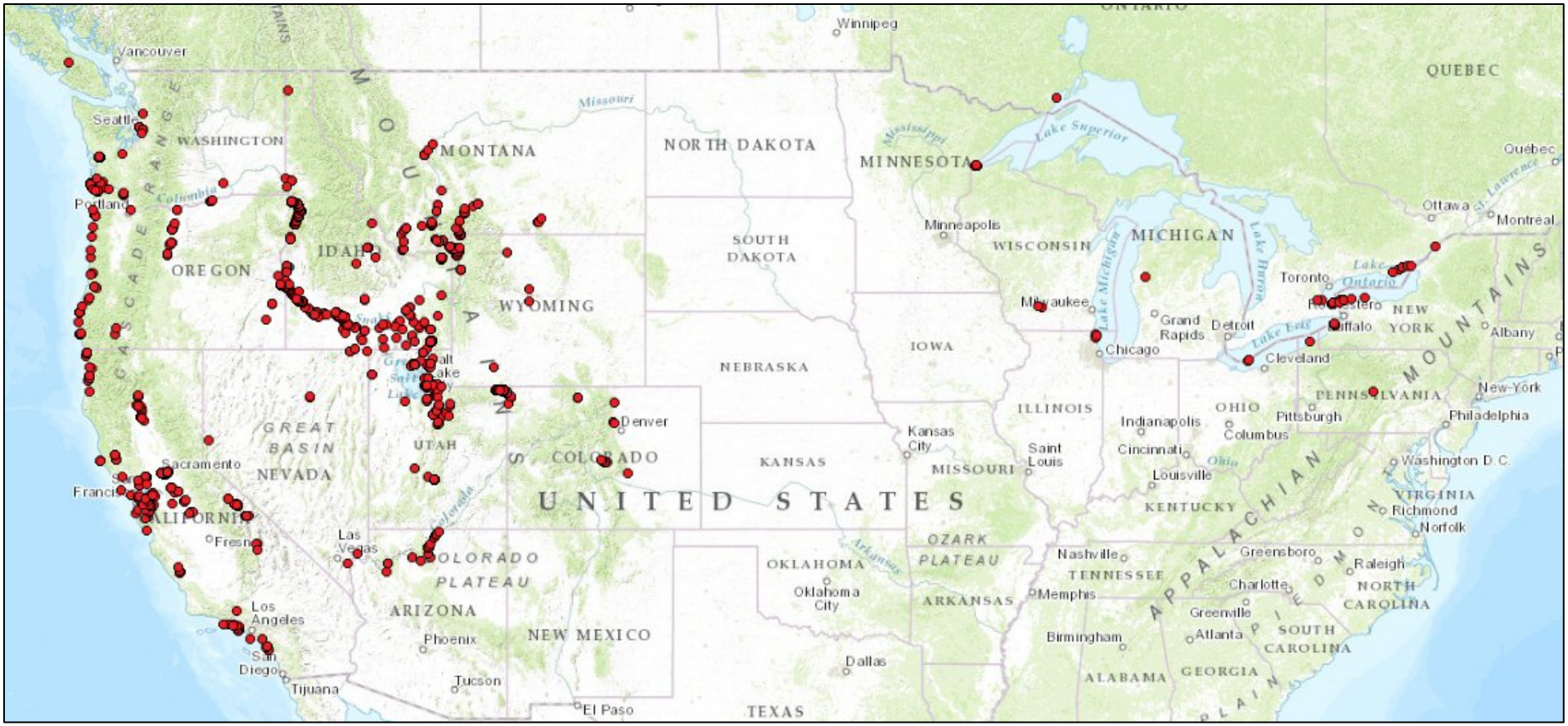


Figure 1. Map of New Zealand mudsnail sightings in the United States and Canada from 1987 through December 2015 (Benson, A. J. 2015).

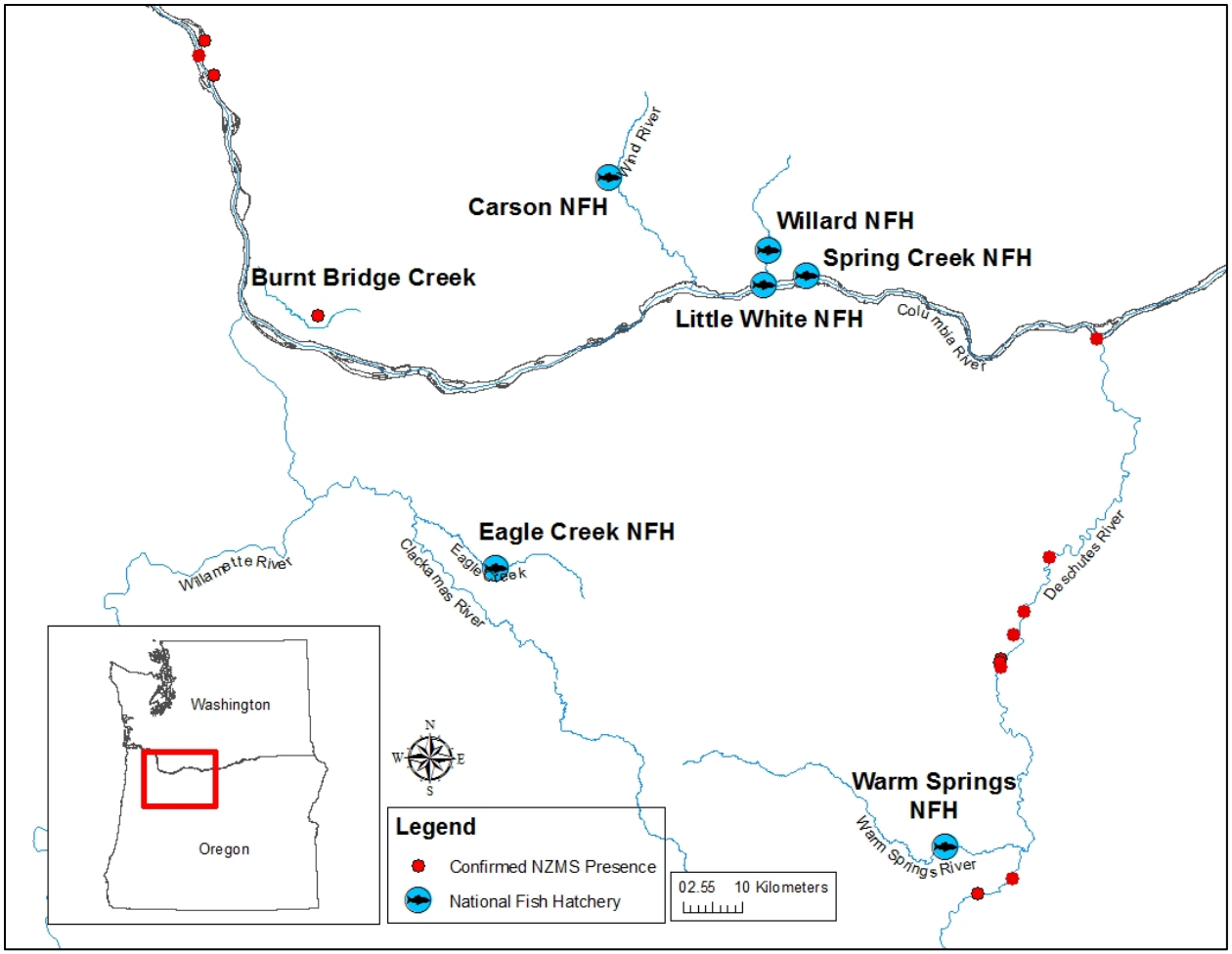


Figure 2. Map of USFWS National Fish Hatcheries surveyed for NZMS and distribution of NZMS populations in the lower Columbia River, 2015.

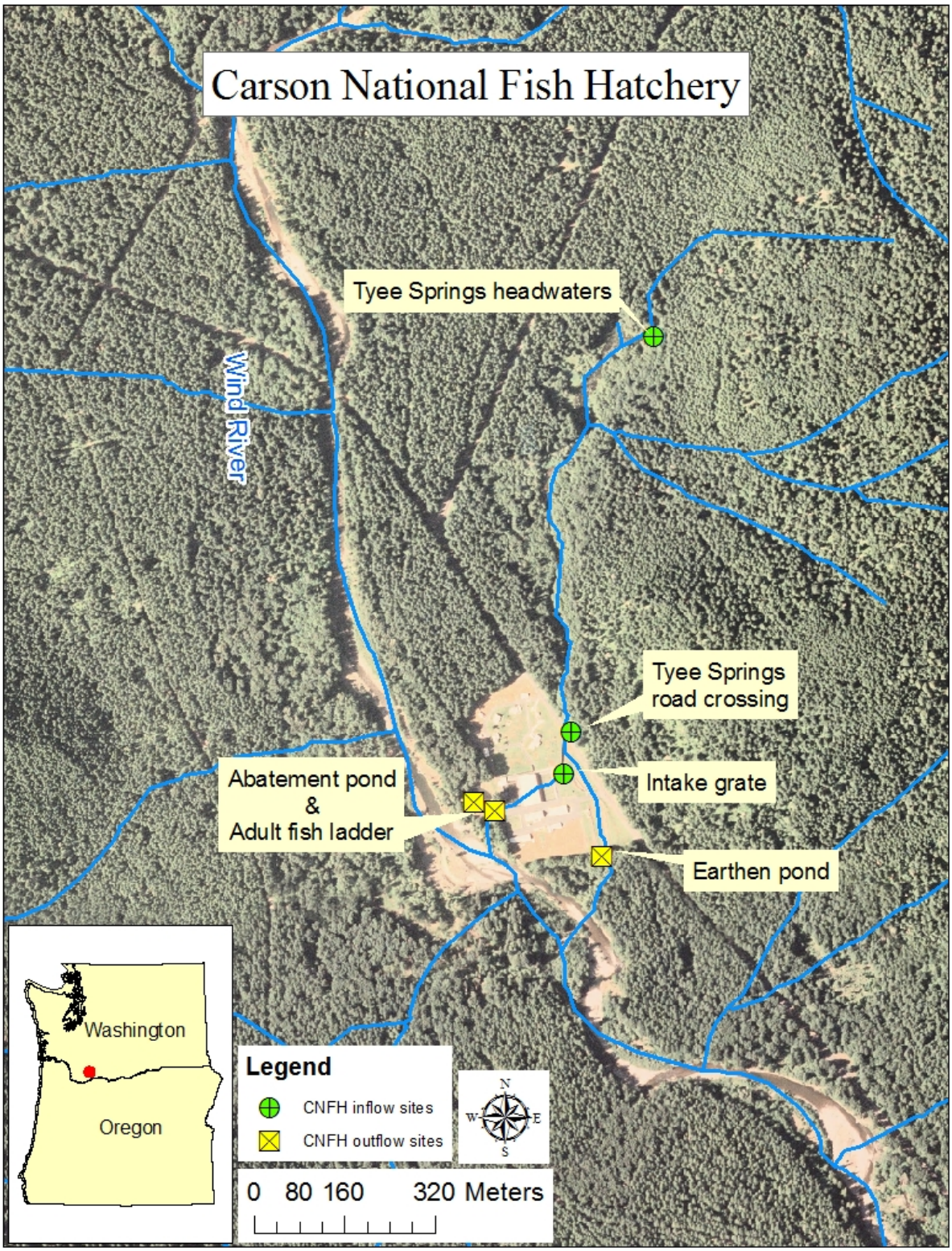


Figure 3. Carson NFH New Zealand mudsnail survey sample site, 2015.

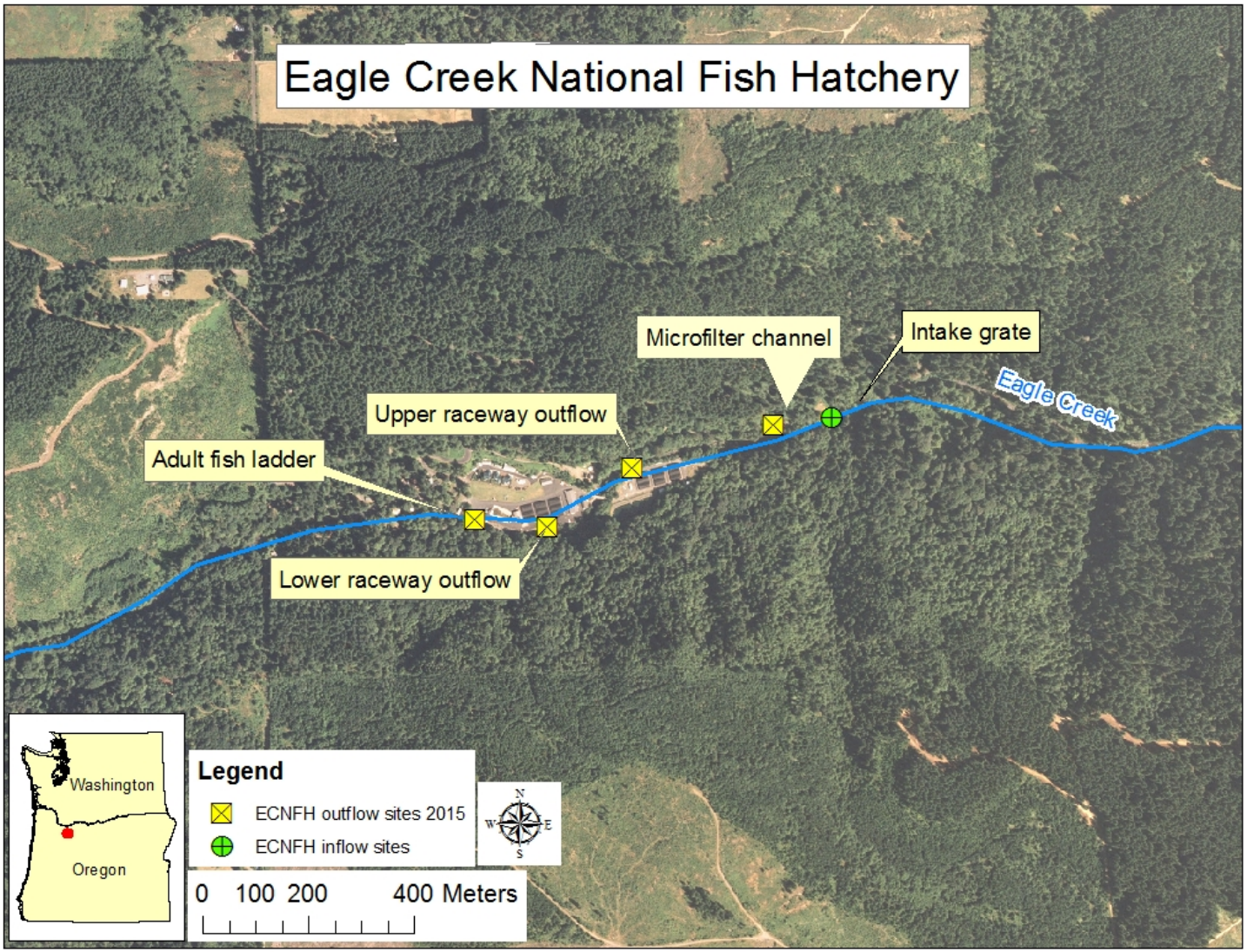


Figure 4. Eagle Creek NFH New Zealand mudsnail survey sample sites, 2015.

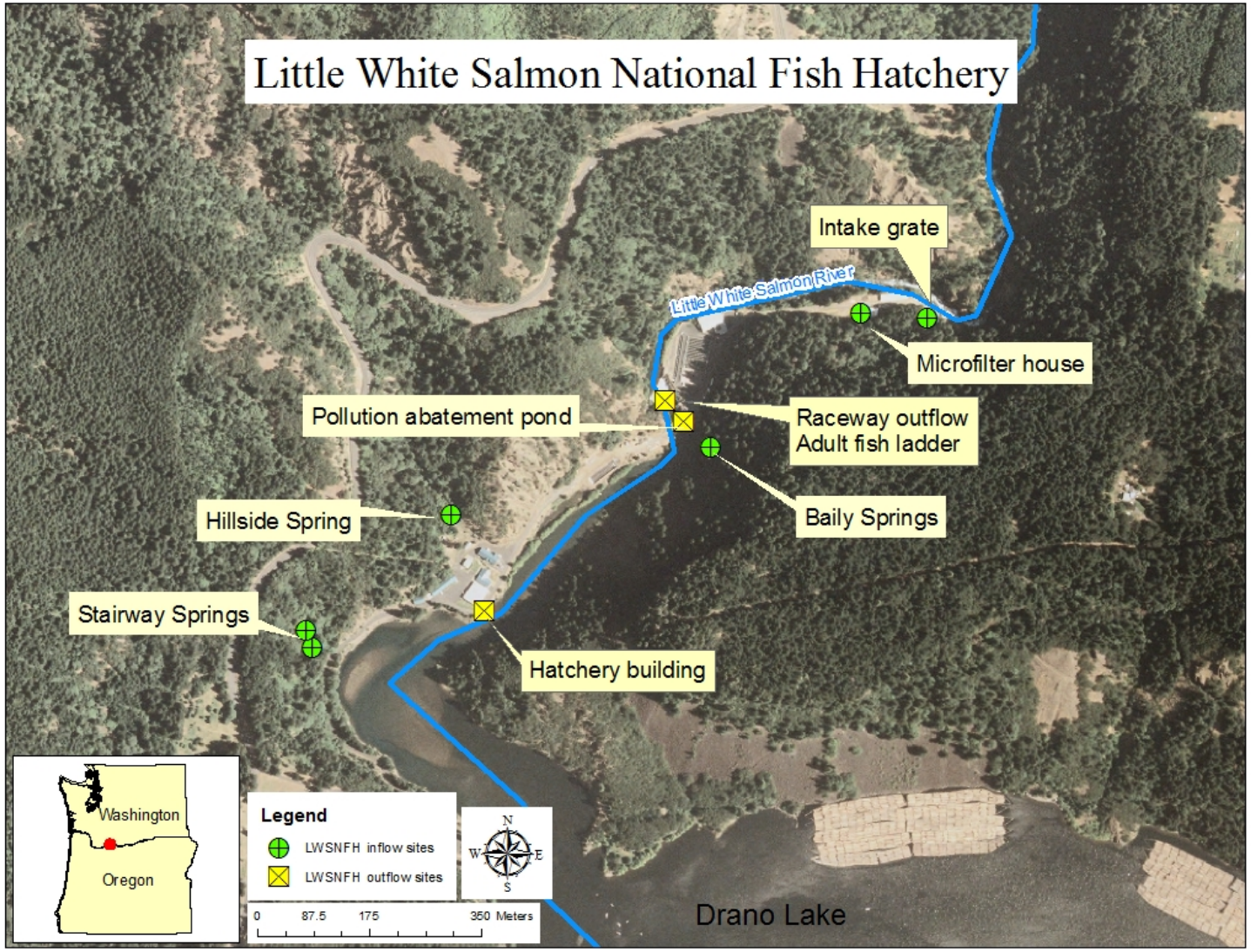


Figure 5. Little White Salmon NFH New Zealand mudsnail survey sample sites, 2015.

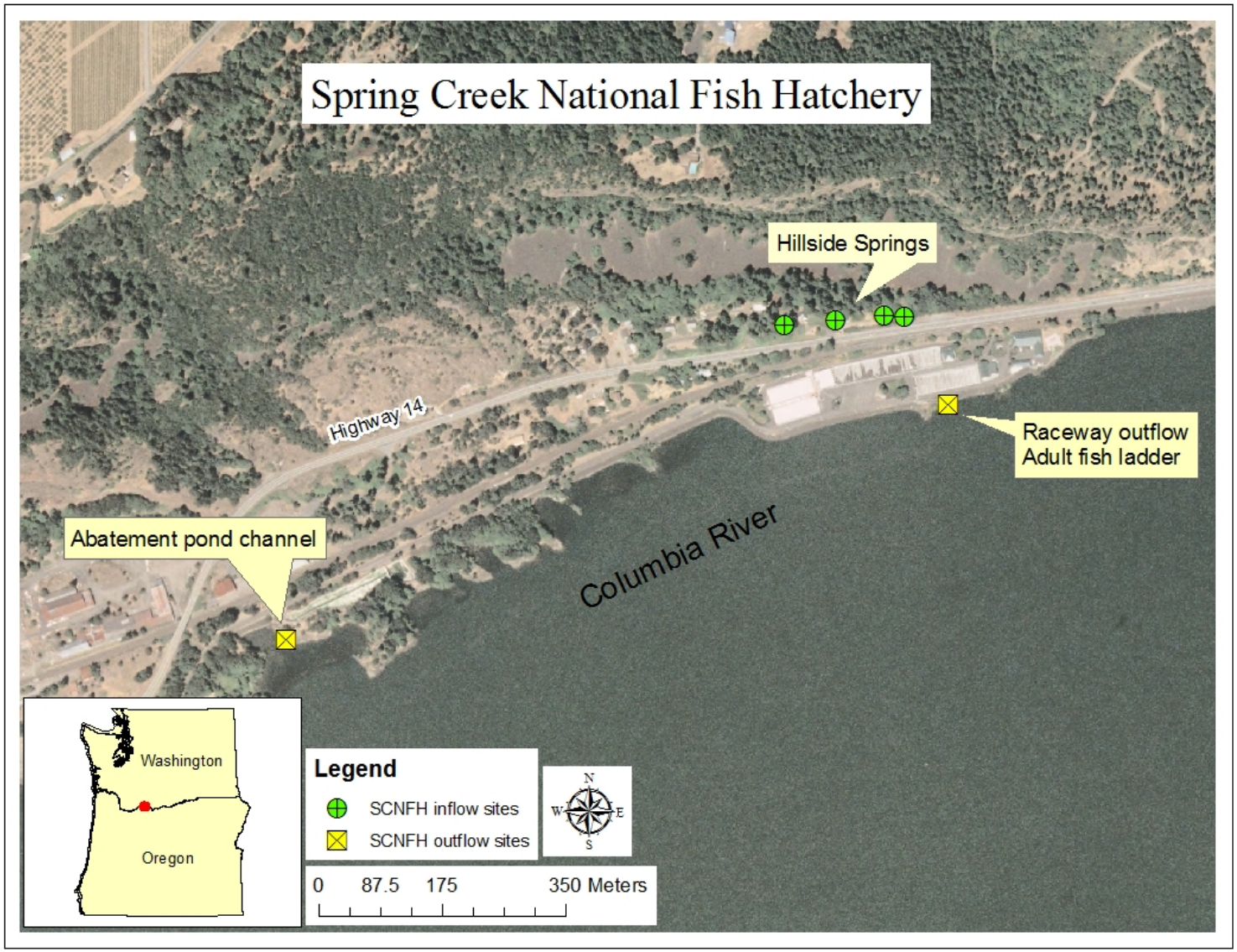


Figure 6. Spring Creek NFH New Zealand mudsnail survey sample sites, 2015.

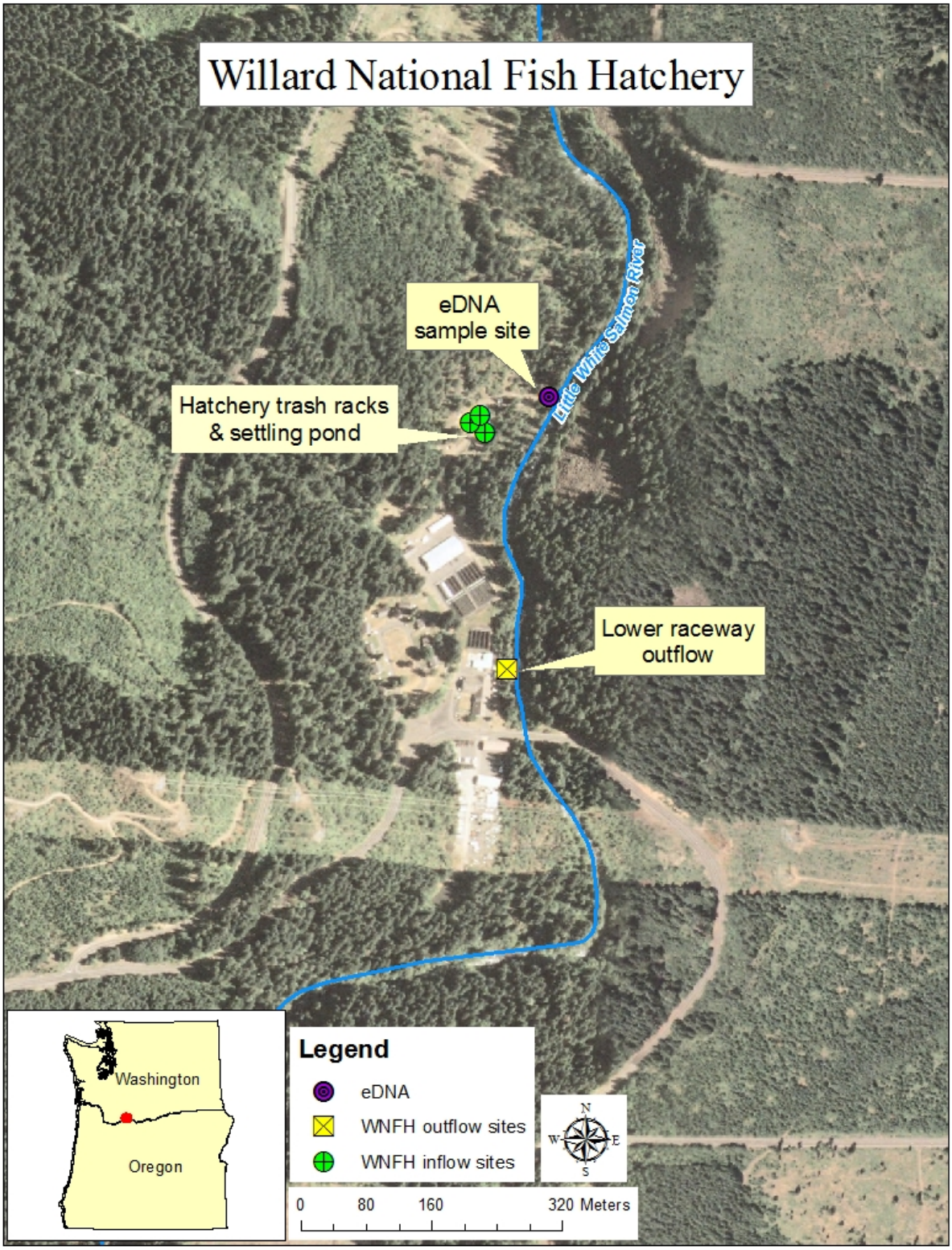


Figure 7. Willard NFH New Zealand mudsnail survey and eDNA sample sites, 2015.

Figure 8. Warm Springs NFH New Zealand mudsnail survey and eDNA sample sites, 2015.

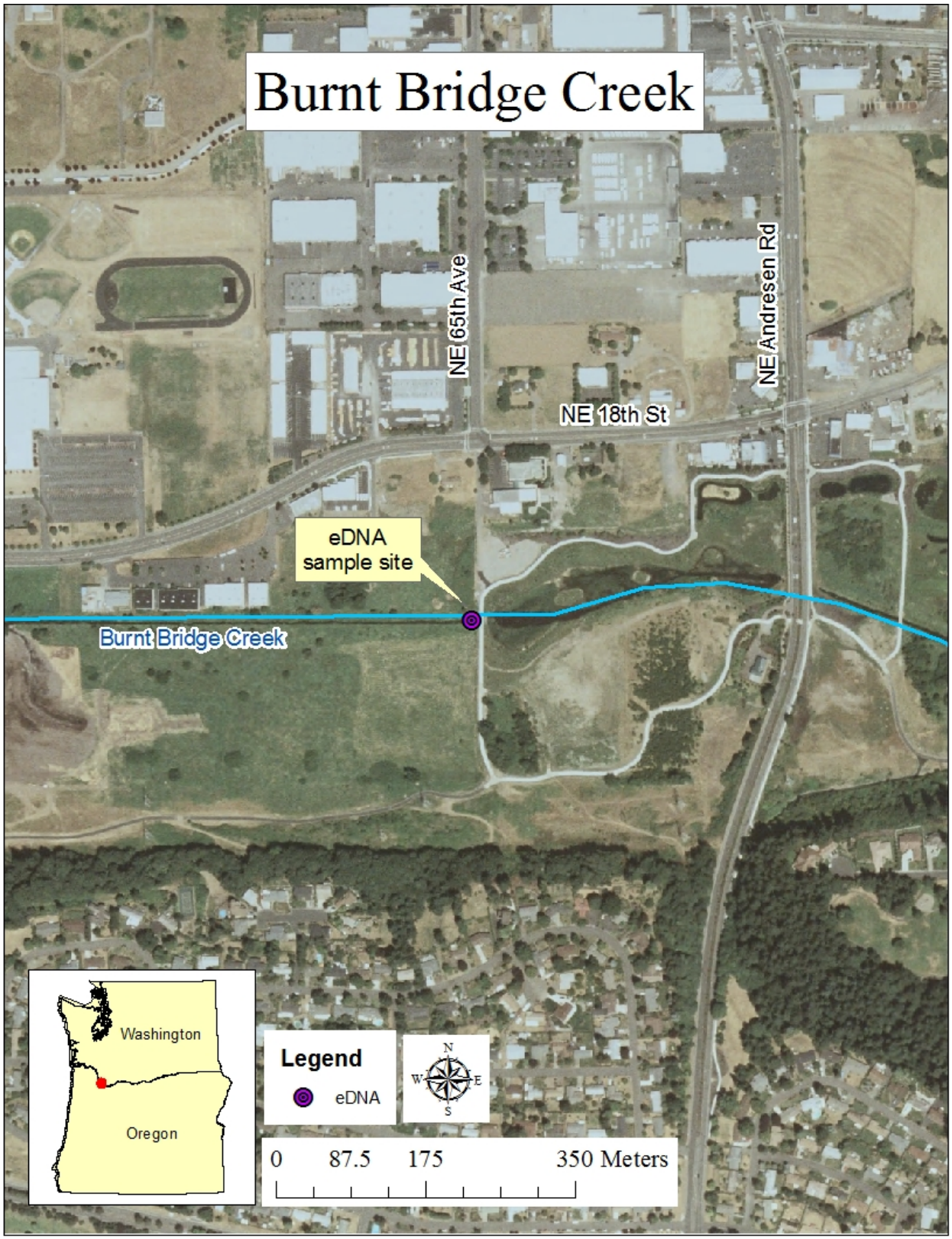


Figure 9. Burnt Bridge Creek New Zealand mudsnail survey and eDNA sample site, 2015.

Table 1. New Zealand mudsnail survey locations at Lower Columbia River Basin National Fish Hatcheries, 2015.

Date	National Fish Hatchery	Location	Specimens Collected	NZMS Found	GPS Coordinate System: UTM			
					Zone	Datum	Northing	Easting
9/8/2015	Carson	**Outflow**- pollution abatement pond	Sample 8	None	10	NAD 1983 (Conus)	5079904.52	579523.87
9/8/2015	Carson	**Outflow-** raceway outflow, adult fish ladder enterance	Sample 9	None	10	NAD 1983 (Conus)	5079887.72	579566.79
9/8/2015	Carson	**Outflow**- earthen pond outflow	None	None	10	NAD 1983 (Conus)	5079810.35	579755.90
9/8/2015	Carson	**Inflow-** hatchery intake grate	None	None	10	NAD 1983 (Conus)	5079956.49	579685.42
9/8/2015	Carson	**Inflow**- Tyee Springs at Wind River Rd. crossing	None	None	10	NAD 1983 (Conus)	5080028.69	579699.17
9/8/2015	Carson	**Inflow**- Tyee Springs headwaters	Sample 10	None	10	NAD 1983 (Conus)	5080730.12	579847.02
9/3/2015	Eagle Creek	**Outflow**- adult fish ladder enterance	None	None	10	NAD 1983 (Conus)	5013906.60	562421.74
9/3/2015	Eagle Creek	**Outflow**- lower raceway outflow	None	None	10	NAD 1983 (Conus)	5013895.52	562559.21
9/3/2015	Eagle Creek	**Outflow**- upper raceway outflow	None	None	10	NAD 1983 (Conus)	5014006.36	562723.31
9/3/2015	Eagle Creek	**Inflow-**microfilter channel	None	None	10	NAD 1983 (Conus)	5014086.22	562989.38
9/3/2015	Eagle Creek	**Inflow**- hatchery water intake grate	None	None	10	NAD 1983 (Conus)	5014099.46	563102.52
9/4/2015	Little White Salmon	**Inflow**- Stairway Springs (2)	Sample 5	None	10	NAD 1983 (Conus)	5063633.15	605223.51
9/4/2015	Little White Salmon	**Inflow**- hillside Springs	None	None	10	NAD 1983 (Conus)	5063841.55	605439.42
9/4/2015	Little White Salmon	**Outflow-** egg house outflow	Sample 6	None	10	NAD 1983 (Conus)	5063689.47	605490.11
9/4/2015	Little White Salmon	**Inflow-** Baily Springs	None	None	10	NAD 1983 (Conus)	5063946.69	605846.84
9/4/2015	Little White Salmon	**Outflow-** raceway outflow, adult fish ladder enterance	None	None	10	NAD 1983 (Conus)	5064018.04	605775.50
9/4/2015	Little White Salmon	**Outflow-** pollution abatement pond (old location)	Sample 7	None	10	NAD 1983 (Conus)	5063984.24	605805.54
9/4/2015	Little White Salmon	**Inflow-** hatchery water intake grate	None	None	10	NAD 1983 (Conus)	5064147.59	606184.79
9/4/2015	Little White Salmon	**Inflow-** microfilter channel	None	None	10	NAD 1983 (Conus)	5064155.10	606081.53
9/9/2015	Spring Creek	**Outflow-** raceway outflow, adult fish ladder enterance	Sample 13-15	None	10	NAD 1983 (Conus)	5064795.99	613286.11
9/9/2015	Spring Creek	**Outflow-** abatement pond channel	Sample 12	None	10	NAD 1983 (Conus)	5064464.42	612347.21
9/9/2015	Spring Creek	**Inflow-** hillside spring #1	None	None	10	NAD 1983 (Conus)	5064912.53	613051.39
9/9/2015	Spring Creek	**Inflow-** hillside spring #2	Sample 11	None	10	NAD 1983 (Conus)	5064917.46	613126.89
9/9/2015	Spring Creek	**Inflow**- hillside spring #3	None	None	10	NAD 1983 (Conus)	5064925.67	613194.19
9/9/2015	Spring Creek	**Inflow**- hillside spring #4	None	None	10	NAD 1983 (Conus)	5064924.02	613223.74
8/31/2015	Willard	**Inflow-** hatchery water intake grate	None	None	10	NAD 1983 (Conus)	5069171.79	606561.39
8/31/2015	Willard	**Inflow-** hatchery water trash rack #1	None	None	10	NAD 1983 (Conus)	5069128.52	606480.41
8/31/2015	Willard	**Inflow-** hatchery water trash rack #2	None	None	10	NAD 1983 (Conus)	5069108.23	606463.50
8/31/2015	Willard	**Inflow-** hatchery water settling pond	None	None	10	NAD 1983 (Conus)	5069138.00	606462.99
8/31/2015	Willard	**Outflow-** lower raceway outflow	None	None	10	NAD 1983 (Conus)	5068838.25	606507.24
9/1/2015	Warm Springs	**Inflow-** hatchery water intake grate	None	None	10	NAD 1983 (Conus)	4968957.62	638644.45
9/1/2015	Warm Springs	**Outflow-** adult holding pond outflow	None	None	10	NAD 1983 (Conus)	4968988.35	638658.23
9/1/2015	Warm Springs	**Outflow-** raceway outflow, adult fish ladder enterance	None	None	10	NAD 1983 (Conus)	4968973.52	638652.93
9/1/2015	Warm Springs	**Outflow**- perimeter of pollution abatement pond	Sample 3,4	None	10	NAD 1983 (Conus)	4969124.03	638722.88
9/1/2015	Warm Springs	**Outflow**- abatement pond discharge pipe	Sample 1,2	None	10	NAD 1983 (Conus)	4969153.71	638752.56

Table 2. Summary of freshwater mollusk genera observed at lower Columbia River National Fish Hatcheries, 2015.

Survey Location	Freshwater Mollusk Genera										
	Ancylidae	**Hydrobiidae**		**Lymnaeidae**		**Physidae**	**Planorbidae**			**Pleuroceridae**	
	Ferrissia rivularis	*Colligyrus greggi*	*Fluminicola sp.*	*Galba parva*	*Radix auricularia*	*Physella gyrina*	*Planorbella subcrenata*	*Menetus opercularis*	*Vorticifex effusa*	*Juga (Juga) sp.*	*Juga (Oreobasis) n.sp.*
Carson: abatement pond perimeter	X							X			
Carson: fish ladder/ raceway outflow		X						X			
Carson: earthen pond outflow								X			
Carson: hatchery intake grate								X			
Carson: Tyee Springs Wind River R. culvert											
Carson: Tyee Springs headwaters				X				X			
Eagle Creek: fish ladder outflow	X										
Eagle Creek: lower raceway outflow											
Eagle Creek: upper raceway outflow	X										
Eagle Creek: microfilter channel											
Eagle Creek: hatchery intake grate											
Little White Salmon: Stairway springs (2)										X	X
Little White Salmon: hillside springs										X	
Little White Salmon: egg house outflow				X							
Little White Salmon: Baily Springs											
Little White Salmon: fish ladder/raceway outflow											
Little White Salmon: abatement pond								X			
Little White Salmon: hatchery intake grate											
Little White Salmon: microfilter house											
Spring Creek: fish ladder/raceway outflow			X			X			X		
Spring Creek: abatement pond channel						X (shell)		X (shell)			
Spring Creek: hillside spring #1										X	
Spring Creek: hillside spring #2								X		X	
Spring Creek: hillside spring #3								X			
Spring Creek: hillside spring #4								X		X	
Willard: hatchery intake grate											
Willard: hatchery trash rack #1											
Willard: hatchery trash rack #2											
Willard: hatchery water settling pond											
Willard: lower raceway outflow											
Warm Springs: hatchery intake grate											
Warm Springs: adult holding pond outflow											
Warm Springs: fish ladder/raceway outflow											
Warm Springs: abatement pond perimeter					X		X				
Warm Springs: abatement pond discharge pipe			X							X	

Table 3. Baseline habitat characteristics at lower Columbia River Gorge National Fish Hatcheries and Burnt Bridge Creek, 2015.

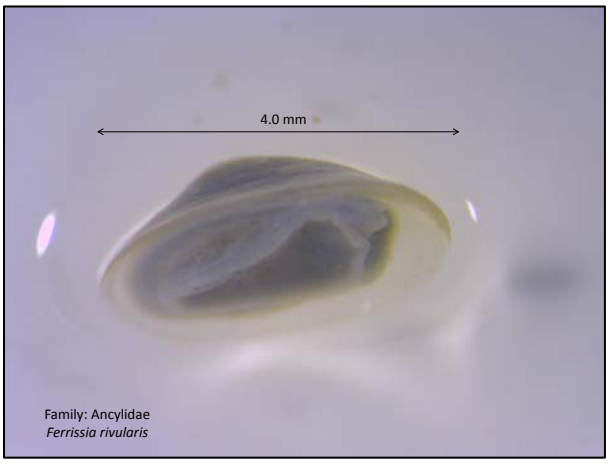
National Fish Hatchery	Site #	Site Description	Survey Begin Time	Survey End Time	Temp (°C)	Max Depth (m)	Dominant Substrate	Dominant Aquatic Vegetation	% Aq. Veg. Cover	Sample methods used	Specimen Collected (Y/N)	Specimen Vial #(s)	Photo taken (Y/N)
Carson	1	Abatement pond	9:34 AM	9:44 AM	13.0	0.50	0	1	1	1,2	Y	8	Y
Carson	2	Adult ladder outflow	9:48 AM	10:06 AM	8.5	1.00	4	1	1	1,2,5	Y	9	Y
Carson	3	Earthen pond outflow	10:12 AM	10:17 AM	9.0	0.50	0	1	2	2	N		Y
Carson	4	Intake grate	10:22 AM	10:32 AM	8.0	2.0+	1	1	3	2,3,5	N		Y
Carson	5	Tyee Springs crossing	10:36 AM	10:44 AM	8.0	1.50	1	1	4	1,2	N		Y
Carson	6	Tyee Springs headw ater	10:56 AM	11:03 AM	7.5	0.20	3	2	2	1,2	Y	10	Y
Eagle Creek	1	Adult ladder outflow	9:14 AM	9:24 AM	13.0	0.70	2	0	0	1,2,5	N		Y
Eagle Creek	2	Low er racew ay outflow	9:29 AM	9:36 AM	13.0	1.10	4	0	0	1,2,5	N		Y
Eagle Creek	3	Upper racew ay outflow	9:41 AM	9:46 AM	12.5	0.50	4	0	0	1,2,5	N		Y
Eagle Creek	4	Microfilter channel	9:52 AM	10:00 AM	13.0	1.10	0	0	0	1,2	N		Y
Eagle Creek	5	Intake grate	10:05 AM	10:15 AM	12.5	2.0+	1	0	0	1,2,5	N		Y
Little White	1	Stairw ay Springs #1	8:22 AM	8:27 AM	9.0	0.05	4	0	0	5	Y	5	Y
Little White	2	Stairw ay Springs #2	8:29 AM	8:34 AM	10.0	0.01	Bedrock	0	0	5	N		Y
Little White	3	Hillside Springs	8:38 AM	8:43 AM	10.0	0.01	4	0	0	5	N		Y
Little White	4	Hatchery building outflow	8:48 AM	8:56 AM	10.0	1.00	1	0	0	1,2	Y	6	Y
Little White	5	Bailey Springs	9:05 AM	9:08 AM	9.0	0.01	5	0	0	5	N		Y
Little White	6	Abatement pond outflow	9:10 AM	9:15 AM	8.0	0.30	4	0	0	1,2,5	Y	7	Y
Little White	7	Adult ladder outflow	9:16 AM	9:24 AM	8.0	0.60	4	0	0	1,2,5	N		Y
Little White	8	Microfilter channel	9:28 AM	9:33 AM	7.0	2.0+	0	0	0	1,2	N		Y
Little White	9	Intake grate	9:36 AM	9:46 AM	7.0	2.0+	4	0	0	1,2,4	N		Y
Spring Creek	1	Hillside Spring #1 (West)	9:17 AM	9:22 AM	9.5	0.25	1	0	0	1,2	N		Y
Spring Creek	2	Hillside Spring #2	9:25 AM	9:34 AM	8.5	0.80	0	3	1	1,2	Y	11	Y
Spring Creek	3	Hillside Spring #3	9:36 AM	9:42 AM	9.0	0.15	0	0	0	1,2	N		Y
Spring Creek	4	Hillside Spring #4 (East)	9:45 AM	9:52 AM	8.0	1.20	0	0	0	1,2	N		Y
Spring Creek	5	Abatement pond outflow	10:04 AM	10:16 AM	13.0	0.60	4	0	0	1,2	Y	12	N
Spring Creek	6	Adult ladder outflow	10:29 AM	10:34 AM	17.0	1.20	4	0	0	1,2	Y	13-16	Y

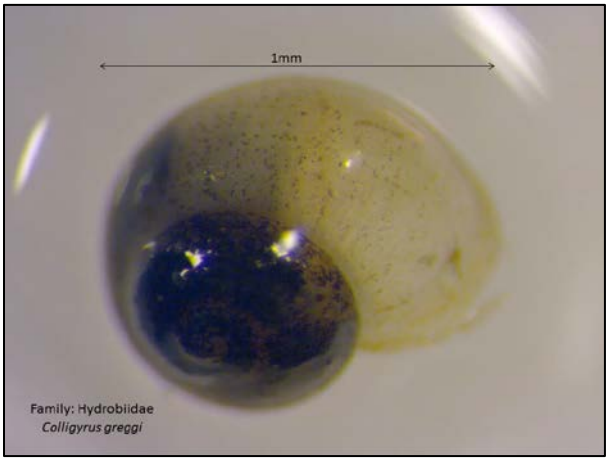
Substrate Type	Aquatic Vegetation Type	% Aquatic Veg. Cover	Sample Method
0 = silt,clay,organic material (<0.059mm)	0 = No vegetation	0 = No vetatation	1 = w ading
1 = Sand (0.06-1mm)	1 = Submerged	1 = 0-25%	2 = aquascope
2 = Gravel (2-15mm)	2 = Emergent	2 = 26-50%	3 = hand net
3 = Pebble (16-63mm)	3 = Floating	3 = 51-75%	4 = D-net
4 = Cobble (64-256mm)		4 = 76-100%	5 = Tactile
5 = Boulder (>256mm)			

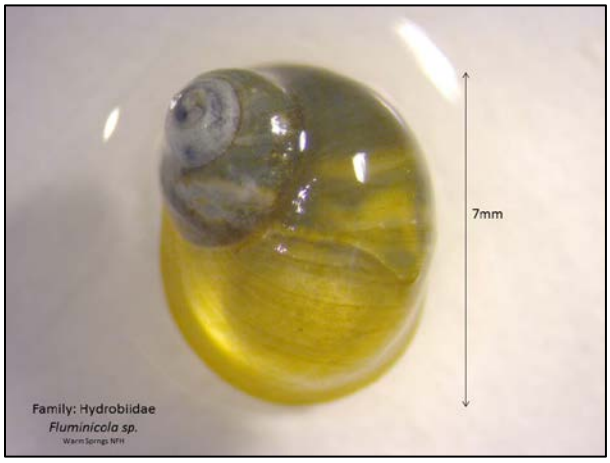
National Fish Hatchery	Site #	Site Description	Survey Begin Time	Survey End Time	Temp (°C)	Max Depth (m)	Dominant Substrate	Dominant Aquatic Vegetation	% Aq. Veg. Cover	Sample methods used	Specimen Collected (Y/N)	Specimen Vial #(s)	Photo taken (Y/N)
Willard	1	Intake grate **(eDNA)**	9:43 AM	9:53 AM	7.5	2.0^{+}	1	0	0	2,4	N		Y
Willard	2	Trash rack #1	9:58 AM	10:03 AM	7.5	1.00	1	0	0	2	N		Y
Willard	3	Upper H_2O settling pond	10:04 AM	10:10 AM	7.5		1	0	0	1,2	N		Y
Willard	4	Trash rack #2	10:12 AM	10:17 AM	7.5	1.32	1	0	0	2,4	N		Y
Willard	5	Low er pond outflow	10:32 AM	10:37 AM	7.5	0.50	4	0	0	1,2	N		Y
Warm Springs	1	Ab. pond outflow **(eDNA)**	10:15 AM	10:30 AM	17.0	0.25	Concrete	0	0	2	Y	1,2	Y
Warm Springs	2	Ab. pond perimeter **(eDNA)**	10:35 AM	10:55 AM	16.5	1.50	0	1	2	1,2,3	Y	3,4	Y
Warm Springs	3	Adult pond outflow	11:00 AM	11:02 AM		0.01	Concrete	0	0	5	N		N
Warm Springs	4	Adult ladder outflow **(eDNA)**	11:05 AM	11:10 AM	14.5	1.90	5	0	0	2	N		Y
Warm Springs	5	Intake grate **(eDNA)**	11:15 AM	11:21 AM	15.0	2.0^{+}	0	1	4	1,2,3,4	N		Y
Burnt Bridge Cr.	1	Ped. bridge near 65th **(eDNA)**	11:30 AM	12:10 PM	17.5	0.80	0	1	4	2,3	Y	48	Y

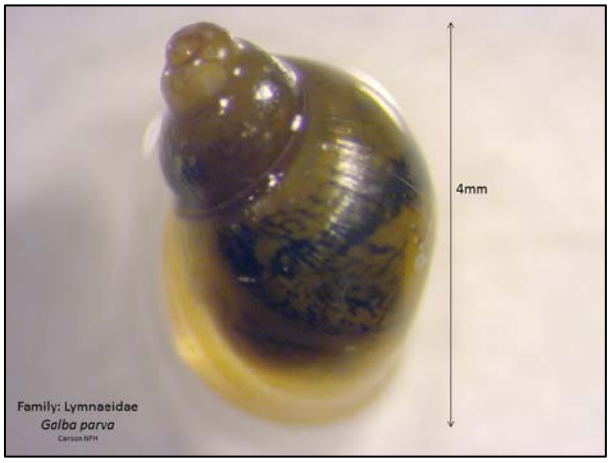
Substrate Type	Aquatic Vegetation Type	% Aquatic Veg. Cover	Sample Method
0 = silt,clay,organic material (<0.059mm)	0 = No vegetation	0 = No vetatation	1 = w ading
1 = Sand (0.06-1mm)	1 = Submerged	1 = 0-25%	2 = aquascope
2 = Gravel (2-15mm)	2 = Emergent	2 = 26-50%	3 = hand net
3 = Pebble (16-63mm)	3 = Floating	3 = 51-75%	4 = D-net
4 = Cobble (64-256mm)		4 = 76-100%	5 = Tactile
5 = Boulder (>256mm)			

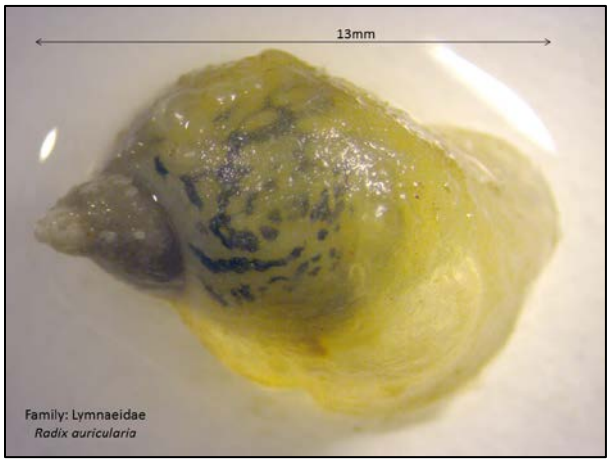
Appendix A: Photographs of Snail Specimen

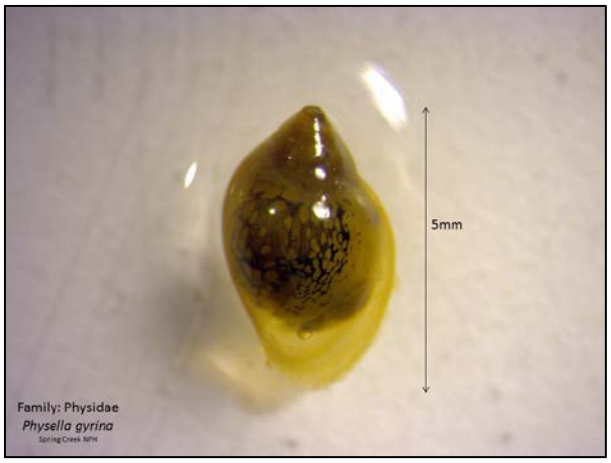


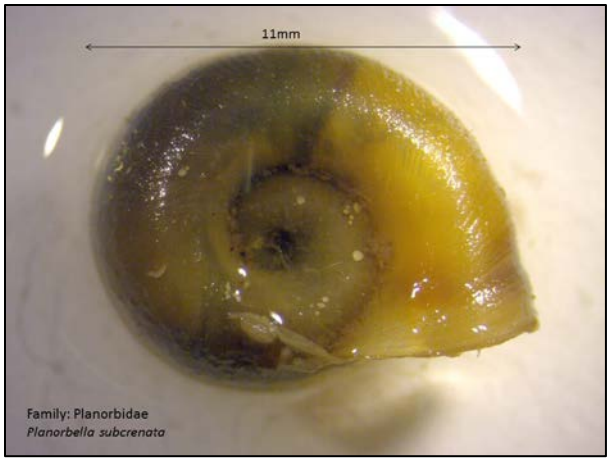








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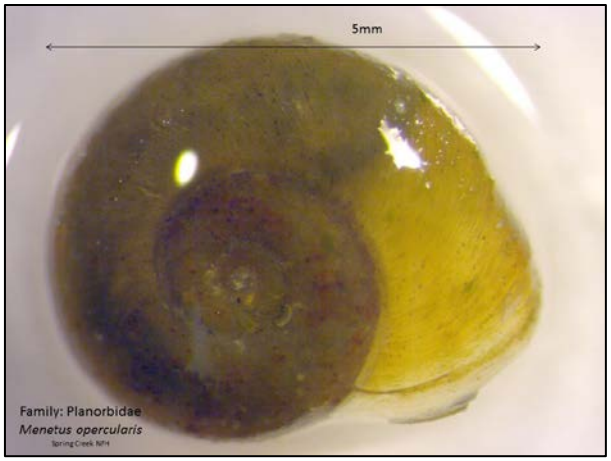
Planorbella subcrenata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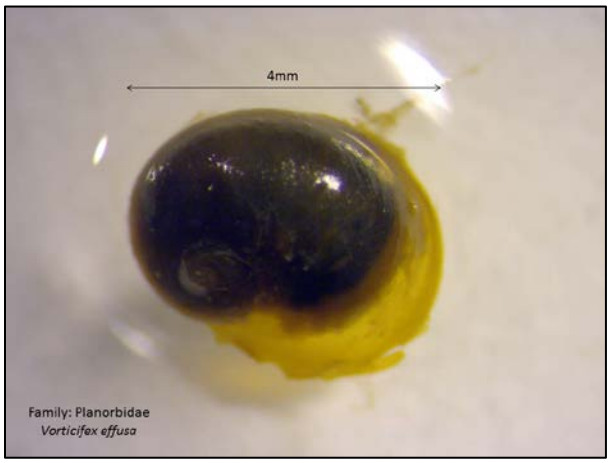
Menetus opercularis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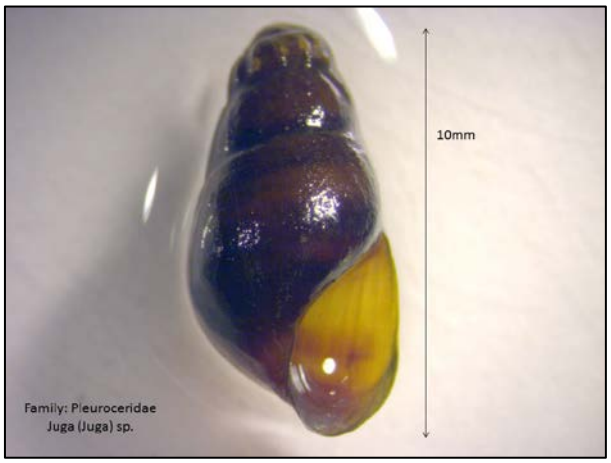
Family: Planorbidae
Vorticifex effusa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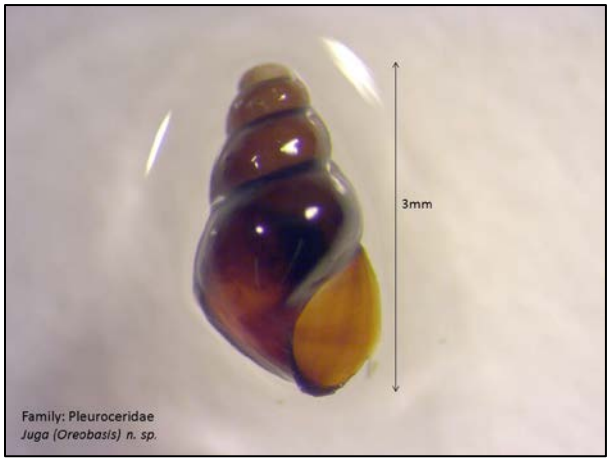
Juga (Juga) sp.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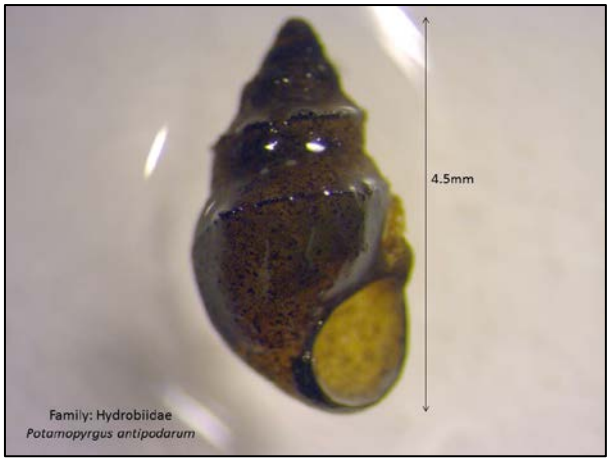
Juga (Oreobasis) n. sp.


4.5mm
Family: Hydrobiidae
Potamopyrgus antipodarum

U.S. Fish and Wildlife Service
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