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Aquatic Invasive Species Monitoring at Lower Columbia River Basin National Fish Hatcheries using eDNA and Visual Surveys

FY 2018 Annual Report

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On the cover: *New Zealand mudsnails clinging to submerged vegetation in Youngs Bay near Astoria, Oregon, photo by Chanice Davies.*

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2018 ANNUAL REPORT

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Abstract

The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum,* zebra mussels *Dreissena polymorpha* and quagga mussels *Dreissena rostriformis bugensis*, are three of the most problematic aquatic invasive species (AIS) in North America today. The potential risk posed by the proximity of these invasive mollusks to lower Columbia Basin National Fish Hatcheries (NFHs) is significant and highlights the need for an effective AIS monitoring program. Conventional monitoring techniques for aquatic snails and mussels can be laborious and may not reliably detect the tiny mollusks when an infestation first occurs or abundance is low. Environmental DNA (eDNA) is gaining popularity as an AIS surveillance tool due to its low environmental impact and increased sensitivity to detect species at low densities. The Columbia River Fish and Wildlife Conservation Office (CRFWCO) has performed visual presence/absence surveys for AIS at lower Columbia River Basin NFHs since 2006. Environmental DNA was incorporated into the annual sampling regime in 2015 to improve our probability of detecting potential invaders. In 2018, 32 sites were visually inspected and 12 locations were surveyed using the eDNA technique at six NFHs. Surveyors observed freshwater snails from six unique families and twelve genera. No target AIS were observed during presence/absence surveys and all eDNA samples (n=36), tested negative for the presence of NZMS, zebra, and quagga mussels. Single-season site-occupancy analysis indicates the probability of NZMS occupancy at these sampled hatcheries is less than 1% (0.00-0.28). Detection probability was higher for eDNA (0.77) than visual surveys (0.54) and in terms of effort, a total of six visual surveys are needed to be 99% confident that a survey location is unoccupied; versus three eDNA samples to obtain the same confidence level. For comparison, visual surveys and eDNA samples were collected from five locations with known NZMS presence: Burnt Bridge Creek, Deschutes River, Columbia River near Kalama, Nestucca River, and Youngs Bay. Visual surveys detected NZMS in three of five locations, while eDNA detected the presence of snail DNA at all five locations. We estimated the probability of occupancy for a natural area with previously documented species presence to be 0.97 with 95% credible intervals ranging from 0.66 to 1.00. The eDNA technique has proven a valuable tool to accurately detect an invasive species that is otherwise difficult to observe in a stream environment, especially when densities are low. The monitoring efforts conducted by the CRFWCO under this project provide valuable early detection data for NZMS, zebra, and quagga mussels at lower Columbia Basin NFHs. Early detection of AIS may improve the success of eradication efforts, or prevent the establishment or unintentional spread of invasive populations to neighboring hatchery facilities or stocking locations.

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Introduction

New Zealand mudsnail, zebra, and quagga mussels are three of the most problematic aquatic invasive species in North America today. The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum* is a small aquatic snail native to New Zealand that was introduced to North America through contaminated ballast water (Zaranko et al. 1997; Gangloff 1998) and the transport of live fish or eggs for the commercial aquaculture industry (Bowler 1991; Bowler and Frest 1992). Since their initial discovery in the Snake River (Idaho) in 1987 (Bowler 1991), NZMS have spread to 20 states (including ten western states), and two Canadian provinces (British Columbia and Ontario) (Benson et al. 2019a; Figure 1). Adult NZMS range from 3-6 mm in length and have an elongate conical shell with 5-8 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 (Appendix A). The zebra mussel (*Dreissena polymorpha*) and closely related quagga mussel (*Dreissena rostriformis bugensis*), are freshwater bivalves native to the Ponto-Caspian region of Eastern Europe/Western Asia. Both species are believed to have been introduced to North America through the discharge of ballast water from commercial cargo ships containing larval or adult mussels. Zebra mussels were first discovered in Lake St. Clair, Michigan in 1988 (Hebert et al. 1989), followed by the detection of quagga mussels near Lake Erie, New York in 1989 (May and Marsden 1992; Mills et al. 1993). The first report of invasive mussels (quagga) west of the Rocky Mountains was from Lake Mead near Boulder City, Nevada in 2007 (Nalepa 2008). Since this time, zebra and quagga mussels have collectively spread to at least 28 states (including five western states) and three Canadian Provinces (Manitoba, Ontario and Quebec) (Benson et al. 2019b; Benson et al. 2019c; Figure 2; Figure 3). As adults, both species are relatively small (<4 cm) with distinct alternating dark and light banding (quagga) or zig-zag patterns (zebra). Although similar in appearance, the mussels can be distinguished by the general shape and structure of the shell. Quagga mussels have a rounded fan-shaped appearance with a convex ventral (hinge) surface and two asymmetrical shell halves that meet to form a curved line. Zebra mussels have a triangular or "D"-shaped appearance with a flat ventral surface and two symmetrical shell halves that meet to form a straight line (Appendix A).

New Zealand mudsnail, zebra and quagga mussels share several biological characteristics that facilitate their success as aquatic invaders including: rapid growth, early sexual maturity and high fecundity (Zaranko et al. 1997; Oregon Sea Grant 2010). They are capable of inhabiting a range of aquatic ecosystems (e.g., estuaries, river, lakes and reservoirs) and tolerate a relatively

broad range of aquatic conditions (see ANSTF 2007 and references therein; Nalepa 2008). Furthermore, they are easily spread to new locations by numerous natural and human-mediated activities. New Zealand mudsnail may be transported on the fur or feathers of terrestrial wildlife and waterfowl, or consumed and dispersed (still alive) in the excrement of local fish species. Long distance dispersal of NZMS has been attributed to ballast water discharge, conveyance of commercial aquaculture products (i.e., fish, eggs, and ornamental plants), hatchery stocking, or the movement of contaminated watercrafts and personal recreational gear between waterbodies. Zebra and quagga mussels are commonly spread during the free-floating larval stage by drifting passively on water currents, through man-made canals and aqueducts, or carried across land in water bearing compartments of boats or other recreational equipment. Juvenile and adult mussels secrete strong byssal threads used to affix themselves to rocks and other surfaces. Mussels routinely attach to boat hulls, motor compartments or boat trailers and may be transported overland and launched in a new water body where they detach or become scraped off during navigation. Once established, all three species have the potential to proliferate quickly and attain extraordinarily high densities that can result in harmful ecological and economic impacts. Heavy infestations can alter aquatic habitat complexity, modify nutrient cycling and food-web dynamics, displace or outcompete native species and inflict significant economic hardship through impacts to fisheries, recreation/tourism, power production, municipal water supply and infrastructure (e.g., encrusting pipelines, pumps, intake screens, dock and pier supports, etc.) (Strayer et al. 1999; Vanderploeg et al. 2002; Hall et al. 2003; Kerans et al. 2005; Hall et al. 2006; Riley et al. 2008; Nalepa 2008; Burlakova et al. 2014).

Early detection is the most important, yet most challenging aspect of aquatic invasive species (AIS) management (Hulme 2006; Harvey et al. 2009). Discovery of a new AIS infestation can be particularly difficult if the organism is small, cryptically colored or occurs in a habitat that is difficult to sample effectively (Harvey et al. 2009). Conventional monitoring techniques for aquatic snail and mussels (e.g., tactile and visual inspections, snorkeling, D-frame dip nets, Hess stream bottom samplers, artificial settlement substrates, benthic grabs, plankton tow sampling, etc.) can be laborious and may not reliably detect the tiny mollusks when an infestation first occurs or abundance is low. Environmental DNA (eDNA) is gaining popularity as an AIS surveillance tool due to its low environmental impact and increased sensitivity to detect species at relatively low densities. 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. Environmental DNA has many potential applications for detecting and monitoring AIS in aquatic environments (Herder et al. 2014; Rees et al. 2014; Davison et al. 2017). Here we describe a less common use of eDNA as a surveillance tool to confirm the absence of target AIS at fish hatchery facilities over time.

National Fish Hatcheries (NFHs) produce fish that provide commercial and recreational fishing opportunities, fulfil tribal trust and mitigation responsibilities and contribute to the recovery of threatened or endangered species. The potential introduction of AIS into a hatchery facility poses two primary concerns. First, routine activities such as fish stocking or transfers can introduce or spread AIS to uninfested waterbodies or hatcheries (ANSTF 2007). Second, AIS may encrust or clog important equipment and infrastructure (e.g., water delivery pipes, filters, screens, pumps, etc.), requiring increased labor and maintenance costs. The U.S. Fish and Wildlife Service (USFWS) has widely adopted the use of Hazard Analysis and Critical Control Point (HACCP) planning to prevent the spread of invasive species through human-mediated pathways. Many NFHs have developed regional HACCP plans to assess and reduce the risk of AIS invasion, identify pathways of potential introduction, or minimize impacts or spread of existing AIS 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 AIS before they become established or spread to new areas.

The USFWS Columbia River Fish and Wildlife Conservation Office (CRFWCO) has performed visual presence/absence surveys for AIS at lower Columbia River Basin NFHs since 2006 (see Allard and Olhausen 2007a, 2007b; Hogle 2009; Poirier 2012; Poirier 2014). Environmental DNA was incorporated into the annual sampling regime in 2015 to improve our probability of detecting potential invaders (Poirier 2015; Poirier 2017; Poirier and Harris 2018). New Zealand mudsnail, zebra and quagga mussels were identified as the focal species for this survey effort because they pose the greatest potential risk to hatchery infrastructure and they have been recently observed in (NZMS), or near (NZMS, zebra, and quagga mussels) the Columbia River Basin (PNWER and PSMFC 2015; Benson et al. 2019a; Benson et al. 2019c). This report presents results of visual AIS presence/absence surveys and eDNA sampling conducted by CRFWCO personnel in 2018. We also present the results of an occupancy analysis used to estimate the detection probability of eDNA sampling and visual surveys. Data in this analysis included eDNA and visual survey detection/non detection information collected at NFHs and five natural areas with documented NZMS presence, from 2015-2018.

Methods

Presence/absence surveys

Six lower Columbia River Basin NFHs were surveyed for NZMS, zebra and quagga mussels including: Carson, Eagle Creek, Little White Salmon, Spring Creek, Warm Springs and Willard National Fish Hatcheries (Figure 4). Visual presence/absence surveys were conducted over a two-week period from 29 August to 6 September 2018. Site selection focused on areas perceived as likely AIS 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 and mussels. In water depths greater than 0.6 m, substrate, aquatic vegetation and hatchery structures were visually inspected using an underwater viewing scope. While searching for invasive mollusks, we also conducted a general inventory of native freshwater snail species present at each sample location. 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. Snails were identified to genera and, whenever possible to species level.

Environmental DNA Sample Collection & Filtration

Environmental DNA sampling was conducted over a two week period (29 August – 13 September 2018), following protocols described in Goldberg and Strickler (2014). Two sites (i.e., hatchery intake grate, raceway/fish ladder outflow, and/or abatement pond outflow) were surveyed at each NFH using the eDNA technique. 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 CRFWCO 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. Immediately following the collection of eDNA water samples at all sites, two personnel performed a visual presence/absence survey for NZMS, zebra and quagga mussels using the methods described above. To validate the performance and reliability of eDNA technology, three water samples and a single field negative were collected at five additional locations with documented NZMS presence: Burnt Bridge Creek, Deschutes River, Columbia/Kalama River, Nestucca River and Young's Bay. These locations were obtained from the Nonindigenous Aquatic Species webpage (Benson et al. 2019a), which tracks confirmed sightings of non-native invertebrates, vertebrates and plants submitted by natural resource professionals, researchers, and citizen scientists. Species observations are spatially referenced and include records of species status (i.e., whether population is sparse or established), potential pathway of introduction and observation date/year.

Environmental DNA water samples were filtered in the CRFWCO laboratory within four hours (or less) of collection. 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 CRFWCO 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. Additionally, waders and boots were placed in a freezer (≈-14°C) overnight between use. Environmental DNA samples were collected and processed on separate but consecutive days beginning with Willard National Fish Hatchery (lowest probability of AIS presence) and ending with Burnt Bridge Creek (confirmed presence of NZMS) to further minimize risk of sample contamination.

Environmental DNA assays

The NZMS assay used in this analysis was developed at the Washington State University eDNA laboratory 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).

Species specific genetic assays for zebra and quagga mussels were also developed using pre-existing sequence data from GenBank. Candidate quantitative PCR assays were tested against tissue derived DNA from 39 zebra mussel specimens from Lake Winnipeg, and 173 quagga mussel specimens from the Colorado River. Each assay was also tested against DNA from 10 native unionid mussel species to ensure specificity (Gingera et al. 2017).

PCR Amplification

Environmental DNA sample processing was performed by the Washington State University eDNA laboratory. 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.

Occupancy Analyses

We used a single-season site-occupancy model (MacKenzie et al. 2006) to estimate the detection probability of NZMS by two methods: eDNA sampling and visual presence/absence surveys. Specific objectives of this modeling were to: 1) estimate the probability of detecting occupancy of NZMS by eDNA and visual surveys; and to 2) calculate the posterior probability of occupancy, if no individuals are detected during a study, given varied levels of sampling effort for each method.

The hierarchical occupancy model has two levels (Kéry and Schaub 2012). The first level is the state process which estimates the true probability that a site (i) is occupied by NZMS (z_i):

$$z_i \sim Bernouli(\psi_i)$$

We sampled two site types: NFHs (n=6) and natural areas with previously documented NZMS presence (n=5). The NFHs are the site type of interest, but may not be occupied by NZMS. Natural areas where NZMS have previously been documented were sampled to provide information on detection probability by field method. To allow for differences in the probability of occupancy between these two site types, we included a covariate for site type in the model. Thus, the true probability that a specific site (i) was occupied (ψ_i) was estimated from the probability of occupancy (ψ_i), which was modeled on the logit scale as a function of site type - either NFH or natural area.

The second level is the observation process, which is the probability that at least one individual will be detected in a replicate survey (j), given that the site is occupied (i.e., given that z_i=1). To model the observation process, replicate eDNA samples and visual surveys at each site ($y_{i,j}$) were conducted. We assumed that occupancy status (i.e., occupied or not) was the same for all replicates collected at a site (i.e., occupancy status was the same over the spatial and temporal distribution of sampling at a site). For each site, 3-12 replicates were collected using each sampling method. Detection probability ($p_{i,j}$) was estimated based on replicate surveys ($y_{i,j}$) by site (i) and survey (j):

$$y_{i,j} \sim Bernouli(z_i * p_{i,j})$$

Similarly, $p_{i,j}$ was modeled on the logit scale as a function of sampling method—either an eDNA sample or a visual survey.

We then used a model developed by Peterson and Dunham (2003) to calculate the posterior probability of occupancy (PPO), if no individuals are detected, using eDNA sampling (below) and visual surveys:

$$PPO_{eDNA} = \frac{P(Co/F)_{eDNA}}{1 + P(Co/F)_{eDNA}}$$

Where $P(Co/F)_{eDNA}$ is the probability of not detecting any NZMS using eDNA when the species is present. In this model, the probability of not detecting any NZMS when they are present at a site is a function of the detection probability of eDNA sampling ($p.eDNA$) and the number of eDNA samples collected (n):

$$P(Co/F)_{eDNA} = (1 - p.eDNA)^n$$

As n increases, the probability that the species has been missed and that the site is truly occupied declines. The posterior probability of occupancy based on the number of visual surveys conducted PPO_{Visual} was similarly calculated using the estimated detection probability of visual surveys ($p.Visual$). For calculation of posterior probability of occupancy, we assumed prior probability of presence was 0.5 (i.e., uninformed) and the probability of false positives was zero (this allowed us to simplify that model in Peterson and Dunham 2003).

The occupancy model was analyzed by Bayesian methods using JAGS software (Plummer 2003) called from Program R (R Core Team 2013). Code for analysis was modified from Kéry and Schaub (2012). Priors for intercept and slope coefficients for occupancy and detection modeled on a logit scale were selected to be uninformative: all were from uniform distributions over the range of -10 to 10. Models were run using Package jagsUI with function autojags (Kellner 2017) for 3 chains, an adaption period of 1,000 iterations, a burn in period of 1,000, and an iteration increment of 1,000, with enough iterations to reach convergence, as assessed by all estimated parameters having an Rhat score of 1.1 or less (Gelman and Hill 2007; Kéry and Schaub 2012).

All posterior distributions are described by the median for central trend (i.e., estimate), and 95% credible intervals for precision.

Results

Presence/absence surveys – National Fish Hatcheries

A total of 32 intake and outflow sites were visually surveyed at six lower Columbia River Basin NFHs (Table 1; Figures 5-10). Native freshwater mollusks were present in 21 (72%) of sites sampled in 2018. Surveyors observed freshwater snail from six unique families and twelve genera, as well as a single freshwater bivalve from the family Sphaeriidae (Table 2). Juga (Juga) sp. was the most common snail genera observed, present in 10 different locations at four NFHs (Eagle Creek, Little White Salmon, Spring Creek and Warm Springs NFH). Juga were also the most abundant snail genera observed at NFHs. The diversity of species was highest at Warm Springs (seven species), Little White Salmon (six species) and Spring Creek (six species), and lowest at Willard (one species). The differences in snail diversity observed at NFHs may be attributable to water chemistry (e.g. temperature, conductivity, DO), habitat complexity (e.g. presence/absence of aquatic vegetation), or the area surveyed. Snail distribution is often patchy and organisms may not be present in the survey area or population density is too low to be detected.

No NZMS, zebra, or quagga mussels were observed in survey locations at lower Columbia River Basin NFHs. However, we did find occurrences of the non-native big-ear radix (*Radix auricularia*) around the perimeter of the abatement pond at Warm Springs NFH. The big-ear radix is a freshwater snail that was introduced to the United States from Eastern Europe through the aquarium plant trade and prefers slow-moving lakes, ponds and rivers with silt or mud substrate. *Radix auricularia* is a vector for a variety of parasites and may outcompete native benthic species for food or habitat. A summary of freshwater mollusk genera and water quality parameters for each sample location can be found in Tables 2 and 3.

Presence/absence surveys – areas with documented NZMS presence

New Zealand mudsnail were observed in three of five locations with documented NZMS presence (Burnt Bridge Creek, Nestucca River and Youngs Bay; Figures 11, 12 and 13). Relative snail abundance was highest in Young's Bay (≈800 snail/m^2), and moderate to low in Burnt Bridge Creek (≈20 snail/m^2) and the Nestucca River (≈2 snail/m^2). No NZMS were observed in the lower Deschutes River, though snails were documented downstream from the Heritage Landing boat launch in 2005 and 2007 (Figure 14; Benson et al. 2019a). Similarly, no NZMS were observed in the Columbia River at the Sportsman's Club boat launch (rkm 117; Figure 15) where snails were last documented in 2002 (Benson et al. 2019a).

Environmental DNA

A total of 68 eDNA water samples were taken at 17 locations in 2018 (Table 3). All eDNA samples taken at National Fish Hatcheries (36 samples) tested negative for the presence of NZMS, zebra and quagga mussels, including 12 field negative samples. Environmental DNA samples taken at five locations with documented NZMS presence tested negative for the presence of zebra and quagga mussels, but had varying results for NZMS. All water samples taken at Young's Bay, the Nestucca River and Burnt Bridge Creek (9 total) tested positive for the presence of NZMS DNA, while only a single sample (out of three) tested positive for NZMS in the Deschutes River as well as in the Columbia River near the mouth of the Kalama River. The amount of NZMS DNA in each positive sample was variable with the lowest number of NZMS DNA copies found in the single positive sample from the mouth of the Kalama River (0.1 copies/ml), and highest density found in Young's Bay (sample average = 120.6 copies/ml; Table 3). Four field negative samples taken at sites with documented NZMS presence all tested negative for the presence of NZMS DNA, indicating disinfection procedures were successful at preventing sample contamination.

Occupancy Analyses

The probability of occupancy for a natural area where NZMS were previously documented was estimated at 0.97 (95%: 0.66 - 1.00 (Table 4; Figure 16). In contrast, the probability of NZMS occupancy at one of the sampled NFHs was estimated as less than 0.02. Detection probability was higher for eDNA than for visual surveys (Table 4; Figure 16). The median estimate from our occupancy model suggested that two representative eDNA samples are required to be at least 95% confident that NZMS are not present in an area of unknown occupancy, if the species is not detected; three samples are needed to be at least 99% confident. The median estimate suggests that three representative visual surveys are required to be at least 90% confident that the species is not present, if none are observed; four to be at least 95% confident and 6 samples are needed to be at least 99% confident that an area is unoccupied, if no individuals are detected during visual surveys (Figure 16).

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, coastal lakes, tributaries and multiple locations along the lower Deschutes River (USGS 2018). Zebra and/or quagga mussels are currently established in five western states including California, Arizona, Nevada, Utah and Colorado. While no zebra or quagga mussels have been found in the Columbia Basin, mussel larvae were recently found in water samples from Tiber Reservoir, Montana, just a few hours east of the headwaters of the Columbia River (Schmidt and McLane 2018) and hitchhiking mussels are routinely found on recreational boats entering Oregon, Washington and Idaho (ISDA 2015; WDFW 2015; Reesman et al. 2018). The potential risk

posed by the proximity of these invasive mollusks to lower Columbia Basin NFHs is significant and highlights the need for an ongoing and effective AIS monitoring program.

Monitoring and surveillance play a vital role in AIS prevention and management. Early and accurate detection of AIS is crucial for effective management and control, but detection may be difficult if the organism occurs at low density, has a patchy distribution, is hard to see, or inhabits an area that is difficult to survey effectively. Many invasive species are only detected once they are abundant and widespread. 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. Conventional sampling techniques may not reliably detect an organism that is small or occurs in low abundance. However, detection may be improved by increasing sampling intensity or frequency (i.e., increase total number of samples or 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). Previous studies have quantitatively compared eDNA with traditional sampling techniques and found that eDNA is more sensitive than other sample methods for detecting the presence of target species, even in low abundance. However, most of these studies had some prior knowledge of the target species presence or distribution before sampling (Goldberg et al. 2011; Dejean et al. 2012; Thomsen et al. 2012; Pilliod et al. 2013; Schmidt et al. 2013; Smart et al. 2015; Dougherty et al. 2016; Wilcox et al. 2016). In this study, we had no knowledge or reason to believe the target AIS were present at NFHs. Monitoring for AIS without knowing whether they are present is inherently challenging because it is unclear whether the organism is truly absent, or simply not detected by the sampling technique. Numerous factors may influence the detectability of a species including its size, population density, distribution, habitat complexity, habitat preference, behavior, time of year, etc. (Ficetola et al. 2008; Jerde et al. 2010; Goldberg et al. 2011; Pilliod et al. 2013). There are also many potential sources of sampling error that may influence the reliability of detection/nondetection results. For example, a false positive detection (i.e. target organism is 'detected' where it is not present) may occur if an organism is misidentified or an eDNA sample is contaminated during field collection or laboratory work. False negative detections (i.e., target organism is not detected, but exists in environment) can occur if the organism is not captured/observed, or in the case of eDNA sampling, if DNA amplification is inhibited, eDNA quality is poor, or is present in very low abundance (see Poirier 2017). Understanding the sources of false positive and false negative detections and taking measures to minimize these risks is an important step toward reducing the uncertainty of detection/nondetection results. Uncertainty can also be reduced by incorporating detection probability into the sample design (Schmidt et al. 2013). In this study we used a single-season site-occupancy model to estimate the probability of detecting the occupancy of NZMS by two different sample methods: eDNA and visual surveys. Our primary interest is the potential occupancy of NZMS at NFHs, but they are presumably not present. Thus we sampled five locations with previously documented NZMS presence which enabled us to evaluate the performance and detection probability of both sample methods as well as the level of sampling

effort needed by each method to feel confident that NZMS are not present at a given location if they are not detected during a survey.

To date, no NZMS, zebra or quagga mussels have been observed or detected at lower Columbia River NFHs. The calculated probability of NZMS occupancy at the NFHs examined was less than 1% (0.00-0.28), providing additional confidence that annual surveillance efforts have been successful (thus far) and NZMS are not currently present at hatchery facilities. The estimated probability of detecting NZMS using eDNA was higher (0.77) than the rate of detection for visual surveys (0.54). Environmental DNA detected NZMS at all five locations with documented snail presence (in 11 of 15 samples), while NZMS were observed at only three of five locations with documented snail presence. Even though eDNA and visual surveys were performed consecutively at each site (i.e., eDNA first followed by a visual survey), it is possible NZMS densities were too low to be detected by visual surveys or the snails were located upstream from the immediate survey area and eDNA was captured as it drifted downstream. The higher detection probability of eDNA indicates a greater sensitivity of the method to detect NZMS compared to visual surveys; however, NZMS DNA was not detected consistently in all sample replicates. In the Deschutes River, eDNA detected NZMS in only one of three samples for the second consecutive year. In both 2017 and 2018, water samples were collected in three locations approximately 10 m apart along the east bank of the river. The most upstream sample was positive for NZMS while the two samples taken further downstream were negative. We suspect the snails may be located near the public boat launch approximately 250 m upstream from the positive sample and the negative samples were potentially too far downstream from the source population to detect the DNA. It is unclear how few NZMS can be reliably detected by the eDNA technique, and how far away snails can be from the sample site. Several studies indicate eDNA can be detected up to several kilometers downstream from a source population (Pilliod et al. 2013; Deiner and Altermatt 2014; Jane et al. 2014), though environmental variables (e.g., water temperature, discharge, UV radiation, water chemistry, bacteria and organic material) and the target organism itself (e.g., species, size, abundance, location in stream) can influence the persistence and quantity of DNA present at a sample location (Pilliod et al. 2013; Herder et al. 2014; Strickler et al. 2014). Detection of NZMS DNA was also inconsistent at the Columbia/Kalama River sample location. Environmental DNA failed to detect NZMS at this location in 2017, but a single water sample out of three tested positive in 2018. This difference in detection is likely due to low abundance and/or patchy distribution, but may also indicate a slight increase in NZMS abundance in or around the sample site over the last year. Based on our estimates of occupancy and detectability, a total of six visual surveys are required to be 99% confident that a survey location is unoccupied; versus three eDNA samples to obtain the same confidence level (Figure 16). We currently collect three 0.5L eDNA samples per site, an effort more than sufficient to reliably detect potential invaders. We also conduct AIS monitoring/surveys in late summer when stream flows are typically at their lowest. This may improve water visibility and increase the concentration of eDNA in the water column, thereby improving the likelihood of detection (Smart et al. 2015).

Environmental DNA sampling has a number of distinct advantages over traditional monitoring techniques (see Poirier 2017), but it still has some major limitations relative to visual surveys. For example, obtaining a positive eDNA sample result does not necessarily mean the organism is currently present in the system, or that the target population is viable. Furthermore, eDNA cannot distinguish whether an organism is dead or alive, its life stage, population structure, habitat utilization, or abundance (Rees et al. 2014; Goldberg et al. 2016; Evans et al. 2017; Trebitz et al. 2017), though, new eDNA techniques are currently being developed with the goal of relating eDNA quantity to relative species abundance. Unlike visual surveys, eDNA is unable to provide real-time information on species presence/absence; eDNA captured within a sample may be days or weeks old. Finally, species-specific eDNA sampling only tests for a few key species and would not detect unanticipated species that might also be present in the system. Both visual surveys and eDNA sampling are valuable AIS monitoring techniques that generate different but complementary information. In this application, eDNA provides valuable early detection information about our target species, while visual surveys remain the primary source of information about snail community composition, can be used to confirm the presence and location of target AIS and could potentially identify new or unanticipated AIS that were not the specific focus of eDNA sampling.

The monitoring and surveillance efforts conducted by the CRFWCO under this project provide valuable early detection data for NZMS, zebra and quagga mussels at lower Columbia Basin NFHs. An efficient and reliable AIS monitoring program is critically important as hatcheries face the ongoing threat of invasive species introductions that could potentially threaten infrastructure, increase maintenance costs, and adversely impact routine hatchery operations. Here we describe a relatively unique AIS monitoring program that uses eDNA together with visual surveys to confirm the absence of three highly invasive mollusks at NFHs. This study adds to the growing body of work that demonstrates the applicability of eDNA as an AIS monitoring tool to accurately detect an organism that is otherwise difficult to detect in low densities. Our work also demonstrates the value of site-occupancy modeling as a tool to evaluate and compare AIS sampling methods and provide increased confidence that our current AIS sampling frequency (once per year), intensity (three eDNA samples and one visual survey per site), and procedures are sufficient to detect NZMS, zebra and quagga mussels at NFH intake and outflow locations. This study could help guide similar AIS monitoring programs make informed decisions regarding field sampling techniques and the effort needed to reliably detect high-risk invaders.

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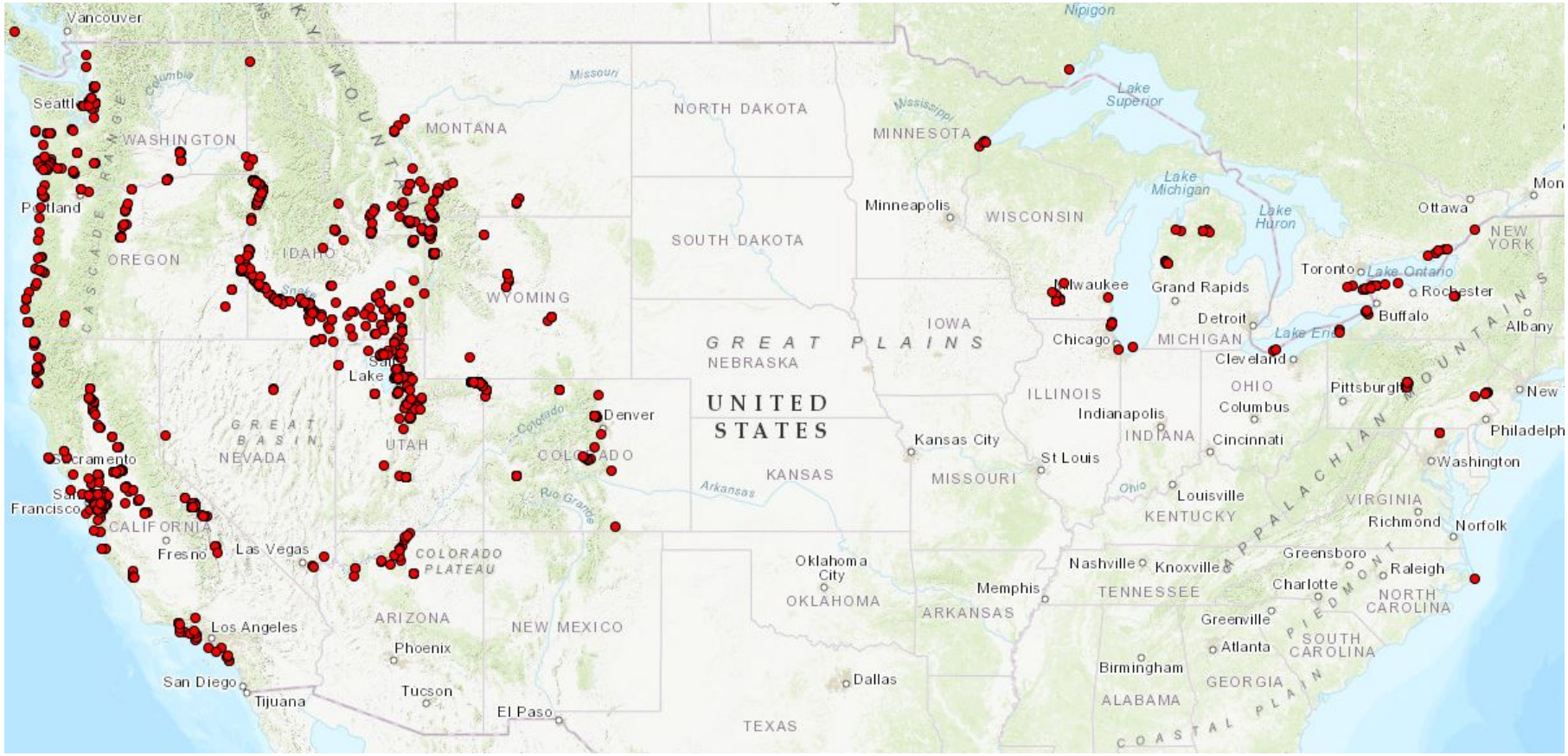


Figure 1: Map of New Zealand mudsnail sightings in the United States and Canada from 1987 through February 2018 (Benson et al. 2019a).

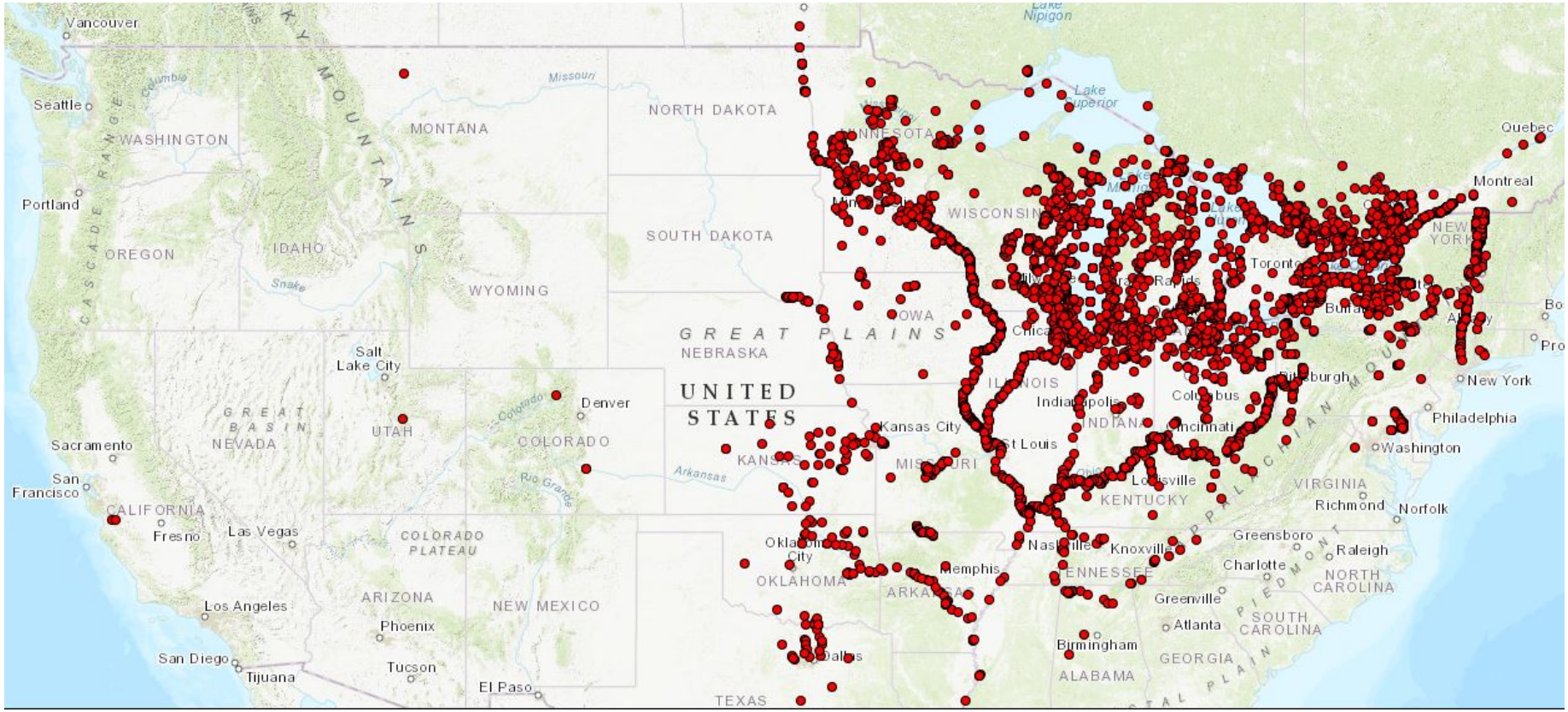


Figure 2: Map of zebra mussel sightings in the United States and Canada from 1987 through April 2019 (Benson et al. 2019b).

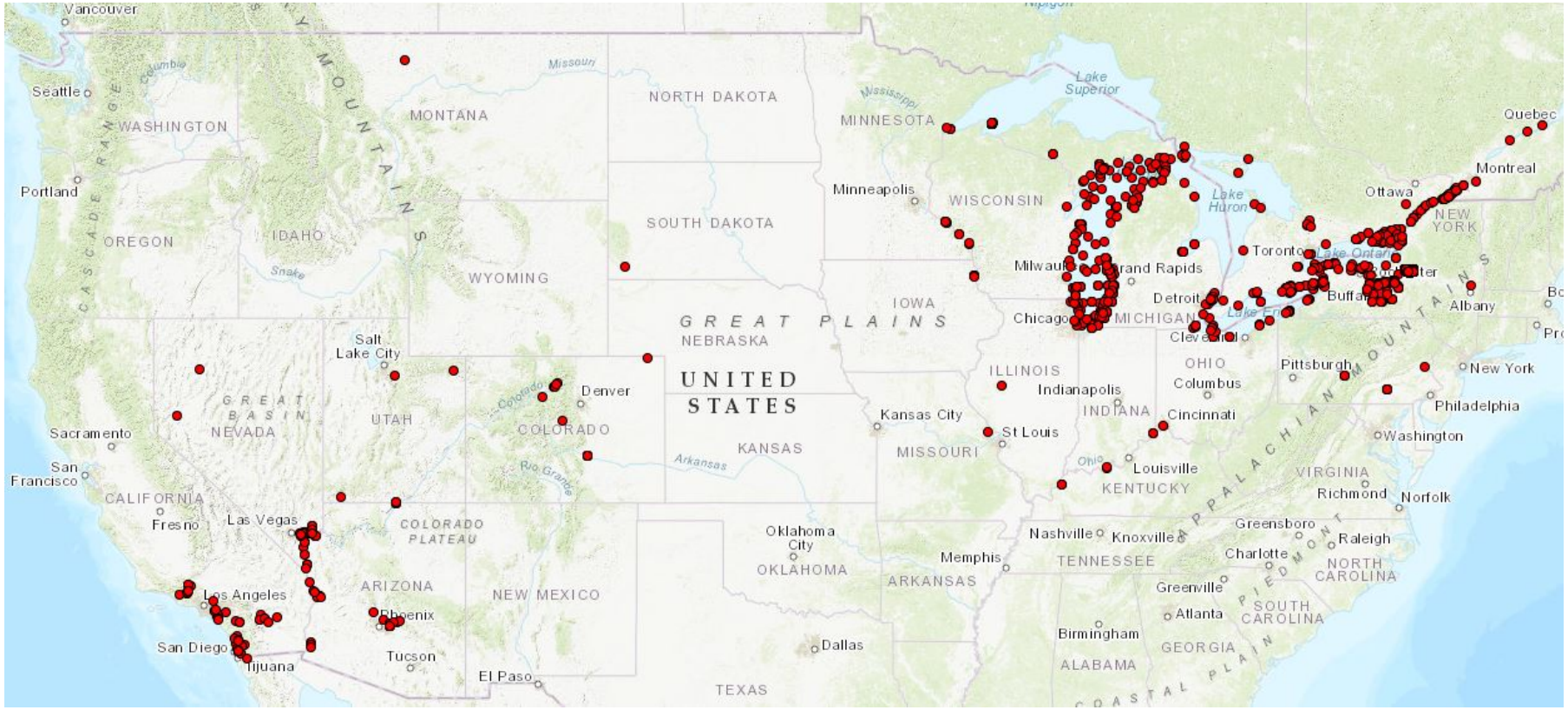


Figure 3: Map of quagga mussel sightings in the United States and Canada from 1987 through April 2019 (Benson et al. 2019c).

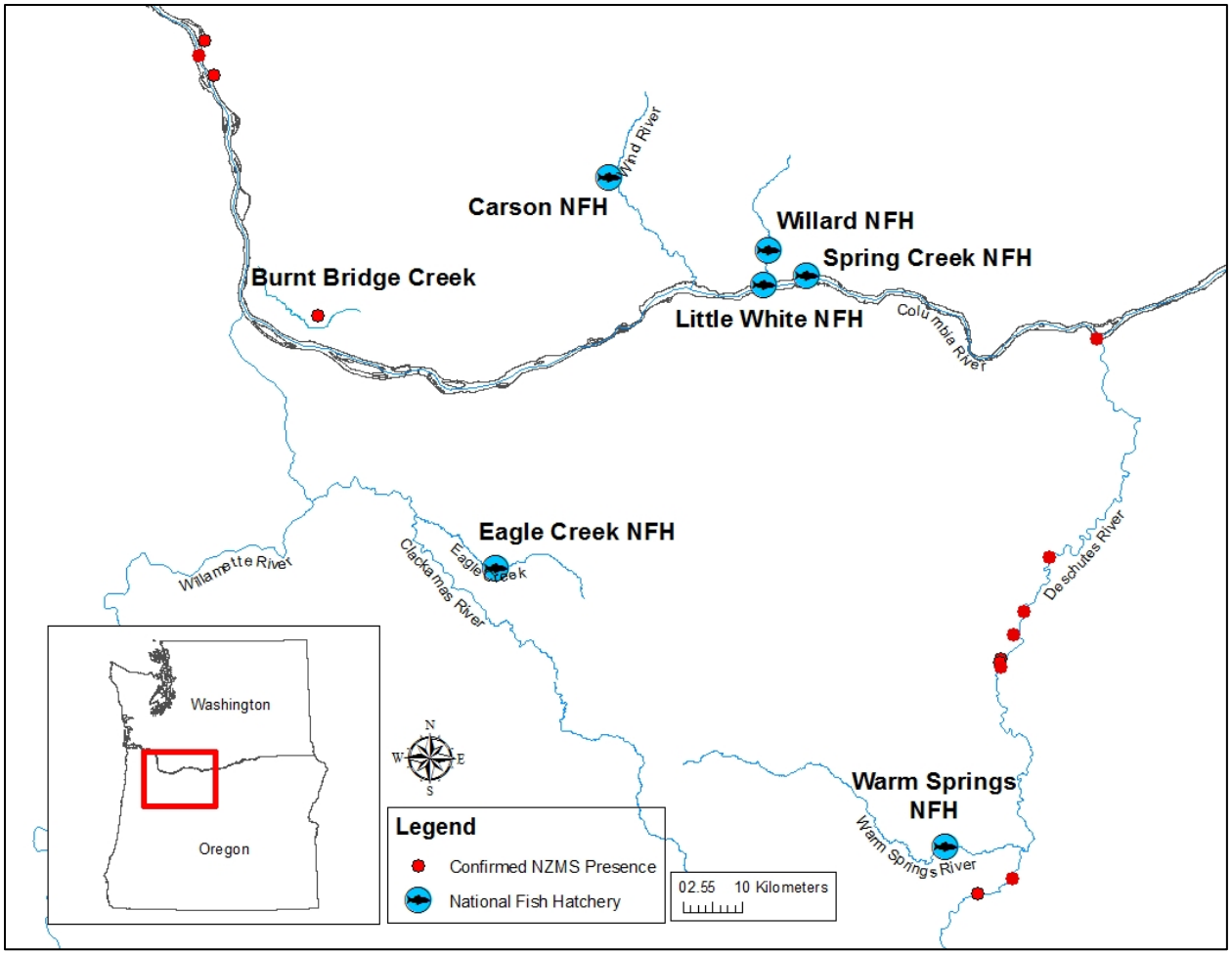


Figure 4: Map of USFWS National Fish Hatcheries surveyed for NZMS, zebra and quagga mussels, and distribution of NZMS populations in the lower Columbia River, 2018.

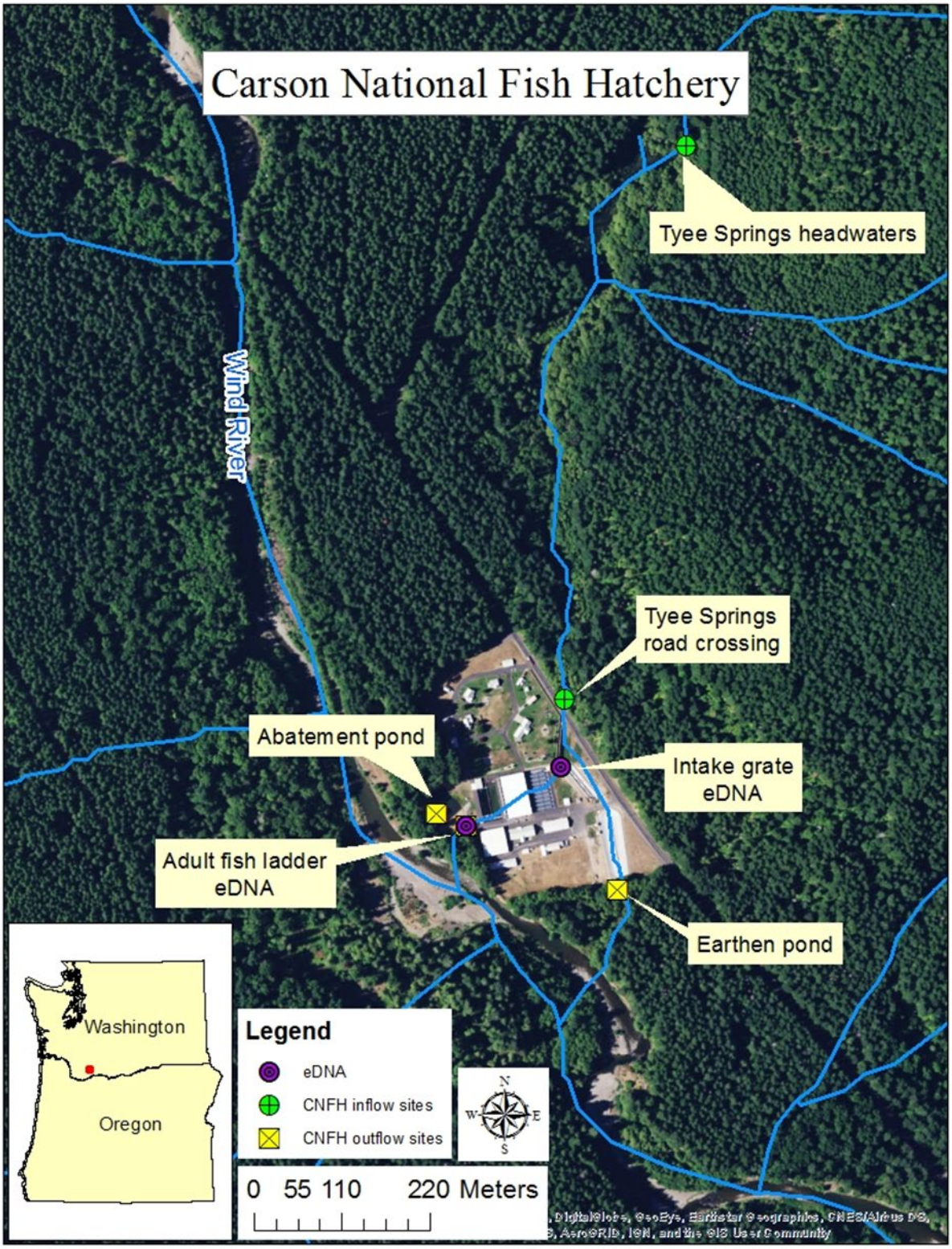


Figure 5: Carson NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

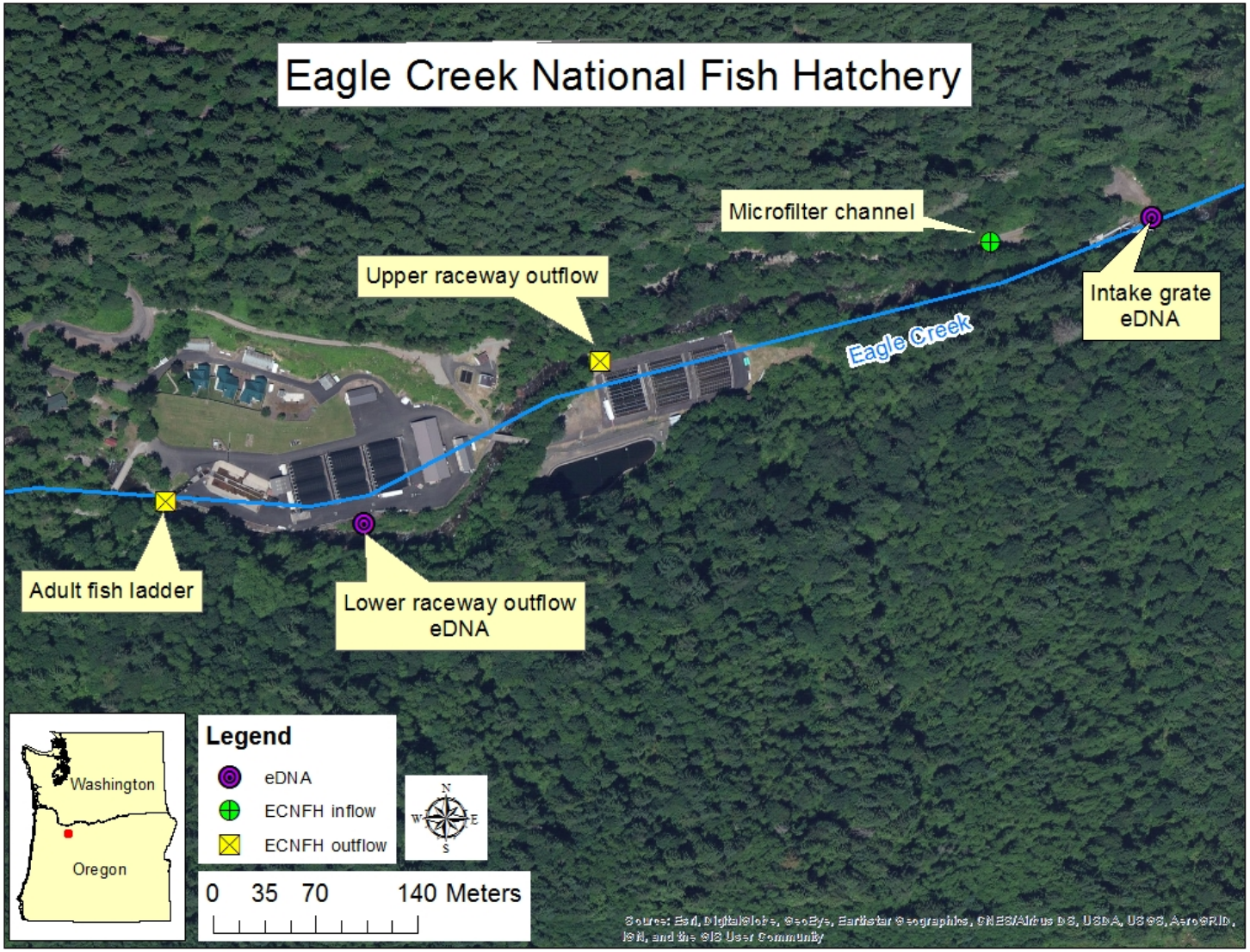


Figure 6: Eagle Creek NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

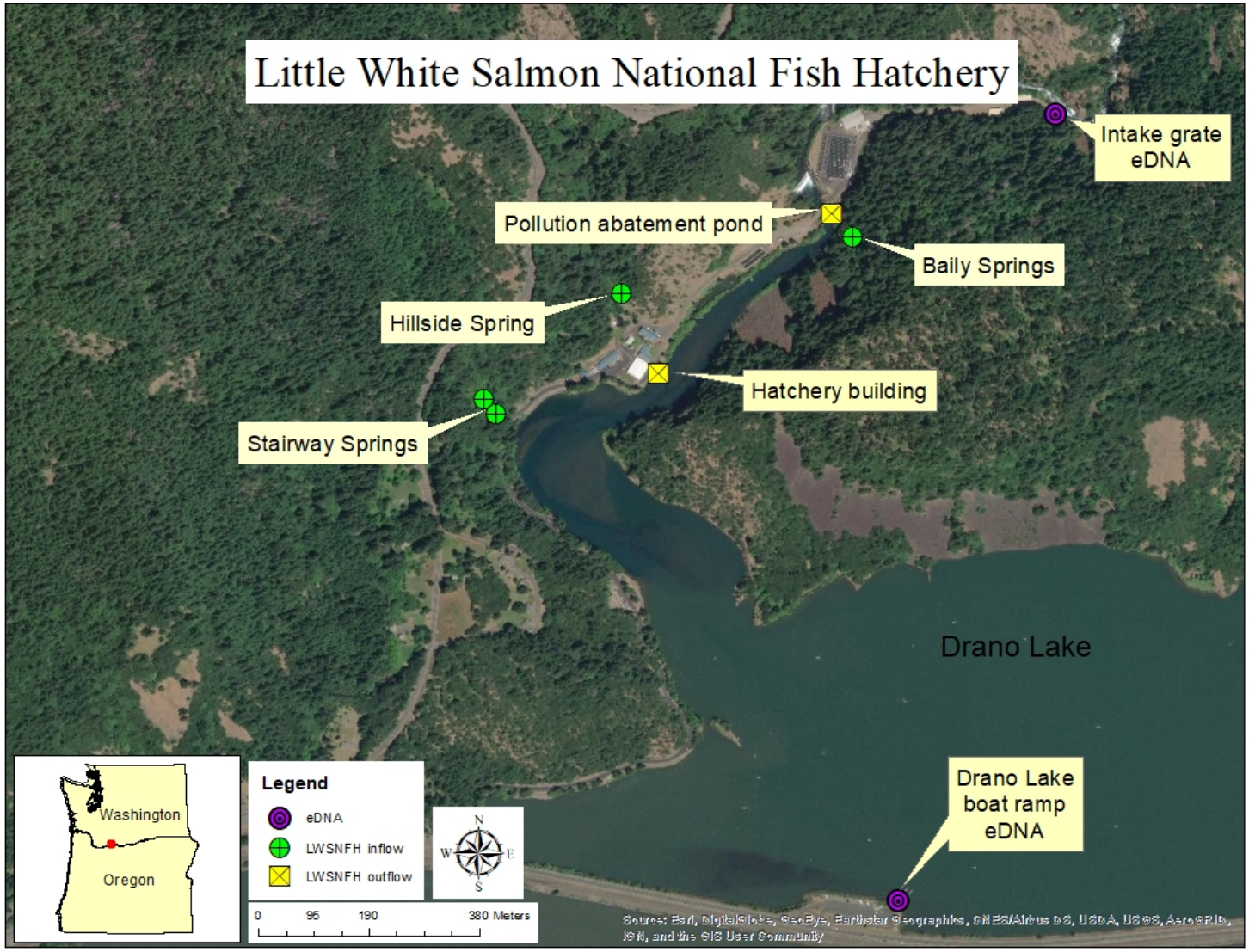


Figure 7: Little White Salmon NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

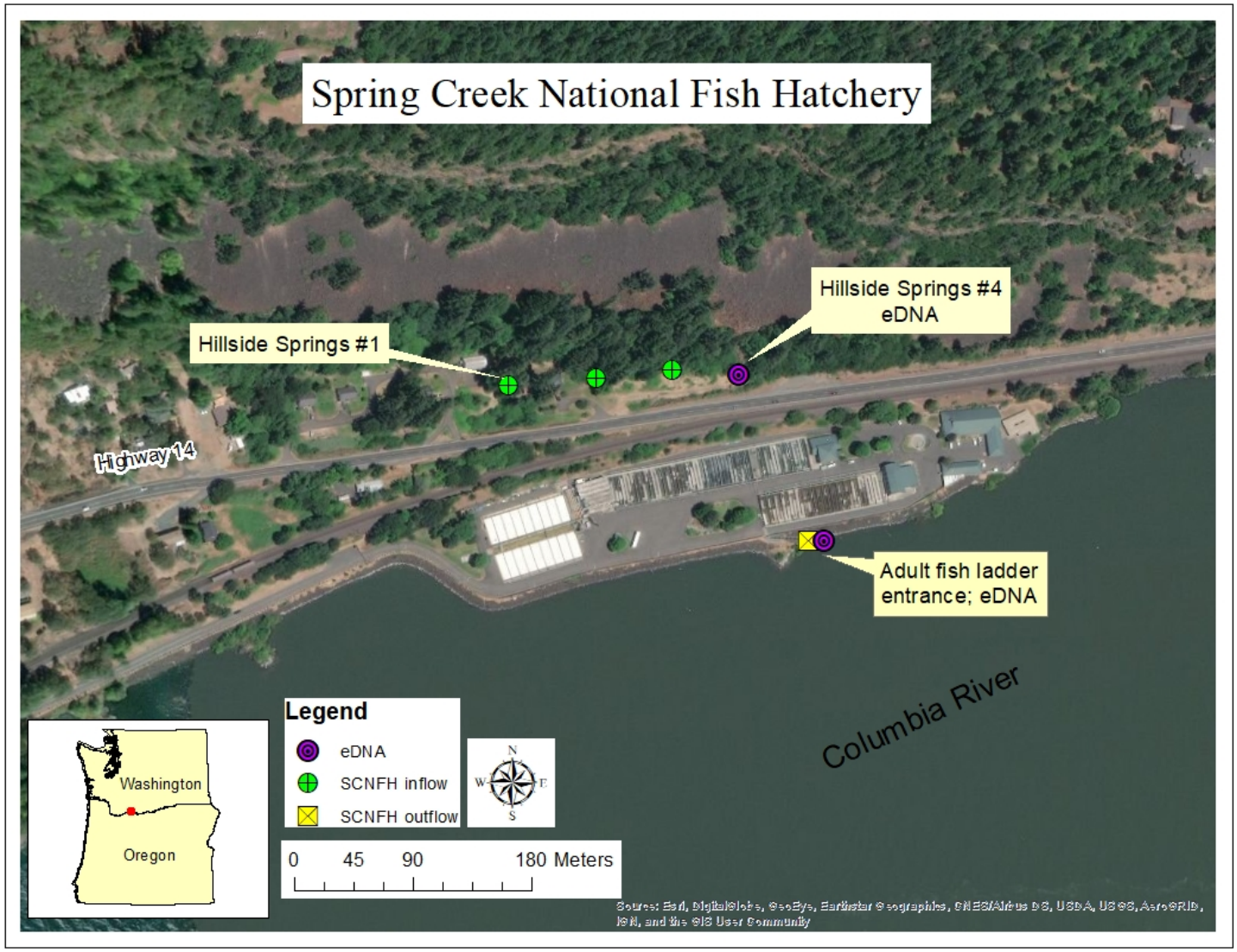


Figure 8: Spring Creek NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

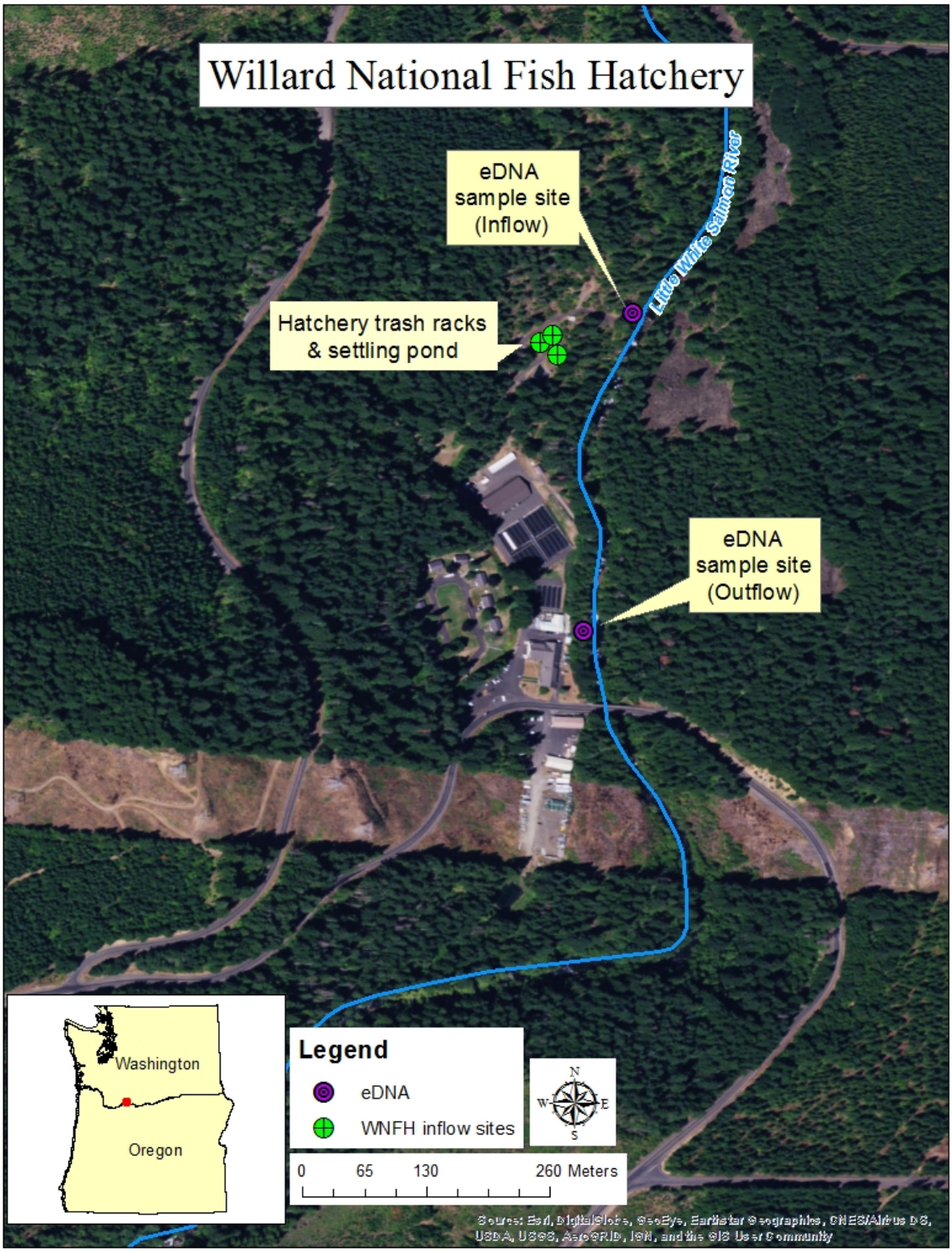


Figure 9: Willard NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

Figure 10: Warm Springs NFH New Zealand mudsnail, zebra and quagga mussel visual survey and eDNA sample locations, 2018.

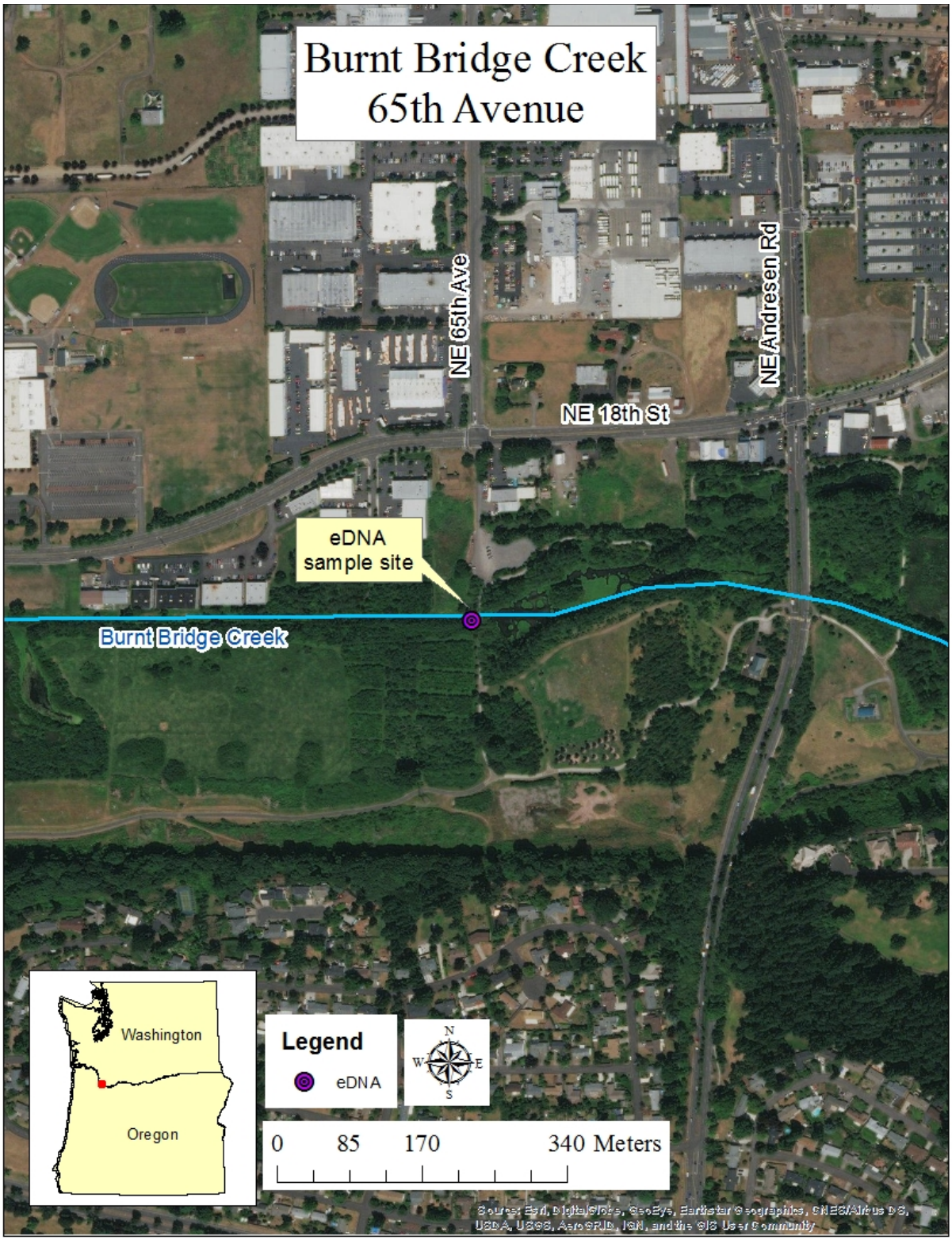


Figure 11: Burnt Bridge Creek (65th Ave.) NZMS visual survey and eDNA sample location, 2018.

Figure 12: Nestucca River NZMS visual survey and eDNA sample location, 2018.

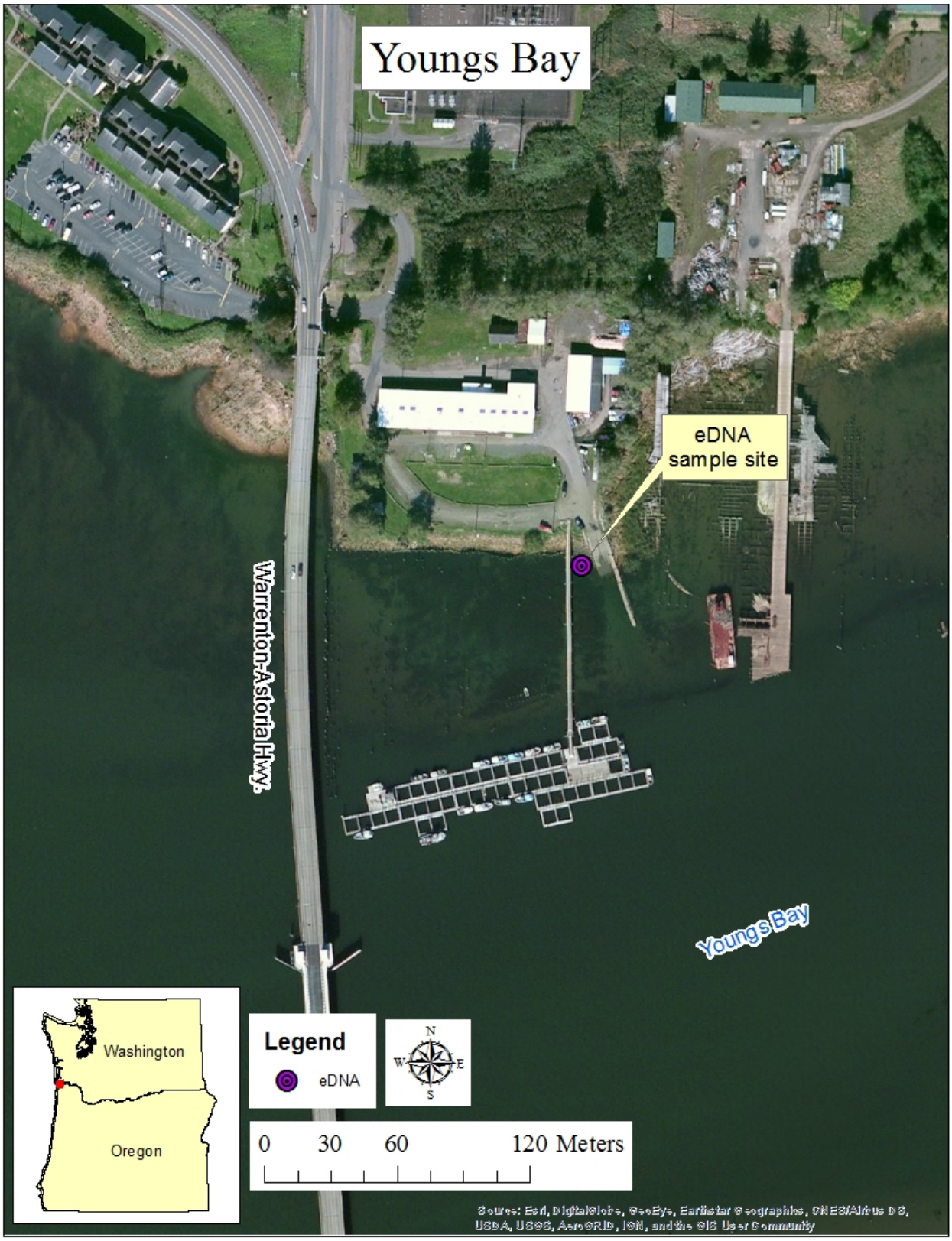


Figure 13: Youngs Bay NZMS visual survey and eDNA sample location, 2018.

Figure 14: Deschutes River NZMS visual survey and eDNA sample location, 2018.

Figure 15: Columbia/Kalama River NZMS visual survey and eDNA sample location, 2018.

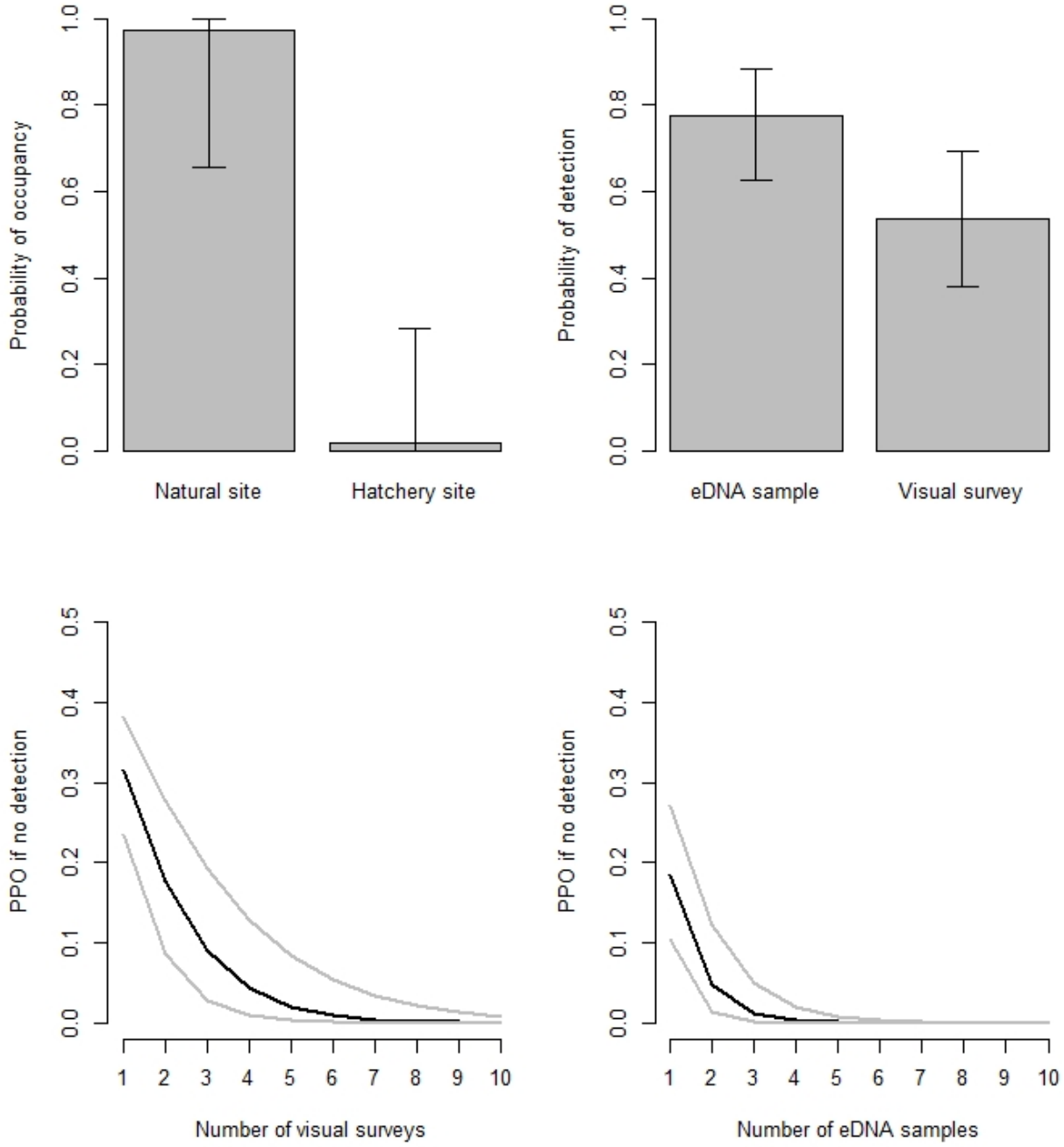


Figure 16: For NZMS, probability of occupancy (upper left panel); probability of detection by sampling method (upper right panel); and the posterior probability of occupancy if no individuals are detected ("PPO if no detection) when 1-10 replicates of either visual surveys (bottom left panel) or eDNA samples (bottom right panel) are completed. Grey bars (upper panels) and black lines (lower panels) represent median posterior estimates. Error bars (upper panels) and grey lines (lower panels) represent 95% credible intervals.

Table 1: New Zealand mudsnail, zebra, and quagga mussel survey locations at lower Columbia River National Fish Hatcheries and five locations with verified NZMS presence, 2018.

Date	National Fish Hatchery/water body	Location	Specimens Collected	NZMS Observed?	GPS Coordinate System: UTM			
					Zone	Datum	Northing	Easting
8/30/2018	Carson	**Outflow**- pollution abatement pond	Sample #3	No	10	NAD 1983 (Conus)	5079904.52	579523.87
8/30/2018	Carson	**Outflow-** raceway outflow, adult fish ladder enterance	None	No	10	NAD 1983 (Conus)	5079887.72	579566.79
8/30/2018	Carson	**Outflow**- earthen pond outflow	Sample #4	No	10	NAD 1983 (Conus)	5079810.35	579755.90
8/30/2018	Carson	**Inflow-** hatchery intake grate	None	No	10	NAD 1983 (Conus)	5079956.49	579685.42
8/30/2018	Carson	**Inflow**- Tyee Springs headwaters	None	No	10	NAD 1983 (Conus)	5080730.12	579847.02
9/5/2018	Eagle Creek	**Outflow**- adult fish ladder enterance	None	No	10	NAD 1983 (Conus)	5013906.60	562421.74
9/5/2018	Eagle Creek	**Outflow**- lower raceway outflow	None	No	10	NAD 1983 (Conus)	5013895.52	562559.21
9/5/2018	Eagle Creek	**Outflow**- upper raceway outflow	None	No	10	NAD 1983 (Conus)	5014006.36	562723.31
9/5/2018	Eagle Creek	**Inflow-**microfilter channel	None	No	10	NAD 1983 (Conus)	5014086.22	562989.38
9/5/2018	Eagle Creek	**Inflow**- hatchery water intake grate	None	No	10	NAD 1983 (Conus)	5014099.46	563102.52
8/31/2018	Little White Salmon	**Inflow-** hatchery water intake grate	None	No	10	NAD 1983 (Conus)	5064147.59	606184.79
8/31/2018	Little White Salmon	**Inflow-** Baily Springs	None	No	10	NAD 1983 (Conus)	5063946.69	605846.84
8/31/2018	Little White Salmon	**Outflow-** clarifier	Sample #5	No	10	NAD 1983 (Conus)	5063984.24	605805.54
8/31/2018	Little White Salmon	**Outflow -** raceway outflow	None	No	10	NAD 1983 (Conus)	5064054.57	605777.68
8/31/2018	Little White Salmon	**Inflow**- hillside Springs	None	No	10	NAD 1983 (Conus)	5063841.55	605439.42
8/31/2018	Little White Salmon	**Inflow**- Stairway Springs (2)	None	No	10	NAD 1983 (Conus)	5063633.15	605223.51
8/31/2018	Little White Salmon	**Drano Lake boat ramp**	Sample #6, 7, 8	No	10	NAD 1983 (Conus)	5062802.74	605922.67
9/4/2018	Spring Creek	**Inflow-** hillside spring #1	Sample #9	No	10	NAD 1983 (Conus)	5064912.53	613051.39
9/4/2018	Spring Creek	**Inflow-** hillside spring #2	None	No	10	NAD 1983 (Conus)	5064917.46	613126.89
9/4/2018	Spring Creek	**Inflow**- hillside spring #3	Sample #10	No	10	NAD 1983 (Conus)	5064925.67	613194.19
9/4/2018	Spring Creek	**Inflow**- hillside spring #4	None	No	10	NAD 1983 (Conus)	5064924.02	613223.74
9/4/2018	Spring Creek	**Outflow-** raceway outflow, adult fish ladder enterance	Sample #11, 12, 13	No	10	NAD 1983 (Conus)	5064795.99	613286.11
8/29/2018	Willard	**Inflow-** hatchery water intake grate	None	No	10	NAD 1983 (Conus)	5069171.79	606561.39
8/29/2018	Willard	**Inflow-** hatchery water trash rack #1 (small)	None	No	10	NAD 1983 (Conus)	5069128.52	606480.41
8/29/2018	Willard	**Inflow-** hatchery water settling pond	Sample #1, 2	No	10	NAD 1983 (Conus)	5069138.00	606462.99
8/29/2018	Willard	**Inflow-** hatchery water trash rack #2 (large)	None	No	10	NAD 1983 (Conus)	5069108.23	606463.50
8/29/2018	Willard	**Outflow-** lower raceway outflow	None	No	10	NAD 1983 (Conus)	5068838.25	606507.24
9/6/2018	Warm Springs	**Inflow-** hatchery water intake grate	sample #14	No	10	NAD 1983 (Conus)	4968957.62	638644.45
9/6/2018	Warm Springs	**Outflow-** raceway outflow, adult fish ladder enterance	sample #15	No	10	NAD 1983 (Conus)	4968973.52	638652.93
9/6/2018	Warm Springs	**Outflow-** adult holding pond outflow pipe	None	No	10	NAD 1983 (Conus)	4968988.00	638661.00
9/6/2018	Warm Springs	**Outflow**- pollution abatement pond	Sample #16, 17	No	10	NAD 1983 (Conus)	4969124.03	638722.88
9/13/2018	Burnt Bridge Creek	Burnt Bridge Creek Trail pedestrian bridge at 65th	Sample #22, 23	Yes	10	NAD 1983 (Conus)	5053521.00	530788.83
9/10/2018	Columbia River	Kalama River Sportsmans Club sand boat launch	Sample #18	No	10	NAD 1983 (Conus)	5098395.00	509714.00
9/11/2018	Deschutes River	Downstream from Celilo Wasco Hwy.	Sample #19, 20	No	10	NAD 1983 (Conus)	5055509.00	662500.00
9/12/2018	Nestucca River	Boat launch near Pacific City	Sample #21	Yes	10	NAD 1983 (Conus)	5006341.28	424560.24
9/7/2018	Young's Bay	Public boat ramp at Astoria Recreation Center	None	Yes	10	NAD 1983 (Conus)	5113355.94	435411.86

Table 2: Summary of freshwater mollusk genera observed at lower Columbia River National Fish Hatcheries, 2018.

Survey Location	Freshwater Mollusk Genera															
	Ancylidae	**Cyrenidae**	**Hydrobiidae**			**Lymnaeidae**		**Physidae**	**Planorbidae**				**Pleuroceridae**		**Sphaeriidae**	**Unionidae**
	Ferrissia rivularis	*Corbicula fluminea*	*Colligyrus greggi*	*Fluminicola sp.*	*Potamopyrgus antipodarum*	*Galba parva*	*Radix auricularia*	*Physella gyrina*	*Planorbella subcrenata*	*Menetus opercularis*	*Vorticifex effusa*	*Unknown Planorbidae*	*Juga (Juga) sp.*	*Juga plicifera*	*Unknown pea clam*	*Oregon floater*
Carson: fish ladder/ raceway outflow	X		X							X						
Carson: abatement pond perimeter	X									X						
Carson: earthen pond outflow			X							X					X (shell)	
Carson: hatchery intake grate			X							X						
Carson: Tyee Springs headwaters			X			X				X						
Eagle Creek: fish ladder outflow	X															
Eagle Creek: lower raceway outflow	X											X				
Eagle Creek: upper raceway outflow	X															
Eagle Creek: microfilter channel													X			
Eagle Creek: hatchery intake grate													X			
Little White Salmon: intake grate																
Little White Salmon: Baily Springs																
Little White Salmon: clarifier						X				X						
Little White Salmon: raceway outflow																
Little White Salmon: hillside springs													X			
Little White Salmon: Stairway springs (2)													X			
Little White Salmon: Drano Lake boat ramp				X				X			X					
Spring Creek: hillside spring #1													X			
Spring Creek: hillside spring #2										X			X		X	
Spring Creek: hillside spring #3										X			X		X	
Spring Creek: hillside spring #4										X			X			
Spring Creek: fish ladder outflow		X		X				X			X		X	X		
Willard: hatchery intake grate																
Willard: hatchery trash rack #1																
Willard: hatchery trash rack #2																
Willard: hatchery water settling pond												X				
Willard: lower raceway outflow																
Warm Springs: hatchery intake grate				X				X	X (shell)							
Warm Springs: fish ladder outflow	X										X		X			
Warm Springs: adult pond discharge																
Warm Springs: abatement pond	X						X	X	X							
Burnt Bridge: 65th pedestrian bridge	X			X	X			X				X				
Columbia R.: Kalama R. Sportsmans launch		X		X												X
Deschutes River: mouth		X		X				X				X				X (shell)
Nestucca River: boat ramp near Pacific City					X											
Youngs Bay: Astoria Rec. Center launch					X											

Table 3: Habitat characteristics and eDNA results at lower Columbia River National Fish Hatcheries and five locations with verified NZMS presence, 2018.

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	eDNA taken (Y/N)	eDNA Results (+/-)	NZMS DNA (copies/mL)
Carson	1	Abatement pond	8:34 AM	8:49 AM	14.0	0.60	0	2	1	1,2	N	-	0.0
Carson	2	Adult ladder outflow	9:00 AM	9:38 AM	10.0	0.80	4	1	2	1,2	Y	-	0.0
Carson	3	Earthen pond outflow	9:49 AM	10:02 AM	10.0	0.75	1	0	0	1,2	N	-	0.0
Carson	4	Intake grate	10:08 AM	10:27 AM	10.0	2.0+	1	1	3	1,2	Y	-	0.0
Carson	5	Tyee Springs headw ater	10:39 AM	10:52 AM	9.0	0.30	4	1	4	1,2	N	-	0.0
Eagle Creek	1	Adult ladder outflow	8:51 AM	9:04 AM	16.0	0.87	4	0	0	1,2	N	-	0.0
Eagle Creek	2	Low er racew ay outflow	9:18 AM	9:37 AM	18.0	0.95	5	0	0	1,2	Y	-	0.0
Eagle Creek	3	Upper racew ay outflow	9:50 AM	9:57 AM	19.0	0.41	4	0	0	1,2	N	-	0.0
Eagle Creek	4	Microfilter channel	10:02 AM	10:12 AM	19.0	1.80	0	0	0	2,5	N	-	0.0
Eagle Creek	5	Intake grate	10:20 AM	10:36 AM	18.0	1.50	0	0	0	1,2	Y	-	0.0
Little White	1	Intake grate	8:56 AM	9:08 AM	13.0	2.0+	concrete	0	0	2	Y	-	0.0
Little White	2	Bailey Springs	9:21 AM	9:25 AM	15.0	0.10	4	0	0	5	N	-	0.0
Little White	3	Clarifier outflow	9:27 AM	9:37 AM	14.0	0.30	4	0	0	1,2,5	N	-	0.0
Little White	4	Racew ay outflow	9:40 AM	9:46 AM	16.0	0.16	concrete	0	0	1,2	N	-	0.0
Little White	5	Hillside Springs	9:54 AM	10:04 AM	18.0	0.11	4	0	0	5	N	-	0.0
Little White	6	Stairw ay Springs (South)	10:09 AM	10:14 AM	17.0	0.06	3	0	0	5	N	-	0.0
Little White	7	Stairw ay Springs (North)	10:16 AM	10:19 AM	18.0	0.05	5	0	0	5	N	-	0.0
Little White	8	Drano Lake boat ramp	10:28 AM	10:55 AM	25.0	2.0*	0	1	1	1,2,5	Y	-	0.0
Spring Creek	1	Hillside Spring #1 (West)	8:39 AM	8:51 AM	11.5	0.49	0	0	0	1,2,5	N	-	0.0
Spring Creek	2	Hillside Spring #2	8:54 AM	9:11 AM	10.5	1.03	0	0	0	1,2	N	-	0.0
Spring Creek	3	Hillside Spring #3	9:13 AM	9:28 AM	10.5	0.10	0	2	4	1,5	N	-	0.0
Spring Creek	4	Hillside Spring #4 (East)	9:38 AM	9:52 AM	14.0	1.31	1	1	2	1,2	Y	-	0.0
Spring Creek	5	Adult ladder outflow	10:18 AM	10:48 AM	23.0	1.71	4	0	0	1,2	Y	-	0.0

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)			

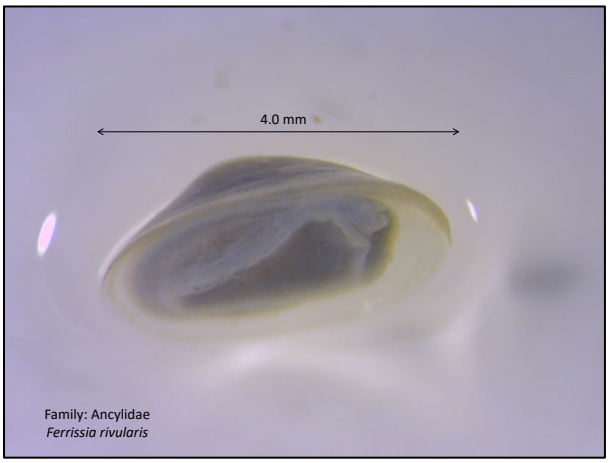
National Fish Hatchery	Site #	Site Description	Survey Begin Time	Survey End Time	Temp (°C)	Max Depth (m)	Dominant Substrate	Dominant Aquatic Vegetation	% Aq. Veg. Cover	Sample methods used	eDNA taken (Y/N)	eDNA Results (+/-)	NZMS DNA (copies/mL)	NZMS DNA in Sample (copies total)
Willard	1	Intake grate	9:07 AM	9:28 AM	6.5	2.0+	1	0	0	1,2	Y	-	0.0	0.0
Willard	2	Trash rack #1	9:36 AM	9:43 AM	7.0	0.62	2	0	0	2,5	N	-	0.0	0.0
Willard	3	Upper H_2O settling pond	9:45 AM	10:00 AM	8.5	0.85	1	0	0	1,2,5	N	-	0.0	0.0
Willard	4	Trash rack #2	10:05 AM	10:26 AM	7.5	2.0+	0	2	1	1,2	N	-	0.0	0.0
Willard	5	Low er racew ay outflow	10:46 AM	10:58 AM	8.0	0.50	4	0	0	1,2,5	Y	-	0.0	0.0
Warm Springs	1	Intake grate	9:15 AM	9:32 AM	20.5	1.80	0	1	4	1,2	Y	-	0.0	0.0
Warm Springs	2	Adult ladder outflow	9:42 AM	9:50 AM	21.0	1.75	5	0	0	1,2	N	-	0.0	0.0
Warm Springs	3	Adult pond outflow pipe	9:52 AM	9:58 AM	18.0	0.05	5	0	0	1,5	N	-	0.0	0.0
Warm Springs	4	Abatement pond outflow	10:06 AM	10:22 AM	20.0	0.85	0	1	2	1,2	Y	-	0.0	0.0
Sample Area	**Sample #**	**Site Description**	**Survey Begin Time**	**Survey End Time**	**Temp (°C)**	**Max Depth (m)**	**Dominant Substrate**	**Dominant Aquatic Vegetation**	**% Aq. Veg. Cover**	**Sample methods used**	**eDNA taken (Y/N)**	**eDNA Results (+/-)**	**NZMS DNA (copies/mL)**	**NZMS DNA in Sample (copies total)**
Burnt Bridge Cr.	1	65th ped. Bridge - North bank	8:14 AM	8:42 AM	16.5	0.62	0	1	2	1,2	Y	+	3.1	3082.7
Burnt Bridge Cr.	2	65th ped. Bridge - mid channel									Y	+	1.9	1943.0
Burnt Bridge Cr.	3	65th ped. Bridge - South bank									Y	+	4.5	4534.5
Columbia River	1	North of sand launch	8:45 AM	9:20 AM	20.5	0.45	1	1	1	1,2	Y	-	0.0	0.0
Columbia River	2	North of sand launch									Y	+	0.1	91.1
Columbia River	3	North of sand launch									Y	-	0.0	0.0
Deschutes River	1	Under Celilo Road bridge	9:15 AM	10:33 AM	17.0	0.70	1	1	1	1,2	Y	+	0.2	227.6
Deschutes River	2	Under railroad bridge									Y	-	0.0	0.0
Deschutes River	3	Slough outlet near Hw y. 84									Y	-	0.0	0.0
Nestucca River	1	North side of boat ramp	9:55 AM	10:23 AM	19.0	0.65	0	0	0	1,2	Y	+	1.8	1839.5
Nestucca River	2	Middle boat ramp									Y	+	3.4	3365.2
Nestucca River	3	South side of boat ramp									Y	+	1.7	1656.5
Young's Bay	1	6 m West of public boat launch	9:41 AM	10:02 AM	19.0	0.48	4	1	1	1,2	Y	+	77.9	77938.4
Young's Bay	2	3 m West Public boat launch									Y	+	215.5	215456.5
Young's Bay	3	0.5 m West Public boat launch									Y	+	68.5	68548.4

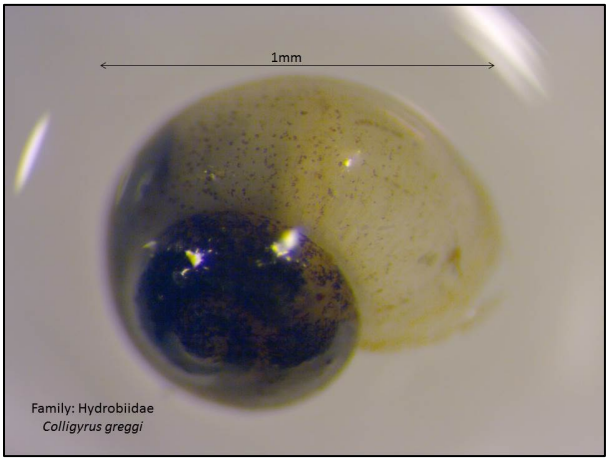
	Substrate Type	Aquatic Vegetation Type	% Aquatic Veg. Cover	Sample Method
	0 = silt,clay,organic material (<0.059mm)	0 = No vegetation	0 = No vetatation	1 = w ading
	1 = Sand (0.06-1mm)	1 = Submerged	1 = 0-25%	2 = aquascope
	2 = Gravel (2-15mm)	2 = Emergent	2 = 26-50%	3 = hand net
	3 = Pebble (16-63mm)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

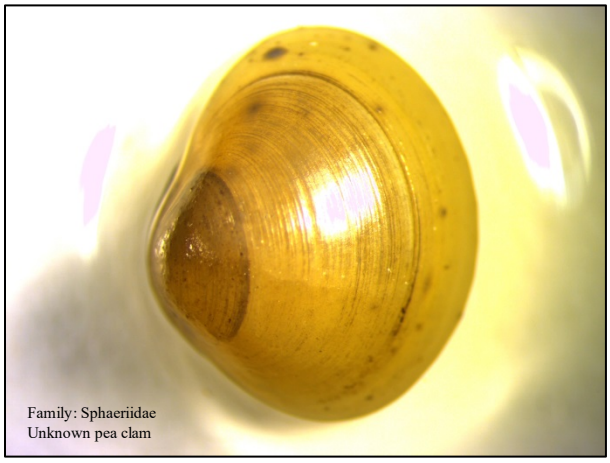
Table 4: Median posterior estimates (and 95% credible intervals) for parameters in the occupancy model of NZMS in hatchery and natural areas detected using eDNA or visual surveys.

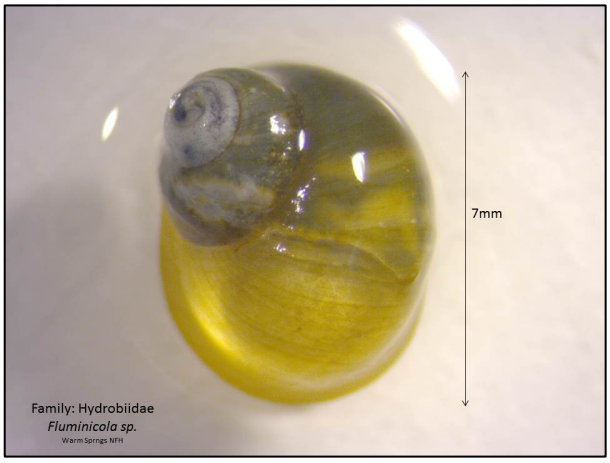
Parameter	Estimate (95% CI)
Intercept for probability of occupancy (logit scale)	-3.89 (-7.83 – -0.93)
Coefficient for natural area (logit scale)	8.17 (3.88 – 9.93)
Probability of occupancy in a natural area	0.97 (0.66 – 1.00)
Probability of occupancy in a hatchery	0.02 (0.00 – 0.28)
Intercept for probability of detection (logit scale)	0.15 (-0.49 – 0.81)
Coefficient for eDNA (logit scale)	1.08 (0.11 – 2.12)
Probability of detection by an eDNA sample (i.e., $p.eDNA$)	0.77 (0.63 – 0.89)
Probability of detection by a visual survey (i.e., $p.Visual$)	0.54 (0.38 – 0.69)

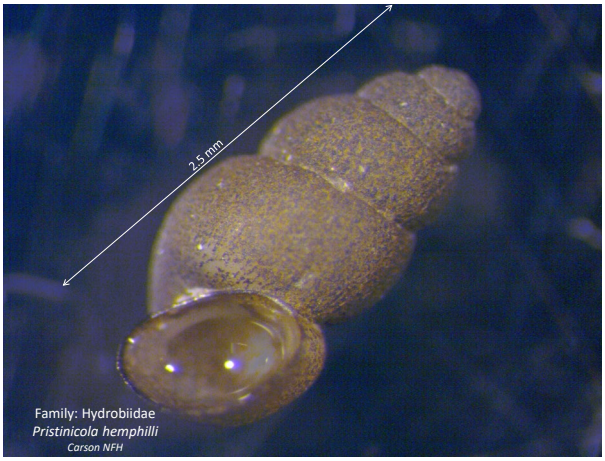
Appendix A: Photographs of Snail Specimen

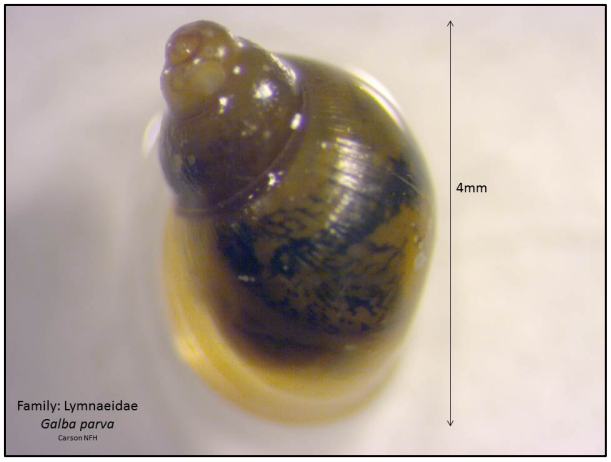


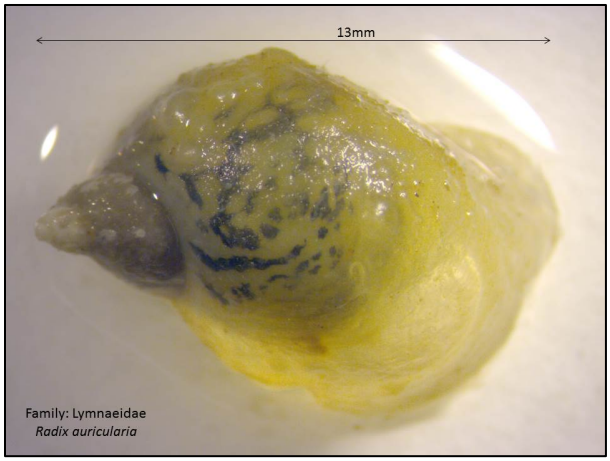








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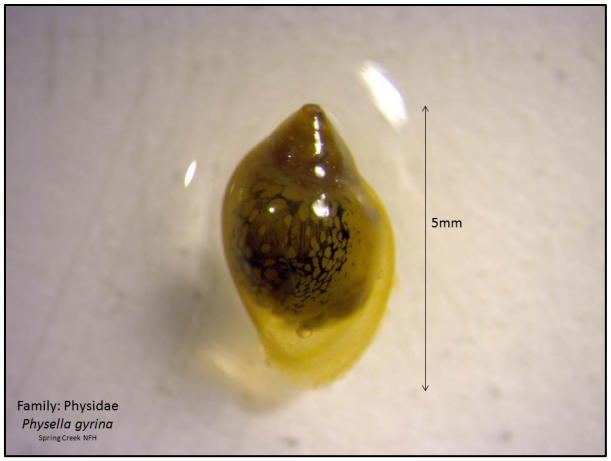
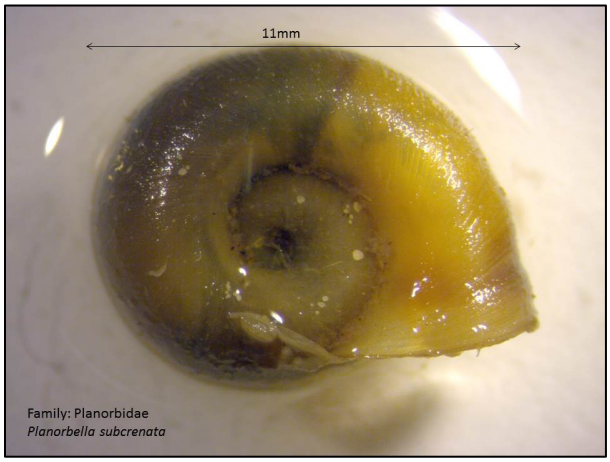
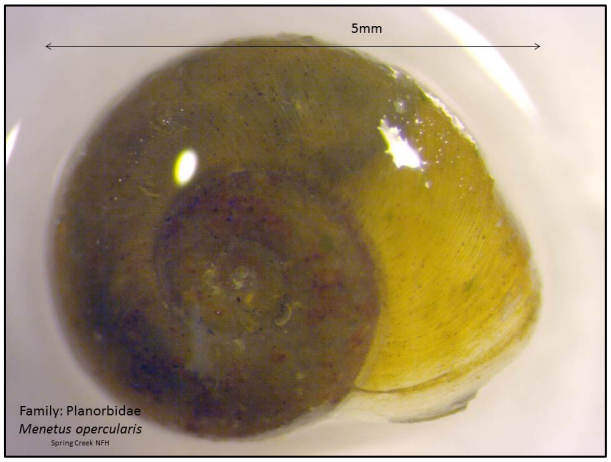
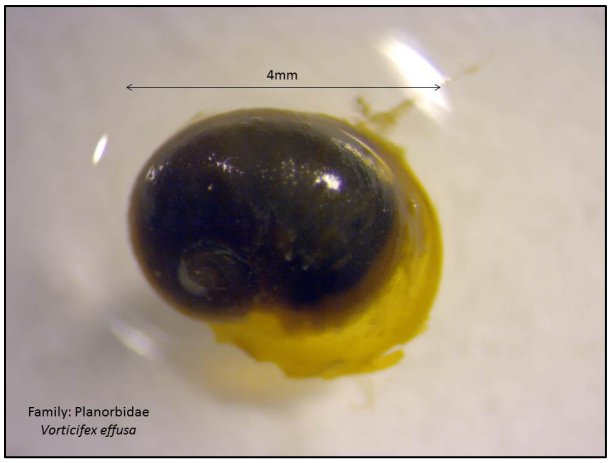
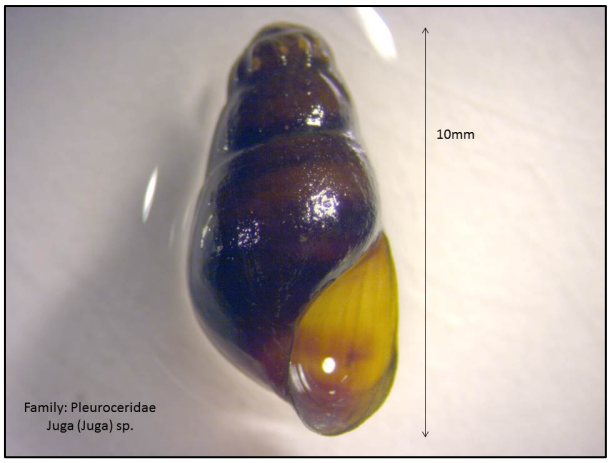
Radix auricularia

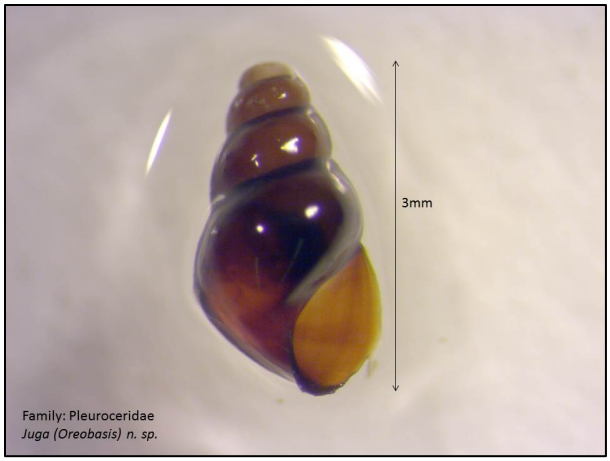

5mm
Family: Physidae
Physella gyrina
Spring Creek NFH

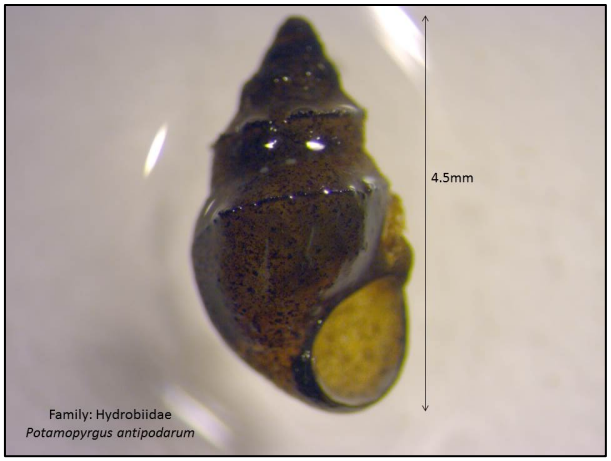

11mm
Family: Planorbidae
Planorbella subcrenata


5mm
Family: Planorbidae
Menetus opercularis
Spring Creek NFH


4mm
Family: Planorbidae
Vorticifex effusa


10mm
Family: Pleuroceridae
Juga (Juga) sp.





New Zealand mudsnail – Burnt Bridge at 65th Ave.

New Zealand mudsnail – Youngs Bay

Zebra Mussels. Photo Credit: ANS Task Force

Corbicula fluminea – Asian clam

Quagga Mussels. Photo Credit: ANS Task Force

U.S. Fish and Wildlife Service
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Vancouver, WA 98683

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