U.S. Fish and Wildlife Service
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Aquatic Invasive Species Monitoring at Lower Columbia River Basin National Fish Hatcheries using eDNA and Visual Surveys

Progress Report: 2021

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On the cover: *Conducting a visual survey for New Zealand mudsnail at Spring Creek National Fish Hatchery. Photo by Jen Poirier.*

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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 and endangered species. Managing the threat of aquatic invasive species (AIS) is one of the ongoing challenges hatchery managers face. Fish hatcheries may be more susceptible to AIS 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 AIS may be spread 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 AIS to new waterbodies or between hatchery facilities (ANSTF 2007). 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 introduction and spread of invasive species through human-mediated pathways. These plans often recommend regular visual inspections of hatchery facilities and grounds to potentially detect AIS before they become established within a facility or inadvertently spread to new areas. Early detection is the most important, yet most challenging aspect of AIS management. 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. Traditional survey techniques may not reliably detect invasive species when an infestation first occurs, or abundance is low. A more sensitive detection tool such as environmental DNA (eDNA) can be employed in addition to visual presence/absence surveys to increase the chances of early detection.

A variety of invasive plants, animals and pathogens potentially threaten NFH management and operations. This ongoing study has primarily focused on the early detection of New Zealand mudsnail (NZMS), a small nonnative snail that has directly impacted hatcheries in the upper Columbia River (Ringold State Hatchery) and Snake River (Hagerman NFH). The USFWS Columbia River Fish and Wildlife Conservation Office (CRFWCO) has performed annual visual (presence/absence) surveys for NZMS at lower Columbia River Basin NFHs since 2006 (see Allard and Olhausen 2007a, 2007b; Hogle 2009; Poirier 2012; Poirier 2014). In 2015, eDNA sampling was incorporated into the annual survey (Poirier 2015; Poirier 2017; Poirier and Harris 2018) and in 2021, we broadened the scope of eDNA sampling to test for zebra mussels, quagga mussels, northern pike and common carp in addition to NZMS. The primary objectives of this study are to 1) conduct visual surveys and eDNA sampling at six National Fish Hatcheries and three reference locations to detect the potential presence of NZMS and four other high-risk AIS and 2), examine the detection probability of eDNA sampling using a multiscale occupancy model (see Kéry and Royle 2016). Early detection of AIS may improve the success of eradication efforts or prevent the establishment and unintentional spread of invasive species to neighboring hatchery facilities and/or stocking locations.

Six lower Columbia River Basin NFHs were surveyed for AIS in 2021 including: Carson, Eagle Creek, Little White Salmon, Spring Creek, Warm Springs and Willard National Fish Hatcheries (Figure 1). Visual presence/absence surveys were conducted over a two-week period in early September. Survey locations focused on areas perceived as likely AIS introduction points (e.g.,

headwater springs, water intake and outflow structures) as well as locations identified by hatchery personnel. 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. A single field biologist visually inspected up to a 20-m long section 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. While searching for invasive mollusks, we also conducted a general inventory of native freshwater snail species present at each sample location

Environmental DNA sampling was conducted over a two-week period in early September, following protocols described in Goldberg and Strickler (2017). One or 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 with ice for transport to the CRFWCO laboratory. Environmental DNA water samples were filtered in the CRFWCO laboratory within two 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. Samples were labeled with a unique site code and stored at room temperature until they were sent to Washington State University eDNA laboratory for analysis. A single field negative water sample was also collected at each eDNA test site and processed in the same manner as field samples to assess the potential for sample contamination associated with handling and transport. Field negatives were collected immediately following the collection of field samples and consisted of filling a sterile 0.5L Nalgene bottle with distilled water and placing it in the cooler on ice alongside field samples.

To validate the performance and reliability of our AIS early detection monitoring efforts, visual surveys and eDNA sampling was also conducted at three reference locations with documented NZMS presence: Burnt Bridge Creek, lower Deschutes River, and the Columbia/Kalama River.

A total of 28 intake and outflow sites were visually surveyed at six lower Columbia River Basin NFHs in 2021. Native freshwater mollusks were present in 20 (71%) of sites sampled. Surveyors observed freshwater snail from six unique families and ten genera, as well as a freshwater bivalve from the family Sphaeriidae (Table 1). *Menetus opercularis* (button sprite) was the most common snail observed, present in 8 different locations at three NFHs (Carson, Little White Salmon, and Spring Creek), while Juga (Juga) sp. was the most abundant snail genera observed at NFHs. The diversity of species was highest at Carson, Spring Creek, and Warm Springs NFHs, each with five snail species. There were no freshwater snails observed at Willard NFH. In

general, hatcheries with the highest diversity and relative abundance of native snails had habitat dominated by silt, sand, or cobble substrates, and submerged aquatic vegetation covering ≥50% of the survey area (Table 2). No NZMS were observed at lower Columbia River Basin NFHs. However, we did find the nonnative snail *Radix auricularia* (big-ear radix) at the Drano Boat ramp (Little white Salmon NFH) and around the perimeter of the abatement pond at Warm Springs NFH. The nonnative clam *Corbicula fluminea* (Asian clam) was also observed at the entrance of the adult fish ladder at Spring Creek NFH.

Five sites were visually surveyed at NZMS reference locations. New Zealand mudsnail were observed in two of the five locations in 2021 (Burnt Bridge Creek and Columbia/Kalama River). Notably, this is the first year NZMS were observed in the Columbia/Kalama River location since visual surveys began in 2017. Relative NZMS abundance was moderate to low in Burnt Bridge Creek (≈20 snail/m^2) and very low in the Columbia/Kalama River (<1 snail/m^2). Most NZMS were observed on submerged vegetation or on the underside of cobble. No NZMS were observed in the lower Deschutes River, though eDNA sampling has detected the snail at this location since 2017. We suspect NZMS abundance is very low, or they may be located deeper in the channel where it is difficult to survey effectively.

A total of 52 eDNA samples were taken at National Fish Hatcheries and NZMS reference locations in 2021. Environmental DNA samples were sent to the Washington State eDNA laboratory for analysis and results will be available in spring 2022. Occupancy modeling results will be available in summer 2022.

The monitoring and surveillance efforts conducted by the CRFWCO under this project provide valuable early detection data for NZMS and other AIS of concern 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. 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 occupancy modeling as a tool to evaluate and compare AIS early detection sampling methods and provide increased confidence that our current AIS sampling procedures are sufficient to detect NZMS and other AIS at NFH intake and outflow locations.

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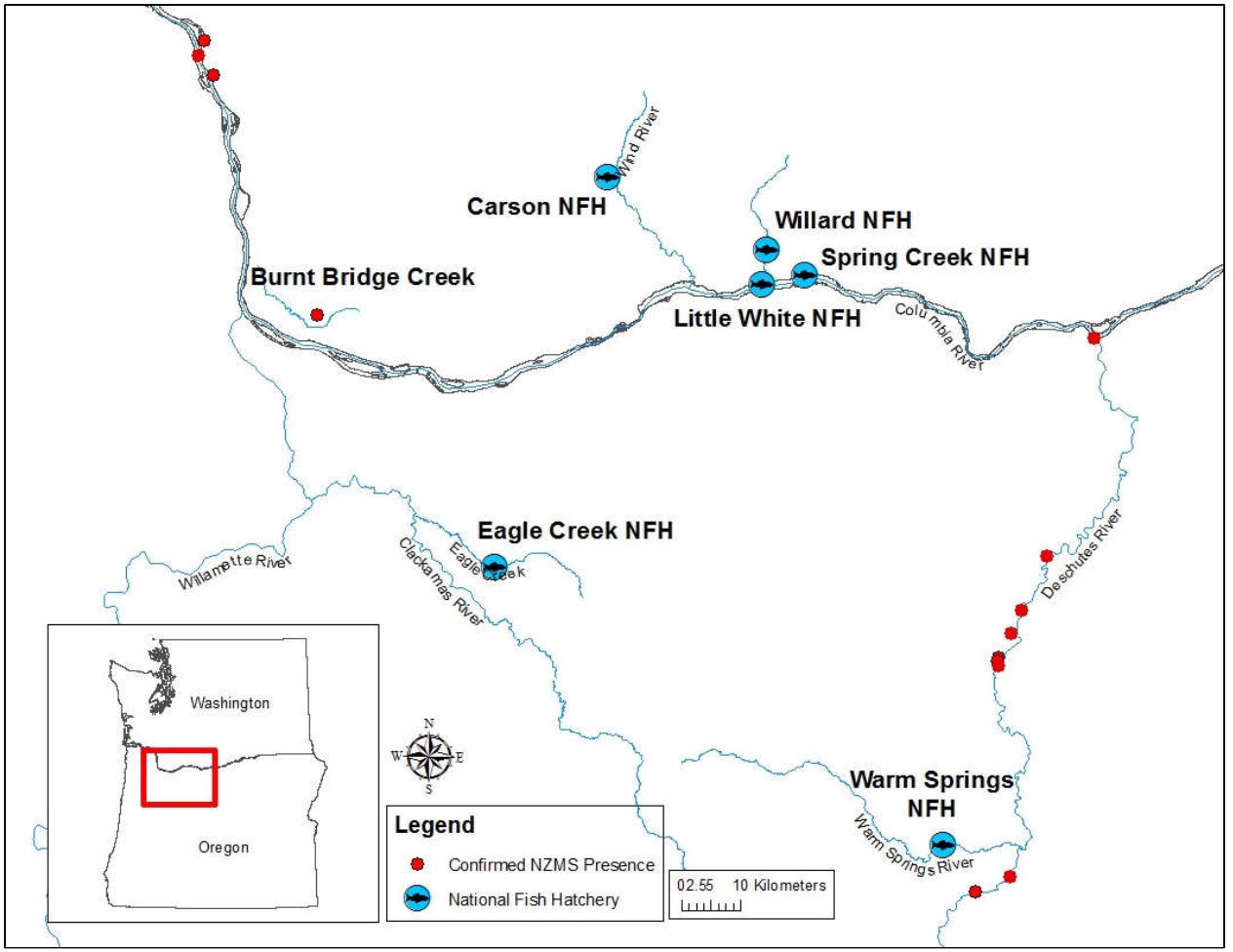

Figure 1: Map of USFWS National Fish Hatcheries surveyed for NZMS and distribution of NZMS populations in the lower Columbia River, 2021.

Table 1: Summary of freshwater mollusk genera observed at lower Columbia River National Fish Hatcheries, 2021.

Survey Location	Freshwater Mollusk Genera														
	Ancylidae	**Cyrenidae**	**Hydrobiidae**				**Lymnaeidae**		**Physidae**	**Planorbidae**			**Pleuroceridae**	**Sphaeriidae**	**Unionidae**
	Ferrissia rivularis	*Corbicula fluminea*	*Colligyrus greggi*	*Fluminicola sp.*	*Pristinicola hemphilli*	*Potamopyrgus antipodarum*	*Galba parva*	*Radix auricularia*	*Physella gyrina*	*Planorbella subcrenata*	*Menetus opercularis*	*Unknown Planorbidae*	*Juga (Juga) sp.*	*Unknown pea clam*	*Oregon floater*
Carson: fish ladder/ raceway outflow	X		X		X						X				
Carson: earthen pond outflow											X			X	
Carson: hatchery intake grate			X								X				
Carson: Tyee Springs headwaters			X				X				X				
Eagle Creek: Hatchery ladder outflow	X														
Eagle Creek: upper raceway outflow	X														
Eagle Creek: microfilter channel															
Eagle Creek: hatchery intake grate	X														
Little White Salmon: intake grate															
Little White Salmon: Baily Springs															
Little White Salmon: clarifier											X				
Little White Salmon: hillside springs													X		
Little White Salmon: Stairway springs (2)													X		
Little White Salmon: Drano Lake boat ramp								X	X						
Spring Creek: hillside spring #1													X		
Spring Creek: hillside spring #2											X		X		
Spring Creek: hillside spring #3											X		X	X	
Spring Creek: hillside spring #4											X		X		
Spring Creek: raceway & fish ladder outflow		X		X					X						
Willard: hatchery intake grate															
Willard: hatchery trash rack #1															
Willard: hatchery trash rack #2															
Willard: hatchery water settling pond															
Willard: lower raceway outflow															
Warm Springs: hatchery intake grate				X						X					
Warm Springs: fish ladder outflow													X		
Warm Springs: abatement pond								X	X	X					
Burnt Bridge: 65th pedestrian bridge	X			X		X			X			X			
Deschutes River: under Celilo Highway				X					X						X (shells)
Deschutes River: btwn Celilo Hwy & boat ramp									X						
Columbia R.: Kalama R. Sportsmans launch		X													X
Columbia R.: South of launch near mouth Kalama R.		X				X									
Rock Creek: upper Rock Creek													X		

Table 2: Habitat characteristics at lower Columbia River National Fish Hatcheries and three locations with verified NZMS presence, 2021.

Date Surveyed	National Fish Hatchery	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)	Native Snail Genera Observed	Nonnative Snail Genera Observed
9/15/2021	Carson	Adult ladder outflow	9:14 AM	9:55 AM	5.0	0.74	4	1	1	1,2,5	**Y**	4	0
9/15/2021	Carson	Earthen pond outflow	10:00 AM	10:08 AM	9.0	0.80	0	0	0	1,5	N	1	0
9/15/2021	Carson	Intake grate	10:12 AM	10:20 AM	5.0	2.0+	1	1	3	2,5	N	2	0
9/15/2021	Carson	Tyee Springs headwater	10:27 AM	10:37 AM	4.5	0.19	4	2	3	1,5	N	3	0
9/17/2021	Eagle Creek	Fish ladder outflow	9:02 AM	9:14 AM	7.0	0.60	4	0	0	1,2,5	**Y**	1	0
9/17/2021	Eagle Creek	Upper raceway outflow	9:20 AM	9:25 AM	7.0	0.56	4	0	0	1,2,5	N	1	0
9/17/2021	Eagle Creek	Microfilter channel	9:28 AM	9:33 AM	6.5	2.0+	0	0	0	2,5	N	0	0
9/17/2021	Eagle Creek	Intake grate	9:36 AM	9:46 AM	6.5	0.80	4	0	0	1,2,5	N	1	0
9/17/2021	Little White	Intake grate	9:06 AM	9:17 AM	4.0	2.0+	concrete	0	0	2	**Y**	0	0
9/14/2021	Little White	Clarifier outflow	9:23 AM	9:30 AM	4.5	0.38	4	0	0	1,2	N	0	0
9/14/2021	Little White	Bailey Springs	9:34 AM	9:39 AM	6.0	0.30	5	0	0	5	N	1	0
9/14/2021	Little White	Hillside Springs	9:47 AM	9:52 AM	7.5	0.10	4	0	0	5	N	1	0
9/14/2021	Little White	Stairway Springs (South)	9:58 AM	10:03 AM	7.0	0.20	5	2	2	5	N	1	0
9/14/2021	Little White	Stairway Springs (North)	10:06 AM	10:10 AM	9.5	0.10	5	0	0	5	N	1	0
9/14/2021	Little White	Drano Lake boat ramp	10:20 AM	10:42 AM	16.0	0.60	4	1	1	1,2,5	**Y**	1	1
9/16/2021	Spring Creek	Hillside Spring #1 (West)	9:14 AM	9:18 AM	7.0	0.20	0	0	0	1,5	N	1	0
9/16/2021	Spring Creek	Hillside Spring #2	9:20 AM	9:28 AM	5.0	0.81	0	0	0	1,2	N	2	0
9/16/2021	Spring Creek	Hillside Spring #3	9:30 AM	9:38 AM	5.0	0.15	0	2	2	1,5	N	3	0
9/16/2021	Spring Creek	Hillside Spring #4 (East)	9:40 AM	9:46 AM	5.0	1.10	0	0	0	1,2,5	N	2	0
9/16/2021	Spring Creek	Adult ladder outflow (Columbia R.)	9:58 AM	10:13 AM	16.0	0.90	4	0	0	1,2,5	**Y**	2	1
9/13/2021	Willard	Lower raceway outflow	9:35 AM	9:45 AM	3.5	0.60	5	0	0	1,2	**Y**	0	0
9/13/2021	Willard	Intake grate (adjacent pool)	9:53 AM	10:00 AM	3.0	0.70	1	0	0	1,2	N	0	0
9/13/2021	Willard	Trash rack #1	10:02 AM	10:08 AM	3.0	1.10	1	0	0	2,5	N	0	0
9/13/2021	Willard	Upper H_2O settling pond	10:09 AM	10:15 AM	3.0	0.18	0	0	0	1,5	N	0	0
9/13/2021	Willard	Trash rack #2	10:16 AM	10:21 AM	3.0	0.58	0	0	0	2	N	0	0
9/20/2021	Warm Springs	Intake grate	9:18 AM	9:29 AM	7.5	0.45	0	1	4	1,2,5	**Y**	2	0
9/20/2021	Warm Springs	Adult ladder outflow	9:37 AM	9:43 AM	8.0	0.41	4	0	0	2,5	N	1	0
9/20/2021	Warm Springs	Abatement pond outflow	9:50 AM	10:00 AM	9.5	0.65	0	1	4	1,5	**Y**	2	1

	Substrate Type	Aquatic Vegetation Type	% Aquatic Veg. Cover	Sample Method	
	0 = silt,clay,organic material (<0.059mm)	0 = No vegetation	0 = No vetatation	1 = wading	
	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)			6 = visual	

Date Surveyed	Sample Area	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)	Native Snail Genera Observed	Nonnative Snail Genera Observed
9/17/2021	Burnt Bridge Cr.	65th ped. Bridge	11:10 AM	11:30 AM	11.0	0.60	0	1	2	1,5	**Y**	4	1
9/22/2021	Columbia River	North of sand launch	8:32 AM	8:52 AM	14.0	0.80	1	1	1	1,2	**Y**	1	1
9/22/2021	Columbia River	North of Kalama River mouth	8:58 AM	9:20 AM	14.0	0.50	1	1	1	1,5	**Y**	1	2
9/21/2021	Deschutes River	Under Celilo Highway	9:20 AM	9:35 AM	11.0	0.75	0	1	1	1,2,5	**Y**	2	0
9/21/2021	Deschutes River	Btwn Celilo Hwy & boat launch	9:40 AM	9:56 AM	11.0	0.75	0	1	4	1,5	**Y**	1	0
9/24/2021	Rock Creek	Upper Rock Creek	9:25 AM	9:50 AM	10.0	0.50	4	2	1	1,5	**Y**	1	0

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	0 = silt,clay,organic material (<0.059mm)	0 = No vegetation	0 = No vetatation	1 = wading	
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	3 = Pebble (16-63mm)	3 = Floating	3 = 51-75%	4 = D-net	
	4 = Cobble (64-256mm)		4 = 76-100%	5 = Tactile	
	5 = Boulder (>256mm)				

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