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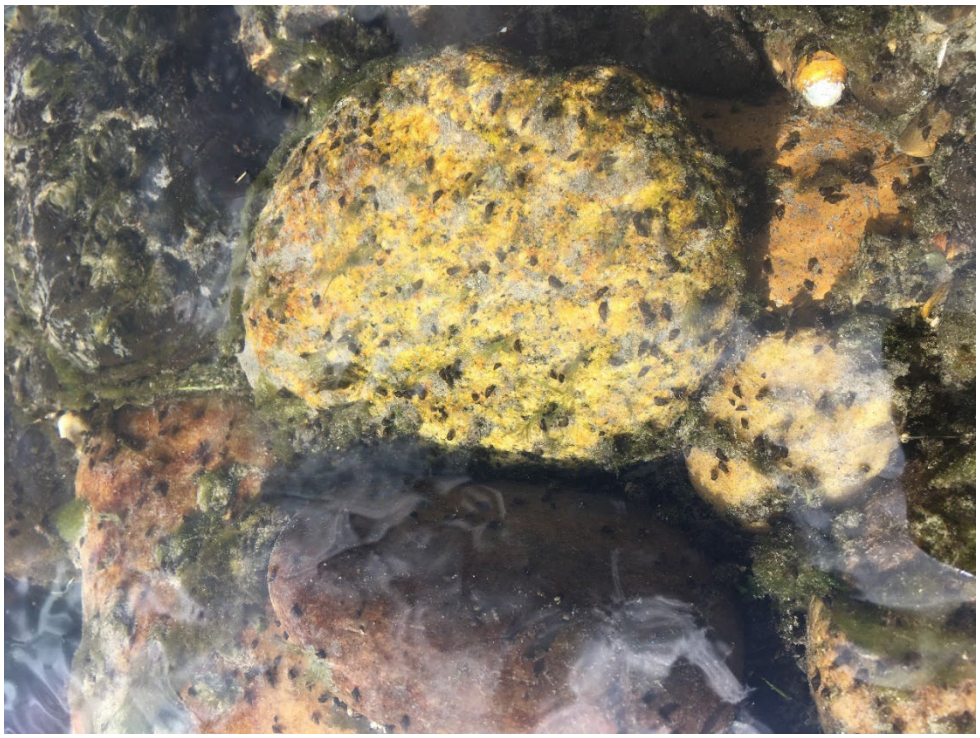
Reliability of visual surveys and eDNA sampling to detect a new invasion of New Zealand mudsnail

2023 Annual Report



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On the cover: *New Zealand mudsnails clinging to cobble in the Upper Columbia River near Mesa, Washington, photo by Jen Poirier.*

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2023 ANNUAL REPORT

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Abstract

Early detection and monitoring of aquatic invasive species (AIS) are vital in preventing their establishment and reducing their spread in aquatic habitats. The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum,* is an invasive aquatic snail that poses a threat to National Fish Hatcheries (NFHs). Conventional monitoring techniques may not reliably detect new infestations of NZMS due to the species' small size and cryptic coloration. Environmental DNA (eDNA) is becoming an increasingly common tool for early detection of AIS, though few studies have quantitatively evaluated the efficacy of eDNA or other early detection techniques for NZMS. In this study, we performed paired eDNA sampling and visual surveys from 15 sites at five waterbodies with documented NZMS occupancy. We used a single-season occupancy model to estimate method-specific detection probabilities and probabilities of occupancy, if NZMS are not detected at an examined site. We also used a multiscale occupancy model to better understand small-scale spatial and temporal patterns in the probability of detecting NZMS by eDNA. A total of 43 visual surveys were conducted and 126 eDNA samples were collected from the 15 sites from 2015 to 2022. New Zealand mudsnail eDNA was detected at 13 sites in all five waterbodies, while visual surveys detected NZMS in five sites and in four of the waterbodies. The probability of detecting eDNA of NZMS was higher than the probability of detecting NZMS with visual surveys in both large (0.69 vs. 0.20) and small (0.94 vs. 0.74) systems. Expected probabilities of occupancy, if NZMS were not detected during sampling, were less than 0.05 when two samples of eDNA were collected in a small waterbody, three samples of eDNA were collected in a large waterbody, three visual surveys were conducted in a small waterbody, and when 14 visual surveys were conducted in a large waterbody. We observed small-scale spatial and temporal variation in eDNA detection in four of six sites where NZMS were detected, potentially due to low abundance, patchy distribution of NZMS or variable environmental conditions. This study adds to the growing body of work demonstrating the effectiveness of eDNA as an AIS early detection and monitoring tool in NFH and other aquatic habitats. Our results provide valuable insight into the nuances of each sampling technique and will help inform future AIS monitoring programs at NFHs.

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Introduction

The introduction and spread of aquatic invasive species (AIS) are concerns for natural resource managers. Given the difficulty and high economic cost of eradicating AIS once they become established, early detection is critical for effective management and control. However, detecting AIS at the onset of invasion can be difficult if the target organism is cryptic, rare, has a limited distribution, or occurs in a habitat that is difficult to survey effectively (Hulme 2006; Harvey et al. 2009). The New Zealand mudsnail (NZMS), *Potamopyrgus antipodarum*, is an AIS that is particularly challenging to detect due to its small size and cryptic coloration.

Conventional monitoring techniques for NZMS (e.g., tactile and visual inspections, snorkeling, D-frame kick nets, Hess stream bottom samplers, artificial settlement substrates, benthic grabs, etc.) can injure benthic invertebrates, are labor intensive, and may not reliably detect snails when an infestation first occurs or when abundance is low. In contrast, the environmental DNA (eDNA) technique has become an increasingly popular tool for the early detection of AIS due to its minimal environmental impact, enhanced sensitivity and potential to detect species at low

densities. Environmental DNA is genetic material that is shed by an organism in 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. In aquatic ecosystems, eDNA could be a valuable technique to detect presence and monitor the spread of AIS (Jerde et al. 2013; Hunter et al. 2015; Davison et al. 2017; Cowart et al. 2018; Chucholl et al. 2021). However, relatively few studies have evaluated the use of eDNA to detect NZMS (Goldberg et al. 2013; Clusa et al. 2016; Thomas et al. 2019; Ponce et al. 2021; Woodell et al. 2021).

National Fish Hatcheries (NFHs) produce fish and other aquatic species, that provide commercial and recreational fishing opportunities, fulfil tribal trust and mitigation responsibilities, and contribute to the recovery of threatened and endangered species. Managing the threat of AIS is one of the many challenges hatchery managers face. Fish hatcheries may be more susceptible to NZMS invasion given their stable environment (i.e., water flow, temperature) and increased nutrient output. Many hatcheries are also located in close proximity to popular river access points such as boat ramps and hiking trails where NZMS may be spread more readily by recreational activities. Routine hatchery operations including the transport of fish or eggs to another hatchery, the movement of fish distribution equipment (e.g., fish hauling truck, tank, nets, transfer water) and fish stocking, each have the potential to introduce or spread NZMS to new waterbodies or hatchery facilities (ANSTF 2007). If NZMS become established within a fish hatchery, they can increase labor and operational costs, damage equipment or infrastructure (e.g., water delivery pipes, filters, screens, pumps, etc.) and require the facility to undergo extensive decontamination and fish depuration procedures (Bruce 2006; Oplinger et al. 2011; Nakano and Strayer 2014). In extreme cases the hatchery may be forced to modify infrastructure (e.g., isolate rearing ponds, add exclusion screens, install hydrocyclones or filter socks), suspend production or close down completely.

Conducting effective AIS surveillance at NFHs is often challenging due to excessive water depth, low visibility, or poor accessibility of hatchery intake and outflow areas. Additionally, the invasive NZMS is naturally cryptic and difficult to detect in low abundance. Environmental DNA is increasingly being used as a monitoring tool for early detection of AIS, though few studies have quantitatively evaluated the efficacy of eDNA or other early detection techniques at NFHs or for NZMS in any aquatic environment. The USFWS Columbia River Fish and Wildlife Conservation Office has performed annual visual (presence/absence) surveys and eDNA sampling for NZMS at lower Columbia Basin NFHs since 2015 (Poirier 2015; Poirier 2017; Poirier and Harris 2019). To assess the reliability of our survey techniques, we performed paired visual surveys and eDNA sampling at five sites with known NZMS occupancy and used an occupancy-modelling framework to estimate site occupancy and method-specific detection probabilities. The purpose of this analysis was to assess effectiveness of our survey techniques to inform future study design for early detection of NZMS and other AIS at NFHs. Specific

objectives were to: 1) estimate the detection probability of eDNA and visual surveys and to estimate probabilities of occupancy, if NZMS are undetected after sampling; and 2) identify potential spatial and temporal patterns in the probability of detecting NZMS by eDNA.

Methods

New Zealand Mudsnail Biology

The New Zealand mudsnail is a tiny freshwater snail that was likely introduced to North America through the discharge of contaminated ballast water (Zaranko et al. 1997; Gangloff 1998) or the shipment of fish or eggs via the commercial aquaculture industry (Bowler 1991; Bowler and Frest 1992). New Zealand mudsnail are highly adaptable and can inhabit both lentic (e.g., lakes, reservoirs) and lotic habitats (e.g., estuaries, rivers, streams, springs, canals), and tolerate a broad range of aquatic conditions (e.g., temperature, salinity, turbidity, water velocity, stream productivity and substrate types; see Geist et al. 2022). Adult NZMS range from 3-6 mm in length and have an elongate conical shell with 5-8 whorls coiled in a clockwise 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 solid operculum that allows the snail to seal off the shell opening making it impervious to mild pollutants and resistant to desiccation (Richards et al. 2004; Schisler et al. 2008; Romero-Blanco and Alonso 2019). The shell wall of NZMS is rigid and difficult for many species of fish to digest. In some instances, the snail may pass through the digestive tract of fish alive and unharmed (Bruce et al. 2009; Oplinger et al. 2009; Brenneis et al. 2011; Butkus and Rakauskas 2020). Fish released from an infested hatchery facility or reared in a contaminated river may introduce or expand the distribution of NZMS by dispersing live snails in their excrement (Vinson and Baker 2008; Bruce et al. 2009). In the United States, NZMS populations are comprised almost entirely of self-cloning parthenogenetic females which become sexually mature at 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). Densities of NZMS often undergo broad fluctuations both seasonally and annually depending on the climate, food resources, water temperature, water velocity, time of establishment, or natural dispersal (Moore et al. 2012; Geist et al. 2022). In the Columbia River estuary, NZMS have been observed in densities ranging from 16,000 to 84,000 snails/m^2 at a single location (Youngs Bay; Litton 2019). Large colonies of NZMS can dominate the invertebrate biomass, consume more than half of the gross primary productivity, and influence nitrogen and carbon cycling in a stream (Hall et al. 2003; Hall et al. 2006; Alonso and Castro-Díez 2008; Moore et al. 2012). In high densities, NZMS may outcompete and deplete available food resources for native snails and other grazing invertebrates (Moore et al. 2012; Larson and Black 2016), potentially impacting higher trophic level predators (Rakauskas et al. 2018).

NZMS Survey Locations

Five waterbodies with documented NZMS occupancy were sampled for NZMS including: Burnt Bridge Creek, Columbia/Kalama River, Lower Deschutes River, Nestucca River and Youngs Bay. One (Nestucca River, Youngs Bay) or four sites (Burnt Bridge Creek, Lower Deschutes River, Columbia/Kalama River) were surveyed within each waterbody using visual surveys and eDNA sampling (Table 1, Figure 1). These locations were obtained from the Nonindigenous Aquatic Species webpage (Benson et al. 2022), 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. Although our five waterbodies were designated as occupied between 2002 and 2013, current NZMS distribution and abundance within sites were unknown.

Visual Surveys

Visual presence/absence surveys were conducted annually over a two-week period in late August or early September (2015-2022). Surveys were focused on areas with suitable NZMS habitat characteristics (e.g., low water velocity, sand, silt, or cobble substrate, presence of aquatic vegetation or other in-channel cover) in water depths ranging from 0.3m-1.2m. One or two field personnel visually inspected up to a 20 meter long section of stream upstream and/or downstream of each survey site for a minimum of 10 minutes. Surface substrate was manually flipped over at haphazard intervals, aquatic vegetation was sifted through by hand and surfaces of structures (i.e., culverts, concrete walls, submerged wood, pilings) were closely examined (visually and by hand) for NZMS. In water depths greater than 0.6 m, substrate, aquatic vegetation and structures were visually inspected using an underwater viewing scope. If field personnel observed NZMS or an aquatic snail that could not be identified, a specimen was collected and taken back to the CRFWCO laboratory for species verification.

Environmental DNA Sample Collection & Filtration

Environmental DNA sampling was conducted over a two-week period in late August or early September (2015-2022), following protocols described in Goldberg and Strickler (2017). Environmental DNA grab samples were collected across the width of the waterbody (Burnt Bridge) or at regular intervals (<10m apart) moving either parallel or perpendicular to a single shoreline (Columbia/Kalama River, Lower Deschutes River, Nestucca River, Youngs Bay). A total of three eDNA grab samples (i.e., spatial replicates) were collected at each site. 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 control water sample was also collected at each site and processed in the same manner as field samples to assess the potential for sample contamination associated with handling and transport. Field negative controls 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 grab samples at all sites, one or two personnel performed a visual presence/absence survey for NZMS using the methods described above.

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 peristaltic 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. In 2020, folded membrane disks were placed in a Ziploc containing silica desiccant beads. Samples were labeled with a unique site code and stored at room temperature (vials with ethanol) or in a freezer (desiccant bags) until they were sent to Washington State University eDNA laboratory for analysis.

A general concern with the eDNA technique 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 laboratory. 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 solution 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) 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 30 minutes. Additionally, waders and boots were placed in a freezer (≈-14°C) overnight between use.

Spatial & Temporal Variation

In 2019, we assessed potential small-scale spatial and temporal variation in eDNA detection by repeatedly sampling eDNA from three separate sites in three of the five waterbodies (Burnt Bridge Creek, Lower Deschutes River, and Columbia/Kalama River; Figures 2, 3, and 4). To explore spatial variability, we collected three grab samples at three different locations within a site (e.g., right bank, midchannel, left bank). Spatial replicates were collected once per week for a total of three weeks to assess temporal variability in eDNA detection. Sites included the one location typically sampled during annual surveys, while the other two sites were selected haphazardly based upon ease of public access to the site (e.g., boat ramp, fishing access trail, pedestrian bridges) or the presence of permanent instream structures (e.g., pilings).

Environmental DNA assay

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).

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.

Introduction to Occupancy Analyses

We examined detection probability of NZMS using two occupancy models: 1) a single-season occupancy model and 2) a multiscale occupancy model. Occupancy models are hierarchical models in which each level of the hierarchy is estimated using a Bernoulli distribution (MacKenzie et al. 2006). A Bernoulli distribution is a discrete probability distribution in which each outcome (i.e., response) can only take on either a value of one or a value of zero, for example, "occupied" or "not occupied" and similarly, "detected" or "not detected". In an occupancy model, detection probability is usually assessed by conducting replicate surveys at multiple sites often at different times (i.e., temporal replicates); however, often when eDNA samples are collected, more than one sample is collected from a site, but from a slightly different location at the site (i.e., spatial replicates). In both cases, it is assumed that the occupancy state of the site (i.e., occupied or not) does not vary among the replicate surveys. First, we used a single-season (two-level) occupancy model (MacKenzie et al. 2006) to estimate detection probability of NZMS sampled by two methods, eDNA and visual survey, and to estimate posterior probabilities of occupancy, if NZMS are undetected, given varied levels of survey effort for each examined method. The purpose of this analysis was to guide future study design for early detection of NZMS. For this first analysis, we sampled at a total of 15 unique sampling sites (e.g., boat ramp, bridge) in five waterbodies occupied by NZMS (Burnt Bridge Creek, lower Deschutes River, Columbia/Kalama River, Nestucca River and Youngs Bay). Sampling was conducted during eight years (2015-2022) and each unique site was sampled 1-8 times (see Table 1). We did not assume occupancy status stayed the same over the years and thus for this analysis, a "site" was defined in the model by unique site and year, resulting in a total of 42 "sites" in this occupancy model. At each site, three grab samples of eDNA were collected and a visual presence/absence survey was conducted. Second, we used a multiscale occupancy model (three-level) described in Kéry and Royle (2016) to assess spatial and temporal patterns in the probability of detecting NZMS by eDNA. Although the amount of water filtered in an eDNA

grab is standardized, unlike visual surveys in which the area examined can be measured and the specific location of any detected species can be recorded, it is often unclear how much area was "examined" spatially by an eDNA grab or where exactly any detections might be geographically. Thus, it is often unknown if and how the distribution of eDNA in a system may differ from the distribution of live organisms in the system. In this second analysis, we evaluated detection of spatial and temporal replication for eDNA. This second analysis was conducted between 8/21 and 9/6 in 2019 in Burnt Bridge Creek, Columbia/Kalama River, and lower Deschutes River.

Detection of eDNA & Visual Surveys (Single-Season Occupancy Model)

To estimate detection probability of eDNA and visual sampling methods, we used a single-season occupancy model with two hierarchical levels (MacKenzie et al. 2006; Kéry and Schaub 2012). The first hierarchical level is the state process to estimate the true probability that a site (i) is occupied by NZMS (z_i):

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

Although all five waterbodies were occupied by NZMS, densities and distributions were largely unstudied and likely vary spatially and temporally. To allow for differences in the probability of site occupancy among the five examined waterbodies, the probability a specific site was occupied (z_i) was estimated from the probability of site occupancy (ψ_i), which was modeled on the logit scale as a function of waterbody (S_i):

$$Logit(\psi_i) = \alpha_0 + \beta_0(S_i)$$

Where α_0 is the intercept for the occupancy portion of the model and β_0 is the slope for the effect of waterbody on the probability of occupancy.

The second level of the model is the observation process or the probability of detecting NZMS in one sample of eDNA ($p_{D_{i,j}}$) or visually ($p_{V_{i,j}}$), given that the site is occupied (i.e., given that z_i=1). For this model, four replicate surveys were completed at each of the 42 sites, three by eDNA ($D_{i,j}$) and one by visual survey ($V_{i,j}$). Detection probability of eDNA ($p_{D_{i,j}}$) was estimated based on three replicate samples (j) collected at each site (i) and the probability that the specific site was occupied (z_i):

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

Detection probability of eDNA ($p_{D_{i,j}}$) was modeled on the logit scale as a function of the size of the waterbody at the site (R_i) which was categorized as large (>100 m) or small (<100 m):

$$Logit(p_{D_{i,j}}) = \alpha_1 + \beta_1(R_i)$$

Where α_1 is the intercept for the eDNA detection portion of the model and β_1 is the slope for the effect of size on the probability of detection by eDNA. We also estimated detection probability of NZMS observed by visual survey of the site ($p_{V_{i,j}}$). Visual detection probability ($p_{V_{i,j}}$) was estimated based on one replicate (i.e., $j = 1$) visual survey ($V_{i,j}$) conducted at each site (i):

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

Detection probability of visual surveys ($p_{V_{i,j}}$) was also modeled on the logit scale as a function of a categorical variable (i.e., large or small) for waterbody size (R_i), as well as a function of the average number of eDNA copies collected per ml of water sampled at the site (N_i):

$$Logit\left(p_{V_{i,j}}\right) = \alpha_2 + \beta_2(R_i) + \beta_3(N_i)$$

As above, α_2 in the intercept and β_2 and β_3 are slopes. We included a slope for the average number of eDNA copies per ml of water sampled to evaluate whether there was a correlation between eDNA copies in the water and the probability of visually detecting NZMS.

We used these estimates of detection probability in a model developed by Peterson and Dunham (2003) from Bayes Theorem to estimate expected probabilities of occupancy (O), if NZMS are not detected during sampling:

$$O = \frac{P(C/F)}{1 + P(C/F)}$$

Where $P(C/F)$ is the probability of not detecting NZMS given different numbers (n) of surveys and our estimates of detection probability (p_t) of eDNA in a small or large waterbody or visual survey in a small or large waterbody (t):

$$P(C/F) = (1 - p_t)^n$$

Estimates of the expected probabilities of occupancy (O) if NZMS are not detected during different levels of survey effort can help guide sampling for early detection of NZMS.

*Spatial & Temporal Patterns in Detection Probability (*M*ultiscale Occupancy Model)*

We used a multiscale model with three hierarchical levels to examine spatial and temporal patterns in detection probability of eDNA for detecting NZMS (Kéry and Royle 2016). The first level was the probability that a site of interest (again, boat ramp, bridge, etc.) was occupied in an occupied waterbody (e.g., lower Deschutes River). The second level was the probability an eDNA grab location at an occupied site was also occupied with NZMS eDNA, which was termed "availability". Since availability is the probability that a grab location in an occupied site is also occupied, we used it in this analysis to assess spatial variability in detection of NZMS by eDNA. The third level is the probability that a grab will detect eDNA of NZMS when it is available (i.e., grab location is occupied). Detection probability was estimated by collecting three temporally replicate eDNA grabs at each grab location within the site. All temporally replicate grabs at each grab location were collected between 8/21 and 9/6 in 2019.

Similar to the first occupancy analysis, the first level is the probability that a specific site (i) in an occupied waterbody is occupied by NZMS (z_i):

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

Similarly, we included a categorical covariate to allow for differences in the probability of site occupancy (due to differences in density or distribution) among the occupied waterbodies examined. Thus, the probability that a specific site was occupied (z_i) was estimated from the probability of site occupancy (ψ_i), which was modeled on the logit scale as a function of waterbody (S_i):

$$Logit(\psi_i) = \alpha_3 + \beta_4(S_i)$$

To estimate the number of occupied sites (out of the 9 sampled) we summed z_i in the model. The second level of the hierarchy, the probability that an eDNA grab location (j) in an occupied site was also occupied by NZMS eDNA or "availability ($a_{i,j}$)" was estimated:

$$a_{i,j} \sim Bernouli(A_{i,j})$$

The probability that a grab location was occupied ($A_{i,j}$) was a function of the probability of occupancy for the specific site (z_i) and the probability that a grab location in an occupied site will be occupied (p_S):

$$A_{i,j} = z_i * p_S$$

Since p_S is the probability that a grab location at an occupied site is occupied, it potentially informs about spatial distribution of NZMS eDNA on a small scale and detection probability of spatial replicates, which could be more efficient in some situations, than temporal replicates. To identify the number of occupied grab locations, we summed $a_{i,j}$ within the model.

The final level is the probability of detecting NZMS eDNA from a temporally replicate survey at a grab location ($L_{i,j,k}$), given that the grab location is occupied (i.e., $a_{i,j}$ = 1) and detection occurs at the time of sampling (p_T):

$$L_{i,j,k} \sim Bernouli(p_T * a_{i,j})$$

Since p_T is the probability of detecting NZMS eDNA at the time of collection at a grab location that is occupied, it potentially informs about temporal patterns in detection of NZMS eDNA.

We analyzed these two occupancy models by Bayesian methods using JAGS software (Plummer 2003) called from Program R (R Core Team 2013) with code modified from Kéry and Royle (2016). Logit scale priors for intercepts ($\alpha_1, \alpha_2, \alpha_3$) and slopes ($\beta_1$, β_2, β_3) were uniform distributions (range -10 to 10). Priors for p_T and p_S were also uniform distributions (range 0 to 1). Inference was completed using Package jagsUI with function autojags (Kellner 2017) for 3 chains, an adaption period of 10,000 iterations, a burn in period of 10,000, and an iteration increment of 50,000. Enough iterations were saved 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). Unless otherwise noted, posterior distributions are described by the median for central trend (i.e., estimate), and 95% credible intervals (CI) for precision.

Results

NZMS visual surveys

Forty-three visual surveys were conducted in five waterbodies with known NZMS occupancy during 2015-2022 (Table 1). New Zealand mudsnail were observed during 14 surveys (33%), at five of the 15 unique sites and in four of the five waterbodies with documented occupancy (Burnt Bridge Creek, Nestucca River, Youngs Bay and Columbia/Kalama River). Field personnel observed NZMS during nine of 13 surveys at Burnt Bridge Creek, one of two surveys at

Nestucca River, three of three surveys at Youngs Bay and one of 12 surveys conducted at Columbia/Kalama River. Relative NZMS abundance was highest in Young's Bay (≈800 snail/m^2), moderate in Burnt Bridge Creek (≈20 snail/m^2) and low in the Nestucca River (≈2 snail/m^2) and Columbia/Kalama River (≈1 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 (Benson et al. 2022).

Environmental DNA

A total of 126 eDNA grab samples were collected in five waterbodies with known NZMS occupancy from 2015-2022 (Table 1). New Zealand mudsnail eDNA was detected in 13 unique sites and in all five waterbodies with documented occupancy. All grab samples collected at Young's Bay and the Nestucca River (15 total) tested positive for the presence of NZMS eDNA, while 36 out of 39 samples tested positive for NZMS in Burnt Bridge Creek, 17 out of 36 samples tested positive in the Deschutes River and 13 of 36 samples tested positive in the Columbia/Kalama River. Concentrations of NZMS eDNA within positive grab samples ranged from 0.04 - 0.90 copies/ml in the lower Deschutes, 0.05 – 9.40 copies/ml in the Columbia/Kalama River, 1.66 – 20.76 copies/ml in the Nestucca River, 0.15 – 146.42 copies/ml in Burnt Bridge Creek and 37.86 - 865.67 copies/ml in Youngs Bay (Table 1). All field negative controls taken at sites with known NZMS occupancy (42 total) tested negative for the presence of NZMS eDNA.

Spatial Temporal Sampling

A total of 81 eDNA grab samples were collected at nine different sites in three occupied waterbodies over a three-week period in 2019. The presence of NZMS eDNA was detected in samples from 12 of 27 grab locations at six sites in the three waterbodies (Table 2). Burnt Bridge Creek was the only waterbody where NZMS eDNA was detected in all three spatial replicates during the entire three-week study period (Sites #1 and #2). Both spatial and temporal distribution of eDNA detection varied widely in four other sites: Burnt Bridge Site #3, lower Deschutes Site #1 and #3, and Columbia/Kalama Site #1. No NZMS eDNA was detected in any sample replicate during week one of the study, but all four sites had one or two positive replicates during weeks two and/or three of the study. All field negative controls collected during the three week period of intensive sampling (27 total) tested negative for the presence of NZMS eDNA.

Detection of eDNA & Visual Surveys (Single-Season Occupancy Model)

The probability that a site examined would be occupied by NZMS ranged from 0.53 (CI: 0.25 – 0.81) in the Columbia/Kalama River to 1.00 (CI: 0.94 – 0.1.00) in Burnt Bridge Creek (Table 3). Detection probability of NZMS sampled by one grab of eDNA was higher in smaller waterbodies compared to larger waterbodies (β_1 = 1.96; CI: 0.67 – 3.59). The probability of detecting NZMS sampled by grab of eDNA (p_D) was 0.94 (CI: 0.85 – 0.99) in small systems and 0.69 (CI: 0.53 – 0.82) in large systems. Similarly, detection probability of NZMS sampled by

visual survey was higher in smaller streams compared to larger streams (β_2 = 2.48; CI: 0.77 – 4.57). Estimates of the probability of detecting NZMS sampled by visual survey (p_V) were lower than those by eDNA with the probabilities being 0.74 (CI: 0.66 – 0.92) in small systems and 0.20 (CI: 0.04 – 0.49) in large systems. Among the two methods, CIs overlapped for small waterbodies, but not for large waterbodies. The probability of detecting NZMS by visual surveys increased with the average number of copies/mL of snail eDNA collected in a grab (β_3 = 2.46; CI: 0.44 – 6.00).

Expected probabilities of occupancy, if NZMS were not detected during sampling, were less than 0.05 when two eDNA grabs were collected in a small waterbody, three eDNA grabs were collected in a large waterbody, three visual surveys were conducted in a small waterbody, and when 14 visual surveys were conducted in a larger waterbody (Figure 5).

Three-level model to examine spatial and temporal patterns in detection probability of eDNA for NZMS

The probability that a site examined would be occupied ranged from 0.48 (CI: 0.03 – 1.00) in the Columbia/Kalama River to 1.00 (CI: 0.72 – 0.1.00) in Burnt Bridge Creek (Table 4). The model suggested that 7 of 9 (95%: 6 – 9) or 78% (95%: 67 – 100) of the examined sites in the three occupied waterbodies were occupied by NZMS. Of the occupied sites, the model estimated that a total of 12 (95%; 12 – 15) grab locations were occupied by NZMS. The probability that eDNA was detected at a grab location (i.e., "spatial replicate") within an occupied site (p_S) was 0.59 (0.34 – 0.83). The probability that eDNA was detected in a temporal replicate collected at specific grab location that was occupied (p_T) was 0.69 (0.51 – 0.83).

Discussion

Accurately detecting presence and identifying distribution is critical for effective control and management of any AIS. Monitoring for AIS without knowing if they are present is inherently challenging because it's unclear whether the organism is truly absent, or it was simply not detected during sampling. Detecting a new infestation of NZMS can be especially challenging because they are very small and difficult to find, particularly when densities are low. Identifying the appropriate sampling effort (i.e., number of eDNA samples or visual surveys) is an important aspect of AIS surveillance design. Based on our estimated detection probabilities, the probability of occupancy if NZMS are not detected at a site in a large waterbody is less than 0.05 if we collect three 0.5L eDNA grab samples or conduct 14 visual surveys. In a small waterbody, our probability of occupancy is less than 0.05 if we collect two eDNA grab samples or conduct three visual surveys. Based on these results, our current level of eDNA sampling at NFHs is likely sufficient to detect an invasion of NZMS, but up to 14 visual surveys may be required to ensure a hatchery facility is unoccupied, which is more than what is currently performed. Our results suggest that the probability of detecting NZMS eDNA is higher than the probability of detecting the snails by visual surveys in both large and small waterbodies, which may make it a

good tool to detect a new invasion. However, our results also suggest that detection probability of eDNA can vary spatially and temporally and may be reduced when water volume is high, snail abundance is low, or distribution is patchy. Detection by visual surveys is also imperfect and may be lower when snail abundance is low or the sample site is large, deep, or complex. However, when detected, visual surveys can pinpoint the precise location of individuals and may be just as reliable as eDNA when sampling in smaller, shallow streams or when NZMS abundance is high. Both eDNA and visual surveys could play a role in detecting the presence, monitoring the spread, and examining patterns in abundance and distribution of NZMS in their introduced range.

Our results indicate that the probability of detecting NZMS eDNA is higher than the probability of detecting NZMS by visual surveys. Ponce et al. (2021) and Woodell et al. (2021), similarly found that eDNA was more effective at detecting NZMS at survey locations than traditional methods, although these two studies did not estimate method-specific detection probabilities. Further, for multiple species, detection probability of eDNA appears higher than that for traditional methods (Smart et al. 2015; Schmelzle and Kinziger 2016; Wilcox et al. 2016; Eiler et al. 2018; Akre et al. 2019; Wineland et al. 2019). The higher probability of detecting NZMS via eDNA may be due to a more wide-spread distribution of NZMS eDNA in water, compared to the actual distribution of snails. Environmental DNA is often distributed more broadly than the physical distribution of the target species though eDNA transport distance from a source population varies widely (Pilliod et al. 2013; Deiner and Altermatt 2014; Jane et al. 2015; Deiner et al. 2016). The lower probability of detecting NZMS via visual surveys could be associated with patchy NZMS distribution or low snail abundance at a given site. In this study, paired eDNA sampling and visual surveys were conducted at spatially referenced locations (i.e., GPS points) with documented NZMS occupancy (see Methods). Although visual surveys were conducted both upstream and downstream from a GPS point, it's possible NZMS were located just outside our search area or occurred in low densities that remained undetected by field personnel. Since eDNA can detect individuals that are upstream or hard to detect by visual surveys due to small numbers or low visibility, it could be an optimal method to detect a new invasion, when abundance could be low and patchy.

We identified correlations between visual detection, NZMS eDNA, NZMS abundance, and waterbody size. We observed higher concentrations of eDNA in sites with high abundances of NZMS, and low or variable eDNA concentrations and eDNA detections (i.e., less than three replicates positive) in locations with low or presumed low NZMS abundance. A similar relationship between NZMS abundance and eDNA concentration was also observed by Thomas et al. (2019) and Ponce et al. (2021). In general, locations where NZMS have been consistently observed during visual surveys tend to be smaller in size (≈7m-90m) or have moderate to high NZMS densities (e.g., Burnt Bridge Creek and Youngs Bay). Tank et al. (2021) developed a qualitative visual sampling methodology for NZMS and evaluated its effectiveness in 12 rivers in Michigan. They found that as relative abundance of NZMS increased, their detectability by visual surveys also increased (Tank et al. 2021). When NZMS were present in low abundance,

we were less likely to encounter them during a visual survey when the spatial area of a survey site was large or the habitat was complex (e.g., dense aquatic vegetation). Conversely, when snail densities were moderate to high, the snails were very conspicuous and easier to detect visually, even in large rivers (e.g., Youngs Bay). Potentially, when NZMS populations reach a certain threshold density, detection probability of visual surveys may approach 1.0, regardless of stream size or environmental conditions. Thus, detection by visual survey appears high when snail abundance is high and when the waterbody is small. Under these conditions, visual surveys could contribute to fine-scale identification of distribution, abundance, or habitat use since field personnel can observe NZMS directly.

Our results suggest that detection probability of eDNA is generally high but can be variable even on a small spatial scale. In the lower Deschutes and Columbia/Kalama River, NZMS eDNA was not detected consistently in all sample replicates (i.e., < 3 replicates positive) at two survey sites. These results were unlikely caused by sample contamination because all field negative controls (10 total) tested negative for NZMS and multiple replicates tested positive at both sites over the duration of the study. Rather, we believe irregular detection of eDNA was due to low densities or patchy distribution of NZMS in these locations. In both waterbodies, sites sampled immediately upstream had a higher rate of eDNA detection (i.e., two or three replicates positive each year) and higher concentrations of NZMS eDNA within each positive replicate. Furthermore, live NZMS were recently discovered in the Columbia/Kalama River approximately 100m upstream from the original sample site. The snails were well hidden on the underside of cobbles and present in very low density (< 5 snail/m^2). Our survey sites are potentially too far downstream from the source population to consistently detect the eDNA. 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. 2015), though environmental variables (e.g., water temperature, discharge, UV radiation, water chemistry, bacteria and organic material) and the target organism (e.g., species, size, abundance, distribution, eDNA shedding rate) can influence the persistence and quantity of DNA present at a sample location (Pilliod et al. 2013; Barnes et al. 2014; Herder et al. 2014; Strickler et al. 2014; Jane et al. 2015; Goldberg et al. 2016). More research is needed to understand eDNA transport for NZMS and how population abundance, snail distribution, and environmental conditions may influence NZMS eDNA detection probabilities. However, what is clear is that when abundance could be low or patchy, sampling effort and design (spatially and temporally) should be carefully considered to ensure a high probability of detection.

Occupancy models estimate and account for less than 100% detection through the process of replicated sampling (MacKenzie et al. 2006). In 2019, we examined the small-scale (weekly) spatial and temporal variation in eDNA detection by sampling the same three sites in three occupied streams over a three-week period. This study allowed us to examine for spatial and temporal patterns in eDNA detection within a site and to consider how spatial and temporal replicates could be used in future occupancy sampling programs using eDNA. Although

temporal replicates are more commonly used in occupancy modeling (MacKenzie et al. 2006), spatial replicates may be more feasible or practical, and may perform equally as well (Whittington et al. 2015; Srivathsa et al. 2018). Detection probability of spatial replicates (0.59; 95%: 0.34 – 0.83) was slightly lower that for temporal replicates (0.69; 95%: 0.51 – 0.83); however, 95% credible intervals overlapped. These estimated detection probabilities suggest that both methods could be used to correct estimates of occupancy for missed detection.

We observed high eDNA detection variability among spatial replicates at four of six sites where NZMS eDNA was detected in 2019 (Burnt Bridge Site #3, lower Deschutes Sites #1 and #3, and Columbia/Kalama Site #1; Table 2). Variability among spatial replicates could be driven by multiple factors including NZMS distribution, abundance, proximity of NZMS to the sampled sites, and waterbody characteristics. Environmental DNA tends to be distributed unevenly in lotic systems, with concentrations highest in close proximity to the source population and decreasing farther downstream (Jane et al. 2015; Laramie et al. 2015; Wilcox et al. 2016; Hinlo et al. 2018; Tillotson et al. 2018; Chucholl et al. 2021). Furthermore, eDNA concentrations are often related to abundance of the organism (Pilliod et al. 2013; Doi et al. 2017; Bracken et al. 2018; Coulter et al. 2019; Chucholl et al. 2021; Ponce et al. 2021; Rourke et al. 2021). At each site noted, NZMS eDNA concentrations in positive replicates were very low (< 0.13 copies/ml), suggesting the eDNA may have originated further upstream and/or that the population is patchy or low density. The variability observed within spatial replicates may also be related to the size of the waterbody. Burnt Bridge Creek is a small (<10m wide), relatively shallow stream, whereas the lower Deschutes and Columbia/Kalama are both large (>100m wide), high volume rivers. Previous studies have suggested that eDNA becomes diluted in high volume lotic systems, reducing eDNA detection probability (Herder et al. 2014; Stoeckle et al. 2017; Rusch et al. 2020; Lam et al. 2022). Chucholl et al. (2021) observed lower eDNA detection of crayfish species in large rivers versus small streams that was attributed to dilution of the eDNA signal. High water volumes in the lower Deschutes and Columbia/Kalama Rivers combined with low concentrations of NZMS eDNA may result in missed detections. Conversely, in Burnt Bridge Creek, NZMS eDNA is confined to a much smaller volume of water and thus easier to capture (Smart et al. 2015). Variability in eDNA detection may also be influenced by the specific physical location (e.g., right bank, midstream, left bank) of eDNA collection within a site. To maximize detection probability, eDNA grab samples should be collected at different locations within a site (i.e., spatial replicates), targeting different microhabitats to optimize eDNA detection (Herder et al. 2014; Goldberg et al. 2016; Xing et al. 2022). In lotic systems, Wood et al. (2021) recommends collecting grab samples from both shorelines or from the shoreline and midstream (rather than a single shoreline) to account for the heterogeneous distribution of eDNA in flowing water. We were only able to access a single shoreline in both the lower Deschutes and Columbia/Kalama Rivers which may have limited our ability to consistently detect eDNA in all spatial replicates.

High temporal (weekly) variation was also observed at four of six sample sites where NZMS eDNA was detected in 2019 (Burnt Bridge Site #3, lower Deschutes Sites #1 and #3, and Columbia/Kalama Site #1; Table 2). Notably, all spatial replicates tested negative for NZMS

during week one of the study. However, in following weeks, one or more of the three replicates tested positive for NZMS eDNA at each site. Within Burnt Bridge Creek (Site #3), NZMS were visually observed during three consecutive surveys, but were only detected within a single spatial replicate (out of 9) during week three of the study. Water depth at this site is lower compared to the other two sites in Burnt Bridge creek (0.2 m vs 0.7 m depth) with higher relative stream flow (i.e., riffle vs pool habitat) that may have dispersed eDNA away from the site more quickly (Stoeckle et al. 2017; Curtis et al. 2020; Gasparini et al. 2020). Additionally, relative abundance of NZMS at site #3 was very low (≈2 snail/m^2) and all snails were observed on the underside of large cobble. Examples of short-term (e.g., hourly, daily, weekly, monthly) temporal variability in eDNA detection are relatively common in the literature (Beentjes et al. 2019; Ely et al. 2021; Troth et al. 2021; Jensen et al. 2022; Searcy et al. 2022). The combined effects of species abundance, distribution, movement (e.g., migration, schooling, diel behavior), eDNA transport, eDNA degradation and sample collection techniques (e.g., number of sample replicates, water volume filtered, filter pore size, etc.) can each result in variable eDNA concentrations and species detection. We theorize that snail abundance, distribution and aquatic conditions (i.e., stream discharge and volume) likely contributed to the temporal variability in these locations. Our results provide insight into the small-scale temporal dynamics of NZMS eDNA detection that can occur within an occupied stream and serves as a caution to not rely on a single spatial or temporal measurement for eDNA monitoring, as it may lead to the false assumption that a site is unoccupied. Incorrectly concluding an invasive species is absent from a site could result in the proliferation or spread of the species to new locations. Given the patchy distribution and often low abundance of NZMS in occupied sites, conducting a single annual eDNA survey may not be the best temporal resolution for detecting a new infestation of NZMS. While our level of sample collection (i.e., three spatial replicates per site) is likely adequate to detect the presence of NZMS at a given site, we recommend collecting eDNA samples two or more times per year to improve early detection monitoring at National Fish Hatcheries.

Conclusion

This project provides estimates of detection probability for two survey methods to aid early detection and monitoring of invasive NZMS, as well as examines spatial and temporal patterns and differences in occupancy and detection probability of NZMS among aquatic systems. An efficient and reliable AIS detection and monitoring program is critically important for NFH in the Columbia Basin and elsewhere as they face the ongoing threat of invasive species introductions that could potentially threaten infrastructure, increase maintenance costs, and adversely impact routine hatchery operations. This study adds to the growing body of work that demonstrates the effectiveness of eDNA as an AIS early detection and monitoring tool and highlights the importance of understanding the relative strengths and weaknesses of our survey methods to improve detection of NZMS in occupied areas. Our results lend more support to suggest that traditional sampling methods such as visual surveys, work well to document occurrence, habitat use, and abundance of a target organism at a localized scale, while eDNA can potentially detect the presence of the target organism both locally and upstream from the sample location and may be especially valuable when abundance is low and distribution is patchy

(Civade et al. 2016; Deiner et al. 2016; Nakagawa et al. 2018; Wood et al. 2021). Additional research to better understand detection probability of these tools under different environmental conditions, for different invasive species, and at different species densities will allow us to adaptively manage our sampling approaches and ensure our procedures are sufficient to detect a potential invasion of NZMS at lower Columbia River NFHs. 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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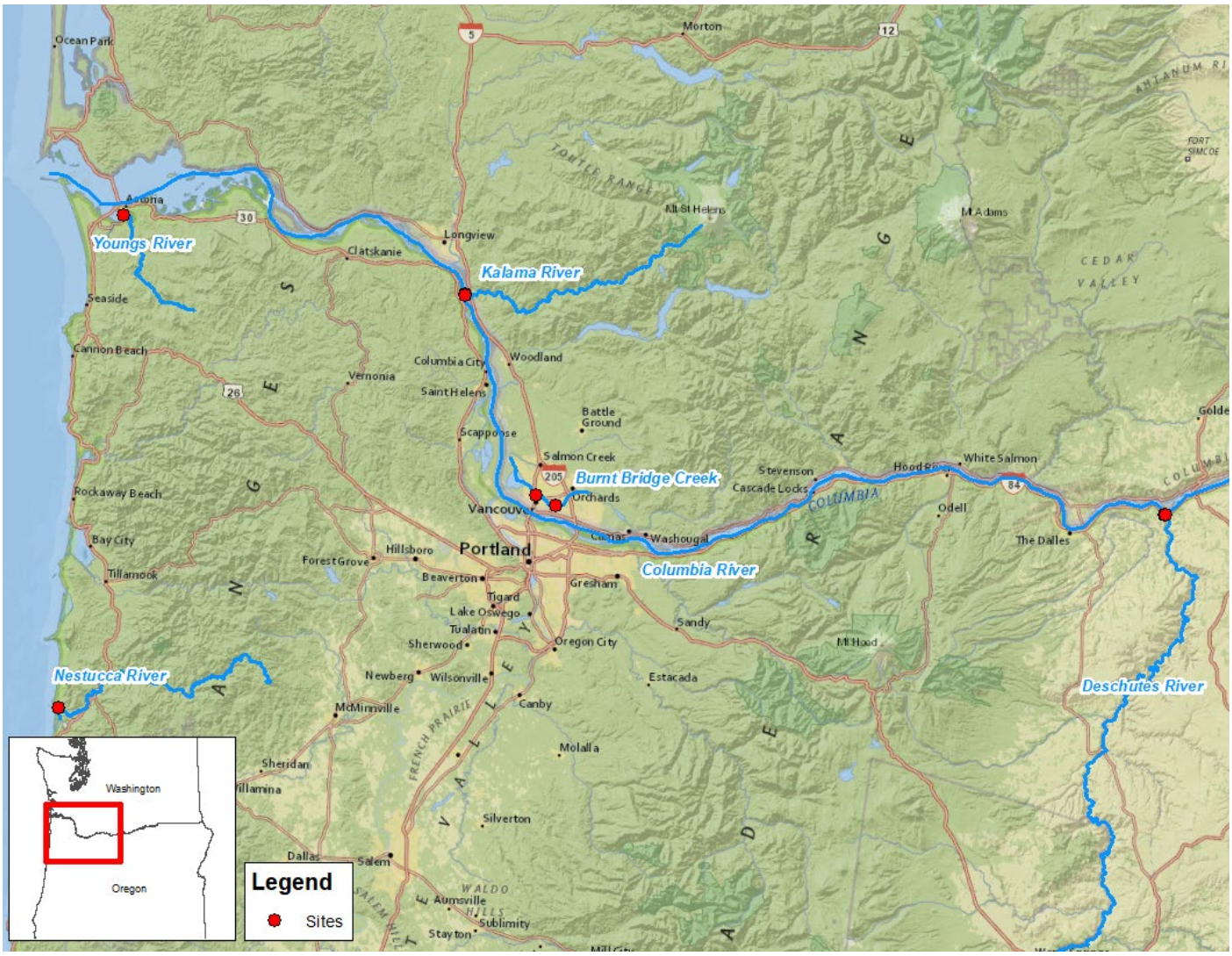

Figure 1: Location of five waterbodies with known NZMS occupancy (red circles). Up to four sites were surveyed within these waterbodies using eDNA and visual surveys from 2015 to 2022.

Figure 2: Three sites sampled weekly for NZMS using eDNA in Burnt Bridge Creek between 8/21 and 9/19 in 2019.

Figure 3: Three sites sampled weekly for NZMS using eDNA in the Columbia/Kalama River between 8/21 and 9/19 in 2019.

Figure 4: Three sites sampled weekly for NZMS using eDNA in the lower Deschutes River between 8/19 and 9/16 in 2019.

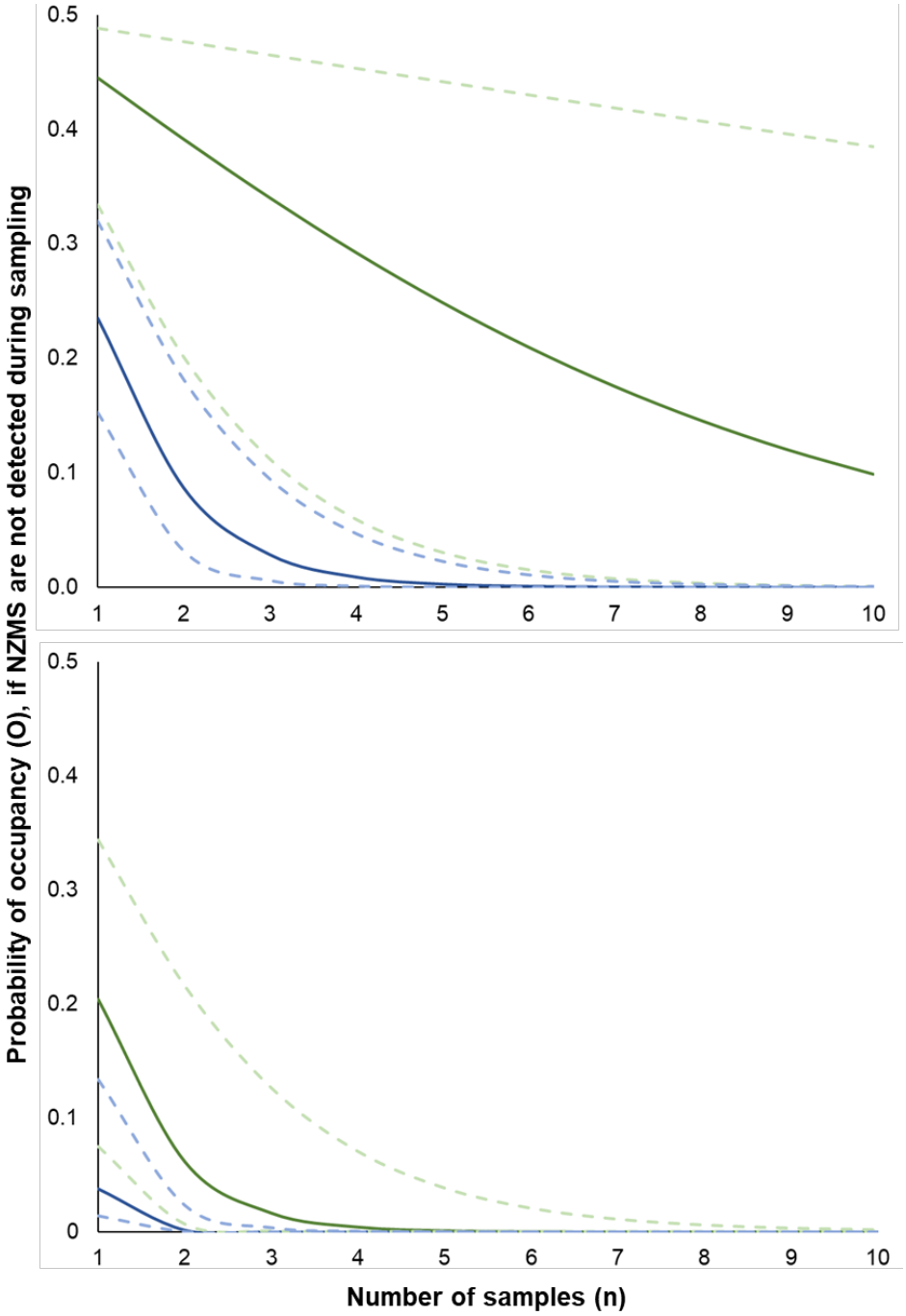

Figure 5: Probability of occupancy (O) if New Zealand Mudsnails are not detected by an eDNA sample (blue solid line) or a visual survey (green solid line) in a large (upper panel) or small (lower panel) occupied waterbody by numbers of samples completed. Estimates of detection probability were produced using a two-level occupancy model. Dashed lines are 95% credible intervals on estimates of detection probability for eDNA samples (blue) and visual surveys (green).

Table 1: New Zealand mudsnail sample site locations, number of times sampled, eDNA and visual survey results and range of eDNA concentrations within positive eDNA sample replicates at five locations with known NZMS occupancy, 2015 to 2021. The Asterisks "*" indicates less than three eDNA sample replicates tested positive at a site.

Year Sampled	Waterbody	Site	Easting NAD 83, UTM 10N	Northing NAD 83, UTM 10N	Number of Times Sampled	NZMS Observed?	NZMS eDNA Detected?	NZMS eDNA (copies/ml)
2015-2022	Burnt Bridge Creek	Burnt Bridge Cr. Trail pedestrian bridge at 65th	530788.83	5053521.00	8	Yes	Yes	0.18 - 146.42
2019, 2022	Burnt Bridge Creek	Burnt Bridge Cr. Trail pedestrian bridge above Andresen	531283.37	5053524.78	2	No	Yes	0.15 - 0.47
2019, 2022	Burnt Bridge Creek	Burnt Bridge Cr. Trail pedestrian bridge below 86th Ave.	532191.42	5053599.92	2	Yes	Yes*	0.18 - 0.33
2016	Burnt Bridge Creek	Leverich Park pedestrian bridge	526444.62	5055460.50	1	No	Yes	4.20 - 6.32
2017-2022	Deschutes River	Under Celilo Highway	662499.60	5055520.52	6	No	Yes*	0.14 - 0.88
2020-2021	Deschutes River	Halfway between Celilo Hwy & Heritage boat launch	662618.90	5055395.24	2	No	Yes*	0.32 - 0.90
2019, 2022	Deschutes River	Heritage boat launch	662675.81	5055305.63	2	No	Yes*	0.23, 0.26
2019, 2022	Deschutes River	Fishing access point	662855.59	5054970.32	2	No	Yes*	0.04, 0.33
2017-2022	Columbia/Kalama River	Pilings N. of sportsmen's Club Boat Launch	509635.25	5098452.27	6	No	Yes*	0.09 - 5.10
2019	Columbia/Kalama River	Pilings to left of tree arch	509636.44	5098559.43	1	No	No	---
2019	Columbia/Kalama River	Next group of pilings downstream	509601.91	5098670.95	1	No	No	---
2020-2022	Columbia/Kalama River	South end of sportsmen's Club property	509931.00	5097852.00	3	Yes (2021)	Yes*	0.05 - 9.40
2022	Columbia/Kalama River	Middle of sportsmen's Club property	509759.61	5098221.13	1	No	Yes	0.08 - 0.11
2016, 2018	Nestucca River	Boat launch outside Pacific City	424560.24	5006341.28	2	Yes (2018)	Yes	1.66 - 20.76
2016-2018	Young's Bay	Public boat ramp at Astoria Recreation Center	435411.86	5113355.94	3	Yes	Yes	37.86 - 865.67

Table 2: Environmental DNA results and NZMS eDNA concentrations at nine sites sampled weekly in Burnt Bridge Creek, lower Deschutes River and Columbia/Kalama River between 8/21 and 9/19 in 2019.

Site #	Date	Waterbody	Location	Replicate #1 NZMS eDNA copies/ml	Replicate #2 NZMS eDNA copies/ml	Replicate #3 NZMS eDNA copies/ml
1	8/21/2019	Burnt Bridge Creek	Pedestrian bridge at 65th	7.59	7.28	11.54
1	8/28/2019	Burnt Bridge Creek	Pedestrian bridge at 65th	5.46	5.55	8.59
1	9/4/2019	Burnt Bridge Creek	Pedestrian bridge at 65th	10.31	5.77	11.07
2	8/21/2019	Burnt Bridge Creek	Pedestrian bridge above Andresen	0.43	0.47	0.16
2	8/28/2019	Burnt Bridge Creek	Pedestrian bridge above Andresen	0.27	0.32	0.16
2	9/4/2019	Burnt Bridge Creek	Pedestrian bridge above Andresen	0.23	0.15	0.27
3	8/21/2019	Burnt Bridge Creek	Pedestrian bridge below 86th Ave.	---	---	---
3	8/28/2019	Burnt Bridge Creek	Pedestrian bridge below 86th Ave.	---	---	---
3	9/4/2019	Burnt Bridge Creek	Pedestrian bridge below 86th Ave.	---	---	0.14
1	8/21/2019	Deschutes River	Under Celilo Hwy.	---	---	---
1	8/28/2019	Deschutes River	Under Celilo Hwy.	---	---	0.08
1	9/4/2019	Deschutes River	Under Celilo Hwy.	0.06	---	---
2	8/21/2019	Deschutes River	Heritage boat launch	---	---	---
2	8/28/2019	Deschutes River	Heritage boat launch	---	---	---
2	9/4/2019	Deschutes River	Heritage boat launch	---	---	---
3	8/21/2019	Deschutes River	Fishing access point	---	---	---
3	8/28/2019	Deschutes River	Fishing access point	0.04	0.05	---
3	9/4/2019	Deschutes River	Fishing access point	0.13	---	---
1	8/21/2019	Columbia/Kalama River	Pilings N. of sportsmen's Club boat launch	---	---	---
1	8/28/2019	Columbia/Kalama River	Pilings N. of sportsmen's Club boat launch	---	---	0.02
1	9/4/2019	Columbia/Kalama River	Pilings N. of sportsmen's Club boat launch	---	---	0.05
2	8/21/2019	Columbia/Kalama River	Pilings to left of tree arch	---	---	---
2	8/28/2019	Columbia/Kalama River	Pilings to left of tree arch	---	---	---
2	9/4/2019	Columbia/Kalama River	Pilings to left of tree arch	---	---	---
3	8/21/2019	Columbia/Kalama River	Next group of pilings downstream	---	---	---
3	8/28/2019	Columbia/Kalama River	Next group of pilings downstream	---	---	---
3	9/4/2019	Columbia/Kalama River	Next group of pilings downstream	---	---	---

Table 3: Parameter estimates with 95% confidence intervals (in parentheses) for the two-level occupancy model to estimate detection probability of New Zealand Mudsnails sampled by eDNA grabs and visual surveys.

Parameter	Estimate
Intercept for site occupancy (α_0)	6.89 (1.43 – 9.79)
Intercept for detection by eDNA (α_1)	0.80 (0.12 – 1.51)
Intercept for detection by visual survey (α_2)	-1.39 (-3.06 – -0.03)
Slope for effect for stream (B_0 Burnt Bridge)	3.58 (-5.08 – 9.68)
Slope for effect for stream (B_0 Nestucca)	2.47 (-7.02 – 9.62)
Slope for effect for stream (B_0 Youngs Bay)	0
Slope for effect for stream (B_0 Kalama mouth)	-6.76 (-9.76 – -1.14)
Slope for effect for stream (B_0 Deschutes)	-4.63 (-8.80 – 7.48)
Probability of site occupancy in Burnt Bridge	1.00 (0.94 – 1.00)
Probability of site occupancy in Nestucca	1.00 (0.65 – 1.00)
Probability of site occupancy in Youngs Bay	1.00 (0.81 – 1.00)
Probability of site occupancy in Kalama mouth	0.53 (0.25 – 0.81)
Probability of site occupancy in Deschutes	0.82 (0.51 – 1.00)

Table 4: Parameter estimates with 95% confidence intervals (in parentheses) for the three-level occupancy model to estimate spatial and temporal patterns in detection probability of New Zealand Mudsnails sampled by eDNA grabs.

Parameter	Estimate
Intercept for site occupancy (α_0)	3.93 (-0.80 – 9.61)
Slope for effect for stream (B_0 Burnt Bridge)	4.01 (-5.57 – 9.70)
Slope for effect for stream (B_0 Kalama mouth)	-3.24 (-9.56 – 8.41)
Slope for effect for stream (B_0 Deschutes)	0
Probability of site occupancy in Burnt Bridge	1.00 (0.72 – 1.00)
Probability of site occupancy in Kalama mouth	0.47 (0.03 – 1.00)
Probability of site occupancy in Deschutes	0.98 (0.31 – 1.00)

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