ENVIRONMENTAL DNA (eDNA) SEDIMENT SAMPLING: A METHOD FOR DETECTING LARVAL LAMPREYS IN RIVERINE HABITAT

by

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ABSTRACT

ENVIRONMENTAL DNA (eDNA) SEDIMENT SAMPLING: A METHOD OF DETECTING LARVAL LAMPREYS IN RIVERINE HABITAT

Jessica J. Olmstead

The Pacific lamprey (*Entosphenus tridentatus*), western river lamprey (*Lampetra ayresii*), and western brook lamprey (*Lampetra richardsoni*) are three sympatric species of lamprey native to the rivers and tributaries of Washington. Pacific lamprey serve important cultural and ecological roles, similar to Pacific salmon, and as such, they face similar challenges to their survival. Concern over the decline of native lampreys in the Pacific Northwest has prompted several collaborative conservation efforts among tribal, federal, state, and local organizations. Environmental DNA (eDNA) is an important indicator in species monitoring, as eDNA detection methods are highly sensitive and non-invasive. eDNA methods have been widely applied to monitor presence of species in aquatic systems and in recent years, eDNA analysis of water samples have been used for lamprey monitoring. Furthermore, one study has demonstrated the ability to detect larval lamprey presence from sediment eDNA in a controlled laboratory experiment. Sampling the sediment may provide site-specific detectability due to the prolonged residency of larval lampreys burrowed in river sediments. Partnering with the Washington State Department of Natural Resources and Washington Department of Fish and Wildlife, the main objective of this research was to determine if eDNA sediment analysis can be applied to detecting larval lampreys from field collected sediments from 8 sites along the Nisqually River. We detected Pacific lamprey and *Lampetra* spp. through sampling eDNA in sediment. *Lampetra* spp. appear to be more prevalent, as they were detected at every site (90% of the sites in sediment and in water). Pacific lamprey were detected at only one site via sediment eDNA. These results indicate that Pacific lamprey are present in the Nisqually River, however, potentially at low numbers. This research demonstrates that analysis of sediment eDNA successfully detects the presence of larval lampreys, in the sites where they were physically detected through electrofishing surveys.

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INTRODUCTION

The Pacific lamprey (*Entosphenus tridentatus*, formerly *Lampetra tridentate*), western river lamprey (*Lampetra ayresii*, formerly river lamprey), and western brook lamprey (*Lampetra richardsoni*) are three sympatric[1] species of lampreys that are native to rivers and tributaries of western Washington. These lampreys each have their own complex life cycle and have co-evolved with the landscape, wildlife, and people for millennia [1]. Pacific lamprey face many of the same challenges as Pacific salmon, such as habitat degradation and fish passage barriers, and have experienced alarming rates of declines in the last few decades [1]. Of these three species, the Pacific lamprey is the largest of the native lampreys and culturally relevant to many Pacific Northwest tribes, making this species the focus of current research, management, and education [2,3]. Western river and western brook lampreys share similar ecological roles and threats to their larval phase as Pacific lamprey (C. Wang, USFWS, personal communication, February 6, 2019). Nonetheless, because of their significantly smaller body size and lower fecundity, they do not provide a major food source to tribal fisheries, and thus historically have been less culturally valued. As a result, less is known about the western river and western brook lampreys, though it is thought that they are experiencing local declines [4].

Pacific lamprey have one of the largest native fish distributions, ranging across the Pacific Rim from Japan, along the west coast of North America to Mexico [5,6].

[1] Sympatric speciation: the evolution of a new species from a surviving ancestral species while both continue to inhabit the same geographic region.

These anadromous[2] and semelparous[3] fish were once abundant throughout rivers and creeks within Washington State, providing ecological and cultural services [4,5]. Lampreys are critical to food web dynamics and nutrient cycling and are First Foods to many Native American tribes in the Pacific Northwest [1,7]. Historical accounts of Pacific lamprey describe rivers that were blackened by their abundance [5]. Recent observations have shown alarming rates of decline throughout rivers in Washington [1,2,8]. The manipulation of river systems by damming and channelization for human use has changed rivers and sediment deposition patterns, altering lamprey spawning and rearing habitat [9,10]. Passage efforts for salmonid species have increased survival for salmonids; however, these alterations do not accommodate lamprey passage [10]. Once adult lampreys spawn in rivers, and eggs hatch, the larvae (ammocoetes) bury into freshwater sediment for extended periods, ranging from 3-9 years, before emerging as juveniles (macropthalmia) and heading downriver to saltwater (L. Porter, CRITFC, pers. comm., April 19, 2018). During the larval phase, lampreys are vulnerable to contaminant exposure and to being dislodged by activities that disrupt sediment or hydrodynamics [11].

Pacific, western river, and western brook lampreys were petitioned to the U.S. Fish and Wildlife Service (USFWS) for listing as threatened or endangered status under the Endangered Species Act (ESA) in 2003. This status was denied due to lack of data on the distribution and age structure of the population [4]. As a result, multiple agencies in Washington, Oregon, Idaho and California have partnered to collect distribution data, and

[2] Anadromous: migrating from saltwater to freshwater to spawn

[3] Semelparous: only reproduce once before death

to restore, enhance and open river habitat for the Pacific lamprey [2,8]. To inform managers on how best to aid in the recovery of lamprey species in Western Washington, fundamental distribution and abundance information must be collected [3]. Estimates of distribution and abundance are ordinarily obtained through physically excavating and electroshocking to release the larvae from the sediment. Traditional methods to perform this work include the use of nets, traps, suction-pumping, and electrofishing [11]. Electrofishing for larval lampreys in freshwater sediment has shown high detection rates, in part due to their prolonged larval stage [12].

The ability to accurately detect rare organisms is imperative to aid in conservation efforts. Cryptic species, such as larval lampreys, are typically present at very low densities, and therefore are often difficult to detect with traditional methods [13]. Environmental DNA (eDNA), the DNA that organisms release into the environment, is an important indicator in species monitoring, as eDNA detection methods are highly sensitive in comparison to traditional detection methods [14]. eDNA methods are widely applied to monitor occurrence of a species in aquatic systems [15], mainly focused on detecting fish or amphibian species in freshwater systems [16–18]. In recent years, eDNA assays have been developed for Pacific lamprey and *Lampetra* spp. [19,20]. Ostberg et al. [20] tested for the presence of Pacific lamprey and *Lampetra* spp. in field collected water samples, which has aided in identifying lamprey distribution throughout Washington State. One limitation of water eDNA analysis in river systems is that the sample may be a signal from upstream and not from the specified area of interest [21]. Additionally, eDNA abundance and persistence in water and sediments vary, and this can influence the objective of a study based on temporal and spatial differences [18,22]. For example,

research by Turner et al. [18] demonstrated that eDNA can be more concentrated (i.e., detectable) and persistent in sediment than in the water and may be able to offer 'current-or-past site occupancy.' Furthermore, recent work (T. Liedtke, USGS, pers. comm., December 6, 2017) shows the ability to detect larval lamprey presence from sediment eDNA in a controlled laboratory experiment. Sampling the sediment would allow for the larval life stage to be detected through non-invasive means rather than through electrofishing, which can cause stress or harm to the larvae. Additionally, the year-long residency of larval lampreys burrowed in riverbed sediments may provide site-specific detectability [18].

The Washington State Department of Natural Resources (WDNR) has been considering what conservation measures the agency can take to support efforts to increase lamprey abundance in river systems. Fine sediment is one of the main habitat requirements to support the larval life stage of the Pacific Lamprey and *Lampetra* spp., potentially occurring on state-owned aquatic lands (SOAL) and managed by the WDNR. More research is needed to better understand the benthic (streambed sediment) habitat conditions and distribution of larval lampreys in Washington State. This habitat can be altered by various uses on SOAL (e.g., dredging, mining, irrigation, etc.) and in order for the WDNR to participate in efforts to protect the species habitat, it is important to find a way to evaluate the probability of larvae presence within a specified area. Our study aimed to address data gaps for species recovery efforts by evaluating whether or not eDNA methods can detect the presence of larval lampreys in field collected river sediment samples.

The main goals of this research were to 1) Determine if eDNA analysis of **sediment** can detect the presence of larval lamprey species: Pacific lamprey (*Entosphenus tridentatus*) and western river and western brook lampreys (*Lampetra* spp.) in the Nisqually River, WA; 2) Compare the detection rates of larval lampreys, using both electrofishing and eDNA analysis of sediment and water; and 3) Use a multi-scale occupancy model [23] to estimate the occurrence of lamprey eDNA in sites, in replicate samples, and in quantitative Polymerase Chain Reaction (qPCR) replicates.

This thesis will begin with an introduction to lampreys to better understand the variations in feeding behavior, morphology, and habitat preferences that occur among species of lampreys and times of life. The literature review will focus primarily on aspects of lamprey life stage and habitat that will be targeted in this study: larval phase and freshwater rearing habitat. Knowledge of the target species biology and ecology is key to interpreting eDNA findings because where they occupy the habitat (e.g., water column or sediments) and the timing of sampling (e.g., spawning, migration, etc.) can all influence what DNA is likely to be detected.

This introduction is followed by a review of the cultural and ecological relevance of native lampreys in the Pacific Northwest and their shared history and uncertain future with Pacific salmon. Next, this thesis will briefly discuss conservation efforts that are underway to address the threats that have contributed to lamprey decline. Lampreys are in decline largely due to habitat disturbances that occur during their larval life stage and barriers to fish passage that restrict adults from reaching historic spawning grounds [24]. Lamprey and salmon face similar anthropogenic pressures and it is essential to

understand these impacts to better implement steps to address the larger issues of lamprey survival.

The literature review will conclude with current and developing methods used to obtain status and distribution information for the conservation of these understudied species. A main objective of this research was to find a method of detection that would have the least amount of impact to the species and surrounding environment, as well as to identify a method that is more practical for management (i.e., less expensive, little associated training, etc.). Collecting water and sediment samples for eDNA analysis of the species need not require direct contact with the animals to confirm presence. Other methods such as electrofishing or sieving require walking through the habitat, shocking and stunning the organism, sifting through the sediment, and physically handling the species. Due to limited knowledge of *Lampetra* spp., the following literature review is focused primarily on Pacific lamprey, with discussion regarding *Lampetra* spp. where applicable.

LITERATURE REVIEW

Introduction to Lampreys

Phylogeny, Description, & Variations in Adult Life History

The lamprey family (Petromyzonidae) belongs to the class agnathan (jawless fishes); one of the oldest groups of vertebrate fish dating back 450 million years [2,25]. Lampreys and hagfish are considered "living fossils" because they are the only remaining members of this ancient class and consist of external parasites, filter-feeders, and scavengers [2,25]. The earliest fossil records of lampreys date back to 400-450 million years ago, a time before the dinosaurs (66 million years ago) and salmon (6 million years

ago) [5] (Figure 1). Approximately 41 species of lampreys have been classified in the world today, 10 of which live in the Pacific Northwest [25]. There are 2 lamprey genera which occur within the study area, *Entosphenus* and *Lampetra.* The genus *Entosphenus* consists of at least 21 species world-wide, 6 of which are found within the northern hemisphere, including Pacific lamprey [25]. Pacific lamprey (*E. tridentatus*) are the only known species of *Entosphenus* to occur outside of Vancouver Island and the Sacramento and Klamath watersheds [26]. The genus *Lampetra* consist of 9 species located within the northern hemisphere, 5 of which are endemic to North America [25]. Western river lamprey (*L. ayresii)* and western brook lampreys (*L. richardsoni)* are the only species within the genera *Lampetra* whose native range encompasses Washington State.

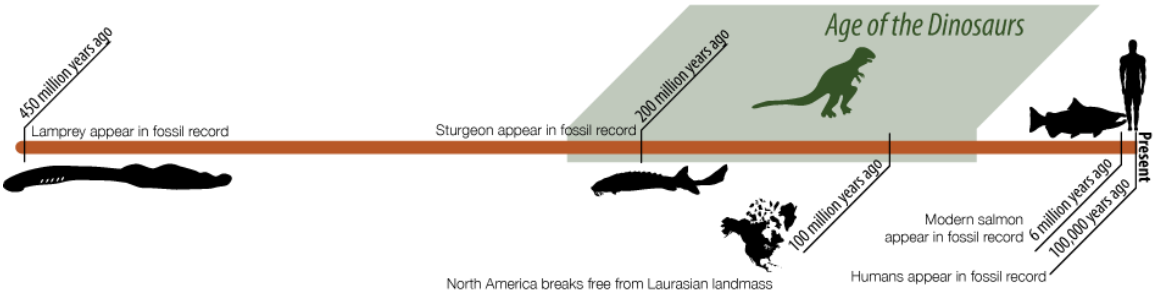


Figure 1. Evolutionary timeline of lampreys, one of the oldest groups of vertebrate fish dating back to 450 million years ago. Pacific lamprey is 1 out of 41 species alive today that represents this ancient line. Illustration obtained from: Columbia River Inter-Tribal Fish Commission (CRITFC). Pacific Lamprey: Pacific Lamprey Evolution and Biology [Internet]. 2019. [27].

Lampreys are commonly called “eels” because of their rounded and elongated eel-like shape, but they are jawless and boneless (cartilaginous) fish, while eels have jaws. Lampreys are unlike many true fishes because they have multiple gill openings and do not have scales or paired fins [2]. As adults, lampreys form a distinctive round, sucker-like mouth, called an oral disc, which they use to “suction” or cling on to rocks

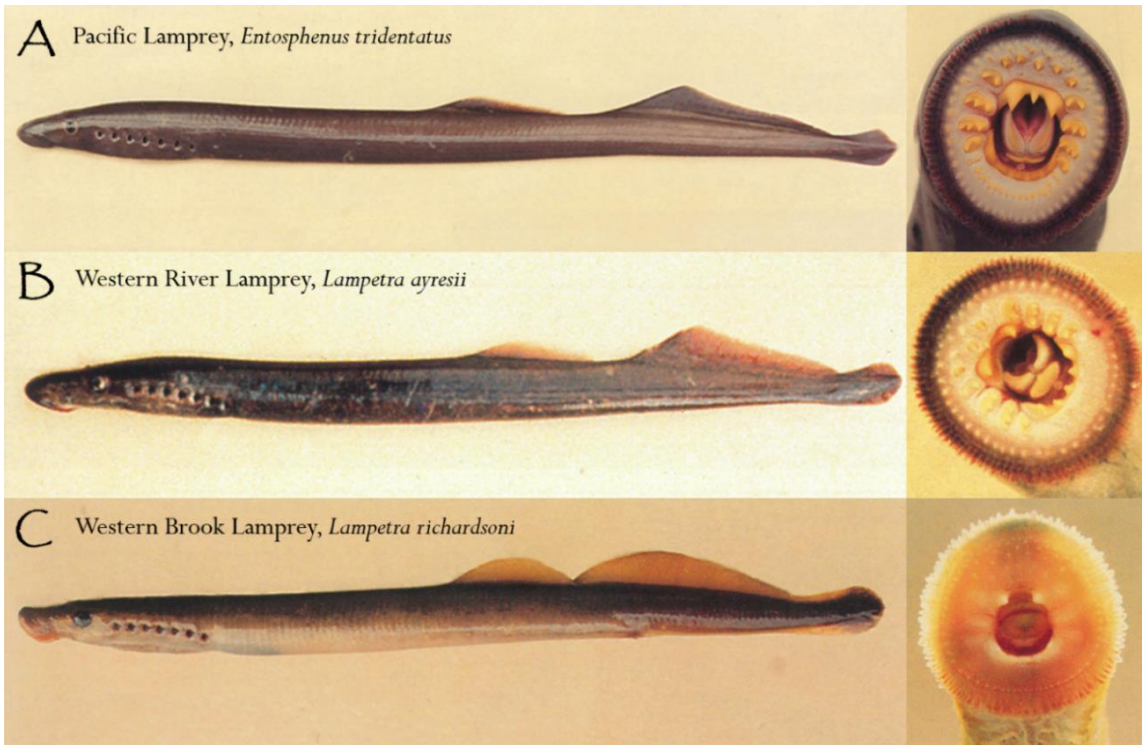


Figure 2. Morphology of adult lampreys. Side view of adult Pacific lamprey (A), western river lamprey (B), and western brook lamprey (C) and corresponding oral discs (mouth). Actual size in relation to each other is not accurately depicted here; adult body lengths of specimens are 26.5 (673.1), 10.5 (266.7), and 4.6 (116.8) inches (mm), respectively. Color plate illustrations adapted from: Wydoski RS, Whitney RR. Inland fishes of Washington. American Fisheries Society in association with University of Washington Press; 2003. 322 p. [28].

and latch onto prey. The characteristics of the teeth (number, structure, and position) are used to identify each species as adults [10]. Adult Pacific lamprey can be identified by the three large teeth anterior to the mouth opening, hence the species' name *tridentatus* (three-toothed lamprey) [2] (Figure 2A). In contrast, adult western river lamprey and western brook lamprey only have two anterior teeth, though the teeth of western brook are small, rounded, and nonfunctional because of their nonparasitic adult life-style [28] (Figure 2B and C). Species identification is typically performed during the adult stage

since lampreys look very similar in their early life stages and differentiating requires specialized training [10].

All species of lamprey are blind and have no teeth during their larval stage. Instead, larvae have an oral hood (oral cirri) that is used for filter-feeding and a light-sensitive eye spot on their forehead [9] (Figure 3). Interestingly, ammocoetes were once considered a distinct species, *Ammocoetes branchialis*, and it was not until the mid-1800's that ammocoetes were recognized as the larval stage of lampreys [29]. This is reasonable considering the difficulty to observe the transformation from larvae into metamorphosed juveniles with developed eyes, oral feeding discs, and coloration change from brown to silver (Figure 4).

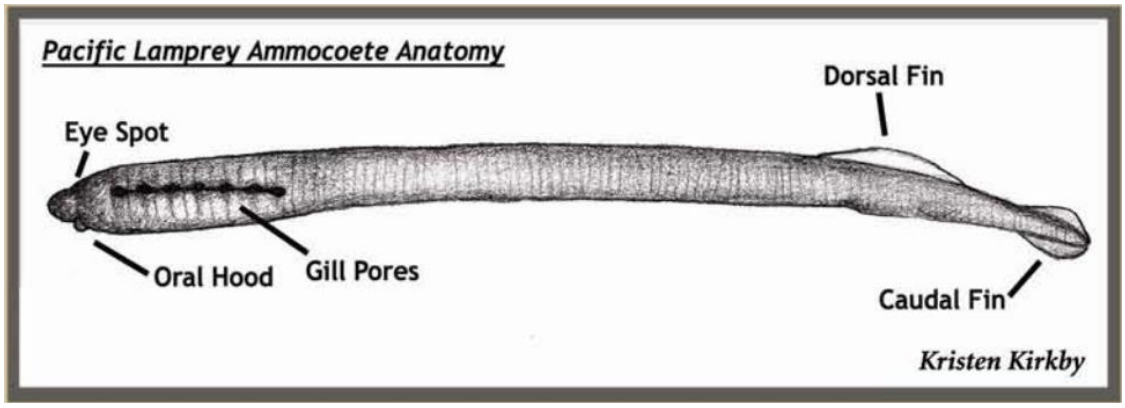


Figure 3. General anatomy of a Pacific lamprey ammocoete (larvae). Different species of larval lampreys can be difficult to identify as early life stages are morphologically similar. Oral hood has specialized structures for filter-feeding and eye spot is light-sensitive. Illustration by Kristen Kirkby (Cascade Columbia Fisheries Enhancement Group) and obtained from: Crandall JD, Wittenbach E. Pacific Lamprey Habitat Restoration Guide. Twisp, Washington; 2015. [9].

Figure 4. Three life stages of the lamprey: larvae (A), juvenile (B), and adult (C). A) Unidentified species of larvae (either *E. tridentatus* or *Lampetra* spp.) under 80 mm in length with undeveloped eyes. Photo taken during field collection of current study. B) Juvenile Pacific lamprey, approximately 150 mm in length with developed eyes, oral disc, and silver coloration. C) Adult Pacific lamprey, approximately 750 mm with blue eyes. Photo B & C taken during visit at Yakama Nation Prosser Hatchery facility in April 2018.

Variations in adult life history exist among different lamprey species (Figure 5). Lampreys can be anadromous or reside in freshwater for their entire life cycle (termed resident). Whether anadromous or resident, lampreys can have parasitic or non-parasitic adult feeding behaviors, either feeding on pieces of flesh, consuming their host's blood and other bodily fluids, or a combination [30]. The mode of feeding influences the preferred prey and habitat that each species utilizes while in the marine environment [31]. For example, Pacific lamprey are parasitic and are often observed latched onto marine hosts that are larger in size and typically found in deeper offshore waters. As a parasite, it is in their best interest to keep their host alive instead of exerting energy to find other prey. Consequently, when Pacific lamprey feed, they create shallow, circular bites that are less likely to cause permanent harm, and can feed on larger prey without killing their host [31]. In contrast, western river lamprey are observed feeding in shallow nearshore waters on the flesh of smaller prey, such as Pacific herring and Pacific salmon [32]. These lamprey create deeper gouges by removing portions of flesh, likely killing their prey [28,31,32]. Because of this behavior, western river lamprey are considered predators instead of parasites [28,32]. Non-parasitic species, like the western brook lamprey, stop feeding after transformation from larvae to juveniles (metamorphosis) and within ten months become sexually mature, spawn, and die [24]. Lamprey also undergo extreme changes in morphology throughout their life cycle and these changes influence their behavior and habitat requirements [9,10].

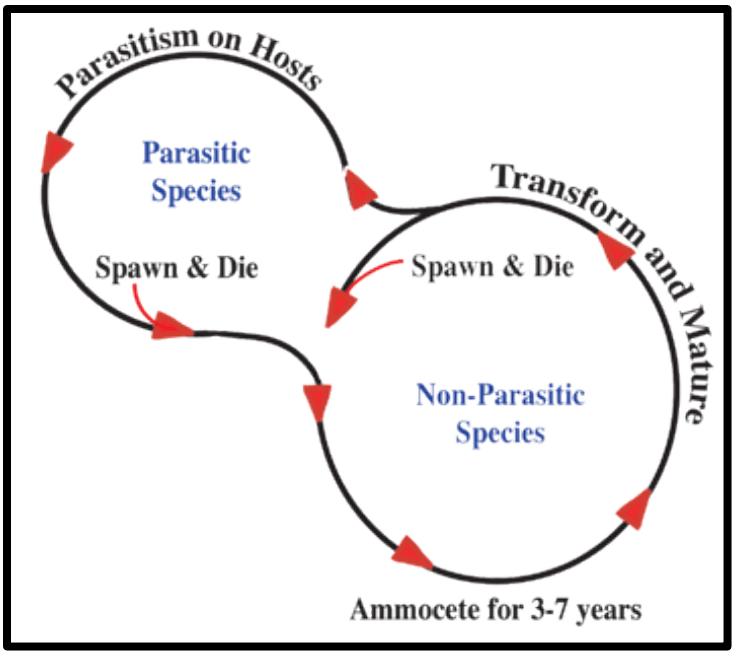


Figure 5. A generalized diagram of the life cycle variation that exists between parasitic and nonparasitic lampreys; such as the western river lamprey and western brook lamprey, respectively. Western brook lamprey do not feed after transformation and within ten months are sexually reproductive, dying shortly after spawning. Diagram adapted from: Mayden RL, Roe KJ. Mayden Lab Lamprey Project [Internet]. [cited 2019 Mar 19]. [33].

Life Cycle & Life History Characteristics

A depiction of the Pacific lamprey life cycle is illustrated in

Figure 6. Variations exist in the adult life history of lamprey species. However, all lampreys share an extended larval phase during which the larvae burrow and filter-feed in freshwater sediments [25]. Eventually, larvae undergo metamorphosis into the juvenile phase with developed eyes and teeth. If anadromous, recently transformed lampreys migrate downstream towards saltwater, where they attach to marine hosts to feed. Once they are ready to reproduce, adults cease feeding and migrate upstream to spawn. Unlike salmon, lampreys do not return to their natal streams. Instead, it appears that lampreys have developed a strategy for locating favorable spawning and rearing habitat by following migratory pheromones produced upstream by larvae [8,34]. The greater the

abundance of larvae, the stronger the chemical trail to follow. Spawning occurs in gravel or cobble substrates, located in riffles and shallow edges of pools, and upstream of larval rearing habitat. During spawning, pairs of lamprey construct nests (or redds) by using their sucker-like mouths to lift and move rocks and their bodies to dig round depressions [35]. The amount of eggs produced is linked to the body size of female lampreys [36]. Following spawning, adult lampreys die within 3-36 days and eggs hatch within 20 days [8]. Detailed information on life history characteristics of Pacific lamprey, western river lamprey, and western brook lamprey is compiled from the literature and viewable for comparison in Table 1**.** To limit the scope of this paper, the following section will only discuss the larval phase and freshwater rearing habitat.

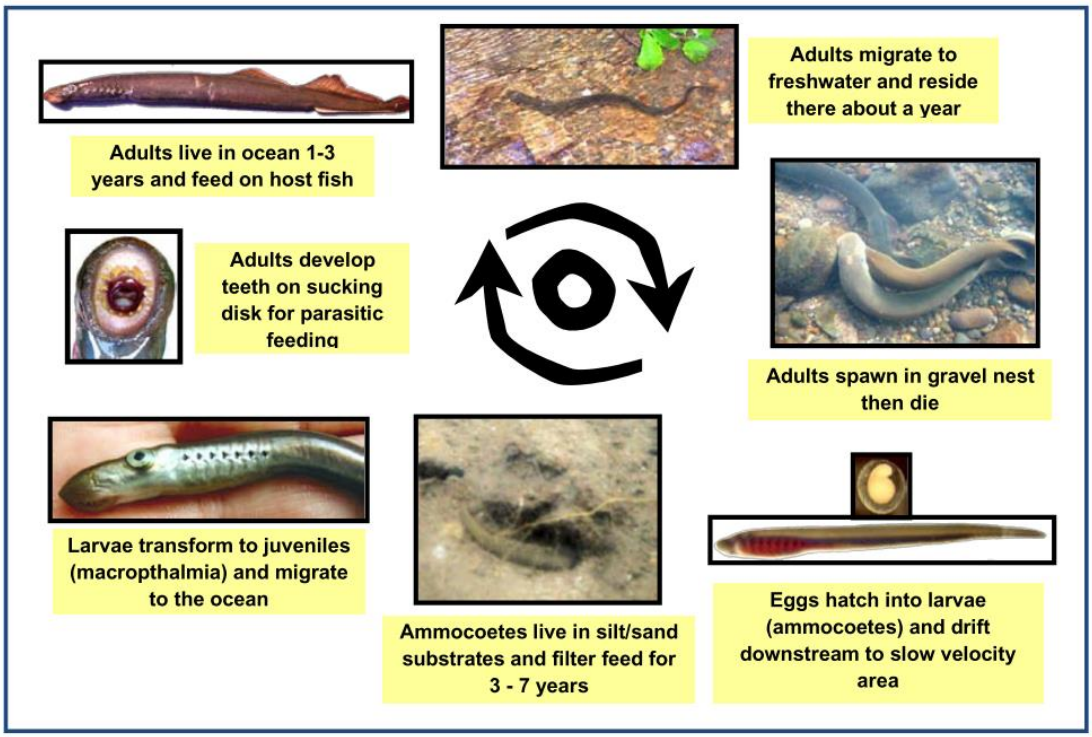


Figure 6. Pacific lamprey life cycle. Figure obtained from: USDA. Pacific Lamprey and NRCS: Conservation, Management and Guidelines for Instream and Riparian Activities. Spokane, Washington; 2011 [cited 2018 Nov 18]. [37].

Larval Phase & Freshwater Rearing Habitat

A few weeks after eggs hatch, tiny wormlike larvae (approx. 10 mm) emerge from their nests and drift downstream into areas with low current velocity and soft substrates, such as shallow pools, alcoves, eddies, and stream edges [8,9]. Slow water environments (< 1 ft/s) and natural obstructions (e.g., abundant large woody debris and boulders) create essential habitat by forming accumulations of fine sediments that provide important burrowing substrate [9,38]. Larvae burrow into the sediments and filter-feed on algae (e.g., diatoms, desmids), plankton, and other microscopic organic matter suspended in the water above and within the sediments [1,24,28]. Larvae prefer fine sediments, including fine sand and silt, but only if there is adequate water flow for food (e.g., suspended particles) and oxygen exchange [9].

Though mostly sedentary and poor swimmers, larvae have been observed moving upstream against slow currents for short distances [24]. Movement can also occur laterally within the sediment and within slow water velocities between patches of preferred habitat [9,24]. In addition, active and passive (e.g., freshets[4]) larval movement downstream can happen all year [9]. Most movement likely occurs at night, as larvae are sensitive to light [10]. Tagging studies have demonstrated that larval movement is influenced by increased current velocity, water levels, water temperature, and larval density [8,24].

[4] Freshet: the flooding of a river from heavy rain or melted snow.

Table 1. Life history characteristics of Pacific lamprey, western river lamprey, and western brook lamprey compiled from the literature.

Common name	**Pacific Lamprey**	**Western River Lamprey**	**Western Brook Lamprey**
Scientific name	*Entosphenus tridentatus*	*Lampetra ayresii*	*Lampetra richardsoni*
Life style type	Anadromous [b]	Anadromous [b]	Resident (freshwater only) [b]
Adult feeding type	Parasitic – Blood [d, f]	Parasitic – Flesh [d, f]	Non-parasitic derivative of *L. ayresii* [d, f]
Adult dentition (Oral Disc)	3 large-anterior (juvenile initially 2), 4 lateral rows (typically 2-3-3-2), 5-6 posterior teeth [h]	2 anterior, 3 lateral rows (typically 2-3-2 or 2-2-2), and 7-10 posterior teeth [h]	2 anterior, 3 lateral rows (typically 1-2-1, 2-2-1, or 2-2-2), and 7-10 posterior teeth; all are small, rounded and nonfunctional [h]
Max larval body size	200 mm or 8 in [b]	200 mm or 8 in [b]	200 mm or 8 in [b]
Adult body size	330-840 mm or 13-33 in [h]; up to 1 lb., females < males [c]	200-330 mm or 8-13 in [h]; females < males [c]	90-200 mm or 3.5-8 in [h]; females < males [c]
Distribution	Drainages of western Canada, USA, Mexico, & Japan; Widespread in the North Pacific Ocean [d, j]	Drainages of North American Pacific Coast [j]	Drainages of Pacific Ocean, British Columbia, Washington, Oregon and Alaska [j]
Washington State distribution	Most large coastal and Puget Sound rivers, and occur long distances inland in the Columbia, Snake, and Yakima Rivers; streams of southern, western, and northern boundaries of the Olympic peninsula [n]	Unknown; likely occurs in major coastal rivers [n]	Coastal and Puget Sound streams; southern and western boundaries of Olympia peninsula but absent from northern and eastern boundaries [n]
Freshwater type	Major river systems and streams [l]	Lower portions of large river systems [l]	Smaller streams with lower gradient than Pacific lamprey [n]

Common name	Pacific Lamprey	Western River Lamprey	Western Brook Lamprey
Larval rearing habitat	Fine silt and mud substrates in backwaters and quiet eddies of cold-water streams with currents less than 1 ft/s and water depths less than 70 cm; downstream of suitable adult spawning habitat [c, n]	Fine silt and mud substrates in backwaters and quiet eddies of cold-water streams with slow current [c, n]	Silty stream bottoms in quiet backwater areas [n]
Juvenile rearing habitat	Unimpeded downstream connection to ocean, deeper water in river channel, larger sediment (gravel) [k]	Unimpeded downstream connection to ocean, deeper water in river channel, larger sediment (gravel) [c]	Gravel beds in streams that are suitable for spawning [n]
Marine habitat	230-820 ft depths typical (up to 2600 ft); up to 62 mi off coast [a, l]	Nearshore surface waters of Pacific Ocean at depths between 85-108 feet [a, n]	Freshwater only [n]
Spawning habitat	Low gradient stream reaches, in gravel, tailouts of pools and riffles [m]	Unknown	Unknown
Duration of larval phase	2-9 years, dependent on habitat conditions and larval densities [c]	Unknown, likely several years (similar to Pacific) [n]	Unknown; likely longer than western river (closely related parasitic species) [c]
Timing of metamorphosis	Early to mid-July (Columbia river) to November; newly transformed lampreys occasionally feed in freshwater [i]	July to April; recently transformed lamprey begin feeding on fish hosts while in freshwater [n]	February to July, burrow over winter and emerge sexually active in spring when water temp > 10 °C [l, n]
Timing of downstream migration	March to July the year following metamorphosis, with peaks during spring and summer freshets; nocturnal movement [c, k]	May to July [n]	Only with downstream drift as larvae [l, n]
Duration of marine phase	1-3 years [l]	4-5 months between May to Sep [a, n]	Freshwater only [l, n]

Common name	Pacific Lamprey	Western River Lamprey	Western Brook Lamprey
Timing of upstream migration	Feb - Sept, peaking Apr - Jun; overwinter (Sept - Mar) and remain in freshwater approx. 1 year before spawning; overwintering areas in riffles and glides, esp. areas containing large boulders; nocturnal movement [k, l]	September or earlier [a, n]	Migrate short distance in freshwater to spawn [l, n]
Timing of spawning	March - July; latitude influences water temperature and timing; populations from coastal streams spawning earlier and inland streams spawning later [k, l]	April to June; peak in May [n]	March to July; peak in May when temperature is approx. 10 °C [l, n]
Life span	minimum 7 years [c, m]	6-7 years [l]	6 years [n]
Predators	Predatory fish, sharks, marine mammals, birds [n]	Predatory fish, sharks, marine mammals, birds [n]	Predatory fish, sharks, marine mammals, birds [n]
Adult prey	Pacific salmon, pollock, flounders, rockfish; scars rarely found on whales [l, n]	Primarily feed on large schools of small fish near surface (e.g., Pacific herring); salmon and other fish as well [l, n]	Mature adults do not feed [l, n]
Mating system	Monogamy, polygyny [e]	Unknown	Polygynandry [e]
Number on nest	1-3 [f]	Unknown	2-12 [f]
Heterospecific with (species found within nest)	Western brook [f]	Unknown	Pacific [f]
Temperature at which spawning occurs	10.1-17.3 °C [f]	12 °C [n]	9.4-16.0 °C [f]

Common name	Pacific Lamprey	Western River Lamprey	Western Brook Lamprey
Fecundity (eggs/female)	20,000 - 240,000; number related to female size and distance of upstream migration [l, m]	11,400 - 37,300 [l]	1,100 - 5,500 [l]
Days for eggs to hatch after fertilization	Approx. 20 [a, f]	Unknown	10 [a, f]
Nest size width	up to 24 in [g]	5.9 in [n]	4-5 in [g]
Nest substrate diameter	Gravel (2-5 cm or 0.8-2 in) [k]	Unknown	1.5 cm [g]
Water depth at nest	30 cm - 4 m [m]	Unknown	20 cm [g]
Velocity at nest	50-100 cm/s [m]	Unknown	12 cm/s [g]
Depth of nest (below substrate)	4-8 cm [m]	Unknown	3 cm [g]
Max upstream migration	Up to 746+ mi [b]	Unknown [b]	Unknown [b]

[a] Beamish [31]
[b] Clemens, ODFW, pers. comm., April 18, 2018: Lamprey Life Stage Lexicon & Anatomy Vocabulary handout
[c] Dawson et al. [24]
[d] Hardisty [29]
[e] Johnson et al. [39]
[f] Johnson et al. [39], Table 6.1, p. 270-274
[g] Johnson et al. [39], Table 6.2, p. 276-277
[h] Lampman [40]
[i] Manzon, Youson, and Holmes [41], Table 4.2, p. 151
[j] Potter et al. [25], Table 2.1, p. 38-42
[k] USDA [37]
[l] USFWS [4]
[m] USFWS [8]
[n] Wydoski & Whitney [28]

Multiple age classes and sizes of larvae can occur within the same habitat year-round [8,9]. Moreover, larval age does not correlate with larval body size and growth rate [24]. This creates difficulty in estimating the duration of the larval life stage and assessing age structures of larval populations. In addition, methods used for age-assessments are complicated and unreliable and can vary among and within species [24]. In general, the age at metamorphosis is estimated between 3-7 years for larval lampreys [3,42]. However, recent genetic testing revealed larval Pacific lamprey were 4 to 9 years before transformation, with on instance of a 2-year-old juvenile (L. Porter, CRITFC, pers. comm., April 19, 2018). The high variability of larval growth is associated with larval density and environmental conditions (e.g., precipitation, water chemistry, and temperature) [24]. Larval density has been shown to have negative effects on growth rates of lampreys in laboratory studies [24]. The eventual body size of adult lampreys does not relate to the final size of larvae when they undergo metamorphosis [29]. This is evident by the comparable larval sizes of Pacific lamprey, western river lamprey, and western brook lamprey and the difference in size as adults (Table 1).

Assessments of larval rearing habitat have found that spatial scale of lotic[5] habitat and larval size can change the relative effects of habitat variables corresponding to larval abundance and distribution within a river system [24,38,43]. At large and small spatial scales (i.e., watershed versus site), larvae are patchily distributed in freshwater environments [24]. Low water velocity, availability of suitable burrowing habitat (e.g., substrate type and composition), and channel morphology (e.g., stream margins, pool

[5] Lotic: (of organisms or habitats) inhabiting or situated in rapidly moving fresh water.

habitats) were positively associated with patchiness of larval occurrence at finer scales [24,35,43]. Moreover, the most important indicators of larval presence were water velocity and sediment grain size [11,24,38]. Aggregations of larvae have been found in fine silt and detritus along stream margins [24,35,43]. Torgersen and Close [43] reported that over 80% of the larvae captured were within the stream margins, indicating that larval abundance and distribution is influenced by habitat heterogeneity. In addition, dissolved organic material (DOM) has recently been implied as a potential indicator for larval abundance and density because of the water-filtering ability of larvae [24].

Large scale patterns of larval distribution and abundance can be attributed to channel gradient, water depth, riparian canopy, and proximity to adult spawning areas [24,38,43]. In terms of large scale processes, stream gradients govern current velocity, and the type of sediment and organic material that is deposited for potential rearing habitat [24]. According to Torgersen and Close [43], water depth was positively associated with larval abundance at large scales because river reaches comprised of large numbers of deep pools provided habitat complexity and refuge for larval lamprey during low flow events. Typically, water depth preference above larval rearing habitat is less than 70 cm for Pacific lamprey, with *Lampetra* spp. preferring shallower depths [24] (Table 1). However, Arntzen and Mueller [44] observed larvae at depths up to 4.5 m and Jolley, Silver and Whitesel [45] collected larvae from sediments at depths up to 16 m. Additionally, Torgersen and Close [43] also found that open riparian canopy was a significant indicator of larval occupancy (> 100 larvae per m^2) due to the increased availability of larval food sources because of improved primary productivity (i.e., increased sunlight for photosynthesis).

Water chemistry parameters – such as temperature, conductivity, dissolved oxygen (DO), and pH – have been observed to affect the development of larval lampreys, though few studies have found it to be important in limiting distribution [24,38,46]. According to Meeuwig et al. [46] for Pacific lamprey larvae, 14ºC is the optimal temperature for larval survival and development, resulting in the lowest incidence of developmental abnormalities. Temperatures above 20ºC increase risk of disease, reproductive irregularities, decreased rates of larval survival, and death at average lethal temperatures of 28ºC [24,36]. Stone and Barndt [38] found that overall conductivity (125 and 175 µS/cm at Middle Fork of the John Day River) was positively correlated with larval abundance ($R^2 = 0.2080$, $P = 0.02$). The authors note that this correlation may be due to water conductivities being closer to that of larvae, resulting in increased sampling efficiencies during electrofishing surveys (discussed later in *Electrofishing Surveys*) when compared to other river environments sampled for larvae (e.g., 76 µS/cm at Cedar Creek, a low order stream with high lamprey use); "A fish will receive the maximum shock through its body when the conductivity of the water approaches that of the fish" (Koltz, 1989 as cited in Stone & Barndt [38]). Furthermore, the authors also observed that the proportion of their sampling units containing larvae per reach were positively associated with dissolved oxygen ($R^2 = 0.34$, $P < 0.05$). Larvae are unable to survive in anoxic (i.e., depleted of dissolved oxygen) conditions, yet they have a very low oxygen consumption rate which allows them to survive in environments with lower levels of oxygen [24]. However, larvae during metamorphosis have a higher oxygen consumption rate and are often found in water with higher dissolved oxygen (Richards & Beamish, 1981 as cited in Dawson et al. [24]). Dawson et al. [24] provide a more comprehensive

description of the various microenvironmental (e.g., substrate size and depth, organic matter in sediment, patchiness at small scales) and macroenvironmental (e.g., channel gradient, water depth, riparian canopy, water chemistry, proximity to spawning habitat, and thermal and oxygen requirements) variables that influence larval lamprey abundance and distribution at different spatial scales.

As mentioned above, the size of larvae can alter the relative importance of habitat variables on larval presence [11,24]. This is because larvae of varying sizes can occupy different types of rearing habitat. Larval preferences for sediment size and composition, burrowing depth and speed, and water velocity and depth are all affected by larval size [24]. For example, larger larvae are stronger than smaller larvae, and therefore have more energy required to burrow through heavier or larger sediment types. Larger larvae (> 100 mm) are often found in larger sediment types and deeper within the sediment (e.g., up to 15 cm deep) [24,44]. In contrast, smaller larvae occupy the upper few centimeters of sediment, including thin layers of fine silt and detritus over gravel and cobble substrate [24,44]. The relationship between larval size and sediment particle size is important when it comes to the speed and depth to which larvae can burrow to flee from predators [24]. Dawson et al. [24] explain that although fine sand is ideal for water flow and burrow construction, finer sediment (e.g., clay and silt) can be difficult to burrow into since it is more compacted. There is also risk to larvae as the packed sediments could clog gills and smother existing burrows.

Finally, biologists conducting larval lamprey surveys use a standardized classification schema to characterize larval habitat preferences into broad categories referred to as Type I, Type II, and Type III [8,11,47] (Table 2). Type I habitat consists of

a loosely compacted mixture of fine sediment and organic matter, located primarily in depositional areas. This habitat type is preferred by larvae because it provides favorable burrowing substrate. Type II habitat consists of shifting coarse sand and small gravel, with low organic matter. This habitat is utilized by larvae but at lower densities. Type III habitat consists of hardpan clay, hard packed gravel, cobble and bedrock. This habitat is rarely occupied by larvae. Type I habitat can occur within patches of Type II and Type III habitat [9,11,24]. The larval habitat preferences are similar among species and multiple species are often found occurring within the same habitat, including Pacific, western river, and western brook lampreys [24,31].

Table 2. Larval habitat classification (Type I, II and III) and level of use by larvae (high, medium, and low). Table adapted from: Crandall JD, Wittenbach E. Pacific Lamprey Habitat Restoration Guide. Twisp, Washington; 2015. [9].

Type	Use	Substrate Composition
I	High	Fine sediment including silt, sand, and detritus; medium-high organic matter
II	Medium	Shifting coarse sand, small gravel; low organic matter
III	Low	Bedrock, boulders, cobble, large gravel; low or no organic matter

Ecological & Cultural Significance: The Intertwined & Unknown Story of Pacific Lamprey & Pacific Salmon

Pacific salmon are recognized as a keystone and iconic species of the Pacific Northwest. The value that salmon have to the ecosystem, tribal peoples, and commercial and recreational harvest is recognized and well-studied. In contrast, many people are unaware of the vital role that Pacific lamprey have in our waters, and their connection to Pacific salmon. Pacific salmon and Pacific lamprey are both anadromous and

semelparous; require similar habitat; are critical to food web dynamics and nutrient cycling; and are First Foods to many Native American tribes in the Pacific Northwest.

The ecological and cultural importance of Pacific lampreys have been described in detail in the literature and reviewed by Close, Fitzpatrick, and Li [1]. The main elements are described below as well as additional information that may apply to other species of lampreys.

- For millions of years, Pacific lamprey have coevolved with native fish communities and are an integral part of the ecosystem; their decline has likely had unseen effects (Kan 1975 as cited in Hayes et al. [48]).
- Native lampreys have significant ecological roles at all stages of their life cycle [30] (Figure 7). They are important contributors to trophic dynamics and nutrient cycling in marine and freshwater ecosystems of the Pacific Northwest [1,5,48]. Where abundant, larval lampreys contribute a large portion of the biomass at the base of the food chain – along with aquatic insects – in freshwater environments [8,30]. For instance, western river lamprey were observed to be the dominant organism by weight in bottom sediments of the Fraser River, B.C. (Beamish & Youson, 1987 as cited in Docker et al. [30]).
- Additionally, larvae are indicators of sediment and water quality, often termed "ecosystem engineers" [30,38,43,49]. This is because larval burrowing and feeding activities can improve water quality and nutrient cycling by increasing bioavailable nutrients, fine particulate organic matter, and substrate oxygen levels [10,24,30,49]. Docker et al. [30] suggests that studies are needed to consider the effects that larvae have on biotic factors in and around their habitat.

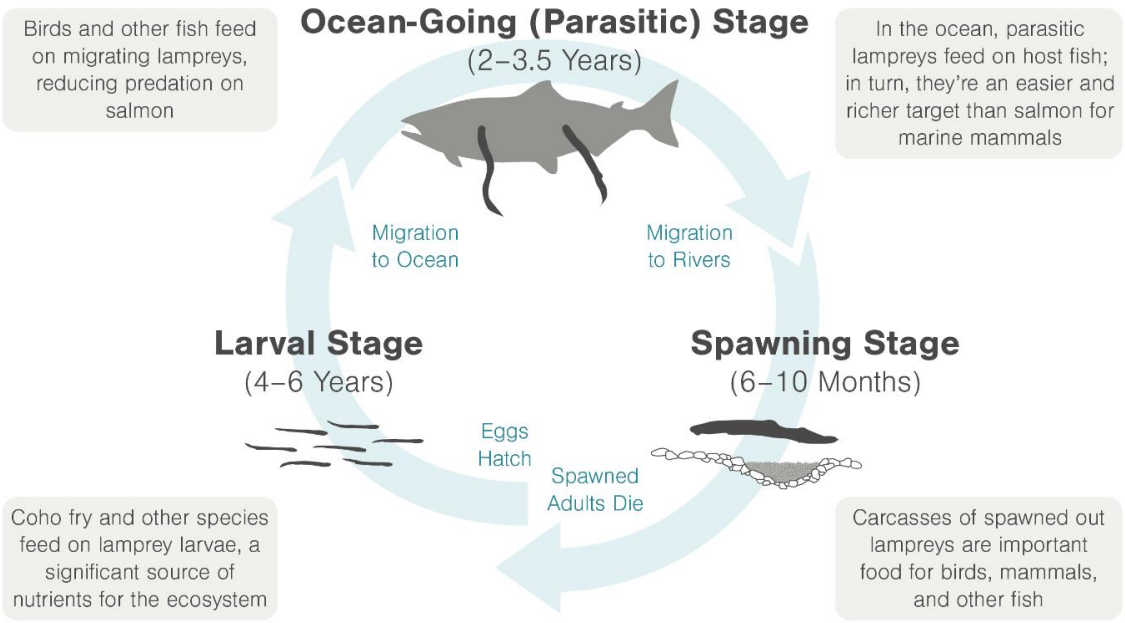


Figure 7. Anadromous lampreys have significant ecological roles in freshwater and marine environments at all stages of their life cycle. Illustration by Mark Garrison and obtained from: Goldfarb B. Defenders of the Forgotten Fish. Hakai Magazine [Internet]. 2015. [50].

- Lampreys at different stages of life are prey for many aquatic organisms ranging from crayfish up to birds and marine mammals [36]. When larvae and juvenile lamprey emerge from their burrows, they are vulnerable to predators, including salmonids, birds and small mammals (e.g., mink, raccoon) [8]. They become particularly exposed during scour events and downstream migration. Historically large numbers of emigrating lampreys may have reduced predation rates on salmon smolts, acting as a buffer from predation by birds, mammals and other fish [1,8]. Merrell (1959) as cited in USFWS [8] found that Pacific lamprey in the Columbia River accounted for 71% by volume of the diets in several species of gull and tern. Moreover, juvenile lampreys have been confirmed as prey for smolts, pikeminnow, channel catfish, and various other fish species [1,28]. In

addition to playing a role as prey, out-migrating juvenile lampreys may begin feeding while still in freshwater. A recently transformed western river lamprey was found attached to a coho salmon at the mouth of the Skeena River in B.C. [28]. In Lake Washington, Warner (2000) as cited in Wydoski and Whitney [28] found that 12% of sockeye smolts had at least one lamprey scar. According to Orlov and Beamish [36], ocean survival of young lampreys is likely influenced by the ability to locate marine hosts.

Anadromous lampreys are an important predator and prey source during their adult life [30]. However, predation is thought to be less during their marine stage, as adults are more dispersed and only congregate together during their upstream migration at which point they are more vulnerable to predation [30]. Pacific lamprey spend a quarter of their lives feeding on a variety of fishes (e.g., Pacific salmon, cod, hake, and herring; pollock, flatfish, and rockfish) and marine mammals while in saltwater environments [37,51]. Docker et al. [30] states, "there is no evidence that native lampreys are detrimental to the ecosystems in which they occur." This may suggest that the net benefits of lamprey to salmon (e.g. as alternative prey for salmon predators, and perhaps other ecosystem services) outweigh their predatory cost to salmon. Additionally, since observations of native lampreys began, "no host decimations have been reported for Pacific lamprey or other parasitic lampreys, suggesting potential co-evolution of these parasitic lampreys with their hosts" [52]. Evidence suggests that Pacific lamprey preference is influenced by the size of the prey; with older and larger-diameter lamprey selecting larger hosts [51]. Pacific salmon are one of the

primary food sources of Pacific lamprey, and Wydoski and Whitney [28] suggest that the declines in Pacific lamprey abundance may be partly attributed to declining salmon populations. Observations of healthy fish captured with visible attachment scars support the theory that Pacific lamprey do not cause mortality of their marine prey, however much of the marine ecology of Pacific lamprey requires further research [9,51,53].

Once anadromous lamprey species are reproductively mature, they begin their upstream migration [8]. At this time, they will lose their appetite and stop feeding. A common misconception is that lampreys will parasitize humans if given the opportunity. However, because immigrating lampreys have lost their appetite, they are unlikely to penetrate the skin if they were to latch on to a person as a convenient surface to rest on while they are swimming upriver [29]. Correspondingly, most upstream migration is nocturnal, with lamprey hiding among the rocks in deeper water during the day [11,34].

Adults returning upstream are also an important food source and potential predation buffer for salmon from predatory fish, birds (e.g., herons, ducks, and seagulls), and marine mammals; including pinnipeds, mustelids, and cetaceans [1,8]. Roffe and Mate (1984) as cited in USFWS [8] observed that Pacific lamprey were the most abundant prey of seals and sea lions in the Rogue River, Oregon. Similarly, stellar sea lions are often observed feeding on immigrating Pacific lamprey at the mouth of the Klamath River [30,31]. According to Wydoski and Whitney [28] and others [1,5,6,8], “there is speculation that declines in Pacific lamprey may have resulted in increased predation on salmon by seals

and sea lions because lamprey may be easier for marine mammals to catch than salmon." Another reason may be because Pacific lamprey are lipid-rich with average caloric values of 5.92 to 6.34 kcal/g wet weight compared to values of 1.26 to 2.87 kcal/g wet weight for Pacific salmon [8]. Furthermore, since adult lamprey die after spawning, their carcasses provide food for scavengers and marine-derived nutrients to freshwater and terrestrial environments as they decompose [8,30,31].

- Pacific lamprey are a tribal trust species and protected under tribal treaty rights [37]. Pacific lamprey, commonly called 'eels,' have been harvested by many Native American tribes (CRITFC: Yakama, Umatilla, Nez Perce, Warm Springs; Yurok, Karuk, Wiyot, Kalapuya, Umpqua, Molalla, Rogue River, Shasta, and other Tribes) over the millennia. Many tribes consider Pacific lamprey to be as valuable as salmon and vital for cultural, ceremonial, medicinal, and subsistence purposes [1,7]. These fish are captured by hand or with nets, to be processed for food and medicine [1]. From a tribal perspective, the decline of lamprey has had negative effects on the ecosystem and tribal way of life, including loss of cultural heritage (i.e., teaching the next generation) and severely depleted fishing opportunities; tribal members in the Columbia basin have to travel long distances from their historic fishing areas because lamprey no longer have upstream passage [1,5].
- In the late 1800s to early 1900s, there was a historic commercial harvest for Pacific lamprey for use as 'premium' feed for salmon hatcheries, fish oil, and protein food for livestock [1]. Larval and adult lamprey have been used as bait for

white sturgeon, trout, and smallmouth bass in the Columbia, Snake, and Fraser Rivers [28]. Historically, lamprey were also used as bait to capture coyotes by fur trappers [1,28].

- Lamprey have been used as academic specimens for teaching material in vertebrate anatomy classes and for scientific research [1,28]. For example, parasitic lampreys secrete a mucous during feeding, acting as an anticoagulant (keeps blood flowing), and this has been researched for the development of medicinal anticoagulants [1].

It is important to recognize the ecological and cultural significance of native lampreys in the Pacific Northwest and their shared history and uncertain future with Pacific salmon. Lampreys and salmon face similar anthropogenic threats to the quality of their environment and availability of food. It is necessary to understand these impacts to better implement actions that will address the larger issues of lamprey survival.

Conservation Efforts

> *"If these most resilient and viable species are starting to go extinct, what is next? It's time that we hear their outcry, see their true beauty, sense their magnanimity, and give them a helping hand."*
>
> – Ralph Lampman (Yakama Nation Fisheries)

Due to their close connection to Pacific lamprey, the tribes were the first to notice and raise awareness of the decline in abundance and reduction in distribution of this species [6]. This awareness led to the USFWS petition in 2003 to list three species of lamprey (Pacific lamprey, western river lamprey, and western brook lamprey) as

threatened or endangered under the ESA in Washington, Oregon, Idaho and California [4]. Although the petition was denied due to lack of data on the abundance and distribution for all three species [2,4], awareness of the declining status prompted the establishment of the Pacific Lamprey Conservation Initiative (PLCI) [2]. USFWS is the lead for the PLCI and has coordinated conservation efforts with Native American tribes and numerous local, state, and federal agencies [6]. The goal of the initiative is to improve the status of Pacific lamprey throughout their U.S. range across Idaho, Oregon, Washington, and Alaska [6]. This area has been divided into 15 regional management units (RMUs) (Figure 8), including the Puget Sound/Strait of Juan de Fuca and Washington Coast (RMU 13 and 14, respectively) [8] (Figure 9). A NatureServe[6] Risk Assessment was performed across the species range, however the analysis did not include RMUs 13 and 14 due to the scarcity of information available on status and distribution of Pacific lamprey in this area [2,54] (Figure 10). NatureServe model parameters needed to complete the Risk Assessment include: 1) population distribution (range extent, area of occupancy), 2) population abundance, 3) short-term population trend (e.g., three generations, approximately 27 years), and 4) threats analysis for each watershed (J. Poirier, USFWS, pers. comm., May 31, 2019).

The result of this data gap has triggered recent work in Puget Sound watersheds [8,20,48]. A revised assessment was updated in 2019[7], though the status of the Puget Sound RMU is largely unchanged since the 2011 assessment [2] due to information

[6] NatureServe, Inc. is a non-profit organization that provides proprietary wildlife conservation-related data, tools, and services.

[7] Risk Assessment is reassessed every five years, next update planned for 2022.

lacking on current and historic population data [8]. Only 4 out of 20 watersheds have received NatureServe status ranks. The Nooksack, Puyallup, Dungeness-Elwha, and Crescent-Hoko watersheds are now categorized as Critically Imperiled (S1)[8]. Moser and Paradis (2017) as cited in USFWS [8] suggest that Pacific lamprey abundance may be increasing in the Elwha River due to the removal of the Elwha Dam in 2012. The status of Pacific lamprey is still unknown in the Nisqually River and the other 15 unranked watersheds within the Puget Sound. Preliminary ranking estimates indicate that the current area of occupancy is 0.1% to 37% of historic distribution throughout Washington [8].

[8] NatureServe Rank Approach, S1 Critically Imperiled – Critically imperiled in the jurisdiction because of extreme rarity or because of some factor(s) such as very steep declines making it especially vulnerable to extirpation from the jurisdiction [8].

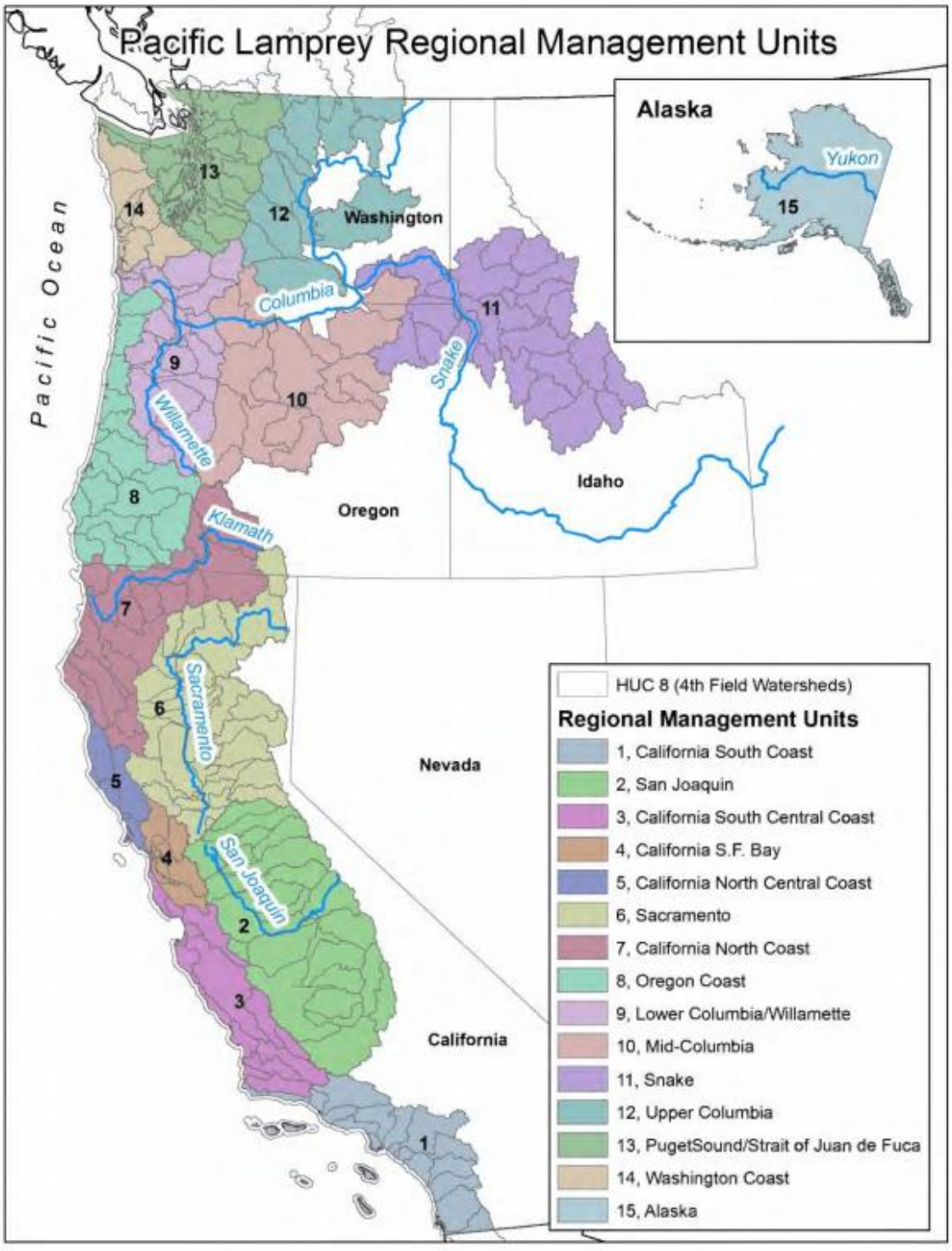


Figure 8. Pacific lamprey range and regional management units (RMUs) across Washington, Oregon, Idaho, California, and Alaska. Figure obtained from: USFWS. Pacific Lamprey Entosphenus tridentatus Assessment. 2019. [8].

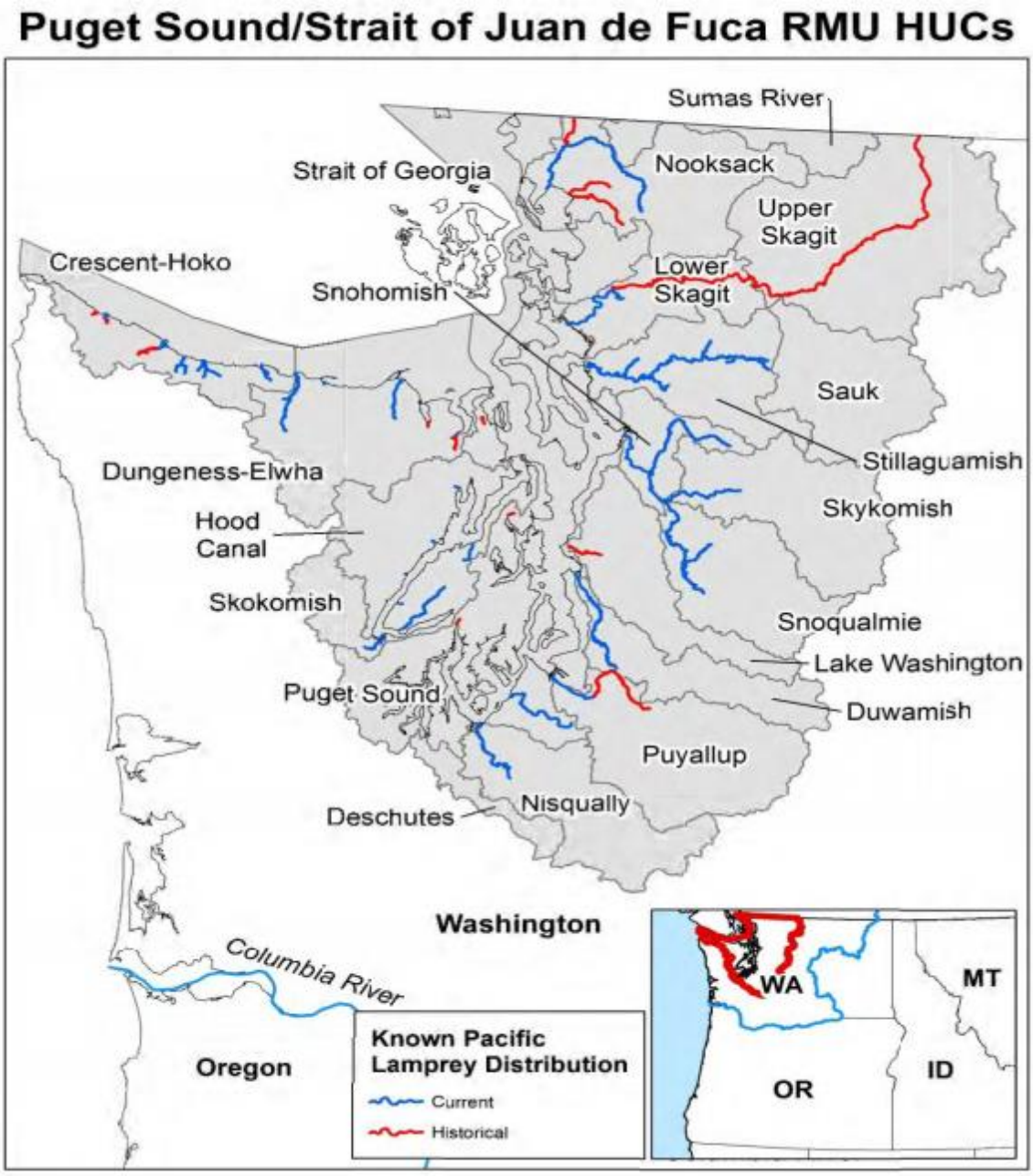


Figure 9. Current (dark blue lines) and historic (red lines) Pacific lamprey distribution across 20 watersheds (4th Field HUCs – Hydrologic Unit Codes) within the Puget Sound/Strait of Juan de Fuca RMU (shaded grey) in Washington State. Figure obtained from: USFWS. Pacific Lamprey Entosphenus tridentatus Assessment. 2019. [8].

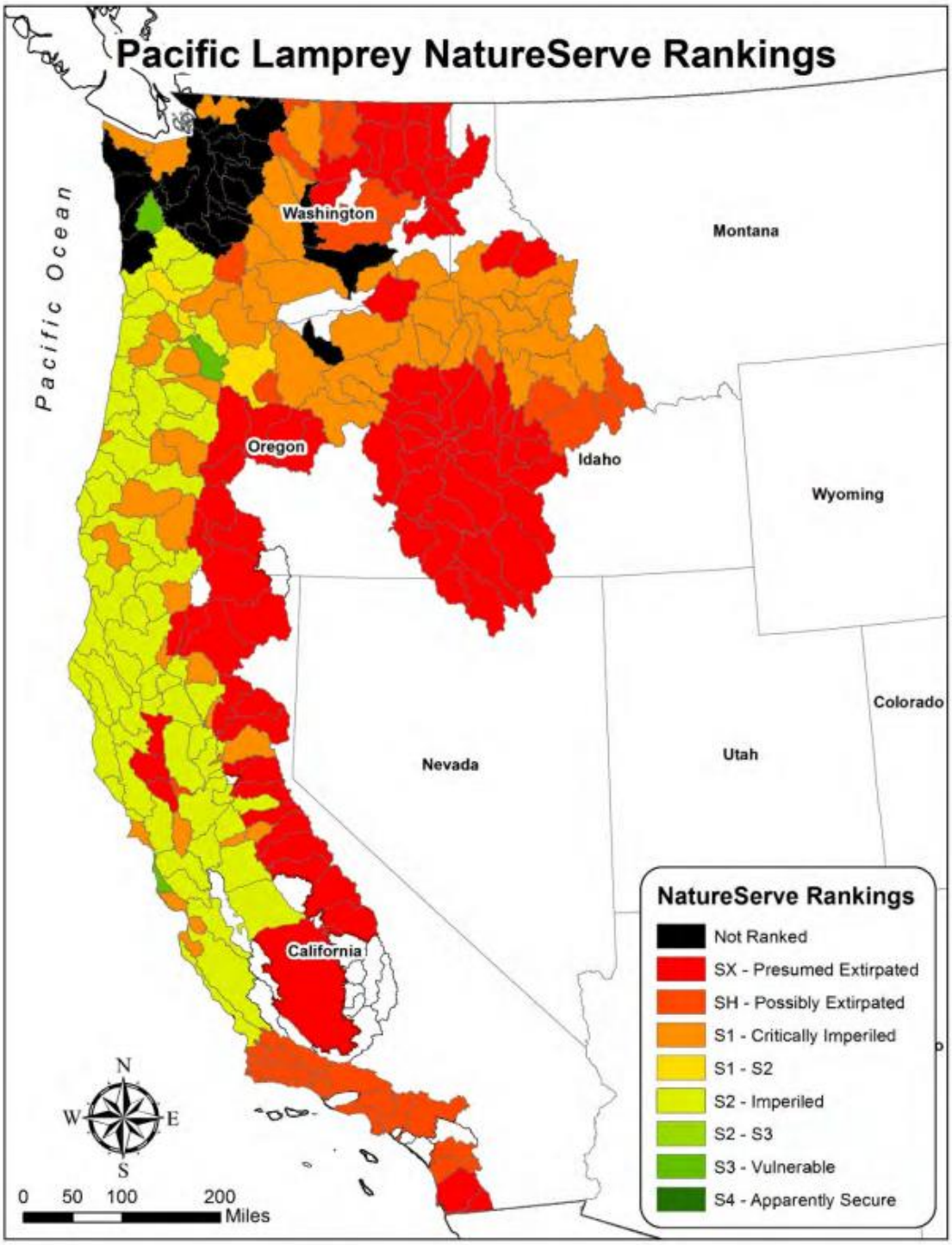


Figure 10. NatureServe risk rankings calculated for Pacific lamprey throughout the species range in Washington, Oregon, Idaho, and California. Note the 'Not Ranked' status (black) in the Puget Sound and Washington Coast RMU. Figure obtained from: USFWS. Pacific Lamprey Entosphenus tridentatus Assessment. 2019. [8].

The new assessment also identified the main threats specific to the Puget Sound/Strait of Juan de Fuca RMU, including dewatering and stream flow management, stream and floodplain degradation, lack of awareness of the status of Pacific lamprey, and climate change [8]. The USFWS assessment ranked major threats that impact Pacific lamprey at various stages of life across their range [8]. The following threat categories were used for the ranking and are not listed by level of severity:

- Passage (dams, culverts, water diversions, tide gates, other barriers)
- Dewatering and flow management (reservoirs, water diversions, instream projects)
- Stream and floodplain degradation (channelization, loss of side channel habitat, scouring)
- Water quality (water temperature, chemical poisoning and toxins, accidental spills, chemical treatment, sedimentation, non-point source)
- Harvest/overutilization
- Predation
- Disease
- Small effective population size
- Lack of awareness
- Climate change
- Mainstem passage (if applicable)

These threats are likely cumulative as no single threat can be identified as the primary reason for lamprey decline [8]. Maitland et al. [55] states that anthropogenic "pressures appear to relate especially to one or more of the three main stages in their life history that occur in freshwater – larval development, downstream and upstream migration, and spawning." Therefore, available stream habitat is essential for lamprey production and survival [38]. Since all lamprey species share a prolonged larval phase in

freshwater sediments, and because larval occupied habitat is comprised of multiple generations, this increases their vulnerability to disruptions to their larval rearing habitat [2,24]. For example, land use practices that restrict accumulations of river sediments can limit the availability of burrowing substrate, and rapid fluctuations in water levels can leave larvae stranded [10]. Artificial barriers that restrict or prohibit mainstem and tributary passage also impact lamprey success by limiting downstream movement of larvae and juveniles and upstream movement of adults returning to spawn [8]. Luzier et al. [2] and USFWS [8] provide summaries of threats and corresponding conservation and restoration actions needed to address the impacts to Pacific lamprey. The primary conservation opportunities to protect and restore Pacific lamprey populations include: 1) provide lamprey passage, 2) protect larval habitat, and 3) restore stream channel complexity [56]. Additionally, the following list provides useful resources and guidelines for lamprey conservation management:

- Best Management Practices to Minimize Adverse Effects to Pacific Lamprey [10]
- Pacific Lamprey and NRCS: Conservation, Management and Guidelines for Instream and Riparian Activities [37]
- Tribal Pacific Lamprey Restoration Plan [5]
- Practical Guidelines for Incorporating Adult Pacific Lamprey Passage at Fishways [53]
- Pacific Lamprey Climate Change Vulnerability Assessment [57]
- Master Plan: Pacific Lamprey Artificial Propagation, Translocation, Restoration, and Research [58]
- Pacific Lamprey *Entosphenus tridentatus* Assessment [8]

The status of *Lampetra* spp. is largely unknown [59]. However, the western river lamprey is the only native lamprey species listed as a state candidate for Washington [60]. Pacific and western river lampreys are both listed as federal species of concern [60].

The statewide general rules for sport fishing currently prohibit the harvest, possession, or use as bait of Pacific lamprey, western brook lamprey, or western river lamprey except for tribal treaty members [61]. While the focus has been on Pacific lamprey, research and restoration efforts will benefit other lamprey species as well (C. Wang, pers. comm., February 6, 2019).

To better understand the status of native lampreys, more research is needed to address lacking distribution and abundance data in Western Washington. The following are potential research barriers: 1) improper field identification of larvae, 2) cryptic larval life history, 3) nocturnal movement throughout most of life cycle, and 4) limited knowledge of lamprey life in the marine environment. The WDNR has contributed to this thesis research to 1) help protect lamprey and their habitat, and 2) address the lamprey distribution data gap that is lacking in Washington State. This research could potentially allow land managers to assess if larval lampreys occupy a project site so that additional measures can be taken to avoid or mitigate the impact to lamprey species.

Detection Methods for Larval Lampreys

The first step towards native lamprey conservation is to determine where lamprey populations occur [9,11]. The type of methods used to obtain occupancy information can vary by life stage and study objective [11]. Currently, the standard methods used to determine lamprey presence in freshwater habitats include electrofishing surveys for larvae and spawning or nesting surveys for adults (Mayfield et al., 2014 as cited in Grote & Carim [22]). A comprehensive review for passive and active methods used to survey early life and adult stages of lampreys is covered in depth in Moser et al. [11]. One common strategy to collect distribution data is to survey for the larval life stage [9,62], as

this stage is less challenging to detect in freshwater environments in comparison to adult lampreys due to the following reasons: 1) occupancy of the stream or reach occurs year-round for larvae (and in multiple age classes) versus seasonally for adults, and 2) most movement is nocturnal for all life stages, however during the day larvae occupy burrows in shallow, more easily accessible habitat and adults are typically in deeper water, hiding under rocks [11,62]. This method assumes that if larvae are present then other life stages are as well [9]. Determining the upstream and downstream extent of larvae occupancy can help identify areas where adults reside, such as where spawning occurs upstream of larval rearing habitat [9].

Our research is specifically centered on larval lampreys. Therefore, the following section will provide a brief background of the detection techniques used for this study: lamprey-specific electrofishing surveys and aquatic eDNA sampling.

Electrofishing Surveys

Electrofishing, or electrical fishing, is a scientific technique used for surveying salmonids and other fish in rivers or streams. Implemented for lamprey monitoring, this surveying method is commonly used for assessing larval lamprey presence in freshwater sediments. However, it is important to use electrofishing settings that are specific to larval lampreys, as higher voltages and frequencies typically used in salmonid surveys can cause stress or harm, even leading to electronarcosis[9] or death [9,59]. USFWS [10] provides electrofishing recommendations for best management practices for monitoring larval lampreys. Surveys are typically performed using backpack or shore-based

[9] Electronarcosis: stun or seize buried larvae causing failure to emerge from the burrow, which can indicate false absence [10].

electrofishing and are best used in waters less than 3 feet (1 meter) deep for safe operations [10,11,63]. Best practices for conducting larval surveys include the use of a two-stage backpack electrofisher [10]. The first stage uses a lower frequency to irritate or "tickle" larvae to come out of their burrows without causing narcosis. The second stage is a higher frequency that immobilizes or "stuns" the larvae for capture by dip netting once they surface from the sediments. This setting is not always needed to capture emerging larvae and should never be used directly on larvae (i.e., avoid using the electrodes to capture larvae). Captured larvae should be handled carefully and placed in a container of cool river water until electrofishing is complete [9,11].

Pace and duration of electrofishing is another consideration for minimizing impact to larvae while sampling. Electrofishing for larvae should be done at a relatively slow and methodical pace over the sample area [9,12]. Electrofishing surveys can be single pass or depletion sampling [11,63]. Depletion sampling is the process of conducting multiple passes over one sample area with a minimum of 15 minutes between passes to reduce the chance of electronarcosis. This method is used when complete removal of larvae is necessary (i.e., quantitative assessments for density estimates, or during salvage or translocation events) [11]. The direction of sampling is also important; researchers should move slowly upstream to minimize disturbance of sediments which can decrease water visibility [9].

Electrofishing capture efficiency is reduced with increased water depth, low visibility (e.g., low light and glare, turbidity, etc.), and vegetative cover [10]. Other variables that may impact electrofishing efficiencies are larval size and density, water temperature, and water conductivity [11,12]. For example, larval sizes less than 40 mm in

length are less efficient for capture [11]. Generally, electrofishing surveys with lamprey-specific settings achieve greater than 90% larval detection rates when used at sites with preferred larval habitat characteristics (i.e., Type I) and in watersheds with known lamprey occupancy [20,62]. Additionally, Dunham et al. [12] observed that lamprey-specific settings had odds of capture approximately 2.66 times greater when compared to standard salmonid settings.

Jolley et al. [64] conducted survival trials on wild-caught and hatchery-sourced Pacific lamprey and *Lampetra* spp. to test the effects of backpack electrofishing. The study also looked at the effects of deep-water electrofishing and suction-pumping, anesthesia, and handling. Deep water electrofishing is often used with a mechanism (e.g., trawl sampler or suction pump) to bring stunned larvae to the surface [11]. Within 96-hours (short-term) following electrofishing, the observed survival rate was greater than 98% (1 mortality), with delayed mortality of 4 (out of 102) larvae a week later (long-term) from signs of a fungal infection and internal hemorrhaging. Stress from handling, shocking, and disturbing habitat are all concerns while sampling and are reasons why eDNA analysis may be a favorable detection method.

Aquatic Environmental DNA – Aqueous & Sedimentary

Thomsen and Willerslev [21] define eDNA as: "genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material." First used to define microbial communities, this approach has been used to detect and evaluate macrobial (animals, plants, and fungi) eDNA collected from terrestrial and aquatic sediments, ice, soil, freshwater, and seawater environments [21]. Genetic material (DNA from cells and tissues) originates from the organism through

excrement, gills, scales, gametes, and decomposing individuals [20–22]. With the development of species-specific primers[10], genetic material present within the sample can be extracted and subsequently amplified by Polymerase Chain Reaction (PCR) and DNA sequencing, allowing for detection of target organisms [19,21]. Quantitative PCR (qPCR; also known as real-time PCR) is a specialized form of PCR that enhances the ability to amplify DNA and quantify gene expression by linking the amplification[11] of DNA to the generation of fluorescence [65]. This is done by using a fluorescently labelled probe (or DNA oligonucleotide) with the species-specific primer pair[12]. This fluorescent signal can be detected in real-time during each PCR run, with levels of fluorescence increasing with the concentration (number of copies per unit) of DNA amplified[13]. A sample is determined to be positive if the fluorescent signal exceeds a certain threshold (Figure 1 of Appendix A). The cycle threshold (C_T) is the number of cycles required for the fluorescent amplification curve to intersect with this threshold [66]. Therefore, each positive qPCR amplification has an associated C_T value. Since the accumulation of the fluorescent signal is positively associated with DNA amplification, the C_T value is also related as higher concentrations will cross the threshold earlier (C_T =

[10] Primers: small pieces of target DNA that prime the DNA sample ready for the polymerase enzyme to bind and begin copying the target gene [65].

[11] During PCR, temperature is used to control polymerase activity and the binding of primers. To start a PCR reaction, the temperature is raised to the "melting temperature." This separates the double stranded DNA into single strands (denaturation). Once this occurs, the temperature is lowered, and this allows for the primers to bind (annealing) and begin copying the DNA strand (synthesis). The temperature change is typically repeated for 40-50 cycles, creating multiple copies of the target DNA, hence amplification [19,20,65,90].

[12] Two primers are required – a forward and reverse pair – to bind to each single DNA strand during denaturation [90].

[13] Standard PCR methodology requires gel electrophoresis with dye to visualize target DNA.

20-30 cycles versus 31-40; Appendix A). Carim et al. [19] and Ostberg et al. [20] have successfully developed qPCR-based assays (primer pair and fluorescent probe) for aquatic detection of Pacific lamprey and *Lampetra* spp., respectively.

Dorazio and Erickson [23] explain that the probability of eDNA from the target species being present in collected samples is dependent on several factors, including 1) source locations of eDNA, 2) the degradation and transport of eDNA from these locations, and 3) the size of the sample. Thomsen & Willerslev [21] provide a thorough assessment of challenges that can arise with this approach during study design, field collection, laboratory analysis and data interpretation (i.e., contamination, PCR inhibition, errors, reference DNA databases). To address some of these issues, it is important to use standardized protocols [67].

Over the past decade, the use of eDNA-based methods have developed as a valuable monitoring tool for conservation, particularly in aquatic environments where it is often more difficult to detect rare, cryptic, or invasive species with traditional sampling methods [20,67]. In contrast to conventional methods, eDNA collection is comparatively easy, non-invasive (no direct handling of animals or harm to habitat), and relatively time- and cost-effective; once on site, approximately 15 minutes suffices to collect and record water samples which reduces staff time and resources in the field considerably [21,22,67]. Likewise, collection of eDNA samples does not require intensive scientific permitting or technical training that is required for electrofishing surveys [68]. In addition, detection is highly sensitive to low animal densities and a single sample can be used to detect multiple species at the time of collection or can be preserved until a later date, if funding is limited [22,67].

eDNA analysis of field collected water samples is a particularly useful method as it can be used in small or large river systems [22]. However, depending on the specific objectives of the study, a few limitations can arise with eDNA analysis in river systems: 1) the DNA sample can be a signal from upstream (downstream drift), 2) analysis is unable to determine from what life history stage(s) the DNA originates, and 3) information on age and size structure of local populations cannot be obtained [21,22]. The species targeted, where they occupy the water column (free-swimming versus benthic-oriented) and the timing of sampling can all influence what DNA is likely to be detected. Hence, knowledge of species' biology and ecology is key to analyzing eDNA results.

Recent studies have expanded the use of eDNA water sampling to apply to a benthically-oriented species, Pacific lamprey [19,20,22]. Results from these studies suggest that eDNA sampling can be a valuable tool for assessing presence and distribution of Pacific lamprey. eDNA analysis is unable to differentiate specific life stages, such as presence of larvae versus adult lamprey. In fact, DNA from adult lamprey present in the water column is more likely to be detected than that of larvae buried in the benthos [22]. However, there are considerations when choosing timing and frequency of sampling. Avoiding lamprey spawning season can limit the amount of adult DNA present, though this timing can vary by species, environmental conditions, and elevation [20]. Moreover, Pacific lamprey can spend up to a year in freshwater before spawning, while larvae of different age-classes occupy sediments year-round [9,24].

Ostberg et al. [20] detected the presence of Pacific lamprey and *Lampetra* spp. through eDNA analysis of water samples collected across 18 Puget Sound watersheds.

The findings from their study found that eDNA detection varied seasonally and by lamprey species; Pacific lamprey eDNA detection rates[14] were 0.26 and 0.90 (of the 14 watersheds detected), while *Lampetra* spp. were 0.62 and 0.93 (of the 16 watersheds detected); fall and spring, respectively. The authors note that the seasonal variation was likely attributed to differences in fall and spring stream flow rates. The sample sites were selected based on a previous study that used incidental catch observations to obtain presence data of Pacific lamprey and *Lampetra* spp. at smolt trap locations across the Puget Sound [20,48]. Pacific lamprey eDNA was detected at two additional sites when compared with the fish trap observations (12 of 18 watersheds). For larval lampreys, aqueous eDNA detection rates are influenced by the density of larval abundance because of their placement in the sediments (Gingera et al., 2016 as cited in Grote & Carim [22]). Ostberg et al. [20] have proposed strategies to improve detection rates of larval lamprey eDNA: 1) collect water samples at locations with suitable larval habitat, and 2) collect sediment samples at these same locations since larvae burrow in the sediment.

The amount of time larvae spend rearing in freshwater and patchy distribution within suitable habitat make eDNA sediment sampling an ideal monitoring tool for Pacific lamprey [20,43]. Sedimentary eDNA analysis has been used to detect other benthic species [18,21,69,70]. A limited number of studies have researched the viability of eDNA analysis to detect larval lampreys in aquatic sediments. The use of this approach to detect larval presence has been conducted in a U.S. Geological Survey (USGS) controlled laboratory experiment at the Western Fisheries Research Center

[14] Probability of detecting lamprey eDNA in water samples. The detection rates were estimated as the frequency of 1-L water samples that tested positive for lamprey eDNA averaged across all locations where lampreys were detected [20].

Columbia River Research Laboratory in Cook, WA (T. Liedtke, pers. comm., December 6, 2017). One objective of the experiment was to evaluate the relationship between eDNA concentration (mean copy number per gram of sediment) and larval biomass (g). Four biomass levels were tested: 1) 25 g = mean of 25 individuals, 2) 50 g = mean of 55 individuals, 3) 100 g = mean of 93 individuals, and 4) 200 g = 206 individuals. They associated a biomass of approximately 1 g, equivalent to 1 larva at a mean larval size of 80 mm in length[15]. The study also tested eDNA detection along a distance gradient from a known larval source and the persistence of eDNA following larval removal. Preliminary results suggest that there may be a positive relationship between biomass and eDNA copy number (i.e., sediments with more larvae had higher concentrations of eDNA); eDNA detection occurs within 2 meters (1.1 m is best) of a known larval source; and eDNA will persist longer if the starting concentration (larval biomass) is higher (28 days to 4 months, 50 g and 200 g, respectively) (T. Liedtke, pers. comm., December 6, 2017). These variables are difficult to control and test in the field as the number and location of larvae within the sediment is unknown. However, these findings provide valuable application for future field studies.

Krieter & Allen [71] is the only other study known to collect sediment samples for eDNA analysis for larval lamprey detection. Their research adapted sampling protocols from Turner et al. [18], Eichmiller, Bajer, and Sorensen [72] and the Center for Genome Research and Bioinformatics (CGRB). Collection was performed from a boat using a Petite Ponar® Grab Sampler, a device commonly used on vessels to take

[15] USGS experiment showed that DNA from larvae smaller than 80 mm in length were not detected as strongly in the sediment.

sediment samples from deeper water. Results from this study found very low eDNA concentrations of Pacific lamprey at all locations, except for one moderate reading at a reference site. To verify eDNA findings, sampling was paired with a custom-built sampling platform, Larval Lamprey Electrofishing System (LLES), that functions as an underwater videography system and electrofishing device to visualize larvae presence. Unfortunately, no larvae were observed to visually confirm eDNA detection results (challenges with water visibility were discussed). When low concentrations of eDNA are detected, it may be challenging to infer presence of target species [71]. Therefore, this approach should be used in combination with other surveying methods since eDNA-based methods are a relatively new technique that will require further fine-tuning [21,22].

Environmental, biological, and demographic factors influence the fate of eDNA in aquatic environments [18,20,22]. Following shedding from the target organism, eDNA immediately begins to decay and is distributed into the environment [15]. Once in the environment, Wilcox et al. [68] states that eDNA can be "lost as a function of degradation, dilution, deposition, and re-suspension." For example, concentrations of DNA in aquatic systems may fluctuate at different times of the year depending on stream morphology, velocity, water depth, temperature, and different life history events (e.g., spawning, juvenile emigration, etc.) [18,68]. Individual fish have been observed to have varying rates of DNA production (shedding), unrelated to body size [68]. Microbial activity, water chemistry, and UV exposure are additional factors that aid in eDNA degradation [15,73]. It is also important to note that eDNA abundance and persistence in water and sediments vary, and this can influence the objective of a study based on temporal and spatial differences [18,22]. Turner et al. [18] compared the presence and

persistence of DNA in aquatic environments. DNA degrades quickly in water – hours to two weeks – therefore eDNA water samples provide time specific inference to species presence [17,68]. In contrast, DNA is more concentrated and persistent in sediment[16], and may be able to offer 'current-or-past site occupancy' [18]. Although sedimentary DNA in rivers is constantly shifting, the year-long residency of larval lampreys burrowed in riverbed sediments may provide site-specific detectability.

Summary of Research Needs

The Pacific, western river, and western brook lampreys are native to rivers and tributaries of Western Washington. Lampreys and Pacific salmon serve important ecological roles and face similar challenges to their survival. Concern over the decline of native lampreys in the Pacific Northwest has prompted several collaborative conservation efforts among tribal, federal, state, and local organizations. These efforts have been focused primarily on Pacific lamprey because of tribal interest that is supported by federal obligation to meet treaty rights (C. Wang, pers. comm., February 6, 2019). This affiliation has facilitated more funding and resources to conserve this species in comparison to other native lampreys, such as western brook and western river lampreys. However, is it recognized that all lampreys share a prolonged larval phase, and therefore other lamprey species will benefit from the implementation of lamprey-specific research, restoration, and management protocols.

[16] "Carp eDNA was 8-1800 times more concentrated per gram of sediment than per milliliter of water and was detected in sediments up to 132 days after carp removal – five times longer than any previous reports of macrobial eDNA persistence in water" [18].

Globally, lampreys are in decline largely due to habitat disturbances that occur during their larval life stage. Consequently, there has been considerable research to understand their freshwater habitat requirements. However, information about lamprey biology and ecology is still poorly understood, especially regarding the estuarine and marine portion of their life cycle. Additionally, research has been focused in limited geographic areas (e.g., Columbia River basin, Fraser River). As such, the status of lampreys within Puget Sound and coastal Washington watersheds is still largely unknown. This data gap has initiated recent studies in this area, including this thesis research.

Recent studies have successfully used eDNA analysis of filtered-water samples to assess lamprey distribution within Washington watersheds [19,20,22]. eDNA detection methods are highly sensitive in comparison to traditional detection methods (e.g., electrofishing, netting, trapping), and are widely used to monitor the occurrence of rare species in aquatic ecosystems. Additionally, eDNA detection methods are non-invasive, providing minimal impact to the species and surrounding environment as sample collection does not require direct contact with the animals to confirm presence.

This literature review focused primarily on aspects of the larval phase and rearing habitat preferences as this life stage is ideal for surveying lamprey occupancy due to their year-round presence in freshwater sediments. However, it is also important to understand the overall lamprey life history, especially within the geographic region of study. This is because eDNA analysis is unable to determine the age of the DNA source, such as presence of larvae, juvenile, or adult lampreys. This knowledge influences sampling decisions, including sample type (e.g., water or sediment), location (e.g., habitat with low

water velocity and fine sediments), and timing (e.g., spawning) of environmental sample collection, and is key to interpreting eDNA findings. For instance, eDNA water sampling is more likely to detect DNA from adult lamprey present in the water column than DNA of larvae buried in sediments. Restrictions to this method include the ability to assess lamprey presence at the site-level, as the eDNA is likely from a source that is located an unknown distance upstream of the sample location.

Research has demonstrated that DNA can be more concentrated in sediment than in water [18]. Furthermore, the ability to detect larval lamprey presence from sediment eDNA has been shown in a controlled laboratory experiment (T. Liedtke, pers. comm., December 6, 2017). The goal of this thesis research was to apply a similar methodology and test the ability of eDNA analysis to detect Pacific lamprey and *Lampetra* spp. from field collected sediments located at sites along the Nisqually River, Washington.

METHODS & ANALYSIS

Introduction

This thesis research was designed to test and validate the use of eDNA analysis of riverbed sediment to identify the presence of larval lampreys, which can live up to nine years buried in freshwater sediments. The following chapter is organized into four sections: Study Design and Field Preparation, Field Data Collection, Laboratory Processing, and Data Analysis.

We selected the Nisqually River (Figure 11) for sampling because it has been documented that all three species of lamprey (Pacific, western river, and western brook lamprey) use the river at all life stages [20,48,74]. Sampling occurred at 8 sites on July

30th – August 2nd, 2018, located between river miles 3.5 and 13 (Figure 12), and each site consisted of three sampling grids; upper (U), middle (M), and lower (L) (Figure 13).

Three field collection methods were used to assess presence and compare the detection rates of larval lampreys at each site and are presented in order of sampling workflow: 1) eDNA sediment sample collection, 2) electrofishing surveys, and 3) eDNA water sample collection. Sediment sampling, electrofishing, and water sample collections were performed at each sampling grid before moving upstream to the next grid. Water sample collection occurred last and upstream of each site to avoid cross contamination between sediment and water eDNA samples. Several environmental parameters linked to larval habitat requirements were also measured during sampling (i.e. river and shoreline description, water quality parameters, sediment grain-size).

From each site, we collected 9 replicate sediment samples[17] and 1 water sample for eDNA analysis. All water (n = 16) and sediment (n = 72) samples were submitted to the Washington Department of Fish and Wildlife (WDFW) for processing in the Molecular Genetics Laboratory (MGL). Samples were tested for the presence of Pacific lamprey and *Lampetra* spp.[18] Additionally, sediment samples for grain-size analysis were submitted to the WDNR Aquatic Assessment and Monitoring Team (AAMT) to be processed at the Marine Station.

eDNA samples were determined positive for detection if a minimum of 2 of 3 qPCR reactions resulted in positive amplification. Positive qPCR reactions were assigned

[17] Each site had 3 sampling grids, with 3 replicate sediment samples each.

[18] Western brook lamprey (*Lampetra richardsoni*) and western river lamprey (*Lampetra ayresii*) cannot be differentiated at this time.

a high, medium, and low eDNA concentration category. To compare detection rates between each detection method, we calculated the proportion of grids with positive lamprey detections. A correlation analysis was run to assess if any relationships exist. Additionally, a multiscale occupancy model was used to estimate the probability of detecting Pacific lamprey and *Lampetra* spp. eDNA at three spatial scales: 1) sites, 2) samples, and 3) qPCR replicates (Figure 18).

Study Design

Duration of Study

We sampled the Nisqually River for lamprey on July 30th – August 2nd, 2018, during annual low flow and outside of the expected spawning activity of lamprey and salmonids. Sampling in August decreases the potential for eDNA signal interference due to adult spawning activity and mortality (i.e., allowed time for any associated DNA to flush down river)[19].

[19] eDNA analysis cannot distinguish between larval and adult lamprey DNA.

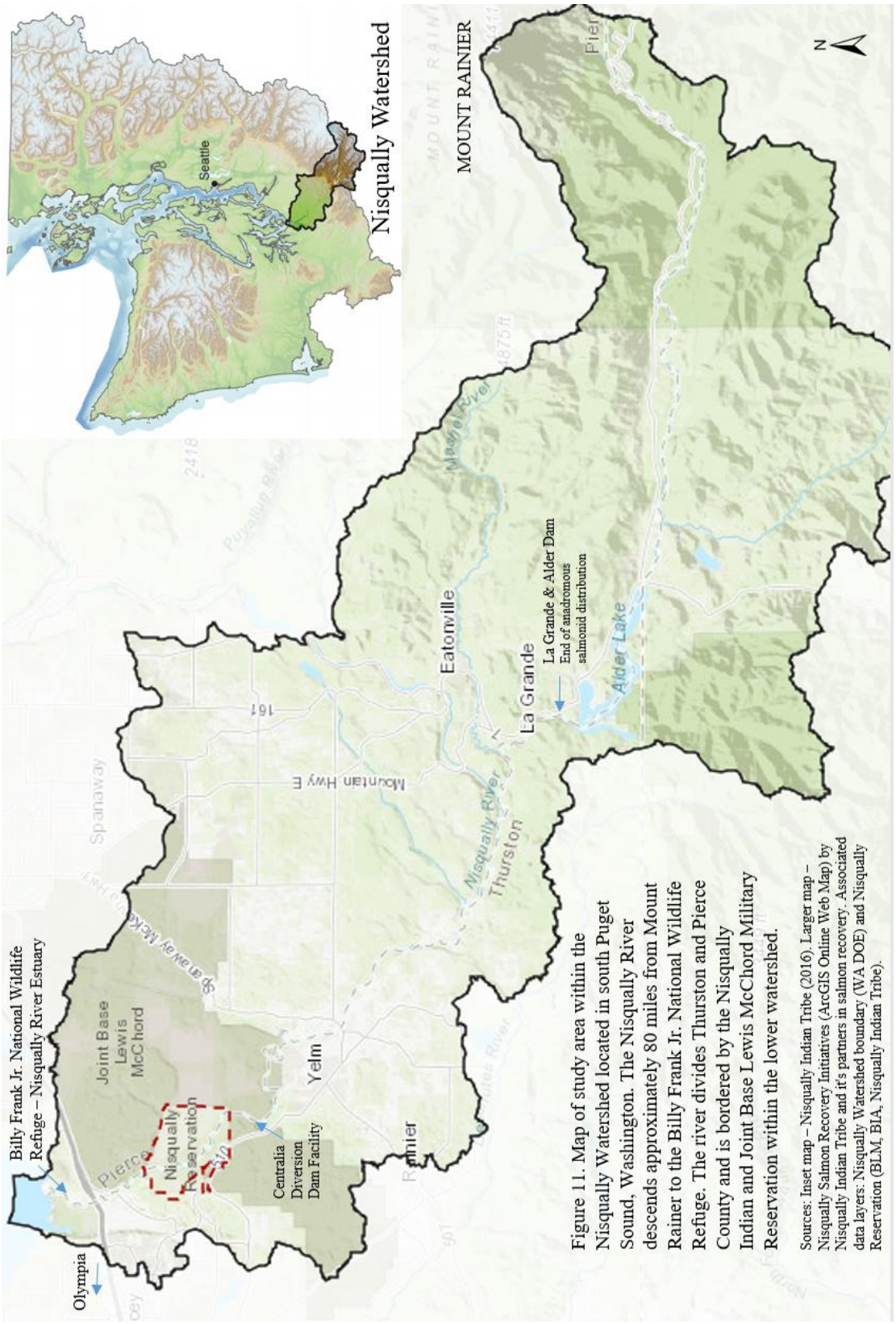


Figure 11. Map of study area within the Nisqually Watershed located in south Puget Sound, Washington. The Nisqually River descends approximately 80 miles from Mount Rainer to the Billy Frank Jr. National Wildlife Refuge. The river divides Thurston and Pierce County and is bordered by the Nisqually Indian and Joint Base Lewis McChord Military Reservation within the lower watershed.

Sources: Inset map – Nisqually Indian Tribe (2016). Larger map – Nisqually Salmon Recovery Initiatives (ArcGIS Online Web Map) by Nisqually Indian Tribe and it's partners in salmon recovery. Associated data layers: Nisqually Watershed boundary (WA DOE) and Nisqually Reservation (BLM, BIA, Nisqually Indian Tribe).

Figure 11. Map of Study Area within the Nisqually Watershed.

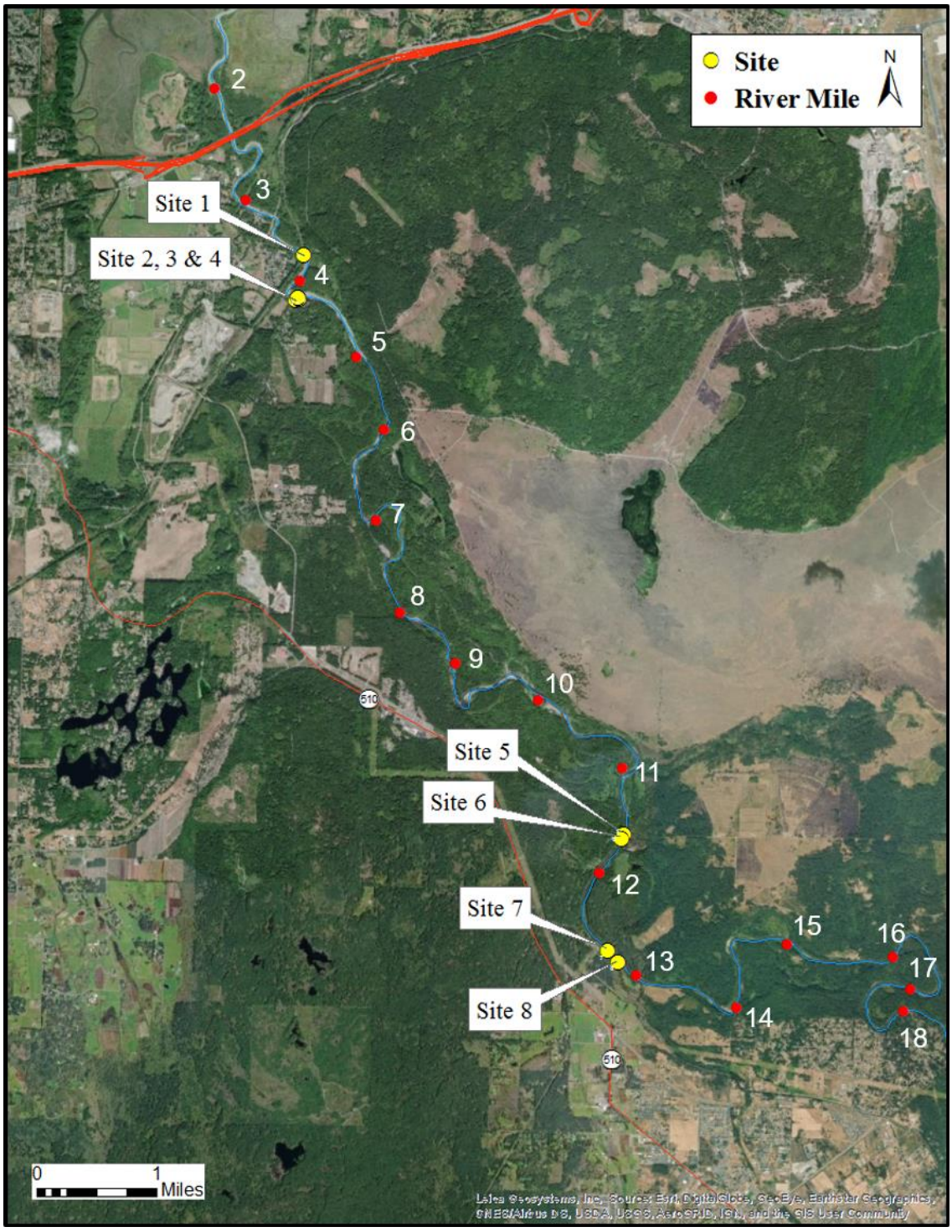


Figure 12. Map of eDNA and electrofishing sampling locations in the Nisqually River. Sampling sites are denoted by yellow circles. The red circles indicate river miles along the Nisqually River.

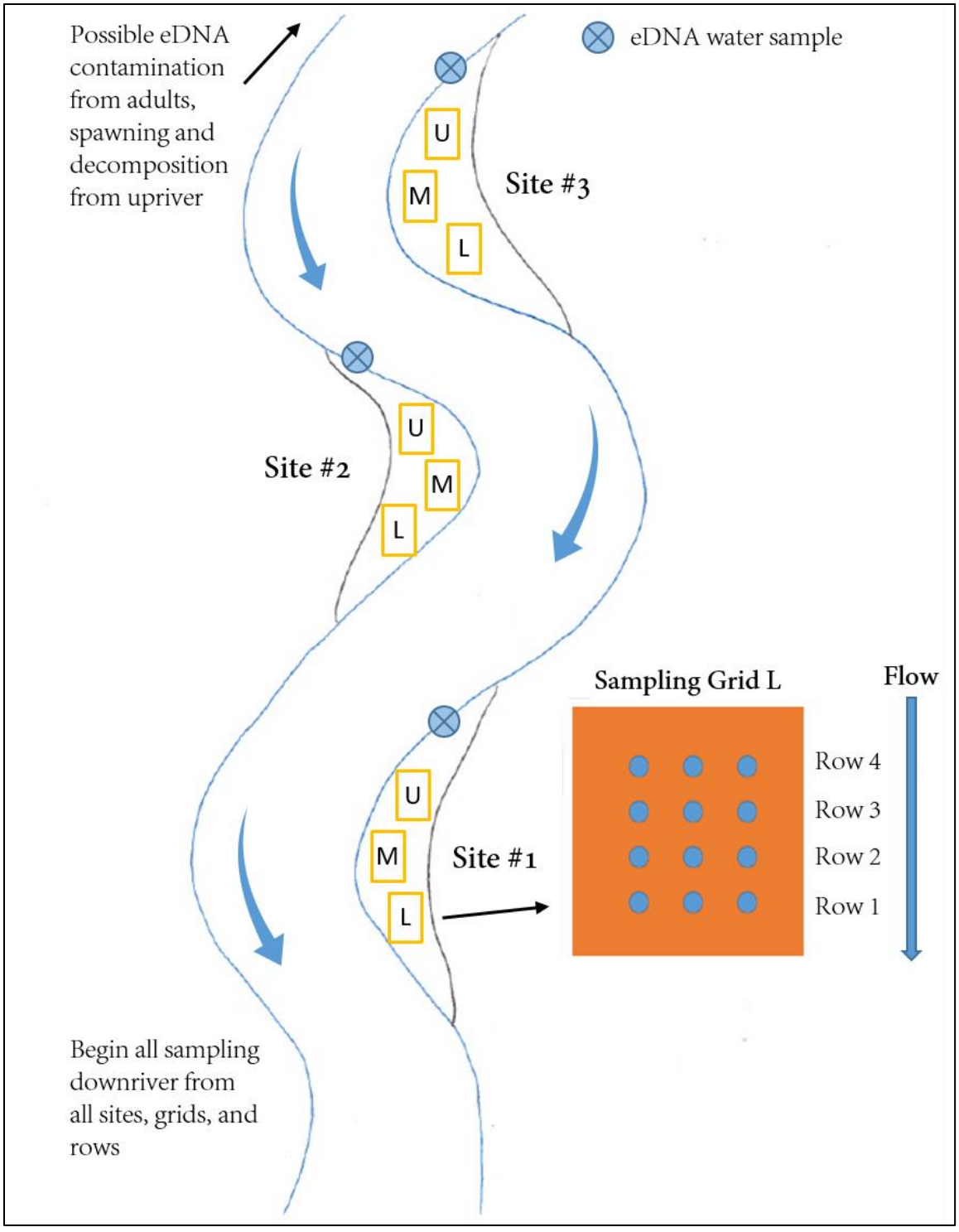


Figure 13. Example of sampling sites and grids. Eight sites were selected (Site 1-8). Each site was divided into three sampling grids (Upper – U, Middle – M, Lower – L) and sampling was performed starting downstream from all locations (sites, grids, and rows). Water samples for eDNA analysis were collected upriver from each site.

Study Area

Glacially fed, the Nisqually River descends from Mt. Rainer National Park and flows approximately 80 miles downriver [75]. At the Billy Frank Jr. Nisqually National Wildlife Refuge, the river merges with the marine waters of the southern Puget Sound [76]. In fact, the Nisqually River is the only river in the U.S. to have its headwaters in a National Park and its estuary in a National Wildlife Refuge [77,78]. The Nisqually Watershed (Figure 11) has a drainage area of approximately 761 square miles [75] and is one of the least developed basins in south Puget Sound [78]. However, observed and predicted population growth in the watershed is a significant concern for the demands on environmental resources (e.g., groundwater) and development[20].

The river has multiple habitat and land use types, flowing through national and state parks and forest, public and private timberlands and agricultural lands, and five urban cities (Eatonville, Yelm, Roy, Lacey, and DuPont) [78]. Within the lower portion of the watershed, the Nisqually River divides Thurston and Pierce County, and is bordered by major public lands, including the Nisqually Indian Tribe, Nisqually Land Trust, Joint Base Lewis McChord Military Reservation and USFWS (Figure 11). There are also two large municipal dams on the Nisqually River, the Alder and La Grande Dams (Figure 11), which provide hydroelectric power and recreation. Distribution of anadromous salmonids is restricted from the upper watershed by the La Grande Dam at river mile 42.5 [75]. The Centralia Diversion Dam facility (Figure 11), located near river

[20] 2016 State of Our Watersheds Report – Nisqually River Watershed: Assessment of key environmental indicators since the 2012 Report show a declining trend in habitat (shoreline modifications/forage fish; water wells; and impervious surfaces). Restoration activities have improved since 2012 but population growth is a major concern.

mile 12.5, diverts water into the Centralia Power Canal and conveys the water 9 miles to the Yelm Hydro Power House [79,80]. The Centralia Diversion Dam has a fish ladder for upriver fish passage and a fish screen that prevents fish from entering the canal.

The lower Nisqually river – particularly between river mile 4.5 and 12.7 – was listed among the top rivers in the Puget Sound for continuous salmon habitat [77]. The less developed shoreline along the Nisqually creates more open space for the river to meander freely, with a healthy riparian zone and abundant large woody debris. The Nisqually River and its tributaries are critical spawning and rearing habitat to several native salmon species including sockeye, chum, coastal cutthroat trout, coho, and pink salmon. Additionally, bull-trout, fall Chinook salmon, and summer and winter steelhead are ESA listed species that utilize the river [77,81].

Site Selection & Description

Selection of potential sites was initially attempted using spatial analysis of satellite orthoimagery[21] in ArcGIS® (©2019 Esri, Inc., version 10.6.1) and Google Earth™ mapping service (©2017 Google, LLC, version 9.0). However, because the river undergoes extreme changes each year during the winter season, imagery data from the previous year was outdated and less reliable. Therefore, local expert knowledge (e.g., known river access and adult lamprey spawning areas) and ground truthing (July 24th and 25th) were required to find favorable site conditions for field collection. Site visits determined that not all access points were feasible due to safety risks (e.g., bank slope, water level and flow) and property rights. Due to restricted river access, all sampling

[21] ArcGIS satellite imagery data layer: 2017 Washington Orthophoto, 3-ft Color

occurred on the Thurston County side of the river (Figure 11) and sites were selected based on larval lamprey habitat requirements (i.e., contained fine sediment and organic matter, side channel habitat and/or shallow pools, low water velocity, abundant woody debris and shade, and occurred downstream of adult lamprey spawning habitat).

A total of 8 sites were selected for our study, between river miles 3.5 and 13 (Figure 12). Sampling was limited to upriver of river mile 3.5, as tidal influence extends upstream to about river mile 3.3, which is not ideal conditions for larval lampreys [76]. Each site consisted of three grids: upper, middle, and lower (Figure 13). The shape of each grid was dependent on shoreline characteristics and water level. The distance between sites and the proximity of the sampling grids to each other was dependent on the area of preferred habitat and river depth, with a minimum of 0.75 m between each grid.

Permissions & Sampling Considerations

This research was funded and staffed by the WDNR in partnership with the WDFW. Staff who assisted with field collection include Joy Polston-Barnes, Elisa Rauschl, Jocelyn Wensloff, and Lydia Mahr (Figure 14). The WDNR manages state-owned aquatic shorelands and bedlands of Washington State. As this was a WDNR project, we had the necessary permissions to conduct work on the shorelands and bedlands of the Nisqually River. Although we were able to gain access to Sites 7 and 8 (Figure 12) with permission from the Nisqually Tribe, we were unable to access any of the river within the reservation (Figure 11). The Nisqually Sportsman Club, Inc. granted access to the river from their private campground (Sites 2, 3, and 4). Other river access locations are public and do not require additional permissions (Sites 1, 5 and 6). Due to the partnership with the WDFW (Inter-Agency Agreement), no additional permits were

needed for eDNA sampling. Electrofishing was permitted under a programmatic WDFW Scientific Research Permit. Christina Wang (formerly Luzier), a Fish Biologist with the USFWS, provided an electrofishing backpack and training in its operation.

Figure 14. WDNR staff who assisted with field collection on the Nisqually River. Left to right: Joy Polston-Barnes, Jocelyn Wensloff, Lydia Mahr, and Elisa Rauschl.

Precautions were taken to mitigate safety risks, such as working in a large group, wearing appropriate equipment (e.g., hats, sunblock, chest-waders), using walking sticks for longer treks in deeper water or over slippery rocks, and performing work in river during annual low flow. To avoid cross contamination of water and sediment samples, we sampled from down-to-upstream at each site, grid, and row. We sterilized all eDNA equipment and personal field gear (boots and waders) before sampling and between each site with Clorox® Healthcare Bleach Germicidal Wipes [82,83]. To ensure that the bleach would not inhibit lamprey DNA, time was allowed for all equipment to air-dry before use. Additionally, nitrile gloves were worn during all eDNA sampling.

Field Data Collection

eDNA Sample Collection: Sediment Samples

To avoid contamination of samples, sediment sampling was performed before any other in-stream work. At each sampling grid, 12 discrete sediment samples (Figure 15A) were taken using a garden trowel every 0.75 m (T. Liedtke, pers comm., Dec. 6, 2017) at less than 5 cm depth [44] and combined into a sterile container (i.e., white dishpan). Each sample location point was flagged with a wood or metal skewer. Efforts were made to sample in homogenous areas without cobble or large organic debris. If this occurred, the sample was taken as close to the 0.75 m measurement as possible. Once all 12 samples from a given grid were collected and combined, the sediment was mixed (with a sterile metal spoon) for 1 minute to form a composite sample. From the composite sample, 3 replicate samples (R1, R2, and R3) were poured into sterile 50 mL tubes (Figure 15B & Figure 16A), wiped with a sterile wipe, and then placed in a 1 L whirlpack bag to prevent leakage and contamination. This yielded a total of 9 replicate sediment samples collected at each of the 8 sites along the Nisqually River. An additional sediment sample (200 to 500 g, approx. half of a Ziploc® sandwich bag) was taken from the remaining composite mixture for sediment grain-size analysis (discussed later under *Sediment Grain-size Analysis*). Sediment samples were stored in a cooler while in the field for no longer than eight hours until delivery the same day to the WDFW MGL, and frozen at -20 ºC for no more than 5 days until DNA extraction.

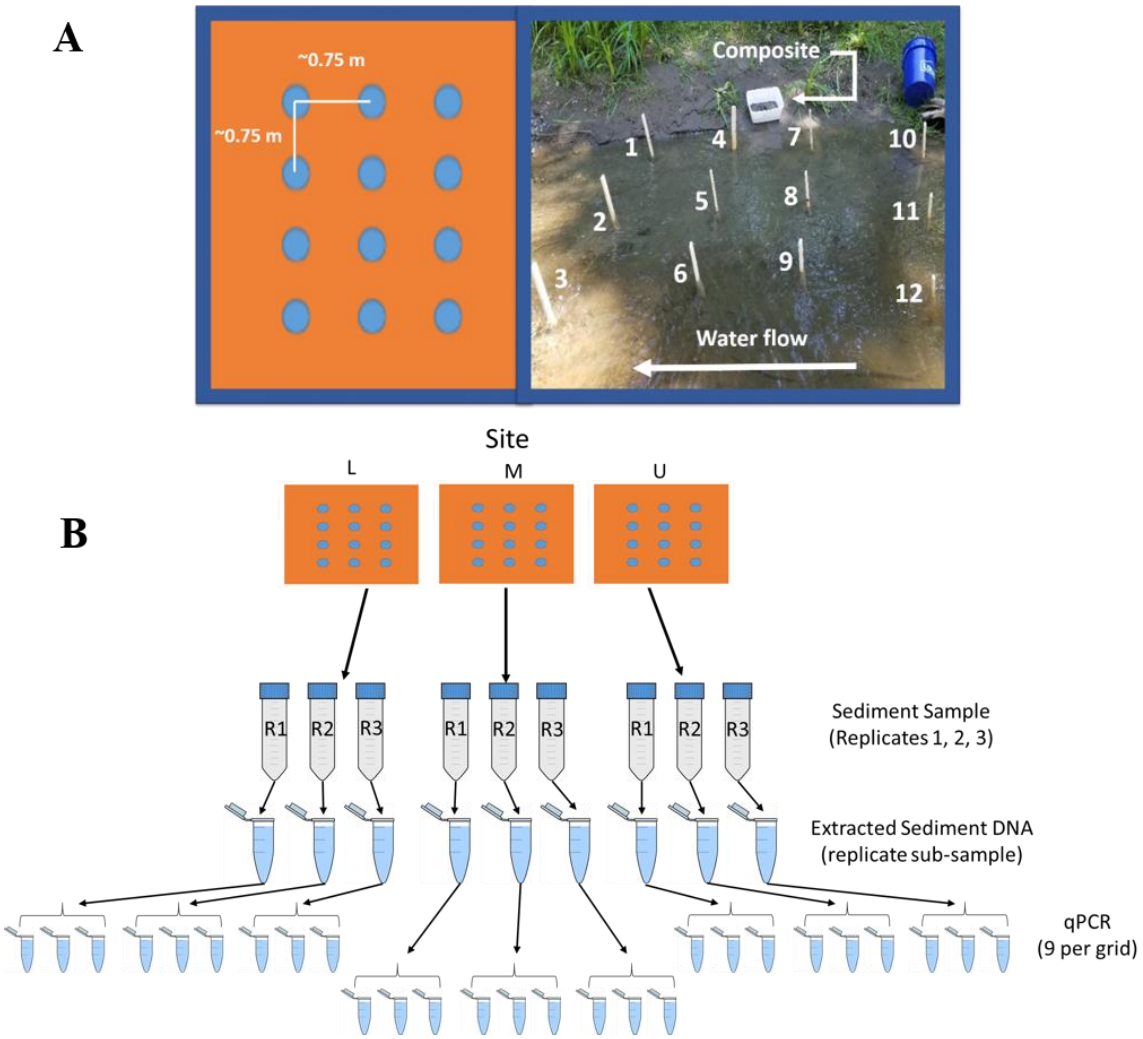


Figure 15. Example of a sample grid and method for sediment eDNA collection. (A) Sediment from 12 discrete locations (shown as blue dots on left and wood stakes on right), taken approximately 0.75 m apart, were mixed together for a composite sample. (B) Each site consisted of 3 sampling grids: upper (U), middle (M), lower (L). The sediment in each grid was mixed and placed in one of 3 replicate 50 mL tubes (R1-3). DNA was extracted from each replicate sediment sample to form the replicate sub-sample. The DNA replicate sub-sample was then qPCR amplified in triplicate for each lamprey assay.

Figure 16. Process for field sampling: A) collecting sediment replicates (R1-3) from composite mixture, B) electrofishing, and C) counting captured larvae.

Electrofishing Survey

Electrofishing is a common and effective technique used for detecting and capturing lamprey larvae [9]. Electrofishing surveys were included in this research to test the quality and accuracy of the sediment eDNA analysis by confirming presence and abundance of larval lampreys. Electrofishing was done the same day, immediately following sediment sampling at each grid (Figure 16B). We conducted single pass electrofishing for lamprey larvae, following a protocol adapted from USFWS & Yakama Nation [10]. This method required a two-stage electrofishing backpack system (AbP-2 model by Engineering Technical Services, Madison, WI). The electrofishing settings for the slow pulse stage applied 125 V, 3 Hz, and a 25% duty cycle with 3 pulses/second to "tickle" the larvae from the substrate. A fast pulse setting of 30 Hz (30 pulses/second) was used on emerged larvae to "stun" and temporarily immobilize larvae for capture. Starting at the lower grid and working upstream, electrofishing surveys were performed at a slow and methodical pace over and within 0.75 m of areas flagged from sediment samples. Three people were required: one to electrofish and two to catch larvae with dip

nets. Additional staff were backups to estimate the number of larvae missed (i.e., escaped) during sampling. Total electrofishing times were recorded for each grid and varied depending on larval density. To standardize the area (m^2) of electrofishing at each grid, electrofishing surveys were performed within a set distance along the outer edges of each grid, depending on habitat features. We performed electrofishing within approximately 0.1 m if there were areas of obstruction (e.g., large wood, rocks, shallow depth) and 0.3 m if there was adequate room to electrofish.

Captured larvae were handled carefully and placed in a container of cool river water[22] until electrofishing at each grid was complete. Larvae were counted, and body lengths binned by size class (< 80 mm and > 80 mm) (Figure 16C & Figure 17). A Wild Fish Conservancy Northwest "photarium" (a small and lightweight plastic fish tank that measures up to 150 mm) was used for length measurements of captured larvae (Figure 17). We were unable to identify the captured larvae down to species, as it is difficult to identify at the larval stage (this requires additional training, and sampling time). Additionally, we could not capture larvae that were less than 40 mm in length because they were too small for our nets[23]. Observations of extremely tiny larvae (< 10 mm) were noted as young of year (YOY) and likely represent recent spawning. All larvae were released immediately following completion of a site sampling, into the grids from which they were collected and carefully placed in slower moving water to avoid the current from sweeping them downriver.

[22] We used a mesh laundry basket weighed down by rocks and placed in river as recommended by Ralph Lampman, Yakama Nation Fisheries Resource Management Program – see Figure 16C)

[23] Dip nets were 8-inch diameter, 6-inch depth and 1/9 mesh.

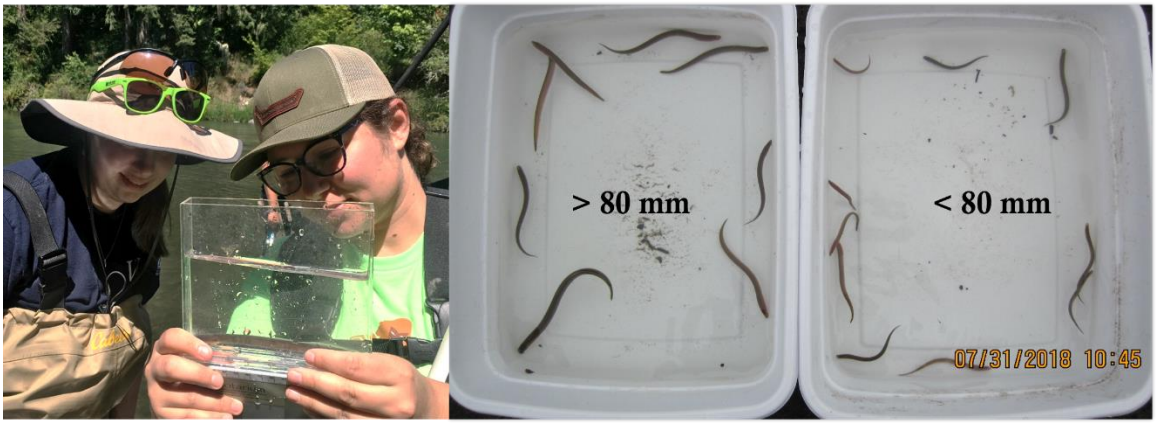


Figure 17. A photarium was used to measure body lengths of larvae and separate into bin size classes (> 80 mm and < 80 mm).

eDNA Sample Collection: Water Samples

Water samples were collected upstream from the upper sampling grid (U), after sediment sampling and electrofishing at each site was completed. The water samples were each taken at a location that appeared to have water flowing across the site, as the sample is meant to represent the potential DNA flowing in the water current to the site. The distance upstream from the site for sample collection varied between sites depending on river characteristics and ranged from 5 to 15 feet.

Water samples were collected by submerging a 1 L sterile Nalgene® bottle upstream from where the sampler was standing. Samples were stored in a cooler (separate from the sediment samples) while in the field for no longer than eight hours. Negative control samples of sterile DI (deionized) water, one per site, were kept in the cooler to be used to detect any contamination from the cooler. Each of the water and negative control sample bottles were sterilized before being stored in the cooler. If the results from qPCR amplification were ambiguous, each of the negative controls were extracted and amplified, to test for contamination. Water samples were delivered the same day to the

WDFW MGL and stored at -20 ºC for no more than 5 days until filtered[24]. Samples were filtered with a 0.45 µm pore size filter. Filters were stored in 100% ethanol in 2 mL tubes until DNA extraction.

Habitat Characterization

We collected environmental data to add to the current literature for lamprey larvae habitat requirements. Habitat and water parameters were measured at each site. A field form and data collection mobile app using Survey123 for ArcGIS® was created for on-site data collection and photograph logging (Appendix B). At each site, photographs were taken of the shoreline, grids, and water sample location (Appendix C). Using a calibrated portable YSI (Yellow Spring Instrument) ProDSS Multiparameter Water Quality Meter, we measured turbidity, temperature, specific conductivity, pH and dissolved oxygen at every eDNA water sample location. Site habitat type (island, side channel, alcove, edge of main channel, or main channel) of each site was qualified and described. We also recorded qualitative observations such as shoreline development, percent cover of aquatic vegetation and organic detritus.

[24] Five days frozen will not change eDNA detection rates, and 8-12 hours kept cool in the field is thought to keep DNA degradation to a minimum, however, you could lose a little bit of DNA. Additionally, 8-12 hours for water in a cooler is the agreed upon time window for other eDNA researchers (S. Brown, WDFW MGL, pers. comm., May 6, 2019).

Laboratory Processing

Sediment Grain-size Analysis

Grain size of sediment is known to be a major indicator of lamprey larvae presence [9,43]. Our samples were processed separately by the WDNR AAMT at the Marine Station [84]. The total percentage of Type I habitat grain size (< 2 mm) was evaluated for all samples and sites. Ralph Lampman (Yakama Nation Fisheries Resource Management Program) defines types of fine sediment that can occur within Type I habitat as "sticky" (Clay, < 0.002 mm), "smooth" (Silt, 0.002 to 0.05 mm), or "gritty" (Sand, 0.05 to 2 mm). Larval lampreys prefer 'smooth' sediment; however, we were only able to filter to < 0.075 mm, and therefore could not calculate which proportion of the samples were classified as 'smooth' sediments. Instead, sediment samples were filtered by the following grain-size fraction: sand (0.5 to 0.075 mm) and very fine sand, silt and clay (< 0.075 mm). 'Smooth' sediments preferred by larval lampreys would fall under the 'very fine sand, silt and clay' category.

eDNA Laboratory Methods

We submitted 72 sediment samples and 8 water samples (plus 8 negative control water samples) to the WDFW MGL. Samples were tested for the presence of Pacific lamprey (*Entosphenus tridentatus*) and *Lampetra* spp. by using the qPCR-based eDNA assays (primer pair and fluorescent probe) developed by Ostberg et al. [20] and Carim et al. [19], respectively. There were 9 sediment replicates per site, as each site had 3 sampling grids with 3 replicates each (R1-3) (Figure 15). Following DNA extraction, triplicate qPCR amplifications were performed from each replicate sample. This yielded 27 qPCR amplifications for each site and for each lamprey assay (Pacific lamprey and

Lampetra spp.). Water samples were analyzed similarly with only one replicate sample resulting in triplicate qPCR amplifications for each site and for each lamprey assay. Negative control samples were only extracted and amplified if the results from water sample qPCR amplifications were ambiguous. Detailed laboratory methods were provided by WDFW and are available for reference in Appendix A.

Data Analysis

We calculated the frequency of positive sediment sample replicates[25] (# positive replicates per site/9 total replicates per site), and the frequency of positive qPCR amplifications (# positive qPCR replicates per site/27 total qPCR replicates per site). Individual *Lampetra* spp. qPCR reactions were assigned a DNA concentration category based on the number of DNA copies per microliter: low (~2.39 copies/µl), medium (23.86 – 238.6 copies/µl), and high (2,386.5 – 131,246.5 copies/µl). Similarly, individual Pacific lamprey reactions were assigned a DNA concentration category: low (~8.85 copies/µl), medium (16.08 copies/µl), and high (16,804.4 copies/µl).

We compared detection rates between eDNA and electrofishing, by calculating the proportion of sites that had lampreys detected by each method. The metric d_{unit} [62,85,86] represents the proportion of grids per site (# positive grids/3 total grids) where lampreys were detected with either method. If 1 out of 3 replicates in a grid were determined to have a positive detection, it counted as that grid being positive. Additionally, the larval density (# larvae/m^2) was also calculated for each grid and site,

[25] Samples were considered positive for detection when two out of three triplicate qPCRs resulted in a positive amplification (see Figure 2 of Appendix A).

using the total number of larvae detected during electrofishing and the average sample area (m^2).

Statistical analyses were performed using the JMP® software package (©1989-2019 SAS Institute Inc., Cary, NC, version 14.3) to test for normal distribution and goodness of fit (Shapiro-Wilk Test) (Appendix D). JMP® was also used to perform parametric and nonparametric multivariate analysis (Pearson or Spearman rank correlation) on a site- and grid-level (n = 8 or 24, respectively) for all collected and processed data (i.e., eDNA, electrofishing, and habitat parameters). However, we found that the Spearman's rank correlation was insufficient for the site-level data that did not fit normal distribution as a sample size of 8 was too small (p-value suspect) (Appendix D). To reduce skew, we log transformed (base 10 logarithm) the eDNA concentration (copies/µl) before performing the multivariate analysis. Additionally, we reported the median and interquartile range (IQR) for nonparametric data and the mean (M) and standard deviation (SD) for normally distributed data.

We modeled probabilities of eDNA occupancy using the R package EDNAOCCUPANCY (Dorazio and Erickson 2018), which fits Bayesian multiscale occupancy models to the data (with or without covariates). Occupancy analysis was performed in RStudio® Desktop (©2018 RStudio, version 1.2.1335) using the programming software R (©The R Foundation, version 3.5.3) (See sample R Script in Appendix E). The models consisted of three nested, hierarchical levels, including: primary sample locations (sites along the Nisqually), samples (replicate sediment and water samples collected at each site), and sub-sample (qPCR replicates for each sediment and water sample). The estimated posterior summary parameters were the probability (ψ)

of eDNA occurrence in a site, conditional probability (θ) of eDNA occurrence in a sediment or water sample, and the conditional probability (*p*) of eDNA detection in a qPCR replicate (Figure 18). We fit models that assumed constant $(\cdot)$[26] parameters ($\psi(\cdot)$, $\theta(\cdot)$, $\rho(\cdot)$) with 11,000 iterations of the Monte Carlo Markov Chain (MCMC) algorithm and 1,000 burn-in steps (Figure 19). We visually assessed convergence of Markov chains with trace plots and autocorrelation plots after each run (Example of 'good' and 'bad' plots in Appendix E). Models with lower values of posterior-predictive loss criterion (PPLC) and widely applicable information criterion (WAIC) functions were used to select the models with the best fit to the data. Additional models were run with covariates to see if other variables (water parameters, vegetation type, grain size of sediment, larval size, etc.) affect estimates of eDNA occupancy.

[26] $(\cdot)$ is a constant parameter that denotes the cumulative distribution function of a standard normal distribution [23].

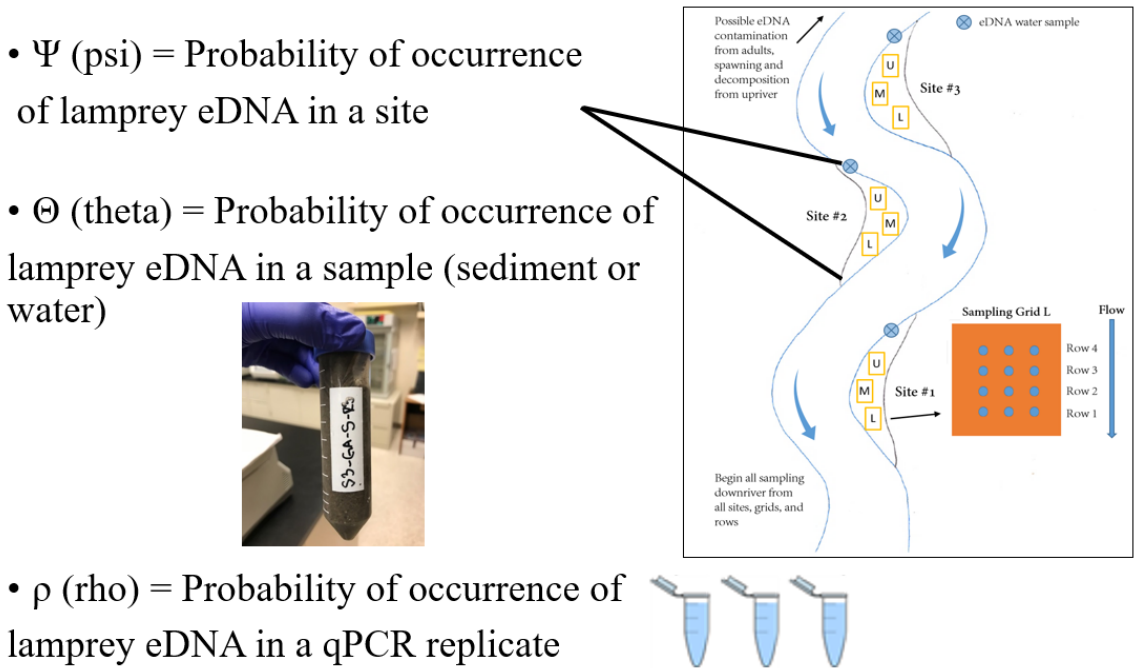
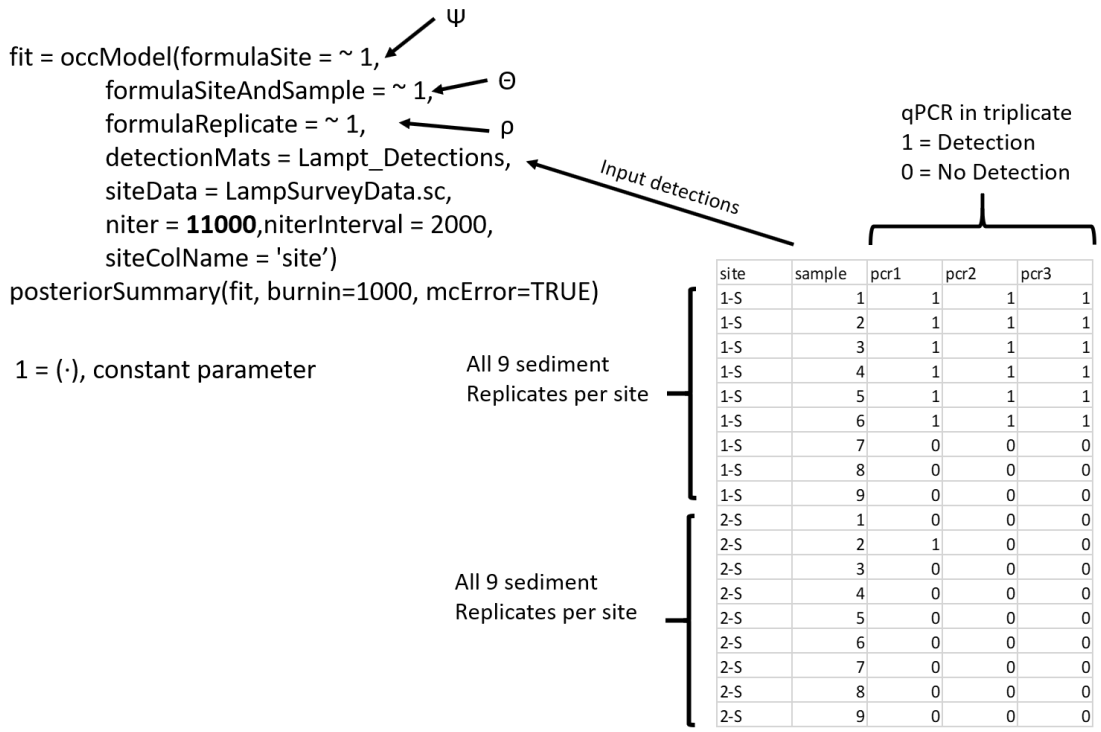


Figure 18. Dorazio and Erickson [23] developed the R package EDNAOCCUPANCY, a software that can be used for fitting Bayesian, multiscale occupancy models with or without covariates. Models consist of three nested, hierarchical levels: the probability (ψ) of eDNA site occupancy (along the Nisqually River), conditional probability (θ) of eDNA sample occupancy (sediment or water), and the conditional probability (*p*) of eDNA detection in a qPCR replicate. Image adapted from: S. Brown, WDFW MGL, pers. comm., March 20, 2019.

To inform future studies, we assessed the certainty of our sampling design. We had 9 replicate sediment samples and 1 water sample per site. We used the equation $\theta^* = 1 - (1 - \theta)^n$, where n = number of sediment or water samples (9 and 1, respectively), to estimate the cumulative probability of sampling lamprey eDNA in the Nisqually River. Next, we used the equation $p^* = 1 - (1 - p)^k$, where k = the number of qPCR replicates (3), to estimate the cumulative probability of detecting lamprey eDNA in replicate qPCR amplifications.



site	sample	pcr1	pcr2	pcr3
1-S	1	1	1	1
1-S	2	1	1	1
1-S	3	1	1	1
1-S	4	1	1	1
1-S	5	1	1	1
1-S	6	1	1	1
1-S	7	0	0	0
1-S	8	0	0	0
1-S	9	0	0	0
2-S	1	0	0	0
2-S	2	1	0	0
2-S	3	0	0	0
2-S	4	0	0	0
2-S	5	0	0	0
2-S	6	0	0	0
2-S	7	0	0	0
2-S	8	0	0	0
2-S	9	0	0	0

Figure 19. Overview of the eDNA Occupancy Model for *Lampetra* sediment detections. We fit occupancy models that assumed constant ((·) = 1) parameters (ψ(·), θ(·), ρ(·)) with 11,000 iterations (niter) of the MCMC algorithm and 1,000 burn-in steps (burnin). We input qPCR triplicate detections (1 = detection; 2 = no detection) from each of the 9 sediment replicates per site (Lampt_Detections). Constant parameters at each hierarchical level were replaced when covariate data was included in the models. Image adapted from: S. Brown, pers. comm., March 20, 2019.

RESULTS

The intention of this thesis research was to 1) test the ability of sediment eDNA analysis to identify the presence of larval lampreys in freshwater sediments, 2) compare this method to traditional detection methods, and 3) use a multi-scale occupancy model to estimate the probability of lamprey eDNA occurrence. The following chapter is organized into four sections: Lamprey Detections, Comparison of eDNA and Electrofishing Detections, Habitat Characterization, and Probabilities of eDNA Occupancy.

Lamprey Detections

Electrofishing Surveys

Electrofishing of larval lampreys in the Nisqually River resulted in positive detections at every site sampled, though in varying abundance (Figure 20, Table 3 & Table 4). As larvae were not identified to species during electrofishing surveys, we are not able to distinguish counts for Pacific lamprey or *Lampetra* spp. We detected[27] a total of 588 larvae during electrofishing surveys of 24 grids within 8 sites (Table 7). In 3 of 24 grids (12.5%) we detected no larvae (S2-U, S3-M, and S3-L) and in 6 of 24 grids (25%) we detected fewer than 10 larvae. Of the total number detected, we physically captured 378 (61%) larvae, averaging 15.8 larvae per grid (48%) and 47.3 per site (61%) (Table 3 & Appendix C). The number of larvae missed (escaped) during surveying, total 210 (39%) for all sites combined. On average, we missed 40% of the larvae per grid, with a range of 0 to 61 larvae missed (Table 3).

[27] Detected = larvae captured with dip nets + estimate of missed larvae (escaped but counted)

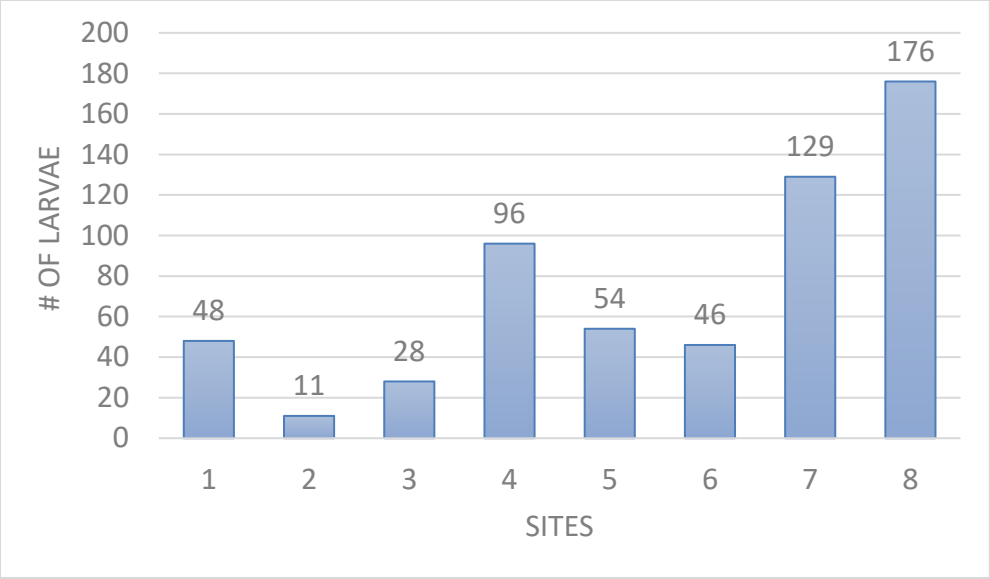


Figure 20. Bar graph demonstrating the site variability of larval detections via electrofishing surveys.

Captured larvae were binned into a small (< 80 mm) and large (> 80 mm) size category (Figure 17, Table 3 & Appendix C). The majority (86%) of the captured larvae were less than 80 mm in length. We captured 333 of 378 small sized and 45 of 378 large sized larvae. Though we did not measure individual lengths, we observed large variation within each size category (Figure 21). We noted very tiny larvae at several grids (S1-M, S3-U, S5-M, and S5-L) occurring in groups of 20 or more. There may have been more throughout our sites, but suspended river sediments and glare made visualizing even larger sized larvae difficult. The largest sized larva we captured was 150 mm in length at Site 7 (Figure 21).

Table 3. Site- and Grid-level descriptive statistics for electrofishing results.

Site	**Small**	**Large**	**Captured**	**Missed**	**Total**
Normal distribution	Y	Y	Y	N	Y
Mean	41.6	5.6	47.3	26.3	73.5
Standard Error	12.2	1.8	12.7	9.3	19.8
Median	32.5	5.0	34.5	19.0	51.0
Mode	n/a	7.0	n/a	10.0	n/a
Standard Deviation	34.5	5.1	35.8	26.2	55.9
Sample Variance	1193.1	26.3	1282.5	686.2	3122.3
Interquartile Range	38.3	4.8	43.8	15.8	62.8
Kurtosis	-0.6	1.6	-0.8	5.9	0.1
Skewness	0.8	1.2	0.6	2.3	1.0
Range	97.0	16.0	104.0	79.0	165.0
Minimum	2.0	0.0	2.0	9.0	11.0
Maximum	99.0	16.0	106.0	88.0	176.0
Sum	333.0	45.0	378.0	210.0	588.0
Count	8.0	8.0	8.0	8.0	8.0
Confidence Level (95.0%)	28.9	4.3	29.9	21.9	46.7
CI Lower	12.7	1.3	17.3	4.3	26.8
CI Upper	70.5	9.9	77.2	48.2	120.2
Grid	**Small**	**Large**	**Captured**	**Missed**	**Total**
Normal distribution	N	N	N	N	N
Mean	13.9	1.9	15.8	8.8	24.5
Standard Error	3.5	0.5	3.7	2.5	5.9
Median	10.0	0.0	13.5	6.0	20.5
Mode	0.0	0.0	0.0	6.0	0.0
Standard Deviation	17.1	2.5	18.3	12.4	28.8
Interquartile Range	19.0	3.0	22.3	9.25	29.0
Sample Variance	294.1	6.2	335.5	152.9	828.3
Kurtosis	4.1	0.4	3.1	14.7	8.3
Skewness	1.9	1.2	1.7	3.5	2.5
Range	69.0	8.0	72.0	61.0	133.0
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	69.0	8.0	72.0	61.0	133.0
Sum	333.0	45.0	378.0	210.0	588.0
Count	24.0	24.0	24.0	24.0	24.0
Confidence Level (95.0%)	7.2	1.1	7.7	5.2	12.2
CI Lower	6.6	0.8	8.0	3.5	12.3
CI Upper	21.1	2.9	23.5	14.0	36.7

Figure 21. We observed large variation in larval sizes. Image on left is of a larva that was approximately 150 mm in length and possibly starting to transform (slight eye development). Tiny larva on right was found in a sediment composite. This size was hard to visualize and impossible to capture with dip nets and could be young of the year indicating recent spawning upriver.

The total number of detected larvae and the total sampling area (m^2) was used to calculate larval density for each grid and site. Site densities are shown below in Table 4. We sampled a total area of 111 m^2 and 424 linear feet of shoreline.

Table 4. Larval density for each site sampled on the Nisqually River. Average length of shoreline sampled is also provided.

Site	Total Larvae Detected Via Electrofishing	Sampled Area (m^2)	Larval Density (#larvae/m^2)	Length of Shoreline Sampled (linear ft)
1	48	16	3	32
2	11	15	1	36
3	28	12	2	59
4	96	14	7	67
5	54	14	4	72
6	46	10	5	69
7	129	16	8	48
8	176	14	13	42
Average	**74**	**14**	**5**	**53**

Average electrofishing time per site was 27.1 minutes, with a range between 12.2 – 48.0 min (Table 5, Table 6 & Appendix C). Electrofishing time and larval detection were strongly correlated (site-level: $r = 0.8$, $p = 0.02$, $R^2 = 0.7$; grid-level: $r_s = 0.7$, $p < 0.001$, $R^2 = 0.7$), as is expected since we observed that sites typically took longer to sample if more larvae were present. However, this does not mean that longer sampling times at sparse sites would result in more larval detection. We also noted the water clarity (Rate 1-5, 1 = no visibility and 5 = very clear) at each grid during electrofishing surveys (Table 5, Table 6 & Appendix C). The average water clarity rating was 4, with a range from 2 – 5. Grids with lower water clarity ratings were mostly due to fine/silty sediments disturbed during electrofishing.

Table 5. Site-level descriptive statistics for larval density, electrofishing time (min), and water clarity (Rate 1-5, 1 = no visibility and 5 = very clear).

Site	Larval Density	Electrofishing Time	Water Clarity
Normal distribution	Y	Y	Y
Mean	5.3	27.1	4.0
Standard Error	1.4	4.0	0.3
Median	4.2	24.0	4.0
Mode	n/a	n/a	4.0
Standard Deviation	3.9	11.3	0.7
Interquartile Range	4.3	12.8	1.3
Sample Variance	15.2	127.1	0.5
Kurtosis	1.2	0.5	-1.6
Skewness	1.1	0.8	0.0
Range	12.2	35.8	2.0
Minimum	0.7	12.2	3.0
Maximum	13.0	48.0	5.0
Sum	42.2	216.5	32.0
Count	8.0	8.0	8.0
Confidence Level (95.0%)	3.3	9.4	0.6
CI Lower	2.0	17.6	3.4
CI Upper	8.5	36.5	4.6

Table 6. Grid-level descriptive statistics for larval density, electrofishing time (min), and water clarity (Rate 1-5, 1 = no visibility and 5 = very clear).

Grid	Larval Density	Electrofishing Time	Water Clarity
Normal distribution	N	N	N
Mean	5.2	9.0	4.0
Standard Error	1.2	1.1	0.2
Median	3.8	10.3	4.0
Mode	0.0	11.0	5.0
Standard Deviation	5.8	5.2	1.0
Interquartile Range	6.8	6.5	2.0
Sample Variance	33.7	27.3	1.0
Kurtosis	7.4	3.6	-0.9
Skewness	2.3	1.3	-0.5
Range	26.6	24.0	3.0
Minimum	0.0	2.0	2.0
Maximum	26.6	26.0	5.0
Sum	124.0	216.5	96.0
Count	24.0	24.0	24.0
Confidence Level (95.0%)	2.5	2.2	0.4
CI Lower	2.7	6.8	3.6
CI Upper	7.6	11.2	4.4

eDNA Sediment and Water Samples

WDFW processed a total of 72 sediment and 8 water samples which we collected from 8 sites along the Nisqually River. Pacific lamprey and *Lampetra* spp. were both detected in the samples via eDNA (Figure 22, Table 7, Table 8 & Appendix F). None of the negative controls were positive for Pacific lamprey or *Lampetra* spp. (Appendix F).

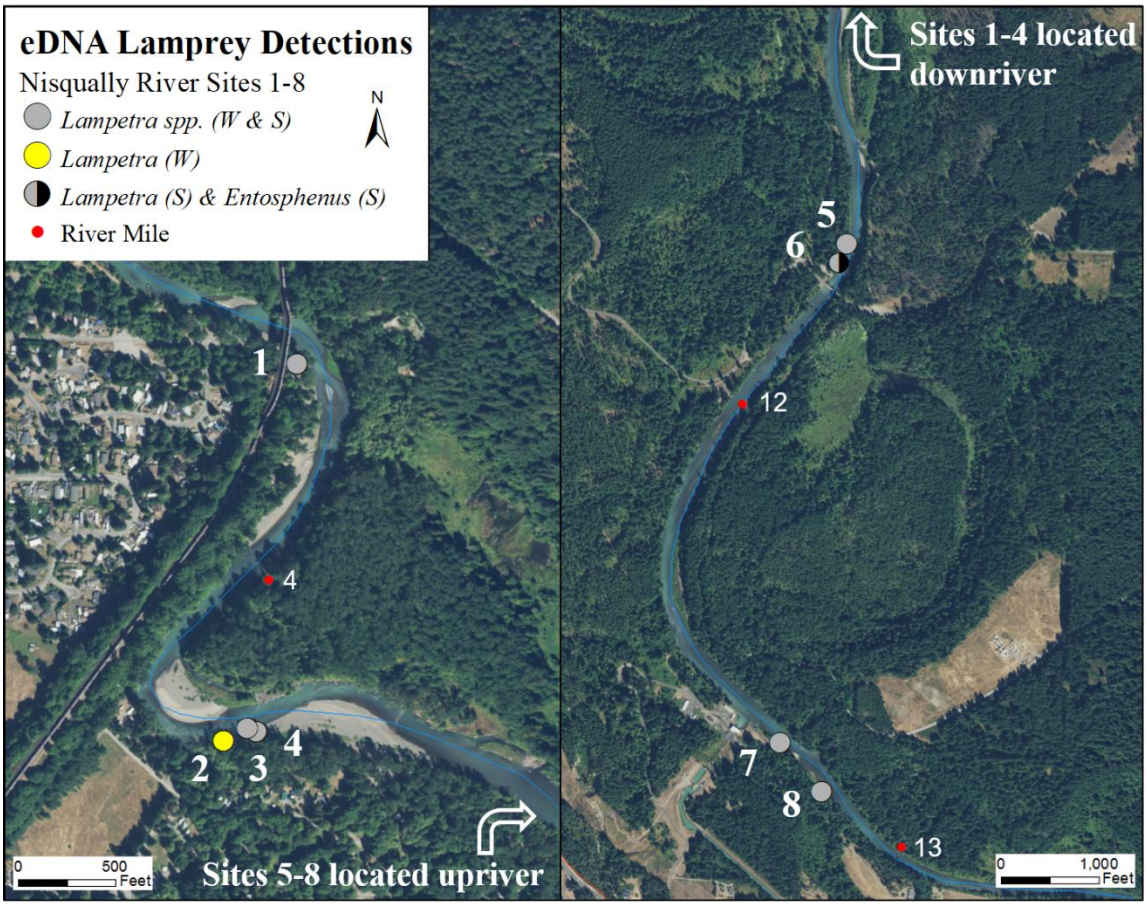


Figure 22. Map of eDNA lamprey detections in both types of samples (water, W; and sediment, S) on the Nisqually River. Detections at each of the sampling sites (Sites 1-4, left; Sites 5-8, right) are denoted by large grey (*Lampetra* spp.) and black/grey (*Lampetra* spp. and *Entosphenus tridentatus*) circles. Site 2 (yellow circle) was only positive for *Lampetra* spp. in the water sample and Site 6 was only positive for *Lampetra* spp. in the sediment sample. *Entosphenus tridentatus* was only positive in one sediment sample collected from Site 6.

Table 7. Detections of *Lampetra* spp. and Pacific lamprey from sediment (S) samples. Sites organized by sampling grid (upper, middle, lower), with positive lamprey detections via qPCR replicates, eDNA concentration, and electrofishing surveys. A total of 9 qPCR amplifications were processed for each grid at a site, table shows how many positives were detected per grid. Grids with 3 or fewer positive detections were re-amplified with another round of qPCR triplicate (See Site 2, 3, & 4 below).

Site, Sample Type & Grid	Sediment Replicates with *Lampetra* spp. Detected Via qPCR	Sediment Replicates with *Lampetra* spp. Positive Detections	Average *Lampetra* spp. eDNA Concentration (copies/µl)	Sediment Replicates with Pacific Lamprey Detected Via qPCR	Sediment Replicates with Pacific Lamprey Positive Detections	Average Pacific Lamprey eDNA Concentration (copies/µl)	Lamprey Detected Via Electro-fishing
1 – S – U	9/9	+	High (43,908)	0/9	-	NA	33
1 – S – M	9/9	+	High (43,836)	0/9	-	NA	14
1 – S – L	0/9	-	NA	0/9	-	NA	1
Total	**18/27**	**+**	**High (29,248)**	**0/27**	**-**	**NA**	**48**
2 – S – U	1/12	-	NA	0/9	-	NA	0
2 – S – M	0/9	-	NA	0/9	-	NA	9
2 – S – L	0/9	-	NA	0/9	-	NA	2
Total	**1/30**	**-**	**NA**	**0/27**	**-**	**NA**	**11**
3 – S – U	9/9	+	Medium (239)	0/9	-	NA	28
3 – S – M	5/9	+	Low (7)	0/9	-	NA	0
3 – S – L	5/12	+	Low (11)	0/9	-	NA	0
Total	**19/30**	**+**	**Medium (85)**	**0/27**	**-**	**NA**	**28**
4 – S – U	6/12	+	Low (10)	0/9	-	NA	20
4 – S – M	7/9	+	Medium (55)	0/9	-	NA	33
4 – S – L	9/9	+	Low (10)	0/9	-	NA	43
Total	**22/30**	**+**	**Medium (25)**	**0/27**	**-**	**NA**	**96**
5 – S – U	9/9	+	Medium (143)	0/9	-	NA	21
5 – S – M	9/9	+	Medium (167)	0/9	-	NA	26
5 – S – L	7/9	+	Low (5)	0/9	-	NA	7
Total	**25/27**	**+**	**Medium (105)**	**0/27**	**-**	**NA**	**54**
6 – S – U	9/9	+	Medium (95)	0/9	-	NA	5
6 – S – M	0/9	-	NA	0/9	-	NA	2
6 – S – L	9/9	+	Medium (143)	4/15	+	High (4,033)	39
Total	**18/27**	**+**	**Medium (80)**	**4/33**	**+**	**High (1344)**	**46**
7 – S – U	2/9	+	Low (13)	0/9	-	NA	22
7 – S – M	9/9	+	Medium (191)	0/9	-	NA	46
7 – S – L	9/9	+	Medium (239)	0/9	-	NA	61
Total	**20/27**	**+**	**Medium (148)**	**0/27**	**-**	**NA**	**129**
8 – S – U	9/9	+	Medium (239)	0/9	-	NA	133
8 – S – M	9/9	+	Medium (119)	0/9	-	NA	34
8 – S – L	9/9	+	High (29,564)	0/9	-	NA	9
Total	**27/27**	**+**	**High (9,974)**	**0/27**	**-**	**NA**	**176**

Pacific lamprey were detected at only one site (Site 6), with sediment sampling (Figure 22), where only 12% of qPCR amplifications were positive (Table 9). Of the 4 positive qPCR reactions successfully amplified for Pacific lamprey, there were 4 of the medium and 1 high category (Appendix F). Associated C_T values ranged from 23.7 – 38.7 (*Median* = 37.1, *IQR* = 3.9). Pacific lamprey were initially positively detected in 3 of 8 water samples collected (Sites 2, 3, and 8) (Appendix F). However, they were detected in only 1/3 qPCR replicates. As per protocol, the WDFW laboratory re-amplified these samples from Sites 2, 3 and 8, and no additional qPCR reactions were positive. The initial detections were determined to be false positives, as only 1/6 qPCR reactions were positive (Table 8).

Table 8. Detections of *Lampetra* spp. and Pacific lamprey from water samples.

Site	Sample Type	Water Replicates with *Lampetra* spp. Detected Via qPCR	Samples with *Lampetra* spp. Positive Detections	Average *Lampetra* spp. eDNA Concentration (copies/µl)	Water Replicates with Pacific Lamprey Detected Via qPCR	Samples with Pacific Lamprey Positive Detections	Average Pacific Lamprey eDNA Concentration (copies/µl)
1	Water	3/3	+	Medium (239)	0/3	-	NA
2	Water	3/3	+	Medium (239)	1/6	-	NA
3	Water	3/3	+	Medium (167)	1/6	-	NA
4	Water	3/3	+	Medium (81)	0/3	-	NA
5	Water	3/3	+	Low (10)	0/3	-	NA
6	Water	0/3	-	NA	0/3	-	NA
7	Water	3/3	+	Low (17)	0/3	-	NA
8	Water	3/3	+	Low (10)	1/6	-	NA

Table 9. Proportion of positive qPCRs for lamprey detections at each site.

Site	Sample Type	Frequency of positive *Lampetra* spp. qPCR replicates	Frequency of positive Pacific Lamprey qPCR replicates
1	Sediment	0.66	0.00
2	Sediment	0.00	0.00
3	Sediment	0.63	0.00
4	Sediment	0.73	0.00
5	Sediment	0.93	0.00
6	Sediment	0.66	0.12
7	Sediment	0.74	0.00
8	Sediment	1.00	0.00
1	Water	1.00	0.00
2	Water	1.00	0.00
3	Water	1.00	0.00
4	Water	1.00	0.00
5	Water	1.00	0.00
6	Water	0.00	0.00
7	Water	1.00	0.00
8	Water	1.00	0.00

Comparison of eDNA and Electrofishing Detections

eDNA surveys of *Lampetra* spp. and electrofishing results were relatively consistent, indicating lamprey presence at every site (Table 7). Larvae were not identified to species, so the electrofishing d_{unit} (proportion of grids with positive detections) potentially includes both Pacific lamprey and *Lampetra* spp. (Figure 23). eDNA surveys indicated the presence of Pacific lamprey at only Site 6 (d_{unit} of 33%), and it is unknown if they were detected via electrofishing. Electrofishing surveys had 25% (2 of 8 sites) higher detection rates when compared to sediment eDNA methods. Electrofishing and sediment eDNA had the same detection rate for 62.5% (5 of 8) of the sites and sediment eDNA had higher detections rates for 12.5% of the sites (1/8). Average detection rate for eDNA in sediment for all *Lampetra* spp. across the sites was 79% (Figure 23).

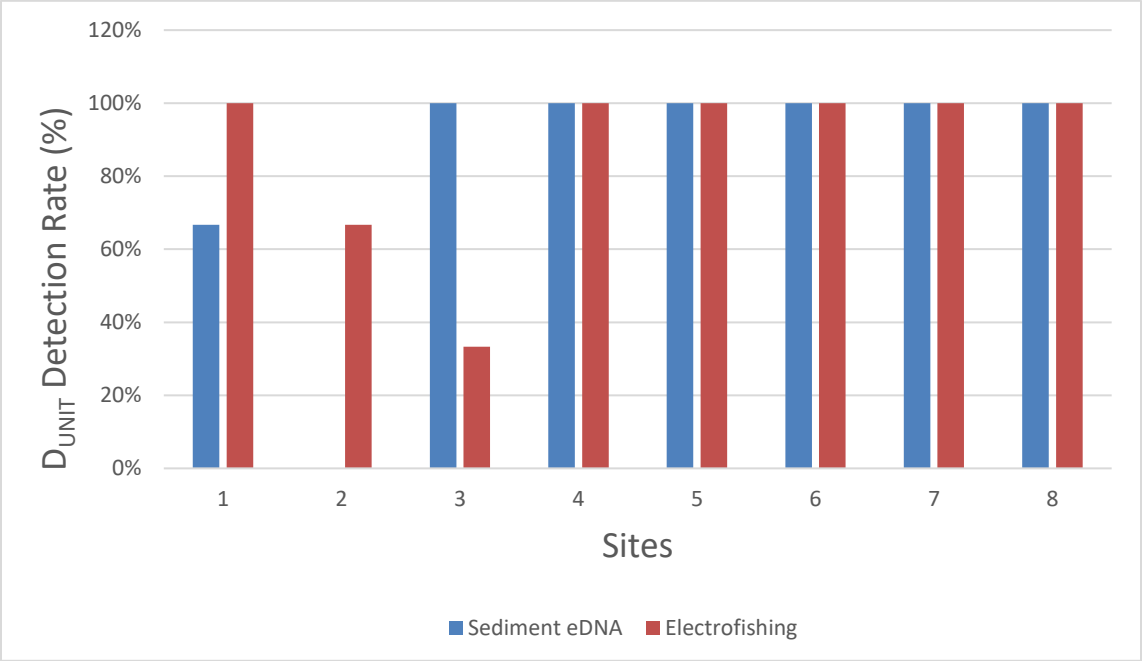


Figure 23. Larval detection (d_{unit}: proportion of grids with positive detections) by sediment eDNA (blue) and electrofishing (red). Site 3 was the only site that sediment eDNA had a higher rate of detection.

The average DNA concentration per grid appeared moderately correlated with the number of larvae detected through electrofishing (r_s = 0.6, p = 0.001), suggesting that increased larval abundance may be an indicator of higher eDNA concentrations. However, closer examination revealed no clear pattern between electrofishing and eDNA concentrations. Grids with the highest eDNA concentrations (S1-U, S1-M, and S8-L) had low (< 10) to medium (< 40) counts of larvae, while grids with the lowest eDNA concentrations had similar counts (Table 7). For example, the two sites with the highest number of larvae counts from electrofishing were Site 7 and 8 with 129 and 176, respectively. Despite both sites resulting in high larval counts, Site 7 sediment eDNA sample average was medium (148), while Site 8 sample average detected high (9,974) concentrations. We also observed detection inconsistencies between sediment eDNA and

electrofishing. For example, there were 4 sampling grids (S1-L, S2-M, S2-L, and S6-M) that had low numbers (<10) of larvae detected by electrofishing but had negative DNA detections. In contrast, there were two adjacent sampling grids (S3-M and S3-L) where we detected no larvae by electrofishing but had positive detections of DNA, though in low concentrations.

Additionally, smaller sized larvae were more strongly correlated ($r_s = 0.7$, $p < 0.001$) with higher sediment eDNA concentrations per grid, while larger larval body size was moderately correlated ($r_s = 0.4$, $p = 0.04$). Although this was unexpected, as larger sized fish have the potential to shed higher amounts of DNA, almost 90% of the larvae we captured were within the smaller sized category (Figure 24).

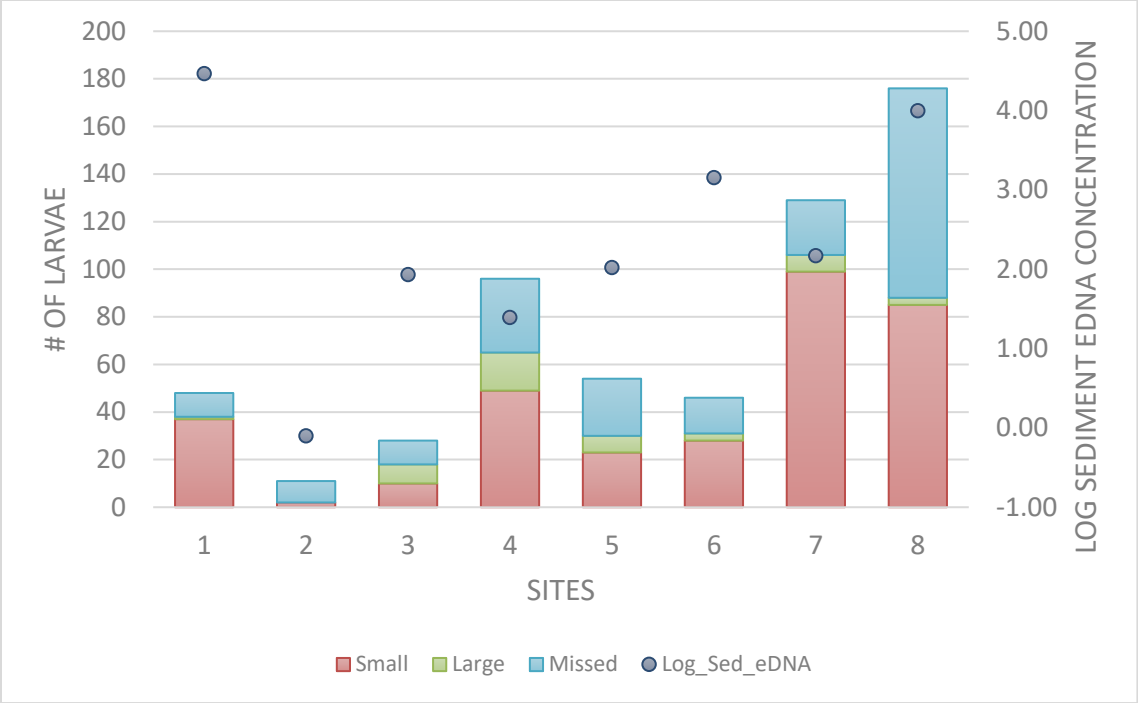


Figure 24. Comparison of larval detections by site. Larval detections via electrofishing (total number of larvae detected) and sediment eDNA concentration (log transformed, dark blue line). Captured larvae were separated into two size categories, small (< 80 mm, red column) and large (> 80 mm, green column). The average number of larvae captured was more than half (61%) of the number that escaped ('missed', blue column) during electrofishing.

Habitat Characterization

Water Parameters

Water measurements collected at each site are shown in Table 10, with descriptive statistics for each parameter. Temperature, dissolved oxygen, conductivity, pH and turbidity were found to have no statistically significant correlation with the number of larvae detected through electrofishing or sediment eDNA concentration (Appendix D).

Table 10. Water parameters taken at each site with corresponding descriptive statistics.

Site	Temperature (ºC)	Dissolved Oxygen (%)	Conductivity (µS/cm)	pH	Turbidity (NTU)
1	17.00	110.10	65.50	8.48	0.50
2	17.10	107.20	66.80	7.65	-0.30[a]
3	15.30	99.50	64.60	7.60	2.30
4	15.80	106.60	64.70	7.68	2.40
5	15.50	104.30	54.60	7.70	2.40
6	14.10	101.20	53.90	7.50	2.70
7	17.30	110.60	62.40	8.40	1.80
8	14.60	104.40	64.80	8.08	1.80
Normal Distribution	Y	Y	N	Y	Y
Mean	15.84	105.49	62.16	7.89	1.70
Standard Error	0.42	1.39	1.78	0.13	0.37
Median	15.65	105.50	64.65	7.69	2.05
Mode	NA	NA	NA	NA	2.40
Standard Deviation	1.20	3.94	5.04	0.38	1.06
Interquartile Range	1.90	4.40	4.52	0.52	0.93
Sample Variance	1.43	15.49	25.36	0.15	1.11
Kurtosis	-1.48	-0.94	-0.29	-1.17	0.54
Skewness	-0.07	-0.18	-1.21	0.83	-1.27
Range	3.20	11.10	12.90	0.98	3.00
Minimum	14.10	99.50	53.90	7.50	-0.30
Maximum	17.30	110.60	66.80	8.48	2.70
Confidence Level (95.0%)	1.00	3.29	4.21	0.32	0.88

[a] Negative values of turbidity are theoretically impossible and are often rounded to 0.00 NTU [87]. The negative value is likely indicative of an issue with the device or operator technique. Low turbidity would possibly increase the ability of DNA to settle on bottom sediments instead of being suspended in the water column.

Habitat Observations

Larval habitat classification was assessed at each site (Table 2). Type I habitat was predominant within 50% of our sites, while the remaining sites consisted of Type II habitat. Sites with Type I habitat detected more larvae (337 of 588) than those with Type II (251 of 588) (Figure 25). We did not find a statistically significant correlation with either total larvae detected (r_s = -0.2, p = 0.6) or sediment eDNA concentration (r_s = -0.4, p = 0.3), perhaps this was in part due to the small sample size for Spearman's rank (n = 8).

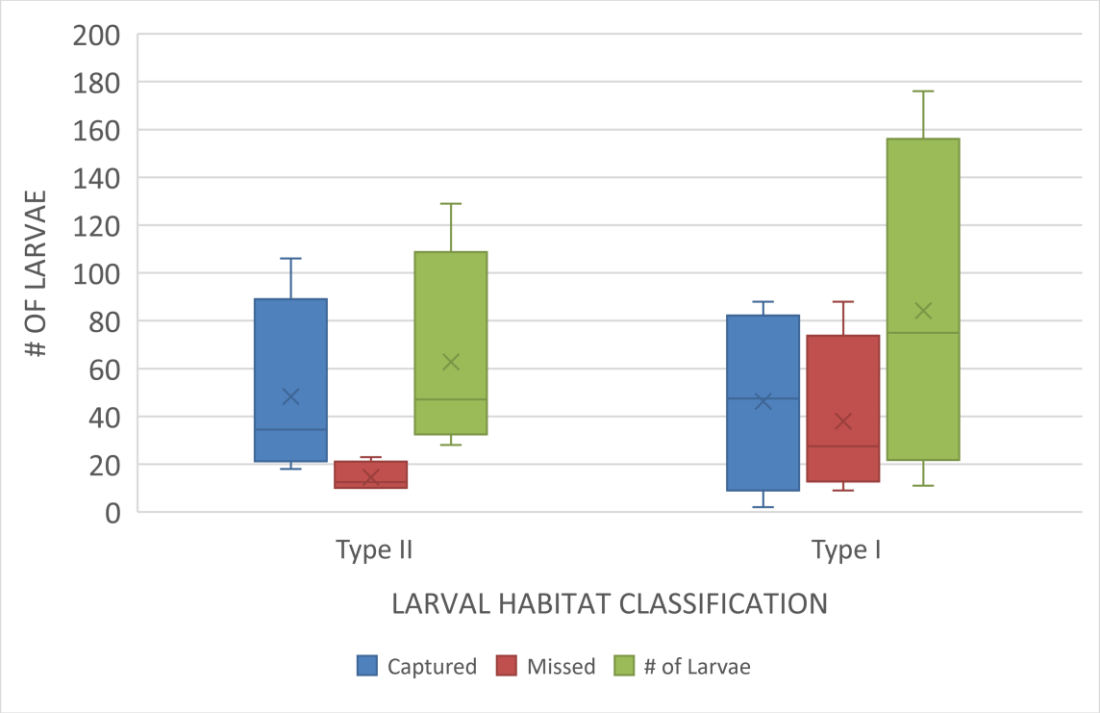


Figure 25. Larval habitat classification (Type I and Type II; Table 2) and number of larvae captured (blue), missed (red), and total detected (green) within each habitat type.

In addition, we evaluated the shoreline habitat of each site and described as either island, side channel, alcove, edge of main channel, or main channel. None of our sites were alcove habitat. Main channel habitat made up 37.5% (3 of 8) of our sites, and 45.9% (270 of 588) of the total number of larvae detected during electrofishing. The remaining sites consisted of edge (25%), side channel (25%), and habitat island (12.5%), with 31.1%, 18.2%, and 4.8% of the larval detections, respectively. Shoreline habitat type also appeared to relate to sediment eDNA concentration (Table 11), however there was no statistically significant relationship (r = -0.5, p = 0.3) (Appendix D). Main channel habitat had the highest concentrations of DNA.

Table 11. Shoreline habitat of at each site and ranked by sediment eDNA concentration.

Site	Habitat Type	Average eDNA Sediment Concentration	Total Larvae Count
1	Main Channel	29248	48
8	Main Channel	9974	176
6	Main Channel	1424	46
7	Edge	148	129
5	Edge	105	54
3	Island	85	28
4	Side Channel	25	96
2	Side Channel	0	11

We also recorded observations of shoreline development, percent cover of aquatic vegetation and organic detritus. Field notes associated with shoreline development were converted to a disturbance scale: none – private (1), none – low (2), low – moderate (3), moderate (4), and moderate – high (5). The average disturbance rating for all sites was 2.6 (SD = 1.6), between ‘none – low’ and ‘low – moderate’ human impact or

development to the shoreline. Site 7 and 8 had the highest number of larvae detected by electrofishing and was private access, with little to no human disturbance along the shoreline. Site 1 was highly disturbed (high-traffic, public recreation) and had the highest eDNA concentrations. Site 1 was also more of a flat beach bar compared to all the others. It may be that larvae or adult lamprey upstream are shedding DNA and it is drifting downstream to this site. However, disturbance had no clear pattern with either number of larvae detected (site-level: r = -0.5, p = 0.2; grid-level: r = 0.4, p = 0.3) or sediment eDNA concentration (site-level: r_s = -0.4, p = 0.07; grid-level: r_s = -0.1, p = 0.7) (Table 12 & Appendix D).

Table 12. Shoreline disturbance, larval count and sediment eDNA concentration. Sites are ranked by low (1) to high (5) disturbance scale.

Site	Disturbance	Total Larvae Count	Average eDNA Sediment Concentration
7	1	129	148
8	1	176	9974
2	2	11	0
3	2	28	85
4	2	96	25
5	3	54	105
1	5	48	29248
6	5	46	1424

Percent cover of aquatic vegetation and detritus were recorded by selecting one of four categories: none (0), low (< 10% or 0.1), medium (10 – 40% or 0.3), and high (> 50% or 0.5). Aquatic vegetation averaged 0.1 (*SD* = 0.1), with a range between 0 – 0.3. Half (4 of 8) of the sites were described as having 'low' percent cover, while the

remaining half had equal parts ‘none’ and ‘medium’ cover. Surprisingly, sites with no aquatic vegetation had the highest number of larvae detected (Figure 26). However, detections in low and medium cover combined outweigh detection in no-cover areas. Percentage of aquatic vegetation cover had no clear pattern with either larval detections (r = -0.1, p = 0.9) or sediment eDNA concentrations (r = -0.5, p = 0.2) (Appendix D).

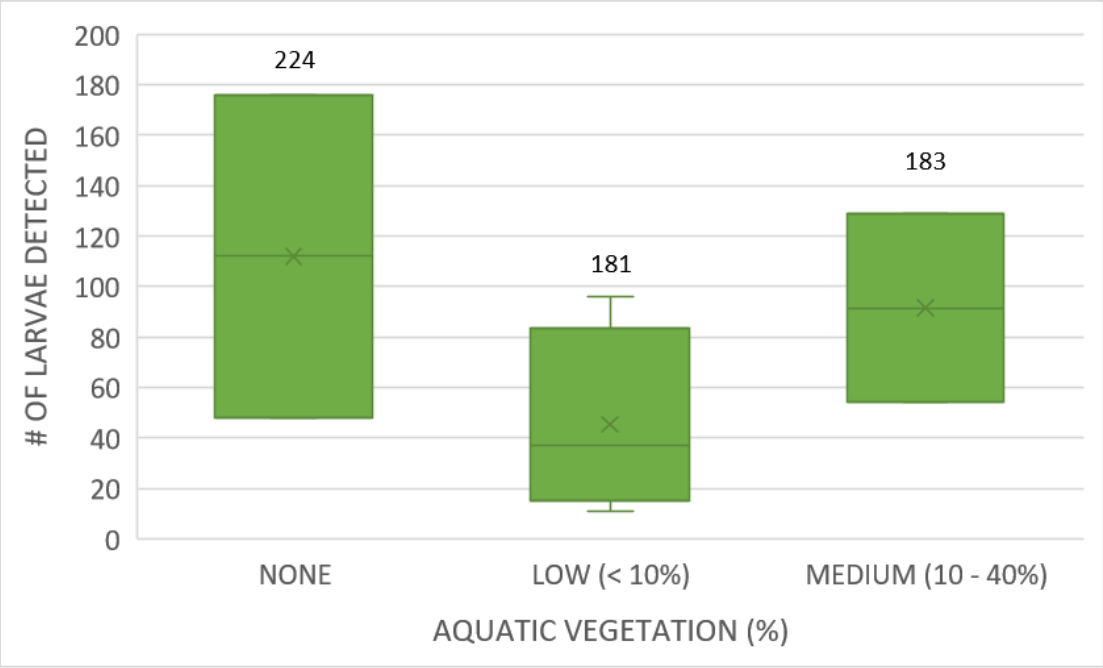


Figure 26. Box and whisker graph illustrating the categories of aquatic vegetation percent cover (none, low, and medium) and the number of larvae detected in each category.

Percent detritus had a median of 0.2 (IQR = 0.2) with a similar range of 0 – 0.3. No pattern was observed between detritus and sediment eDNA concentration (r_s = -0.2, p = 0.7). Increased detritus and larval abundance appeared to be strongly correlated (r_s = 0.8, p = 0.03), however the p-value is suspect due to a small sample size (Table 13 & Appendix D). Detritus provides food for filter feeding larvae and may explain this potential relationship.

Table 13. Detritus percent cover observations, total larvae detected during electrofishing and average eDNA sediment concentration (Pacific lamprey and *Lampetra* spp.). Sites are ranked from highest to lowest larval counts.

Site	Detritus (%)	Total Larvae Count	Average eDNA Sediment Concentration
8	Medium (10-40%)	176	9,974
7	Medium (10-40%)	129	148
4	Medium (10-40%)	96	25
5	Medium (10-40%)	54	105
1	None (0)	48	29,248
6	Low (< 10%)	46	1424
3	Low (< 10%)	28	85
2	Low (< 10%)	11	0

Sediment Grain-Size

The average percentage of very fine sand, silt, and clay type soil for each site was 8% (SD = 7%, range = 1 – 21%). Fine sediment appeared to be an indicator of more larvae ($r_s = 0.5$, $p < 0.01$) and sediment DNA concentration ($r_s = 0.6$, $p = 0.001$) per grid (Table 14). Very fine sediment also appeared to moderately correlate with the number of small sized larvae at each grid (Figure 27). This could be due to a preference of smaller sized larvae to occupy habitats with finer sediments because they are lighter and require less energy to move. In contrast, very fine sediment and larger sized larvae were not correlated ($r_s = 0.2$, $p = 0.3$).

Table 14. Sediment type composition (%), total larvae detected during electrofishing and average eDNA sediment concentration. Sites are ranked by the fine sediment category, from highest to lowest percentages.

Site	Very Fine Sand, Silt, and Clay (< 0.075 mm)	Sand (0.5-0.075 mm)	Total Larvae Count	Average eDNA Sediment Concentration
7	21%	79%	129	148
5	15%	85%	54	105
1	8%	92%	48	29,248
8	8%	92%	176	9,974
6	7%	93%	46	1424
3	2%	98%	28	85
2	1%	99%	11	0
4	1%	99%	96	25

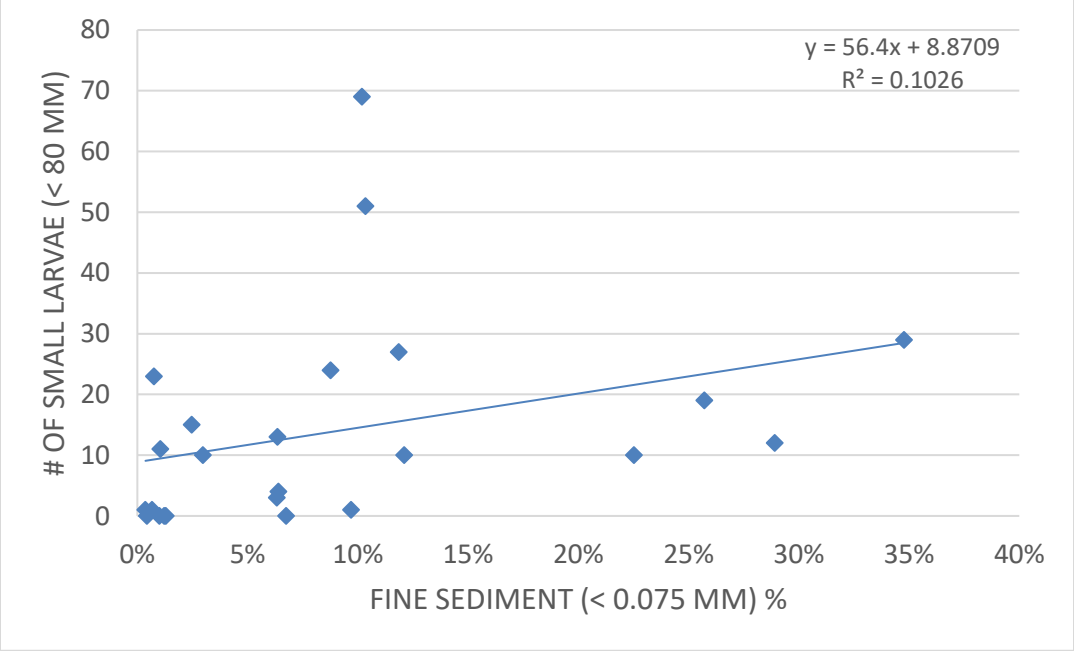


Figure 27. Relationship between small larvae and fine sediment. This scatter plot illustrates a moderate correlation ($r_s = 0.6$, $p < 0.01$) between the number of small (< 80 mm in length) larvae captured during electrofishing and the average percentage of very fine sediments (< 0.075 mm) observed at each grid.

eDNA Occupancy

The multiscale occupancy model for *Lampetra* spp. revealed high probabilities for site occupancy in the Nisqually River ($\psi = 0.93$; range 0.67 – 1.00), sample occupancy in sediment samples ($\theta = 0.76$; range 0.66 – 0.85), and detection probability in qPCR replicates ($\rho = 0.91$; range 0.85 – 0.94) (Table 15). Visual assessment of trace and autocorrelation plots revealed that the occupancy model did not fit for the *Lampetra* spp. water samples (Table 15). This is likely because we did not do enough replicate sampling at the site-level (C. Ostberg, USGS, pers. comm., May 30, 2019). The occupancy model for Pacific lamprey revealed low probabilities of occupancy through sediment sampling, which reflects the lack of Pacific lamprey presence through eDNA sampling (Table 7). As there were no water detections of Pacific lamprey, the model was overfit, and would not run (Table 15).

Table 15. eDNA occupancy model parameter estimates for *Lampetra* spp. and Pacific lamprey in the Nisqually River (all sites combined). All parameters are constant ($\psi(\cdot)$, $\theta(\cdot)$, $\rho(\cdot)$).

Species	Sample Type	Site	Occupancy in site (ψ) (95% CI)	Occupancy in sample (θ) (95% CI)	Occupancy in qPCR replicate (ρ) (95% CI)
Lampetra spp.[a]	Sediment	All	0.93 (0.67 – 1.00)	0.76 (0.66 - 0.85)	0.91 (0.85 - 0.94)
Pacific Lamprey[b]	Sediment	All	0.23 (0.04 -0.80)	0.23 (0.03 - 0.67)	0.55 (0.17 - 0.88)
Lampetra spp.	Water	All	NA	NA	NA
Pacific Lamprey	Water	All	NA	NA	NA

[a] PPLC = 33.93; WAIC = 0.58. [b] PPLC = 5.31; WAIC = 0.09.

In addition to running the models with constant parameters, we ran the occupancy analyses with covariates[28] to determine if estimates of eDNA occupancy were affected. Covariate measurements (14 different parameters) were taken at each site and are potential environmental indicators for predicting probability of *Lampetra* spp. eDNA occupancy in sediment samples. We decided to only run models containing covariates for *Lampetra* spp. in sediment since *Lampetra* spp. were the prevalent species detected with sediment eDNA. Of the 99 models performed, only 55 models were a good fit based on visual assessment of trace and autocorrelation plots. None of the site-level covariate models (ψ(covariate), $\theta(\cdot)$, $\rho(\cdot)$) fit the data. Model selections were performed using the PPLC and WAIC values and the top 10 covariate models are listed in Appendix G. From this analysis, we determined that the two best models for predicting eDNA occupancy were: $\psi(\cdot)$, θ(Lamprey+AquaticVeg), ρ(Lamprey+Large+Per_Fine) and $\psi(\cdot)$, θ(Lamprey+AquaticVeg), ρ(Lamprey+Large) (Table 16). That is, combinations of the number of larvae (Lamprey) and percentage of aquatic vegetation cover (AquaticVeg) in sediment samples, with larger sized larvae (Large), and percentage of fine sediments (Per_Fine) in qPCR replicates were found to be indicators of eDNA occupancy. Both models produced similar probabilities and criterion values, therefore both are considered to have the best fit (Table 16).

[28] Environmental parameters taken during sampling and discussed under Habitat Characterization.

Table 16. Multiscale occupancy models with covariates to refine estimates of *Lampetra* spp. in sediment eDNA detection probabilities, sample occupancy, and site occupancy.

Model	Occupancy in site (ψ) (95% CI)	Occupancy in sample ($\overline{\theta}$) (95% CI)	Occupancy in qPCR replicate ($\overline{\rho}$) (95% CI)	PPLC	WAIC
ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large+Per_Fine)	0.93 (0.67- 1.00)	0.76 (0.66 -0.85)	0.91 (0.85 - 0.94)	30.60	0.54
ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large)	0.23 (0.04 -0.80)	0.23 (0.03 - 0.67)	0.55 (0.17 - 0.88)	30.63	0.52

Our cumulative probability estimates for sampling *Lampetra* spp. eDNA in at least 1/9 sediment samples (θ*) were high (Table 17), indicating high probabilities of obtaining eDNA from *Lampetra* spp. when 9 sediment samples are collected independently of other variables. Similarly, cumulative probability estimates for detecting *Lampetra* spp. in 1/3 qPCR replicates (ρ*) were high, with a median estimate of 100%, in sediment samples (Table 17).

Despite low probabilities of Pacific lamprey occurrence in sediment samples, the cumulative probability estimates for sampling Pacific lamprey eDNA in sediment were fairly high, though median estimates were extremely variable (Table 17). Similarly, the cumulative probability estimates of detecting Pacific lamprey in at least 1/3 replicate qPCRs were also high despite low probabilities for detecting eDNA in qPCR replicates.

Table 17. Cumulative probabilities (%) of sampling lamprey eDNA in sediment or water samples (θ*), and in at least one of 3 qPCR replicates (ρ*).

Species	Sample Type	Cumulative Prob. of Occupancy in Sample θ* (95% CI)	Cumulative Prob. of Occupancy in Replicate ρ* (95% CI)
Lampetra spp.	Sediment	1.00 (1.00 – 1.00)	1.00 (1.00 – 1.00)
Pacific Lamprey	Sediment	0.90 (0.24 – 1.00)	1 (0.81 – 1.00)
Lampetra spp.	Water	NA	NA
Pacific Lamprey	Water	NA	NA

DISCUSSION

Lamprey Detections in the Nisqually River

The first objective of this study was to determine if eDNA analysis of sediment can detect the presence of lampreys in the Nisqually River. We detected both Pacific lamprey and *Lampetra* spp. through sampling eDNA in the sediment. *Lampetra* spp. were detected at every site in either sediment or water eDNA samples and appear to be more abundant throughout the sites sampled than Pacific lamprey. Pacific lamprey were detected at only one site through sediment eDNA. Larvae that were detected through electrofishing were not identified to species, so we were unable to assess eDNA detection rates for both methods per species. Pacific lamprey and *Lampetra* spp. are known to be sympatric (through trapping efforts and water eDNA analysis) in only seven rivers in Puget Sound, these include: Nooksack, Green, Puyallup, Nisqually, Skokomish, Tahuya, and Little Quilcene rivers [20,48,74]. Although both species are present in the Nisqually

River, trapping data indicate that *Lampetra* spp. might be more abundant, particularly in the lower portions of these rivers [31,48].

We were expecting to find more Pacific lamprey, given our conversations with the Nisqually tribal biologists, and did not consider the *Lampetra* spp. life history to the extent we might have. As such, the sampling design was set up for Pacific lamprey life history. Our water eDNA results are what would be expected if Pacific lamprey had spawned, died, and had time to decay before sampling for water samples. Sediment samples would then have shown that larvae are in the sediment and not the water. There is evidence of eDNA remaining in sediment from fish for up to 132 days [18]. If eDNA came from Pacific lamprey adults from upriver, it would have likely settled in the grids, but it was not present. Future research should consider selecting areas with extensive Pacific lamprey data to test the accuracy of eDNA assay in sediment eDNA samples (such as the Columbia River). The spawning period for *Lampetra* spp. in the Nisqually River is unknown, however they typically spawn between March – July (could vary depending on location). We sampled at the end of July, so there could have still been adult DNA present, which may explain the reason why *Lampetra* spp. were found in both water and sediment samples throughout most of the sites.

Pacific lamprey were minimally detected at one site in a sediment sample. These results indicate that Pacific lamprey are present in the Nisqually River, however, potentially at low numbers. Beamish [31] noted that Pacific lamprey, in comparison to *Lampetra* spp., have "exceptional migratory instincts" and typically migrate and spawn in the upper most tributaries and headwaters. If Pacific lamprey are migrating to the upper reaches of the Nisqually River, it is likely that our surveys missed them, as our sites did

not extend further than river mile 13. It would be of interest to conduct eDNA surveys in the upper reaches of the Nisqually River, to better determine the occupancy of Pacific lamprey.

Interestingly, a similar project called the Basin-wide Lamprey Inventory and Monitoring Project (BLIMP) and managed by the U.S. Forest Service Department of Agriculture and National Genomics Center, collected three water eDNA samples from the Nisqually River on August 29th, 2018; just a few weeks after our sampling (K. Carim, USFS, pers. comm., Feb. 13, 2019). Their samples had positive detections for Pacific lamprey[29] near river mile 3, 12 and 22. River mile 3 and 22 were outside of our sampling area (Figure 12), however the positive detections at this site demonstrate Pacific lamprey presence in the upper reaches of the Nisqually, and possibly in the lower reach as well; though this signal may have drifted downriver. Also, the sample taken near river mile 12 was within the vicinity of Site 6, yet our samples had positive detections only in sediment eDNA at this site. Furthermore, one additional water sample was taken further upstream and confirmed no presence of Pacific lamprey above the Alder and La Grande Dams.

We demonstrated that analysis of sediment eDNA successfully detects the presence of larval lampreys. Recent work by Ostberg et al. [20] detected the presence of Pacific lamprey and *Lampetra* spp. in Puget Sound through water eDNA. This method of detection showed seasonal variation in detection rates, most likely due to differences in stream flow rates between fall and spring sampling dates [20]. Both Pacific lamprey and *Lampetra* spp. larvae are present in freshwater systems year-round, suggesting that

[29] They did not test for *Lampetra* spp. during the sampling effort.

detections should occur year-round. Water samples may capture DNA from both adult and larval lampreys, while only larval detection may occur when sediment is disturbed. Thus, sediment eDNA may be a better tool than filtered water to detect the year-round presence of larval lampreys. Future work will be needed to determine if there is a similar seasonal difference with sediment eDNA, though it may be difficult to access larvae habitat to sample when the water level is higher. Our work suggests that sediment eDNA may be a non-invasive method to detect the presence of larval lampreys, without the risks associated with electrofishing [88]. Additionally, larvae are difficult to identify to species [26] and may require anesthetization when handled [48], causing potentially further risk to the larvae.

The C_T values for our eDNA samples typically ranged higher than what was reported for Ostberg et al. [20] in water eDNA samples sampled from the Puget Sound watersheds. This indicates that their water samples had relatively high eDNA concentrations when compared to ours since lower CT values reflect higher levels of DNA during each qPCR reaction. However, it is important to note that the timing of sampling and lamprey life history can alter the amount of available DNA in the system as their study sampled in spring (June) and fall (October), while we sampled in late summer (August). We did not have any detections for Pacific lamprey in water samples, therefore we cannot compare C_T values directly between each sample type. Additionally, we used the Pacific lamprey eDNA assay developed by Carim et al. [19], and this may change our ability to compare as well. In contrast, we used the same *Lampetra* spp. eDNA assay. Their study found *Lampetra* spp. C_T values ranged from 19.4 – 27.0 in water samples, in comparison to ours which ranged higher at 33.1 – 43.4. These comparisons are not

conclusive, as many variables likely affect these values, including different methods, sampling timing, human error, and other factors that affect lamprey DNA concentrations.

The second objective was to compare the detection rates of larval lampreys, using both electrofishing and eDNA analysis of sediment, and water. Larvae detected during the electrofishing surveys were not identified to species, making the comparison of methods per species impossible. Taking additional steps for species identification (visual or genetic) and larval biomass (measurements of individual larval body lengths and weight) would potentially require additional permitting because of anesthetization, however having this information would assist with comparisons of larval abundance and eDNA concentrations. All methods of detection, eDNA (sediment and water) and electrofishing, detected the presence of Pacific lamprey and *Lampetra* spp., and eDNA detections were consistent with electrofishing detections. Generally, electrofishing surveys with lamprey-specific settings achieve greater than 90% larval detection rates when used at sites with preferred larval habitat and known lamprey occupancy [20,62]. This was consistent with our research, as all the sites had larvae observed during electrofishing.

Additionally, when larval lampreys were captured, they were separated into two categories based on size (< 80 mm and > 80 mm)[30]. These sizes were selected based off the USGS lab-controlled experiment showing larvae eDNA was not detected as strongly in the sediment if they were smaller than 80 mm in length (T. Liedtke, pers. comm., Dec. 6, 2017). Looking at the total number of captured larvae for all sites combined, 88%

[30] We suggest using a smaller mesh size for the dip nets used during electrofishing surveys to better aid in the capture of smaller sized larvae. The diameter and depth of the dip nets worked well, and anything smaller would make it much harder to catch the larvae (such as an aquarium net size).

were smaller than 80 mm in length. On average, 86% of the larvae captured at each site were in this smaller size class. The average number of smaller larvae captured per sample site was 42 when compared to 6 per site for larvae greater than 80 mm. Based on the USGS findings, it was unexpected to have higher concentrations of eDNA in our sediment and water samples when compared to the proportion of smaller sized larvae under 80 mm.

Larval Habitat Characterization

Knowing how to characterize preferred larval habitat is important for traditional sampling methods as well as sediment eDNA sampling. If we can collect samples at sites with environmental indicators of larval rearing, then we have a higher likelihood of detecting larvae. Furthermore, it is important to understand how the environment, such as water chemistry, affects the development of larval lampreys. Optimum temperature for larval development is 14ºC, with temperatures above 20ºC increasing risk of survival [46]. The average water temperature observed at our sites was within a safe range (14.1 – 17.3ºC); however, water depth, speed, and availability of shade affect water temperature (and eDNA longevity) and there may have been locations with higher temperatures. We observed an average conductivity of 62 µS/cm, which is closer to the conductivity observed at Cedar Creek (a low order stream with extensive lamprey use, 76 µS/cm) [38], versus at the Middle Fork of the John Day River (high order stream, 125 and 175 µS/cm) [43]. Larval density may be related to conductivity, as larvae will "receive the maximum shock through its body when the conductivity of the water approaches that of the fish" (Koltz, 1989 as cited in Stone & Barndt [38]). We observed an overall average of 5 larvae per m^2 and a maximum abundance of 13/m^2 in the Nisqually River. Stone and Barndt

[38] observed an average density of 1/m^2 (44/m^2 max.) in Cedar Creek and Torgersen and Close [43] observed an average of 4/m^2 (118/m^2 max.) in the Middle Fork of the John Day River. Few studies have found pH to impact larval distribution [24]. Goodwin et al. (2008) as cited in Dawson et al. [24] found that pH was associated with larval abundance of European river lamprey and European brook lamprey at a regional scale (i.e., Northern Ireland), as more larvae were detected at sites with pH > 8.16 than those with lower pH. The authors note that this relationship could be a result of other factors that influence pH, such as climate, bedrock type, land use, and water capacity. None of our water parameters were found to have a statistically significant correlation with numbers of larvae detected during electrofishing or eDNA concentrations. A potential next step would be to collect a larger sample size with more frequent water and habitat measurements, at each site and/or sampling grid. As well as look at different water depths, velocity, habitat types, seasonal differences, and comparison to a less healthy water body.

Probabilities of Lamprey Occupancy

Occupancy modeling estimates could improve conservation efforts of protecting lamprey species by identifying changes in population abundance, identifying key habitats to larval lamprey, and by providing decision support for long term management, through estimation of occurrence and detectability. Our model provided site (ψ) estimates for the Nisqually River, as a whole. The results of our occupancy model estimates indicate high occupancy probabilities for *Lampetra* spp., but not Pacific lamprey, in sediment. As mentioned above, future research that include sampling in the further reaches of the Nisqually River will be needed to determine the range and occupancy of Pacific lamprey

in this river. Additionally, we are not sure why the model did not fit for *Lampetra* spp. water samples. We speculate that we did not have enough replicate sampling at the site-level and perhaps adding another water sample in the future will produce better success.

We included covariates in the multiscale occupancy models to refine estimates of *Lampetra* spp. in sediment eDNA detection probabilities, sample occupancy, and site occupancy. Of the top 10 models we compared, the two models with the lowest PPLC and WAIC scores with the best fit included: the number of larvae detected during electrofishing and percent cover of aquatic vegetation as predicators of sample occupancy; and the number of larvae detected, larger sized larvae, and percentage of fine (< 0.75 mm) sediments as predictors of detection probability (Table 16). These predictors appear to consist of recognized indicators of larval presence and eDNA longevity. Increased larval abundance and larger larvae, have the potential to shed more DNA, increasing the probability of occurrence. Aquatic vegetation may provide food (as it breaks down), and oxygen, and the shade from the plants could provide protective effects on the DNA by cooling water temperatures and shielding from UV exposure. Acidic pH, increased water temperature, and UV exposure have been demonstrated to reduce the lifespan of eDNA [73]. Fine sediments are preferred by larvae and percent fines have been found to influence larval distribution at small spatial scales (1 m^2 quadrats) [38]. Furthermore, fine particles have a protective effect since they are more able to bind to DNA than larger sized particles. Buxton et al. [89] detected great crested newts from water and sediment samples collected from a pond during every season and found that the probability of detecting eDNA varied seasonally in both sample types; though eDNA detection was lower in sediment in all season when compared to water. “Unbound DNA

within sediments has been found to be broken down more quickly than DNA bound to sediments. DNA that has been incorporated into sediments through the settling of cellular material but remains unbound may explain why samples did not show a constant level of detection all year" [89].

We used occupancy models to determine the certainty of our sampling design to better inform future research planning. We collected 9 replicate sediment samples and 1 water sample, and each sample was qPCR amplified in triplicate. We detected high cumulative probability estimates for detecting *Lampetra* spp. in at least 1/9 sediment samples and 1/3 qPCR replicates (Table 17). With a cumulative probability estimate of 100%, future studies could suffice with fewer replicate sediment samples, and still have a high probability of detection.

The initial reasoning behind our sampling design to include three sampling grids per site, evolved from a combination of: 1) applying the composite methodology from the USGS lab-controlled experiment, and 2) wanting a robust sampling design (3 replicate sediment samples per grid/9 replicate sediments samples per site). We also knew finding larval habitat and access on the Nisqually would be difficult, so it was a way to get more information and samples from the sites we found. However, based on the high certainty of our sampling design, future sampling should explore the possibility of not using a composite or triplicates. Furthermore, future work should attempt sampling at a larger scale, such as sampling sediment further apart to experiment with the idea that an eDNA sediment sample can represent a larger sample area of habitat use or species distribution.

Sediment eDNA Implications for WDNR Management of SOAL

I currently work for the WDNR Aquatics Division as an Aquatic Land Manager. This position assists with aquatic leasing and licensing of state-owned aquatic lands (SOAL). I negotiate these contracts with applicants based on what type of activities occur and make sure that the uses meet agency and state policy and regulatory standards. Sediment eDNA analysis is a potential tool that can be applied to all aquatic systems, not only on SOAL. The goal of this research is to incorporate our findings into the WDNR's habitat stewardship measures to be used to determine if larval lampreys are present at a proposed project site. Wildlife and aquatic land managers will potentially be able to take a sediment sample and determine if lamprey occur at a project site. If larvae are determined to be present before a proposed project, a recommendation for future research is to perform Before-After-Control-Impact (BACI) monitoring; monitor before and after the activity and see if there is a change in either larval abundance or use. Examples of uses include overwater structures, boat launches, water access points, bulkhead armoring, restoration, dredging, irrigation, outfalls/pipes. Additionally, evaluate if there are disturbed areas where larvae are known to occur in a water body and if there is a disturbance threshold.

Certain proposed activities may be denied or modified to protect the species if implemented into policy and management. Applicants who propose uses, such as dredging or mining, will most likely be affected or inconvenienced. This may be the case for new projects and existing uses. For example, if a new project is proposed in an area where lamprey are detected, several actions may occur, depending on the state and/or agency's authority to do so: 1) application may be denied, 2) applicant may be asked to

modify their proposal to accommodate lamprey presence, and/or 3) mitigation and/or resource damages may be paid as compensation for the impact their use may cause. Another possibility might be for the WDNR to work with USFWS, WDFW, and the tribes to relocate larvae if the habitat will be impaired. This may also be the situation for tenants that have existing agreements and need to renew their use but now are required to consider updated obligations and regulations, including potential permit requirements. Depending on the situation of the proponent, they may not be equipped – financially or otherwise – to make these accommodations and may face challenging decisions. This may be particularly frustrating to the public since lamprey are not a charismatic species and their ecological and cultural value is not well understood. Nonetheless, the implementation of lamprey-specific research, restoration, and management protocols, along with increasing public awareness, will directly impact the conservation efforts towards restoring lamprey species in Puget Sound.

CONCLUSION

Common to all the research objectives identified is a need to obtain information on the current distribution and abundance of lamprey and the status of their current habitat. For the WDNR Aquatics, the benthic habitat conditions and distribution of larvae is particularly relevant. It is recognized that all species of lamprey share a prolonged larval phase and thus, globally, lamprey are in decline largely due to habitat disturbances that occur during their larval life stage. The larval stage is ideal for surveying lamprey occupancy due to their year-round presence in freshwater sediments. A goal of this work was to find a method of detection that would have the least amount of impact to the

species and surrounding environment, while providing an efficient tool for management. Although recent studies indicate that traditional detection methods have low mortality rates, non-invasive sampling methods have been investigated using eDNA. eDNA methods have been used for lamprey monitoring, however these previous studies used water samples which provide a river reach signal in flowing systems, rather than site-level detectability. The main purpose of this thesis research was to test the ability of eDNA analysis to detect Pacific lamprey and *Lampetra* spp. from field collected sediments by applying methodology developed in a USGS lab-controlled experiment.

Our study detected Pacific lamprey and *Lampetra* spp. through sampling eDNA in sediment collected from the Nisqually River, Washington. *Lampetra* spp. appear to be more prevalent, as they were detected at every site, while Pacific lamprey were detected at only one site via sediment eDNA. These results indicate that Pacific lamprey are present in the Nisqually River, however, potentially at low numbers. This research demonstrates that analysis of sediment eDNA successfully detects the presence of larval lampreys, in the sites where they were physically detected through electrofishing surveys.

In 2003, the Pacific lamprey, western river lamprey or western brook lamprey were petitioned for listing under the Endangered Species Act. The petition was denied because of insufficient information on the distribution and age structure of the population [4]. Since then, other federal, state, and local entities in the Pacific Northwest have been partnering to design and implement studies to characterize lamprey habitat and their population dynamics at all life stages. Additionally, the Puget Sound regional management area was not included in the Pacific Lamprey Conservation Initiative risk assessment due to the lack of available abundance and distribution data; though local

experts and other studies indicated declining populations across the Washington Coast and Puget Sound. Our work suggests the utility in using sediment eDNA to aid in better assessing lamprey species occupancy and distribution in the Puget Sound. The findings of this research and subsequent use within the WDNR management will help add to this knowledge gap and build the case for lamprey protection.

REFERENCES

1. Close DA, Fitzpatrick MS, Li HW. The Ecological and Cultural Importance of a Species at Risk of Extinction, Pacific Lamprey. Fisheries 2002;27:19–25.

2. Luzier CW, Schaller HA, Brostrom JK, Cook-Tabor C, Goodman DH, Nelle RD, et al. Pacific Lamprey (*Entosphenus tridentatus*) Assessment and Template for Conservation Measures. US Fish and Wildlife Service. Portland, OR (United States): 2011.

3. Clemens BJ, Beamish RJ, Coates KC, Docker MF, Dunham JB, Gray AE, et al. Conservation Challenges and Research Needs for Pacific Lamprey in the Columbia River Basin. Fisheries [Internet] 2017 [cited 2018 Nov 18];42:268–80. Available from: http://doi.wiley.com/10.1080/03632415.2017.1305857

4. USFWS. Endangered and threatened wildlife and plants; 90-day finding on a petition to list three species of lampreys as threatened or endangered. US Fish and Wildlife Service. Fed. Regist. 2004;69:77158–67.

5. CRITFC. Tribal Pacific Lamprey Restoration Plan for the Columbia River Basin. Columbia River Inter-Tribal Fish Commission. 2011.

6. Wang C, Schaller H. Conserving Pacific Lamprey through Collaborative Efforts. Fisheries 2015;40:72–9.

7. Close DA, Jackson AD, Conner BP, Li HW. Traditional ecological knowledge of Pacific lamprey (*Entosphenus tridentatus*) in northeastern Oregon and southeastern Washington from indigenous peoples of the Confederated Tribes of the Umatilla Indian Reservation. J. Northwest Anthropol. [Internet] 2004 [cited 2018 Nov 18];38:141–62. Available from: https://aboriginalfisheriesresearchunit.files.wordpress.com/2012/06/traditional-ecological-knowledge.pdf

8. USFWS. Pacific Lamprey Entosphenus tridentatus Assessment [Internet]. US Fish and Wildlife Service. 2019 [cited 2019 Mar 8]. Available from:

https://www.fws.gov/pacificlamprey/Documents/PacificLamprey_2018Assessment_final_02282019.pdf

9. Crandall JD, Wittenbach E. Pacific Lamprey Habitat Restoration Guide. Twisp, Washington: 2015.

10. USFWS. Best management practices to minimize adverse effects to Pacific Lamprey (*Entosphenus tridentatus*). US Fish and Wildlife Service. 2010.

11. Moser ML, Butzerin JM, Dey DB. Capture and collection of lampreys: the state of the science. Rev. Fish Biol. Fish. [Internet] 2007;17:45–56. Available from: http://link.springer.com/10.1007/s11160-006-9037-3

12. Dunham JB, Chelgren ND, Heck MP, Clark SM. Comparison of Electrofishing Techniques to Detect Larval Lampreys in Wadeable Streams in the Pacific Northwest. North Am. J. Fish. Manag. [Internet] 2013;33:1149–55. Available from: http://doi.wiley.com/10.1080/02755947.2013.826758

13. Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshw. Sci. [Internet] 2013 [cited 2019 Mar 20];32:792–800. Available from: https://www.journals.uchicago.edu/doi/10.1899/13-046.1

14. Baldigo BP, Sporn LA, George SD, Ball JA. Efficacy of Environmental DNA to Detect and Quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New York. Trans. Am. Fish. Soc. [Internet] 2017 [cited 2019 Mar 20];146:99–111. Available from: http://doi.wiley.com/10.1080/00028487.2016.1243578

15. Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, et al. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol. Evol. 2016;7:1299–307.

16. Pilliod DS, Goldberg CS, Arkle RS, Waits LP. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can. J. Fish. Aquat. Sci. [Internet] 2013 [cited 2019 Mar 20];70:1123–30.

Available from: http://www.nrcresearchpress.com/doi/10.1139/cjfas-2013-0047

17. Thomsen PF, Kielgast J, Iversen L, Wiuf C, Rasmussen M, Gilbert M, et al. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. [Internet] 2012 [cited 2019 Mar 15];21:2565–73. Available from: http://doi.wiley.com/10.1111/j.1365-294X.2011.05418.x

18. Turner CR, Uy KL, Everhart RC. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol. Conserv. 2015;183:93–102.

19. Carim KJ, Dysthe JC, Young MK, McKelvey KS, Schwartz MK. A noninvasive tool to assess the distribution of Pacific lamprey (Entosphenus tridentatus) in the Columbia River basin. PLoS One 2017;12:e0169334.

20. Ostberg CO, Chase DM, Hayes MC, Duda JJ. Distribution and seasonal differences in Pacific Lamprey and *Lampetra* spp eDNA across 18 Puget Sound watersheds. PeerJ 2018;6:e4496.

21. Thomsen PF, Willerslev E. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. [Internet] 2015 [cited 2018 Oct 14];183:4–18. Available from: https://www.sciencedirect.com/science/article/pii/S0006320714004443

22. Grote A, Carim K. Using eDNA Sampling to Detect Pacific Lamprey in a Large River: 2016 Wenatchee River Pilot Study [Internet]. Leavenworth, WA: 2017 [cited 2018 Nov 15]. Available from: https://www.fws.gov/leavenworthfisheriescomplex/MidColumbiaFWCO/pdf/PacificLamprey_WenatcheeRiverEDNAPilot.pdf

23. Dorazio RM, Erickson RA. ednaoccupancy: An r package for multiscale occupancy modelling of environmental DNA data. Mol. Ecol. Resour. 2018;18:368–80.

24. Dawson HA, Quintella BR, Almeida PR, Treble AJ, Jolley JC. The Ecology of Larval and Metamorphosing Lampreys [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov

15]. page 75–137.Available from: http://link.springer.com/10.1007/978-94-017-9306-3_3

25. Potter IC, Gill HS, Renaud CB, Haoucher D. The Taxonomy, Phylogeny, and Distribution of Lampreys [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov 15]. page 35–73. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_2

26. Goodman DH, Kinziger AP, Docker M. Morphological diagnosis of *Entosphenus* and *Lampetra* ammocoetes (Petromyzontidae) in Washington, Oregon, and California [Internet]. 2009. Available from: https://www.researchgate.net/publication/239730987

27. CRITFC. Pacific Lamprey: Pacific Lamprey Evolution and Biology [Internet]. Columbia River Inter-Tribal Fish Commission. 2019 [cited 2019 Mar 20]; Available from: https://www.critfc.org/fish-and-watersheds/columbia-river-fish-species/lamprey/

28. Wydoski RS, Whitney RR. Inland fishes of Washington [Internet]. American Fisheries Society in association with University of Washington Press; 2003 [cited 2019 Feb 22]. Available from: https://books.google.com/books?id=36l3QgAACAAJ&source=gbs_book_other_versions

29. Hardisty M. Lampreys: life without jaws [Internet]. Forrest Text; 2006 [cited 2018 Nov 18]. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C48&q=hardisty+2006+lampreys&btnG=&oq=Hardisty+2006

30. Docker MF, Hume JB, Clemens BJ. Introduction: A Surfeit of Lampreys [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov 15]. page 1–34. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_1

31. Beamish RJ. Adult Biology of the River Lamprey (*Lampetra ayresi*) and the

Pacific Lamprey (*Lampetra tridentate*) from the Pacific Coast of Canada. Can. J. Fish. Aquat. Sci. 1980;37:1906–23.

32. Beamish RJ, Neville C-EM. Pacific salmon and Pacific herring mortalities in the Fraser River plume caused by river lamprey (*Lampetra ayresi*) . Can. J. Fish. Aquat. Sci. 2008;52:644–50.

33. Mayden RL, Roe KJ. Mayden Lab Lamprey Project [Internet]. [cited 2019 Mar 19]; Available from: http://bio.slu.edu/mayden/lamprey/lifehistory.html

34. Moser ML, Almeida PR, Kemp PS, Sorensen PW. Lamprey Spawning Migration [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov 15]. page 215–63. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_5

35. Gunckel S, Jones KK, Jacobs SE. Spawning Distribution and Habitat Use of Adult Pacific and Western Brook Lampreys in Smith River, Oregon. Am. Fish. Soc. Symp. 2009;72:173–89.

36. Orlov A, Beamish R. Jawless Fishes of the World: Volume 2 [Internet]. 2016 [cited 2019 Feb 22]. Available from: https://books.google.com/books?hl=en&lr=&id=yTT5DAAAQBAJ&oi=fnd&pg=PR5&ots=gWVNjL5-Ai&sig=rGchDT3K8oHM74ylYR5anoC3llQ

37. USDA. Pacific Lamprey and NRCS: Conservation, Management and Guidelines for Instream and Riparian Activities This Technical Note is divided into two parts: Part I Conservation and Management of Habitats for Pacific lamprey (Entosphenus tridentatus) and Part II [Internet]. Spokane, Washington: 2011 [cited 2018 Nov 18]. Available from: https://efotg.sc.egov.usda.gov/references/public/WA/Bio_TN_25_0411.pdf

38. Stone J, Barndt S. Spatial distribution and habitat use of Pacific lamprey (*Lampetra tridentata*) ammocoetes in a western washington stream. J. Freshw. Ecol. 2005;20:171–85.

39. Johnson NS, Buchinger TJ, Li W. Reproductive Ecology of Lampreys [Internet].

In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2019 Feb 22]. page 265–303. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_6

40. Lampman R. Columbia Basin Lamprey Identification Guide. 2017.

41. Manzon RG, Youson JH, Holmes JA. Lamprey Metamorphosis [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov 15]. page 139–214. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_4

42. Clemens BJ, van de Wetering S, Sower SA, Schreck CB. Maturation characteristics and life-history strategies of the Pacific lamprey, *Entosphenus tridentatus*. Can. J. Zool. 2013;91:775–88.

43. Torgersen CE, Close DA. Influence of habitat heterogeneity on the distribution of larval Pacific lamprey (*Lampetra tridentata*) at two spatial scales. Freshw. Biol. 2004;49:614–30.

44. Arntzen E V., Mueller RP. Video-based electroshocking platform to identify lamprey ammocoete habitats: Field validation and new discoveries in the Columbia river basin. North Am. J. Fish. Manag. 2017;37:676–81.

45. Jolley JC, Silver GS, Whitesel TA. Occurrence, Detection, and Habitat Use of Larval Lamprey in Columbia River Mainstem Environments: Bonneville Reservoir and Tailwater. 2010 Annual Report. 2011.

46. Meeuwig MH, Bayer JM, Seelye JG. Effects of Temperature on Survival and Development of Early Life Stage Pacific and Western Brook Lampreys. Trans. Am. Fish. Soc. 2005;134:19–27.

47. Crandall J, Wittenbach E. Pacific Lamprey Habitat Restoration Guide. 2015.

48. Hayes MC, Hays R, Rubin SP, Chase DM, Hallock M, Cook-Tabor C, et al. Distribution of Pacific Lamprey Entosphenus tridentatus in Watersheds of Puget Sound Based on Smolt Monitoring Data. Northwest Sci. 2013;87:95–106.

49. Shirakawa H, Yanai S, Hydrobiologia AG-, 2013 U. Lamprey larvae as ecosystem engineers: physical and geochemical impact on the streambed by their burrowing behavior. Springer [Internet] 2013 [cited 2019 Mar 8]; Available from: https://link.springer.com/article/10.1007/s10750-012-1293-8

50. Goldfarb B. Defenders of the Forgotten Fish. Hakai Mag. [Internet] 2015; Available from: https://www.hakaimagazine.com/features/defenders-forgotten-fish/

51. Murauskas JG, Orlov AM, Siwicke KA. Relationships between the abundance of Pacific lamprey in the Columbia River and their common hosts in the marine environment. Trans. Am. Fish. Soc. 2013;142:143–55.

52. Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA. Similarities, Differences, and Unknowns in Biology and Management of Three Parasitic Lampreys of North America. Fisheries 2010;35:580–94.

53. Pacific Lamprey Technical Workgroup. Technical White Paper: Practical Guidelines for Incorporating Adult Pacific Lamprey Passage at fishways [Internet]. 2017 [cited 2018 Oct 22]. Available from: https://www.fws.gov/oregonfwo/Documents/Lamprey/2017.06.20 LampreyPsgFINAL.pdf

54. Wang C, Schaller H, Jackson A. Pacific lamprey 2015 Regional Implementation Plan for the Mid-Columbia Regional Management Unit [Internet]. 2016. Available from: http://www.epa.gov/wed/pages/ecoregions/level_iii.htm

55. Maitland PS, Renaud CB, Quintella BR, Close DA, Docker MF. Conservation of Native Lampreys [Internet]. In: Lampreys: Biology, Conservation and Control. Dordrecht: Springer Netherlands; 2015 [cited 2018 Nov 15]. page 375–428. Available from: http://link.springer.com/10.1007/978-94-017-9306-3_8

56. USFWS. Fact Sheet Pacific Lamprey (*Lampetra tridentata*) [Internet]. US Fish and Wildlife Service. Portland, Oregon: 2008 [cited 2019 Mar 21]. Available from: https://www.fws.gov/oregonfwo/species/data/pacificlamprey/documents/012808pl

-factsheet.pdf

57. USFWS. Pacific Lamprey Climate Change Vulnerability Assessment [Internet]. US Fish and Wildlife Service. Vancouver, WA: 2017 [cited 2019 Mar 23]. Available from: https://www.sciencebase.gov/catalog/item/59e100c0e4b05fe04cd000c4

58. CRITFC. Master Plan: Pacific Lamprey Artificial Propagation, Translocation, Restoration, and Research. Columbia River Inter-Tribal Fish Commission. 2018.

59. Jolley JC, Uh CT, Silver GS, Whitesel TA. Low Mortality of Larval Lampreys from Electrofishing, Suction-pumping, Anesthesia, and Handling. J. Fish Wildl. Manag. 2017;8:640–7.

60. WDFW. Threatened and endangered species [Internet]. Washington Department of Fish and Wildlife. 2019 [cited 2019 Mar 31]; Available from: https://wdfw.wa.gov/species-habitats/at-risk/listed

61. WDFW. Sport Fishing Rules Pamphlet Corrections and Updates/Clarifications [Internet]. Washington Department of Fish and Wildlife. 2018 [cited 2019 Mar 21]. Available from: https://wdfw.wa.gov/publications/01998/wdfw01998.pdf

62. Reid SB, Goodman DH. Detectability of Pacific Lamprey Occupancy in Western Drainages: Implications for Distribution Surveys. Trans. Am. Fish. Soc. 2015;144:315–22.

63. Harris JE, Jolley JC, Silver GS, Yuen H, Whitesel TA. An Experimental Evaluation of Electrofishing Catchability and Catch Depletion Abundance Estimates of Larval Lampreys in a Wadeable Stream: Use of a Hierarchical Approach. Trans. Am. Fish. Soc. 2016;145:1006–17.

64. Harris JE, Jolley JC. Estimation of occupancy, density, and abundance of larval lampreys in tributary river mouths upstream of dams on the Columbia River, Washington and Oregon. Can. J. Fish. Aquat. Sci. [Internet] 2017;74:843–52. Available from: www.nrcresearchpress.com

65. Primerdesign Ltd. Beginner's Guide to Real-Time PCR [Internet]. Chandlers Ford, U.K.: [cited 2019 Mar 30]. Available from: http://www.primerdesign.co.uk/learn/learn-about-qpcr

66. Thermo Fisher Scientific I. Real-Time PCR: Understanding Ct [Internet]. 2016 [cited 2019 Mar 30]; Available from: https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/real-time-pcr-basics/real-time-pcr-understanding-ct.html

67. Carim KJ, Mckelvey KS, Young MK, Wilcox TM, Schwartz MK. A Protocol for Collecting Environmental DNA Samples From Streams. 2016.

68. Wilcox TM, McKelvey KS, Young MK, Sepulveda AJ, Shepard BB, Jane SF, et al. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char Salvelinus fontinalis. Biol. Conserv. [Internet] 2016 [cited 2019 Mar 15];194:209–16. Available from: https://www.sciencedirect.com/science/article/pii/S0006320715302020

69. Bhadury P, Austen MC, Bilton DT, Lambshead PJD, Rogers AD, Smerdon GR. Molecular detection of marine nematodes from environmental samples: Overcoming eukaryotic interference. Aquat. Microb. Ecol. 2006;44:97–103.

70. Chariton AA, Court LN, Hartley DM, Colloff MJ, Hardy CM. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Front. Ecol. Environ. 2010;8:233–8.

71. Krieter J, Allen W. DRAFT 2017/2018 Lamprey Study Report: An Investigation of Larval Lamprey - Sampling Methods and Use of Portland Shipping Terminals. Portland, OR (United States): 2018.

72. Eichmiller JJ, Bajer PG, Sorensen PW. The Relationship between the Distribution of Common Carp and Their Environmental DNA in a Small Lake. PLoS One [Internet] 2014 [cited 2018 Nov 18];9:e112611. Available from: https://dx.plos.org/10.1371/journal.pone.0112611

73. Orzechowski Id SCM, Frederick PC, Dorazio RM, Hunter ME. Environmental

DNA sampling reveals high occupancy rates of invasive Burmese pythons at wading bird breeding aggregations in the central Everglades. 2019 [cited 2019 May 3]; Available from: https://doi.org/10.1371/journal.pone.0213943

74. Cook-Tabor C. Fishes of the Nisqually River, Estuary, and Reach. Lacey, WA: 1999.

75. Klungle MM, Anderson JH, Zimmerman MS. Nisqually River Juvenile Salmonid Monitoring [Internet]. 2018 [cited 2019 Mar 31]. Available from: https://wdfw.wa.gov/sites/default/files/publications/02008/wdfw02008.pdf

76. USFWS. Nisqually National Wildlife Refuge Final Comprehensive Conservation Plan. 2005.

77. Native Fish Society. Nisqually River [Internet]. 2019 [cited 2019 Feb 18];Available from: https://nativefishsociety.org/watersheds/nisqually-river

78. Nisqually Indian Tribe. 2016 State of Our Watersheds Report Nisqually River Watershed [Internet]. 2016 [cited 2019 Mar 31]. Available from: http://geo.nwifc.org/sow/SOW2016_Report/Nisqually.pdf

79. City of Centralia. Yelm Hydroproject [Internet]. 2019 [cited 2019 Feb 18]; Available from: http://www.cityofcentralia.com/Page.asp?NavID=359

80. Nisqually River Council. Centralia City Light Yelm Hydroelectric Project Presentation [Internet]. 2018 [cited 2019 Feb 18]; Available from: https://www.slideshare.net/Nisqually/centralia-city-light-yelm-hydroproject-presentation

81. WDFW. State Wildlife Action Plan Update (Appendix A-4): Species of Greatest Conservation Need Fact Sheets - FISH [Internet]. Washington Department of Fish and Wildlife. 2015 [cited 2019 Mar 31]. Available from: https://wdfw.wa.gov/sites/default/files/publications/01742/13_A4_Fish.pdf

82. Kemp B, Smith D. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci. Int. [Internet] 2005 [cited 2019 Mar

20];154:53–61. Available from: https://www.sciencedirect.com/science/article/pii/S0379073804008229

83. Champlot S, Berthelot C, Pruvost M, Bennett EA, Grange T, Geigl E-M. An Efficient Multistrategy DNA Decontamination Procedure of PCR Reagents for Hypersensitive PCR Applications. PLoS One [Internet] 2010 [cited 2019 Mar 20];5:e13042. Available from: http://dx.plos.org/10.1371/journal.pone.0013042

84. Pope LC, Ward CW. Manual on Test Sieving Methods: Guidelines for establishing sieve analysis procedures (4th edition) [Internet]. West Conshohocken, PA: 1998 [cited 2019 Mar 30]. Available from: https://epdf.tips/manual-on-test-sieving-methods-astm-manual-series.html

85. Jolley JC, Silver GS, Whitesel TA. Occurrence, Detection, and Habitat Use of Larval Lamprey in the White Salmon River Basin: Pre-Condit Dam Removal 2011 Annual Report. 2012.

86. Jolley JC, Silver GS, Harris JE, Whitesel TA. Pacific lamprey recolonization of a Pacific Northwest river following dam removal. River Res. Appl. [Internet] 2018 [cited 2018 Oct 14];34:44–51. Available from: http://doi.wiley.com/10.1002/rra.3221

87. Egan JW (LaMotte). Turbidity – Clarifying Low Level Measurements [Internet]. 2019 [cited 2019 Nov 5]; Available from: http://www.lamotte.com/en/blog/test-factors/83-turbidity-low-level-measurments

88. Reynolds JB, Holliman FM. Guidelines for assessment and reduction of electrofishing-induced injuries in trout and salmon. Manag. new Millenn. are we ready [Internet] 2000 [cited 2019 May 20];235–40. Available from: https://training.fws.gov/courses/CSP/CSP2C01/resources/8-Electrofishing-Effects/2_Guidelines for Assessment and Reduction of EF Injury.pdf

89. Buxton AS, Groombridge JJ, Griffiths RA. Seasonal variation in environmental DNA detection in sediment and water samples. 2018 [cited 2019 May 3]; Available from: https://doi.org/10.1371/journal.pone.0191737

90. Dragoni C. Molecular Methods in Clinical Microbiology: Polymerase Chain Reaction (PCR). [Internet]. 2019 [cited 2019 Mar 30]; Available from: https://www.labce.com/spg538191_polymerase_chain_reaction_pcr.aspx

APPENDICES

Appendix A. WDFW eDNA Laboratory Methods

Project: Environmental DNA monitoring for Pacific, River and Western Brook Lamprey from the Nisqually River

02/22/2019

Dr. Sarah Brown, WDFW Molecular Genetics Laboratory

eDNA Laboratory Methods

All laboratory work was performed in AirClean 600 Work Stations (ISC Bioexpress, Utah, USA), equipped with HEPA air filters and UV lights. All work surfaces were decontaminated with 50% bleach and exposed to UV light for at least one hour before work began. DNA extraction of sediment samples were performed with the DNeasy Powersoil kit and manufacturer's protocol (Qiagen, Inc.). Sediment samples were thawed, vortexed, and 275 µl of sediment/water were transferred to a new tube to begin the extraction process. DNA extraction of filtered water samples were performed on half of the filter sample, using the Qiagen DNeasy Blood & Tissue and Qiashredder kits (Qiagen, Inc., as per Pilliod et al., 2013). The other half of the filter was stored for potential future use. Post extraction, each filter sample was processed in triplicate.

We used quantitative Polymerase Chain Reaction (qPCR) to detect minute levels of DNA, using species-specific primers, and a fluorescently labeled reporter molecule (probe), which yields increased fluorescence with an increasing amount of product DNA (Figure 1). A sample is determined "positive" or "negative," based on whether or not the sample crossed the threshold (dashed line in Figure 1). When a sample crosses the threshold, this is referred to as the C_T, "Cycling Threshold." Samples with higher concentration of DNA typically cross the threshold earlier in the cycling (~cycle 20-30) than samples with lower concentration (~ cycle 31-40) (Figure 1). We used a C_T threshold of ≤ 50 for positive detections as suggested by Turner et al. (2015).

We tested samples for the presence of Pacific lamprey (*Entosphenus tridentatus*) using the primer pair and probe (Table 1) developed by Carim et al. (2017). qPCR products were obtained by amplifying DNA in 10 µl reaction volumes, containing 5 µl of Taqman gene expression master mix, 0.5 µl of 20X primer/probe mixture, 1.5 µl of molecular grade water and 3 µl of DNA. Cycling conditions consisted of 2 minutes at 50 ºC, then 95 ºC for 10 minutes, followed by 45 cycles of 95 ºC for 15 seconds, and 60 ºC for 1 minute. Additionally, we tested for the presence of Western Brook lamprey (*Lampetra richardsoni*), and Western River lamprey (*Lampetra ayresii*), using a genus (*Lampetra* spp.) level primer pair and probe (Table 1), developed by Ostberg et al. (2018). qPCR products were obtained by amplifying DNA in 15 µl reaction volumes, containing 7.5 µl of Taqman gene expression master mix, 0.7 µM, of each primer, 0.2 µM of the probe, 2.97 µl of molecular grade water and 3 µl of DNA. Cycling conditions

consisted of 50 ºC for 2 minutes, 95 ºC for 10 minutes, followed by 50 cycles of 95 ºC for 60 seconds, and 60 ºC for 1 minute.

For quantification of Pacific lamprey qPCR products, a 125-base pair (bp) gBlock gene fragment of Cytochrome Oxidase I (COI) was synthesized based on the Genbank accession KX389871 – KX389877, from bp 195 to 319 (Table 2). Similarly, a 126 bp gBlock gene fragment of Cytochrome b (Cytb) was synthesized based on the Genbank accession KU672486 – KU672508 (*Lampetra* spp.) from bp 747 to 872 (Table 2). All gBlocks were synthesized by Integrated DNA Technologies (IDT; Coralville, Iowa). To assess the amplification success of each qPCR, we developed a standard curve from 1:10 serial dilutions of these synthetic fragments 10^7 to 10^0 (for both Pacific lamprey and *Lampetra* spp. assays). The Limit of Quantification (LOQ, the lowest concentration at which at least 90% of the replicates amplified), and the Limit of Detection (LOD, the lowest concentration that was 10-fold below the LOQ) were determined for each assay by running the standard curve dilution with twelve replicates. The LOQ of the Pacific lamprey assay was 10^1 (16.08 copies/µl), and the LOD was 10^0 (1.61 copies/µl). The LOQ of the *Lampetra* spp. assay was 10^1 (23.86 copies/µl), and the LOD was 10^0 (2.39 copies/µl).

Samples were considered positive for detection when two out of three triplicate qPCRs resulted in a positive amplification (e.g. C_T of 50 or below), as per Turner et al. (2015; Figure 2). If qPCR samples were positive for only one of three replicates, the samples were re-amplified, in triplicate. If the results from the re-amplification were still ambiguous, the paired equipment blank was extracted and amplified, to test for contamination.

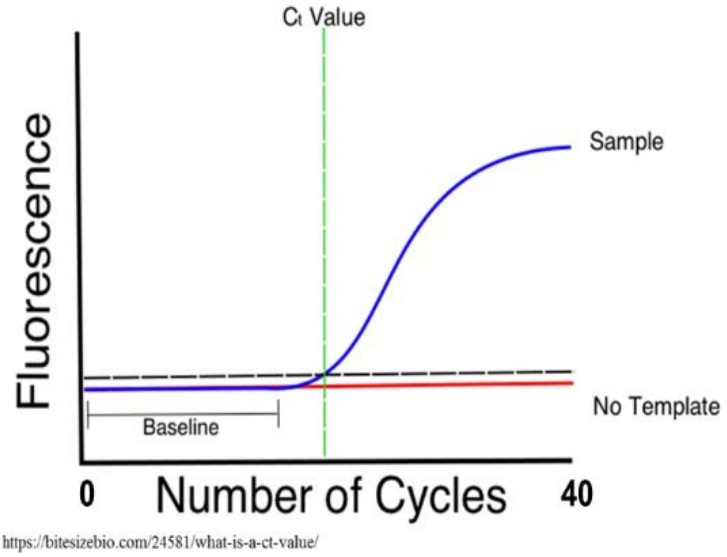


Figure 1. Diagram of qPCR real-time output. The Y-axis denotes fluorescence, and the X-axis denotes the number of cycles (from 0-40 in this instance). A sample replicate (blue line) is deemed a detection, if the PCR cycle at which the fluorescence of a sample crosses the threshold (dashed line; C_T), is before the termination of thermal cycling (cycle 40). The point at which the sample crosses the threshold is referred to as C_T.

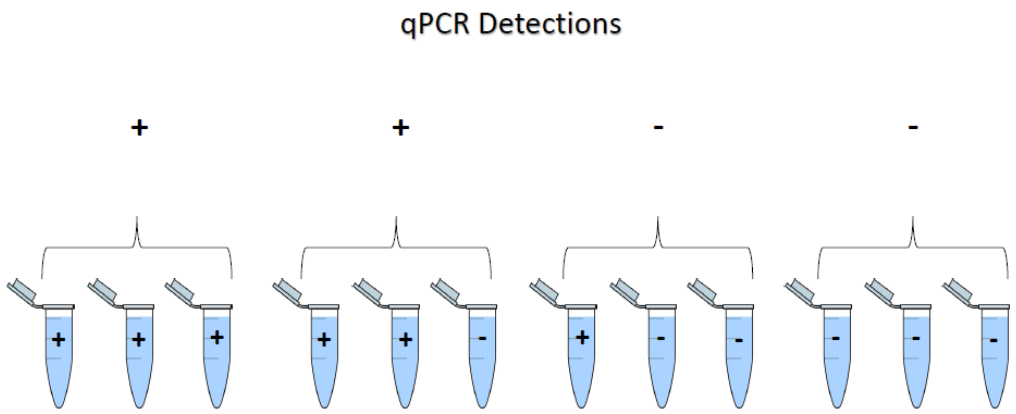


Figure 2. Example of possible combinations that can result in positive (+) or negative (-) detections of lamprey eDNA for each qPCR. Filter samples were considered positive for detection when two out of three triplicate qPCRs per filter resulted in a positive amplification.

Table 1. A genus (*Lampetra* spp.) and species (Entosphenus tridentatus) level primer pair (Forward, Reverse) and fluorescent probe, developed by Ostberg et al. (2018) and Carim et al. (2017), respectively.

Species	Lampetra Spp.	Pacific Lamprey
Forward (5'-3')	CTTTAGCAGCAGCCATCATA	TACCACTCATACTTAGTGCCCCTG
Reverse (5'-3')	GTAGTGCTAGATCAGCAATTAGAA	CTGTGCCAGCCCCTGCT
Probe (5'-3')	6FAM-CAT+TCAATT+TCG+TCC+GC-3IABkFQ	FAM-TTTGATTACTTCCACCCTCAC-MGBNFQ
Reference	Ostberg et al. 2018	Carim et al. 2017
DOI	https://doi.org/10.7717/peerj.4496	https://doi.org/10.1371/journal.pone.0169334

Table 2. IDT gBlock Sequences for quantification of qPCR products. For Pacific lamprey, a 125 bp gBlock gene fragment of COI was synthesized based on the Genbank accession KX389871 – KX389877, from bp 195 to 319. For *Lampetra* spp., a 126 bp gBlock gene fragment of Cytb was synthesized based on the Genbank accession KU672486 – KU672508 from bp 747 to 872.

Species	**IDT gBlock Sequence (5'-3')**
Lamptera spp .	CTTTAGCAGCAGCCATCATAATTCTCCTAGTTATCCCCTTTACCCACACCTCTAAACAACGTGGC ATTCAATTTCGTCCGCTTGCCCAAATTACATTCTGRATTCTAATTGCTGATCTAGCACTAC
Pacific Lamprey	TACCACTCATACTTAGTGCCCCTGATATAGCCTTCCCTCGTATAAACAACATAAGCTTTTGATTA CTTCCACCCTCACTACTCCTACTTTTAGCCTCCGCAGGAGTTGAAGCAGGGGCTGGCACA

Literature Cited:

Carim, K. J., Dysthe, J. C., Young, M. K., McKelvey, K. S., & Schwartz, M. K. (2017). A noninvasive tool to assess the distribution of Pacific Lamprey (*Entosphenus tridentatus*) in the Columbia River Basin. *PloS one*, *12*(1), e0169334.

Ostberg, C. O., Chase, D. M., Hayes, M. C., & Duda, J. J. (2018). Distribution and seasonal differences in Pacific Lamprey and *Lampetra* spp eDNA across 18 Puget Sound watersheds. *PeerJ*, *6*, e4496.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S. and Waits, L.P., 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Canadian Journal of Fisheries and Aquatic Sciences, 70(8), pp.1123-1130.

Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, *183*, 93-102.

Appendix B. Field form for surveying larval lampreys.

Survey123 version available at: https://arcg.is/1CGr4m

Lamprey Larval Survey Form			
Site No.:	Date:	Start time:	End Time:
Crew:			RM:
Location Description (e.g., landmarks):			
1) Collect eDNA water sample ☐		# of samples:	Lat:
Label, example: S1-GA-W-1 (Site 1, Grid A, Water sample 1)		Photo of sample area ☐	Long:
2) Collect water quality profile with YSI Meter at eDNA water sample location			*(decimal degrees)*
Temperature (°C):		DO (%):	
Sp Conductivity (µS/cm): @ °C		pH:	
Turbidity (NTU):		Daily Streamflow (cfs): (USGS, McKenna, WA)	
3) Qualitative habitat characterization of site from shore (do not disturb shoreland sediment)			
General Shoreline Description: *Circle all that apply (Describe shoreline on side of river where sampling is occurring*) (Shaded) / (LWD) / (Vegetated) / (Coniferous) / (Deciduous) / (Shrub/grass) / (Pasture) / (Bare) / (Developed (e.g., armored) / Other:			
Larval Habitat Classification: *Circle one for site*		Photo of shoreline ☐	
High (I)	Fine sediment including silt, sand, and detritus; medium-high organic matter		
Medium (II)	Shifting coarse sand, small gravel; low organic matter		
Low (III)	Bedrock, boulders, cobble, large gravel; low or no organic matter		
Habitat Type: (*circle one)*	Island / Side Channel / Alcove / Edge / Main		
Aquatic Veg *(including algae on rocks)* : *(circle one)*	None / L (<10%) / M(10-40%) / H(>40%)		
Detritus: *(circle one)*	None / L (<10%) / M(10-40%) / H(>40%)		
Complete each grid, if applicable:	**Grid A**	**Grid B**	**Grid C**
Center Point GPS: *(decimal degrees)*	Lat:	Lat:	Lat:
	Long:	Long:	Long:
4) Collect composite sediment samples ☐		*Label, example: S1-GA-S-R1*	
Replicate 1 *(R1)*	☐	☐	☐
Replicate 2 *(R2)*	☐	☐	☐
Replicate 3 *(R3)*	☐	☐	☐
Sediment Sample, *Label, example: S1-GA-G*	☐	☐	☐
Photo of grid area	☐	☐	☐
Sediment type: *(circle one)*	Clay / Silt / Sand / Coarse	Clay / Silt / Sand / Coarse	Clay / Silt / Sand / Coarse
5) Electrofishing	☐	☐	☐
Start Time:			
End Time:			
Total Shocking Time:			
Number of <80 mm:			
Number of ≥ 80 mm:			
# Captured:			
# Missed:			
Total Observed:			
Water Clarity (*Rate* between *1-5, where 1 = no visibility and 5 = very clear)*			
List all other species encountered during survey:			
Comments:			

Appendix C. Field data & Sampling Photographs

Table 1. Header and description key for data collection and analysis.

Header	Description
% Captured	Percent captured out of total detected
% Fine	Percent fine sediment (< 0.075 mm)
% Large	Percent large out of total captured
% Missed	Percent missed out of total detected
% Sand	Percent sand sediment (0.5-0.075 mm)
% Small	Percent small out of total captured
Aquatic Veg	Aquatic vegetation percent cover: 0 = none, 0.1 = low, 0.25 = medium, 0.5 = high
Captured	Total number of larvae captured during electrofishing
Cond	Conductivity (µS/cm) measurement taken once for each site
Detritus	Detritus percent cover: 0 = none, 0.1 = low, 0.25 = medium, 0.5 = high
Disturbance	Shoreline Use: 1 = none-private, 2 = none to low, 3 = low to moderate, 4 = moderate, 5 = moderate to high
DO	Dissolved Oxygen (%) measurement taken once for each site
d_{unit}	Proportion of grids per site (# positive grids/3 total grids) with positive lamprey detections
Electrofishing d_{unit}	Larval detections during electrofishing, d_{unit} = # positive grids/3 total grids
Electrofishing Time	Total electrofishing time (min)
Lampetra spp. eDNA d_{unit}	Proportion of grids per site (# positive grids/3 total grids) with positive *Lampetra* spp. detections via sediment eDNA
Lampetra spp. qPCR Detections	*Lampetra* qPCR detections (# positive qPCRs/total qPCRs per site)
Large	# of large (> 80 mm) larvae captured
Larval Density	Number of larvae detected during electrofishing over sampled area (larvae/m2)
Larval Habitat	Larval Habitat Classification: Type 1 or Type 2
Missed	Total number of larvae missed (escaped) during electrofishing
Pacific & Lampetra spp. eDNA d_{unit}	Proportion of grids per site (# positive grids/3 total grids) with positive lamprey detections via sediment eDNA
Pacific Lamprey eDNA d_{unit}	Proportion of grids per site (# positive grids/3 total grids) with positive Pacific lamprey detections via sediment eDNA

Header	Description
Pacific Lamprey qPCR Detections	Pacific lamprey qPCR detections (# positive qPCRs/total qPCRs per site)
pH	pH measurement taken once for each site
Sediment Type	Field observation of sediment type: 1 = silt, 2 = sand
Shoreline Habitat	1 = main, 2 = side channel, 3 = island, 4 = edge, 5 = alcove
Small	Number of small (< 80 mm) larvae captured
Streamflow	Streamflow (cfs) measurements taken at the McKenna station
Temp	Water temperature (˚C) measurement taken once for each site
Total	Total number of larvae detected during electrofishing (captured + missed)
Turbidity	Turbidity (NTU) measurement taken once for each site
Water Clarity	Clarity of water during electrofishing at each grid, averaged for each site (rating 1-5, 1 is no visibility and 5 is very clear)

Table 2. Coordinates (decimal degrees) for general site and water sample locations.

Site	Site Location (Lat)	Site Location (Long)	Water Sample Location (Lat)	Water Sample Location (Long)
1	47.0575585	-122.6908584	47.0561756	-122.6904584
2	47.0522194	-122.6921616	47.0522194	-122.6919711
3	47.0524101	-122.6916733	47.0523991	-122.6916459
4	47.0523682	-122.6914825	47.0524707	-122.6913834
5	46.9887206	-122.6315840	46.9886292	-122.6315725
6	46.9881592	-122.6318665	46.9876660	-122.6322480
7	46.9746399	-122.6337585	46.9746176	-122.6335900
8	46.9732800	-122.6320000	46.9732497	-122.6318990

Table 3. D_{unit} (proportion of grids within sites with positive detections) for each lamprey species and combined by sediment eDNA and electrofishing surveys.

Site	*Lampetra* spp. eDNA dunit	Pacific Lamprey eDNA dunit	Pacific & *Lampetra* spp. eDNA dunit	Electrofishing dunit
1	0.67	0.00	0.67	1.00
2	0.00	0.00	0.00	0.67
3	1.00	0.00	1.00	0.33
4	1.00	0.00	1.00	1.00
5	1.00	0.00	1.00	1.00
6	0.67	0.33	1.00	1.00
7	1.00	0.00	1.00	1.00
8	1.00	0.00	1.00	1.00

Table 4. Results of electrofishing surveys at each site.

Site	Captured	% Captured	Missed	% Missed	Total	Small	% Small	Large	% Large
1	38	0.79	10	0.21	48	37	0.97	1	0.03
2	2	0.18	9	0.82	11	2	1.00	0	0.00
3	18	0.64	10	0.36	28	10	0.56	8	0.44
4	65	0.68	31	0.32	96	49	0.75	16	0.25
5	30	0.56	24	0.44	54	23	0.77	7	0.23
6	31	0.67	15	0.33	46	28	0.90	3	0.10
7	106	0.82	23	0.18	129	99	0.93	7	0.07
8	88	0.50	88	0.50	176	85	0.97	3	0.03

Table 5. Additional parameters measured or observed at each sample site.

Site	Electrofishing Time	Water Clarity	Aquatic Veg	Detritus	Streamflow	Larval Habitat	Shoreline Habitat	Disturbance
1	12.20	4.00	0.00	0.00	540.00	2.00	1.00	5.00
2	20.30	5.00	0.10	0.10	545.00	1.00	2.00	2.00
3	20.25	4.67	0.10	0.10	545.00	2.00	3.00	2.00
4	27.20	3.33	0.10	0.25	545.00	1.00	2.00	2.00
5	35.50	4.00	0.25	0.25	545.00	1.00	4.00	3.00
6	20.75	3.33	0.10	0.10	545.00	2.00	1.00	5.00
7	32.30	3.00	0.25	0.25	540.00	2.00	4.00	1.00
8	48.00	4.67	0.00	0.25	495.00	1.00	1.00	1.00

Table 6. Site and shoreline habitat description.

Site	Habitat Shoreline Description	Site Description
1	Developed, bare, shrub grass	River access under railroad bridge at WDFW public access site and Riverbend campground lower bar
2	Shaded, LWD, deciduous, vegetated, shrub grass	Riverbend campground side channel area
3	LWD, vegetated, shrub grass	Other side of side channel area at Riverbend, along mainstream channel
4	LWD, shrub grass, bare, vegetated, deciduous	Upstream side channel at Riverbend
5	shaded, LWD, vegetated, deciduous, shrub grass	Downstream edge habitat from Tank Bridge
6	shrub grass, vegetated, coniferous, deciduous	Upstream of Site 5, grids distanced along shoreline down from Tank Bridge
7	shaded, shrub grass, deciduous, vegetated	River mile 12.5, upstream of Centralia Diversion Dam, private access
8	shaded, LWD, vegetated, coniferous, deciduous, shrub grass	Upstream of Site 7 and Centralia Diversion Dam, private

Table 7. The following table includes all measurements and observations taken at each sampling grid (U, M, L), including coordinates for each sampling grid, eDNA qPCR detections, sediment type, larval counts via electrofishing, and field comments.

Site, Grid, & Sample Type	Grid Location (Lat)	Grid Location (Long)	*Lampetra* spp. qPCR Detections	*Lampetra* spp. Detected	Pacific Lamprey qPCR Detections	Pacific Lamprey Detected in Sediment
S1-U-S	47.0562706	-122.6904602	1.0	Yes	0.0	No
S1-M-S	47.0563011	-122.6904602	1.0	Yes	0.0	No
S1-L-S	47.0578966	-122.6913549	0.0	No	0.0	No
S2-U-S	47.0522003	-122.6921234	0.1	No	0.0	No
S2-M-S	47.0522194	-122.6921616	0.0	No	0.0	No
S2-L-S	47.0522308	-122.6922226	0.0	No	0.0	No
S3-U-S	47.0523911	-122.6915665	1.0	Yes	0.0	No
S3-M-S	47.0524101	-122.6916733	0.6	Yes	0.0	No
S3-L-S	47.0523682	-122.6917267	0.4	Yes	0.0	No
S4-U-S	47.0523796	-122.6914673	0.5	Yes	0.0	No
S4-M-S	47.0523682	-122.6914825	0.8	Yes	0.0	No
S4-L-S	47.0523605	122.6915131	1.0	Yes	0.0	No
S5-U-S	46.9886818	-122.6316071	1.0	Yes	0.0	No
S5-M-S	46.9886589	-122.6316071	1.0	Yes	0.0	No
S5-L-S	46.9888000	-122.6315918	0.8	Yes	0.0	No
S6-U-S	46.9877205	-122.6322632	1.0	Yes	0.0	No
S6-M-S	46.9881592	-122.6318665	0.0	No	0.0	No
S6-L-S	46.9885292	-122.6316833	1.0	Yes	0.3	Yes
S7-U-S	46.9746094	-122.6337280	0.2	Yes	0.0	No
S7-M-S	46.9746399	-122.6337585	1.0	Yes	0.0	No
S7-L-S	46.9746895	-122.6338272	1.0	Yes	0.0	No
S8-U-S	46.9732600	-122.6319000	1.0	Yes	0.0	No
S8-M-S	46.9732800	-122.6320000	1.0	Yes	0.0	No
S8-L-S	46.9733400	-122.6321000	1.0	Yes	0.0	No

Site, Grid, & Sample Type	Pacific & *Lampetra* Sediment eDNA Concentration	Sediment Type	% Fine	% Sand	Larval Density	Small	% Small	Large
S1-U-S	43907.9	silt	12%	88%	5.5	27	96%	1
S1-M-S	43836.3	silt	12%	88%	2.8	10	100%	0
S1-L-S	0.0	sand	1%	99%	0.2	0	0%	0
S2-U-S	2.4	sand	0%	100%	0.0	0	0%	0
S2-M-S	0.0	sand	1%	99%	1.8	1	100%	0
S2-L-S	0.0	sand	0%	100%	0.4	1	100%	0
S3-U-S	238.6	sand	3%	97%	9.0	10	56%	8
S3-M-S	6.7	sand	1%	99%	0.0	0	0%	0
S3-L-S	11.0	sand	1%	99%	0.0	0	0%	0
S4-U-S	10.0	sand	1%	99%	3.8	11	79%	3
S4-M-S	54.5	silt	2%	98%	8.1	15	68%	7
S4-L-S	9.5	sand	1%	99%	9.5	23	79%	6
S5-U-S	143.2	silt	23%	77%	3.8	10	71%	4
S5-M-S	167.0	silt	29%	71%	5.7	12	80%	3
S5-L-S	5.5	sand	10%	90%	1.8	1	100%	0
S6-U-S	95.5	sand	6%	94%	1.4	4	100%	0
S6-M-S	0.0	sand	7%	93%	0.9	0	0%	0
S6-L-S	4176.2	silt	9%	91%	9.6	24	89%	3
S7-U-S	13.1	silt	26%	74%	4.2	19	100%	0
S7-M-S	190.9	silt	35%	65%	8.1	29	91%	3
S7-L-S	238.6	silt	10%	90%	11.4	51	93%	4
S8-U-S	238.6	sand	10%	90%	26.6	69	96%	3
S8-M-S	119.3	silt	6%	94%	7.4	13	100%	0
S8-L-S	29563.9	silt	6%	94%	2.3	3	100%	0

Site, Grid, & Sample Type	% Large	Captured	% Captured	Missed	% Missed	Total	Electrofishing Time	Water Clarity	Disturbance Rating
S1-U-S	4%	28	85%	5	15%	33	5.0	3	5
S1-M-S	0%	10	71%	4	29%	14	3.8	4	5
S1-L-S	0%	0	0%	1	100%	1	3.5	5	5
S2-U-S	0%	0	0%	0	0%	0	5.8	5	2
S2-M-S	0%	1	11%	8	89%	9	10.3	5	2
S2-L-S	0%	1	50%	1	50%	2	4.3	5	2
S3-U-S	44%	18	64%	10	36%	28	12.0	4	2
S3-M-S	0%	0	0%	0	0%	0	3.3	5	2
S3-L-S	0%	0	0%	0	0%	0	5.0	5	2
S4-U-S	21%	14	70%	6	30%	20	4.0	4	2
S4-M-S	32%	22	67%	11	33%	33	12.0	3	2
S4-L-S	21%	29	67%	14	33%	43	11.2	3	2
S5-U-S	29%	14	67%	7	33%	21	11.5	2	3
S5-M-S	20%	15	58%	11	42%	26	15.0	5	3
S5-L-S	0%	1	14%	6	86%	7	9.0	5	3
S6-U-S	0%	4	80%	1	20%	5	6.0	3	5
S6-M-S	0%	0	0%	2	100%	2	2.0	4	4
S6-L-S	11%	27	69%	12	31%	39	12.8	3	3
S7-U-S	0%	19	86%	3	14%	22	10.3	2	1
S7-M-S	9%	32	70%	14	30%	46	11.0	3	1
S7-L-S	7%	55	90%	6	10%	61	11.0	4	1
S8-U-S	4%	72	54%	61	46%	133	26.0	4	1
S8-M-S	0%	13	38%	21	62%	34	11.0	5	1
S8-L-S	0%	3	33%	6	67%	9	11.0	5	1

Site, Grid, & Sample Type	Human Disturbance
S1-U-S	High (Kid swimming/fishing)
S1-M-S	High (Kid swimming/fishing)
S1-L-S	High (Public river access)
S2-U-S	None to low (side channel area, unlikely)
S2-M-S	None to low (side channel area, unlikely)
S2-L-S	None to low (side channel area, unlikely)
S3-U-S	None to low (side channel area, unlikely)
S3-M-S	None to low (side channel area, unlikely)
S3-L-S	None to low (side channel area, unlikely)
S4-U-S	None to low (side channel area, unlikely)
S4-M-S	None to low (side channel area, unlikely)
S4-L-S	None to low (side channel area, unlikely)
S5-U-S	Low or moderate (trash found but further downriver from trails)
S5-M-S	Low or moderate (trash found but further downriver from trails)
S5-L-S	Low or moderate (trash found but further downriver from trails)
S6-U-S	High (Adjacent to public boat launch and under bridge)
S6-M-S	Moderate to high (trail that comes down to water mid-grid)
S6-L-S	Low or moderate (trash found but further downriver from trails)
S7-U-S	None - private
S7-M-S	None - private
S7-L-S	None - private
S8-U-S	None - private
S8-M-S	None - private
S8-L-S	None - private

Site, Grid, & Sample Type	Grid Comments
S1-U-S	1 lamprey collected in eDNA sample, too small. Disturbed sediment from eDNA collection drifted upstream. Couldn't visualize smaller larvae due to water visibility (unmeasurable size).
S1-M-S	3 or more lamprey in eDNA sediment sample, too small. Grid M, countless tiny larvae observed during efishing, unable to capture (50+).
S1-L-S	1 larvae, outside of grid area. No capture.
S2-U-S	Glare on water made it hard to see, likely no larvae.
S2-M-S	3 of the number missed were unmeasurable (too small to capture).
S2-L-S	
S3-U-S	Approx. 20 tiny larvae.
S3-M-S	
S3-L-S	
S4-U-S	Cobbles in sediment sample.
S4-M-S	Water was clear until electrofishing, during electrofishing fine sediment got disturbed and remained suspended for several minutes limiting visibility.
S4-L-S	Cobbles in sediment sample. Lots of LWD along grid. Fine sediment layered on gravel/cobble in some areas.
S5-U-S	Water was very clear before electrofishing, fine silt got suspended during electrofishing which reduced visibility. Silt remained suspended for several minutes.
S5-M-S	There were at least 20 that were very small and could not be captured with a net.
S5-L-S	All that were missed were too small for the net.
S6-U-S	3 of 4 captured were from sed sampling, sed got kicked up and glare was bad during efish.
S6-M-S	A lot of cobble, sculpin and smaller fish. Grid M generally had less fine sediment than Grid L. There is a foot traffic trail that comes down to mid grid.
S6-L-S	Water started clear, fine sed got kicked up over time, lots of glare.
S7-U-S	Not included in efish time: found larvae near small freshwater input above grid.
S7-M-S	
S7-L-S	1 lamprey measured at 150 mm.
S8-U-S	Relatively strong current. Woody debris made it difficult to catch in the net.
S8-M-S	Relatively strong current on the edge of Grid M. Difficult to net due to woody debris.
S8-L-S	Fairly strong current moving through Grid L. Difficult to net due to woody debris.

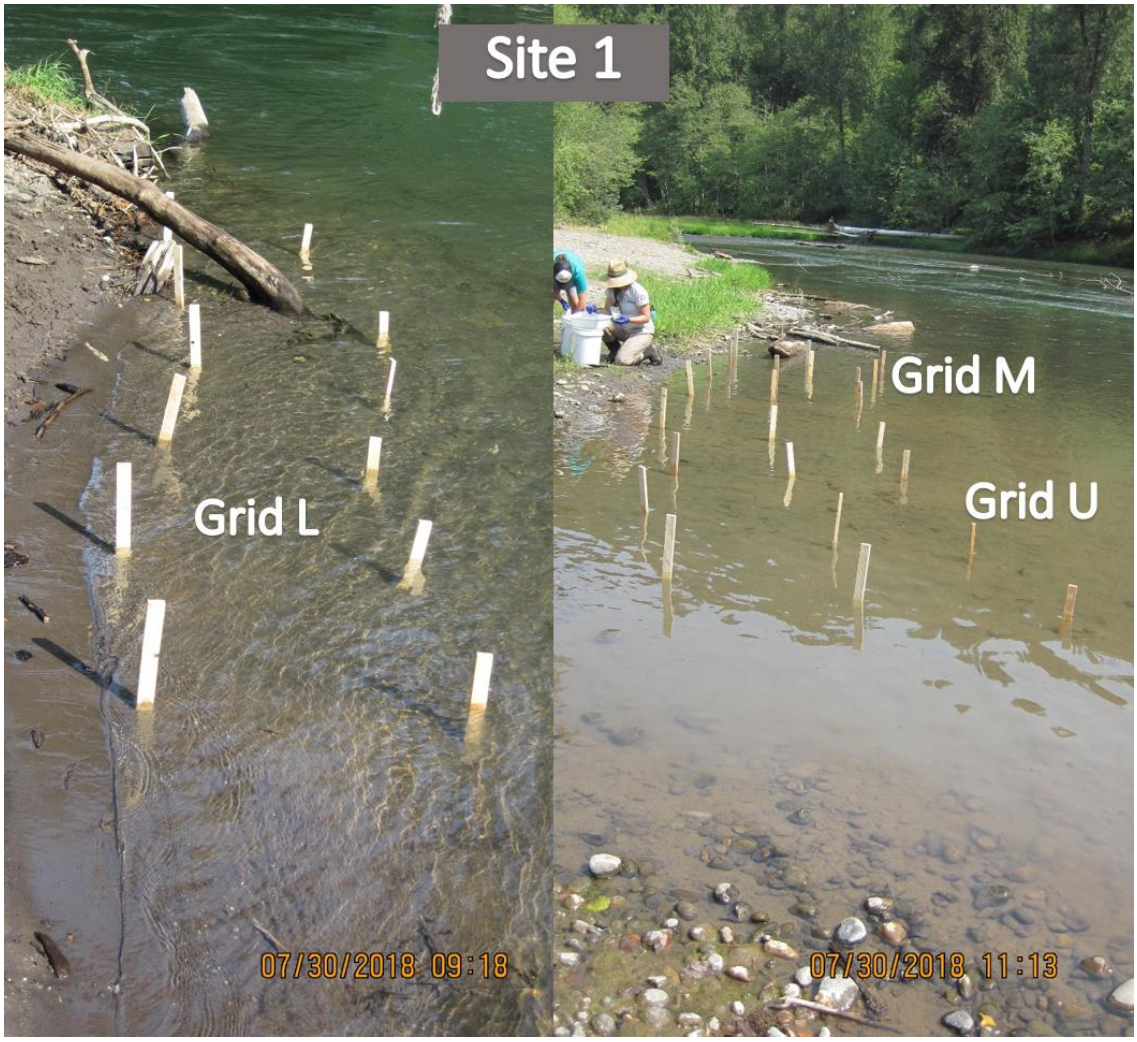


Figure 1. Site 1 photographs of sampling grids L, M, and U.

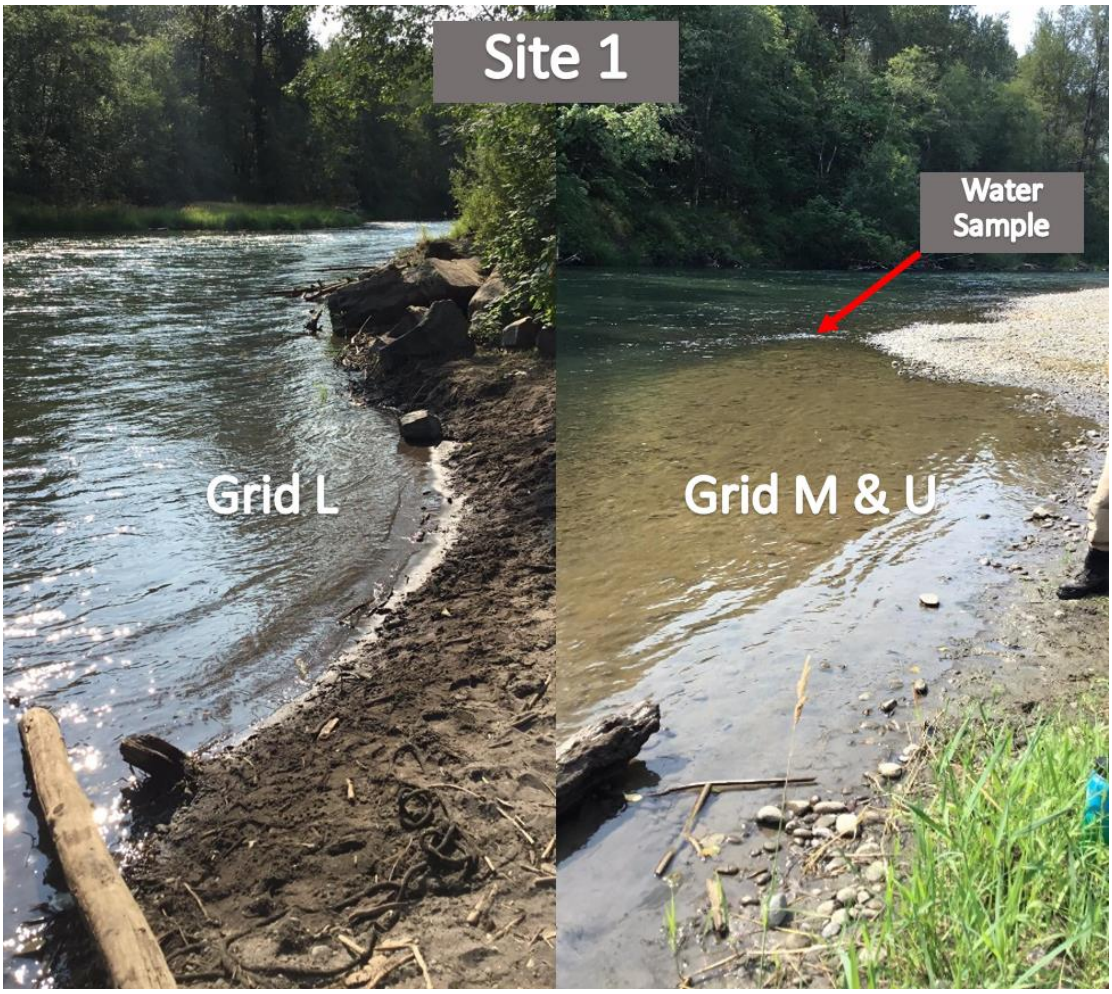


Figure 2. Site 1 photographs of the shoreline and approximate upstream location of the eDNA water sample.

Figure 3. Site 2 photographs of sampling grids L, M, and U.

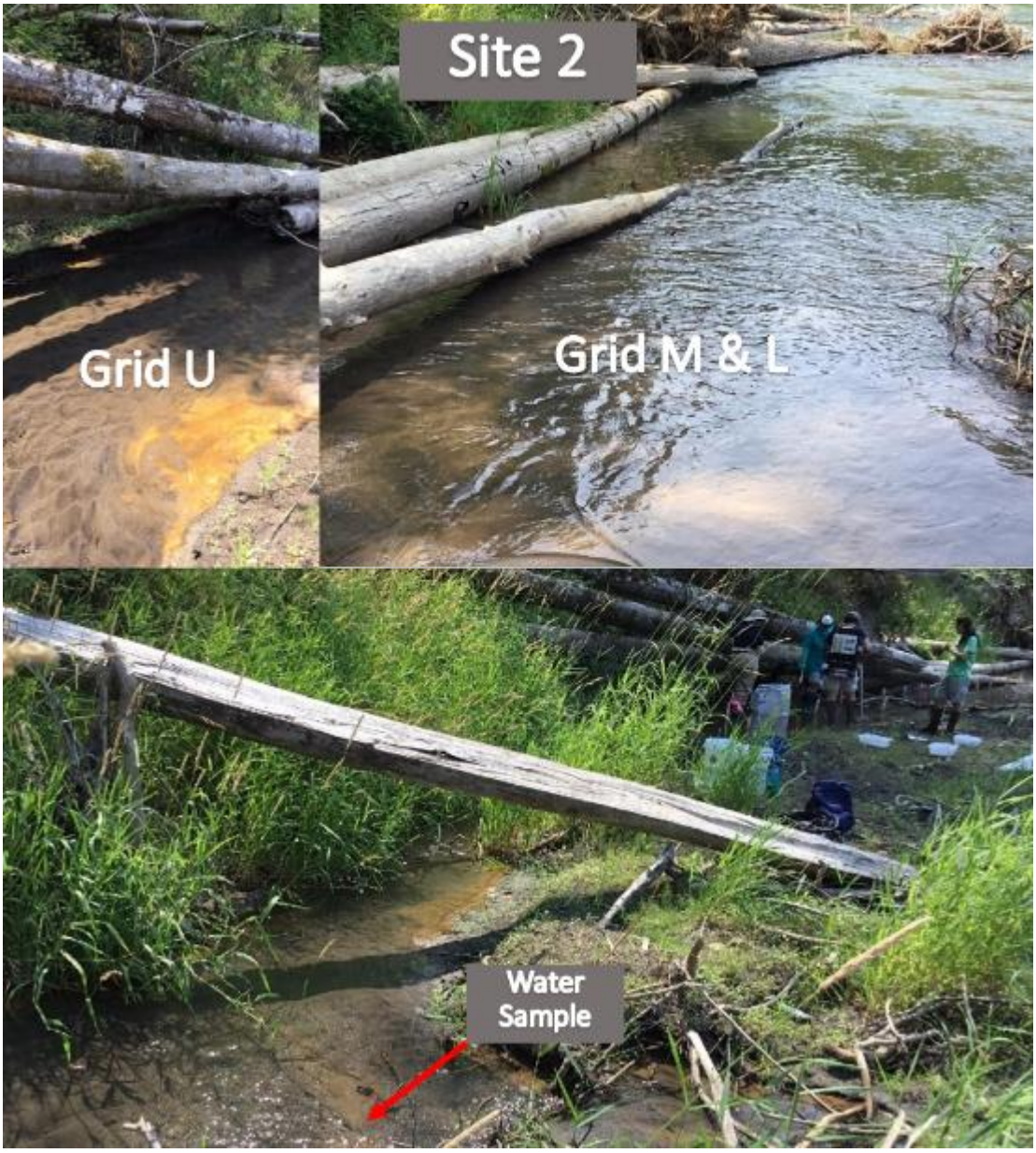


Figure 4. Site 2 photographs of the shoreline and approximate upstream location of the eDNA water sample.

Figure 5. Site 3 photographs of sampling grids L, M, and U.

Figure 6. Site 3 photographs of the shoreline and approximate upstream location of the eDNA water sample.

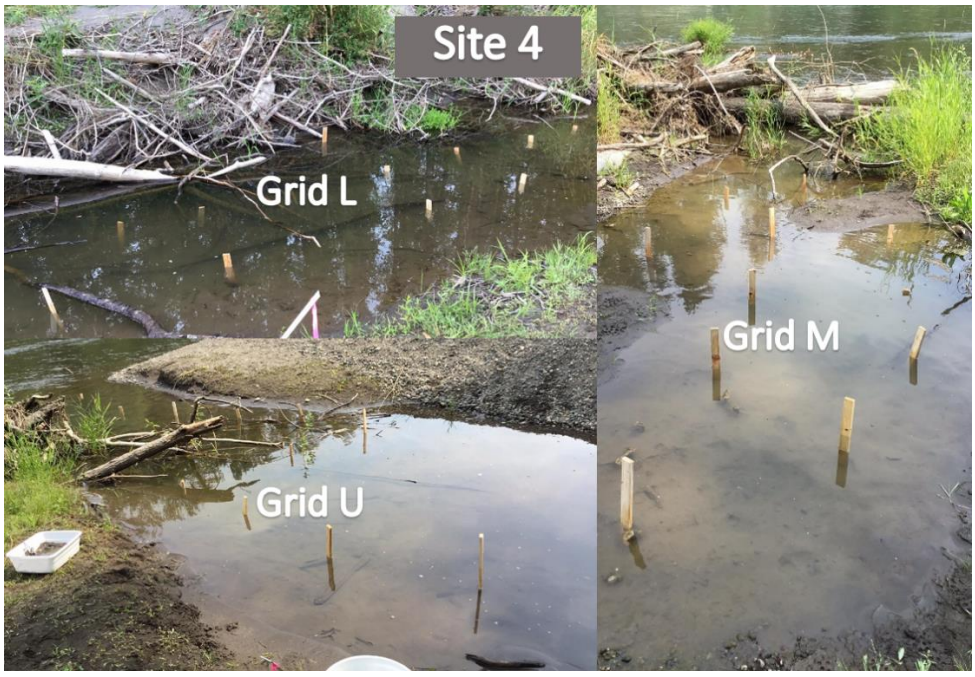


Figure 7. Site 4 photographs of sampling grids L, M, and U.

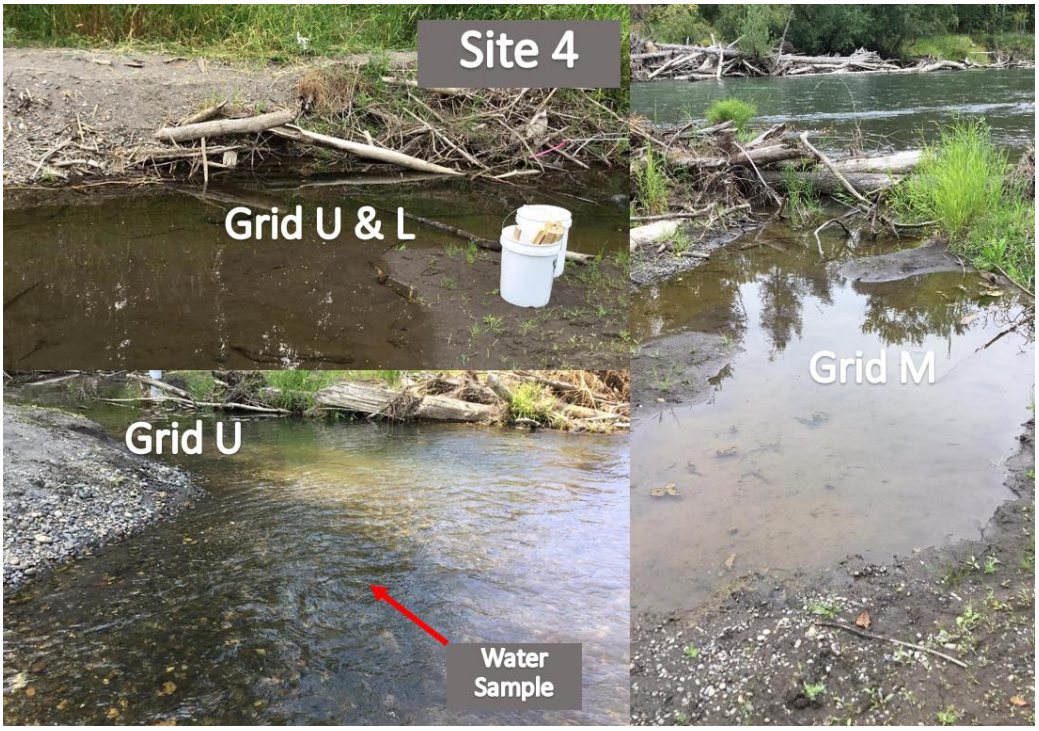


Figure 8. Site 4 photographs of the shoreline and approximate upstream location of the eDNA water sample.

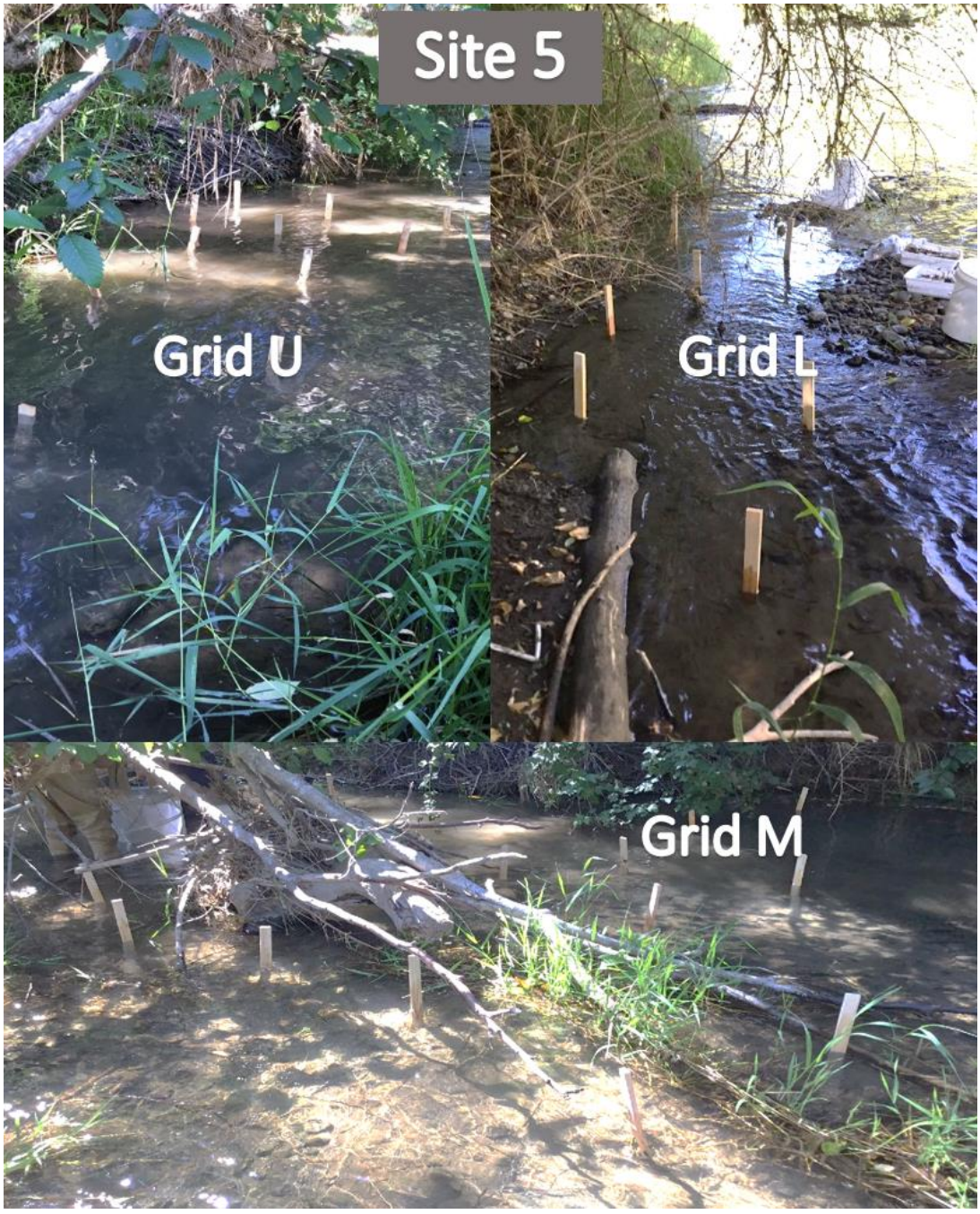


Figure 9. Site 5 photographs of sampling grids L, M, and U.

Figure 10. Site 5 photographs of the shoreline and approximate upstream location of the eDNA water sample.

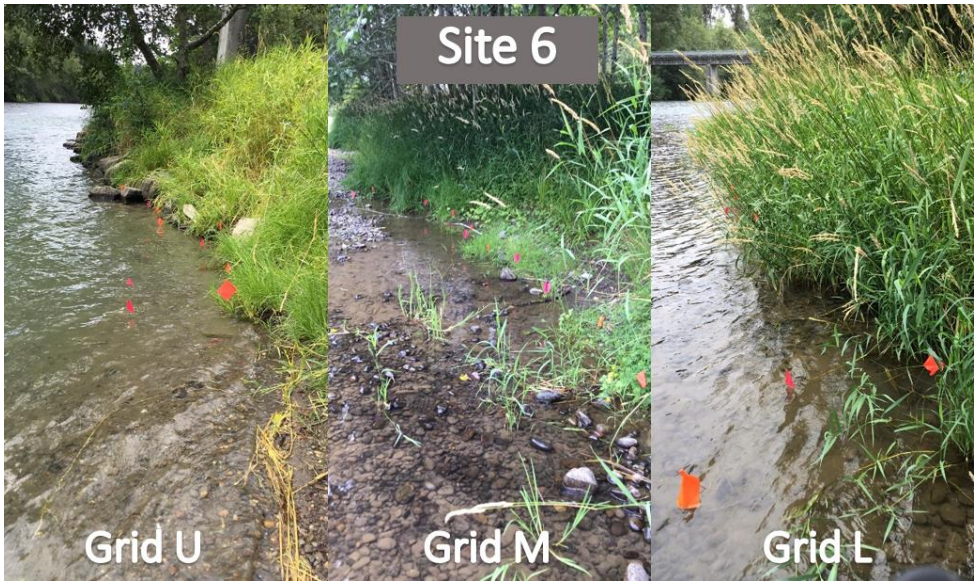


Figure 11. Site 6 photographs of sampling grids L, M, and U.

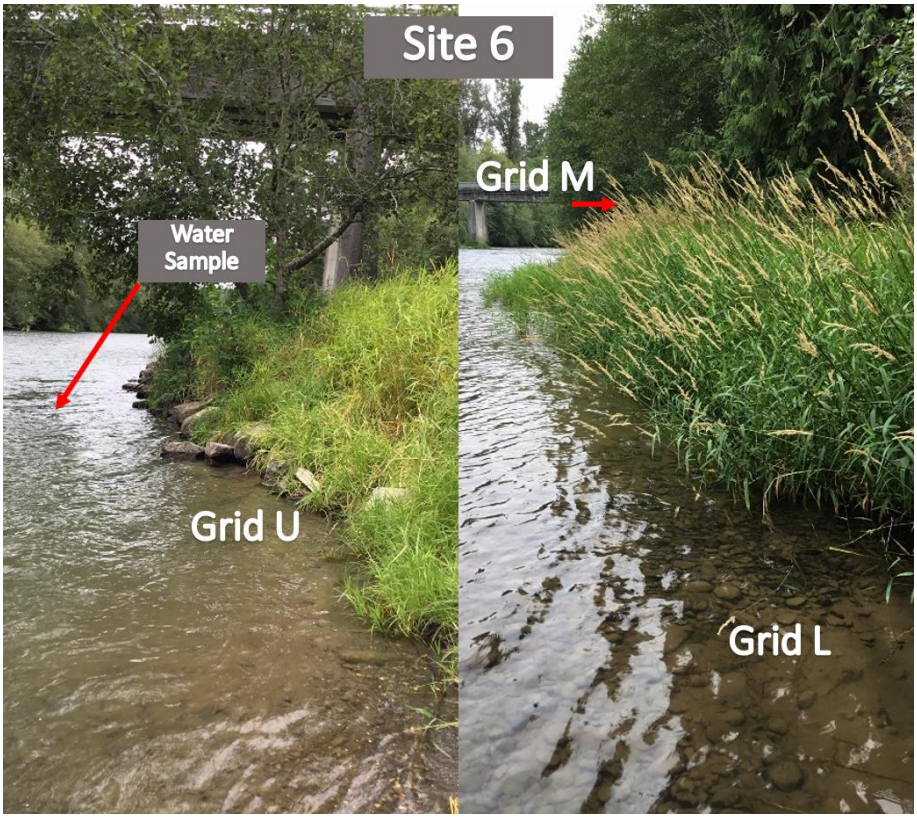


Figure 12. Site 6 photographs of the shoreline and approximate upstream location of the eDNA water sample.

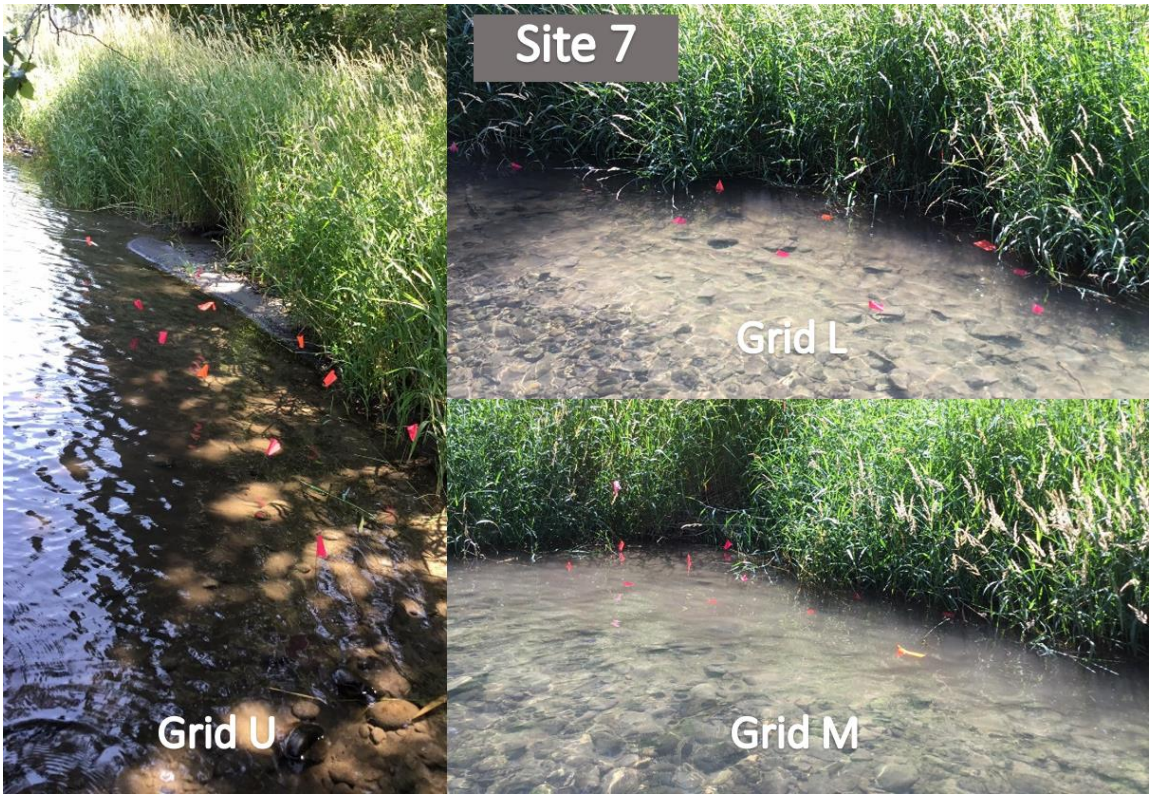


Figure 13. Site 7 photographs of sampling grids L, M, and U.

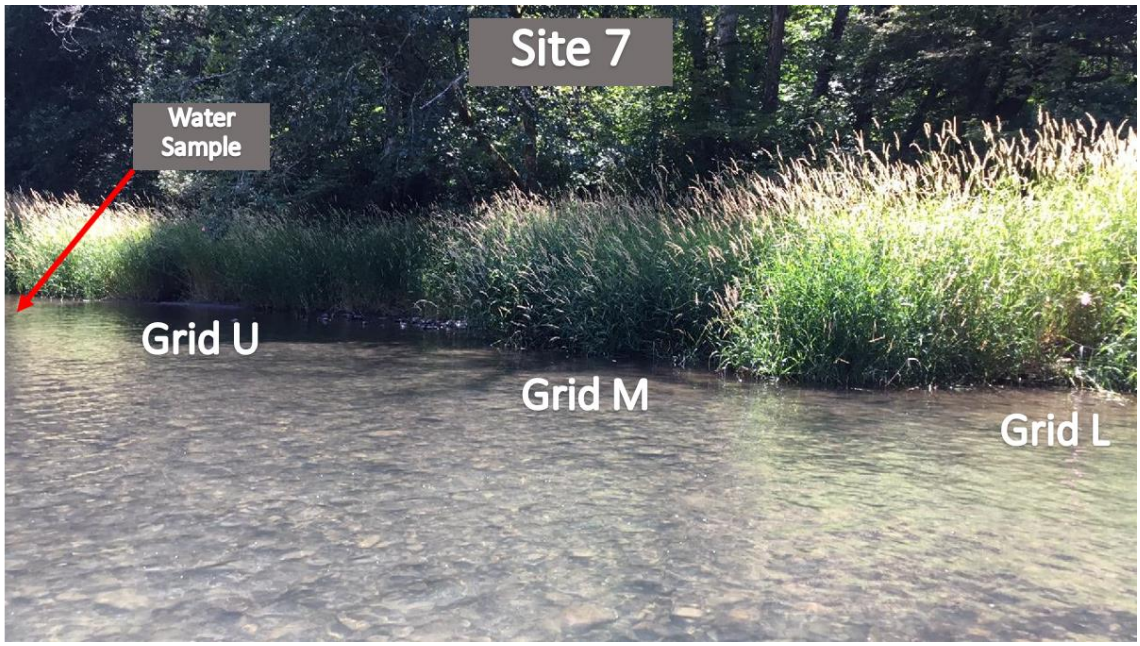


Figure 14. Site 7 photograph of the shoreline and approximate upstream location of the eDNA water sample.

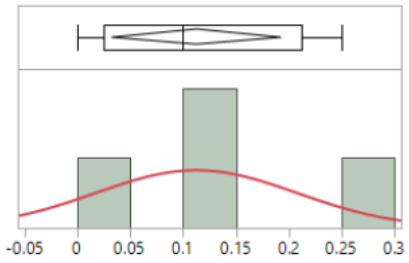
Figure 15. Site 8 photographs of sampling grids L, M, and U.

Figure 16. Site 8 photograph of the shoreline and approximate upstream location of the eDNA water sample.

Appendix D. JMP Data Analysis

1. Site-level Statistics: Normal distribution & Goodness-of-fit tests.

Aquatic Veg



Normal(0.1125,0.09543)

Quantiles

100.0%	maximum	0.25
99.5%		0.25
97.5%		0.25
90.0%		0.25
75.0%	quartile	0.2125
50.0%	median	0.1
25.0%	quartile	0.025
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	0.1125
Std Dev	0.0954314
Std Err Mean	0.0337401
Upper 95% Mean	0.1922826
Lower 95% Mean	0.0327174
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.1125	0.0327174	0.1922826
Dispersion	σ	0.0954314	0.0630967	0.1942288

Measure	
-2*LogLikelihood	-15.88655
AICc	-9.486553
BIC	-11.72767

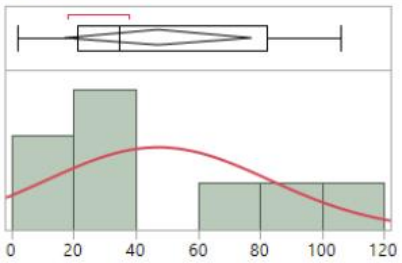
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.832265	0.0627

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Captured



Normal(47.25,35.812)

Quantiles

100.0%	maximum	106
99.5%		106
97.5%		106
90.0%		106
75.0%	quartile	82.25
50.0%	median	34.5
25.0%	quartile	21
10.0%		2
2.5%		2
0.5%		2
0.0%	minimum	2

Summary Statistics

Mean	47.25
Std Dev	35.812009
Std Err Mean	12.661457
Upper 95% Mean	77.189589
Lower 95% Mean	17.310411
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	47.25	17.310411	77.189589
Dispersion	σ	35.812009	23.677975	72.887183

Measure	
-2*LogLikelihood	78.955549
AICc	85.355549
BIC	83.114432

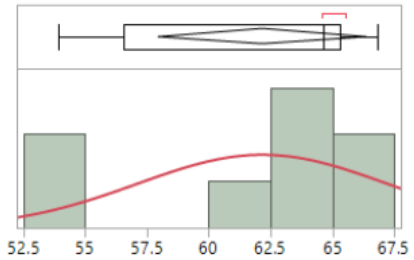
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.934453	0.5575

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Cond



Normal(62.1625,5.03557)

Quantiles

100.0%	maximum	66.8
99.5%		66.8
97.5%		66.8
90.0%		66.8
75.0%	quartile	65.325
50.0%	median	64.65
25.0%	quartile	56.55
10.0%		53.9
2.5%		53.9
0.5%		53.9
0.0%	minimum	53.9

Summary Statistics

Mean	62.1625
Std Dev	5.0355699
Std Err Mean	1.7803428
Upper 95% Mean	66.372342
Lower 95% Mean	57.952658
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	62.1625	57.952658	66.372342
Dispersion	σ	5.0355699	3.3293886	10.248755

Measure	
-2*LogLikelihood	47.567444
AICc	53.967444
BIC	51.726327

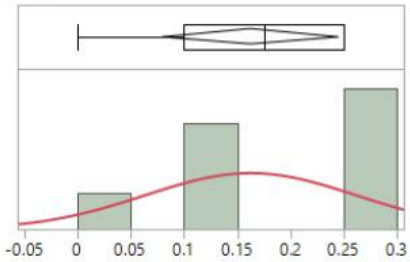
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.768437	0.0130*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Detritus



Normal(0.1625,0.0991)

Quantiles

100.0%	maximum	0.25
99.5%		0.25
97.5%		0.25
90.0%		0.25
75.0%	quartile	0.25
50.0%	median	0.175
25.0%	quartile	0.1
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	0.1625
Std Dev	0.0991031
Std Err Mean	0.0350382
Upper 95% Mean	0.2453523
Lower 95% Mean	0.0796477
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.1625	0.0796477	0.2453523
Dispersion	σ	0.0991031	0.0655244	0.2017018

Measure	
-2*LogLikelihood	-15.28249
AICc	-8.882493
BIC	-11.12361

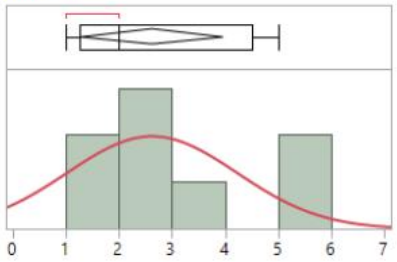
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.792710	0.0239*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Disturbance



Normal(2.625,1.59799)

Quantiles

100.0%	maximum	5
99.5%		5
97.5%		5
90.0%		5
75.0%	quartile	4.5
50.0%	median	2
25.0%	quartile	1.25
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Summary Statistics

Mean	2.625
Std Dev	1.5979898
Std Err Mean	0.5649747
Upper 95% Mean	3.9609529
Lower 95% Mean	1.2890471
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	2.625	1.2890471	3.9609529
Dispersion	σ	1.5979898	1.0565496	3.2523441

Measure	
-2*LogLikelihood	29.20296
AICc	35.60296
BIC	33.361843

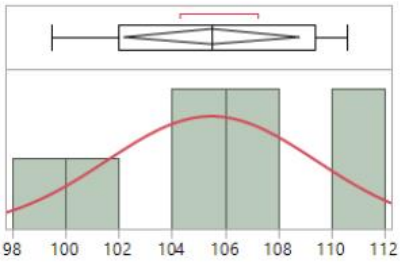
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.833788	0.0650

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

DO



Normal(105.488,3.93535)

Quantiles

100.0%	maximum	110.6
99.5%		110.6
97.5%		110.6
90.0%		110.6
75.0%	quartile	109.375
50.0%	median	105.5
25.0%	quartile	101.975
10.0%		99.5
2.5%		99.5
0.5%		99.5
0.0%	minimum	99.5

Summary Statistics

Mean	105.4875
Std Dev	3.9353481
Std Err Mean	1.3913556
Upper 95% Mean	108.77753
Lower 95% Mean	102.19747
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	105.4875	102.19747	108.77753
Dispersion	σ	3.9353481	2.6019504	8.0095041

Measure	
-2*LogLikelihood	43.623006
AICc	50.023006
BIC	47.781889

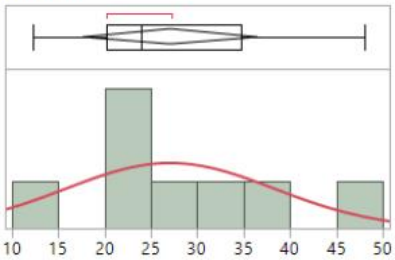
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.952818	0.7396

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Efish Time



Normal(27.0625,11.2752)

Quantiles

100.0%	maximum	48
99.5%		48
97.5%		48
90.0%		48
75.0%	quartile	34.7
50.0%	median	23.975
25.0%	quartile	20.2625
10.0%		12.2
2.5%		12.2
0.5%		12.2
0.0%	minimum	12.2

Summary Statistics

Mean	27.0625
Std Dev	11.275154
Std Err Mean	3.9863691
Upper 95% Mean	36.488765
Lower 95% Mean	17.636235
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	27.0625	17.636235	36.488765
Dispersion	σ	11.275154	7.4548406	22.948007

Measure	
-2*LogLikelihood	60.464642
AICc	66.864642
BIC	64.623525

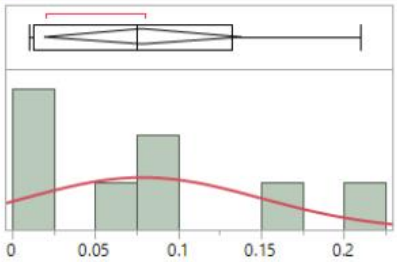
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.941819	0.6291

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Fine



Normal(0.07875,0.071)

Quantiles

100.0%	maximum	0.21
99.5%		0.21
97.5%		0.21
90.0%		0.21
75.0%	quartile	0.1325
50.0%	median	0.075
25.0%	quartile	0.0125
10.0%		0.01
2.5%		0.01
0.5%		0.01
0.0%	minimum	0.01

Summary Statistics

Mean	0.07875
Std Dev	0.0710005
Std Err Mean	0.0251025
Upper 95% Mean	0.1381079
Lower 95% Mean	0.0193921
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.07875	0.0193921	0.1381079
Dispersion	σ	0.0710005	0.0469437	0.1445053

Measure	
-2*LogLikelihood	-20.61808
AICc	-14.21808
BIC	-16.45919

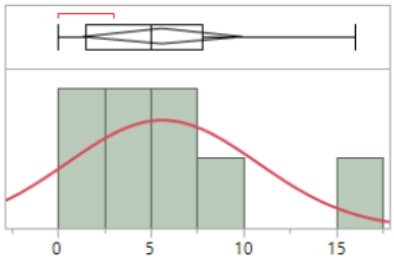
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.881472	0.1945

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Large



Normal(5.625,5.12522)

Quantiles

100.0%	maximum	16
99.5%		16
97.5%		16
90.0%		16
75.0%	quartile	7.75
50.0%	median	5
25.0%	quartile	1.5
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	5.625
Std Dev	5.1252178
Std Err Mean	1.8120381
Upper 95% Mean	9.9097893
Lower 95% Mean	1.3402107
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	5.625	1.3402107	9.9097893
Dispersion	σ	5.1252178	3.3886615	10.431213

Measure	
-2*LogLikelihood	47.849785
AICc	54.249785
BIC	52.008668

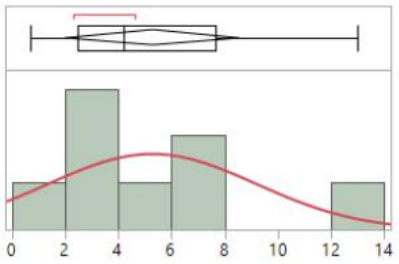
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.894032	0.2550

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Larval Density



Normal(5.275,3.90595)

Quantiles

100.0%	maximum	13
99.5%		13
97.5%		13
90.0%		13
75.0%	quartile	7.65
50.0%	median	4.2
25.0%	quartile	2.475
10.0%		0.7
2.5%		0.7
0.5%		0.7
0.0%	minimum	0.7

Summary Statistics

Mean	5.275
Std Dev	3.9059478
Std Err Mean	1.3809611
Upper 95% Mean	8.5404541
Lower 95% Mean	2.0095459
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	5.275	2.0095459	8.5404541
Dispersion	σ	3.9059478	2.5825117	7.9496666

Measure	
-2*LogLikelihood	43.503024
AICc	49.903024
BIC	47.661907

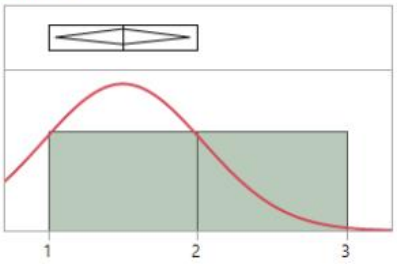
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.925480	0.4759

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Larval Habitat



Normal(1.5,0.53452)

Quantiles

100.0%	maximum	2
99.5%		2
97.5%		2
90.0%		2
75.0%	quartile	2
50.0%	median	1.5
25.0%	quartile	1
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Summary Statistics

Mean	1.5
Std Dev	0.5345225
Std Err Mean	0.1889822
Upper 95% Mean	1.946872
Lower 95% Mean	1.053128
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.5	1.053128	1.946872
Dispersion	σ	0.5345225	0.3534124	1.0878987

Measure	
-2*LogLikelihood	11.680913
AICc	18.080913
BIC	15.839796

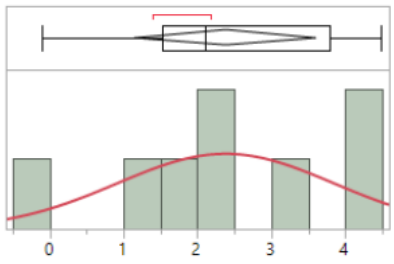
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.664656	0.0009*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Log Sed eDNA



Normal(2.37875,1.46834)

Quantiles

100.0%	maximum	4.47
99.5%		4.47
97.5%		4.47
90.0%		4.47
75.0%	quartile	3.7875
50.0%	median	2.095
25.0%	quartile	1.525
10.0%		-0.1
2.5%		-0.1
0.5%		-0.1
0.0%	minimum	-0.1

Summary Statistics

Mean	2.37875
Std Dev	1.4683366
Std Err Mean	0.5191354
Upper 95% Mean	3.6063102
Lower 95% Mean	1.1511898
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	2.37875	1.1511898	3.6063102
Dispersion	σ	1.4683366	0.9708262	2.9884646

Measure	
-2*LogLikelihood	27.8491
AICc	34.2491
BIC	32.007983

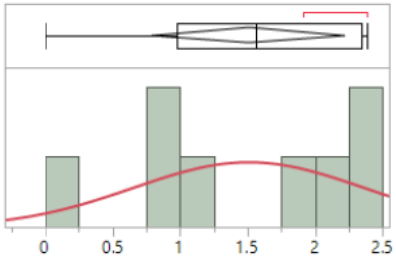
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.963242	0.8404

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Log Water eDNA



Normal(1.50875,0.85389)

Quantiles

100.0%	maximum	2.38
99.5%		2.38
97.5%		2.38
90.0%		2.38
75.0%	quartile	2.34
50.0%	median	1.565
25.0%	quartile	0.98
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	1.50875
Std Dev	0.8538892
Std Err Mean	0.3018954
Upper 95% Mean	2.2226192
Lower 95% Mean	0.7948808
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.50875	0.7948808	2.2226192
Dispersion	σ	0.8538892	0.5645695	1.7378969

Measure	
-2*LogLikelihood	19.175755
AICc	25.575755
BIC	23.334638

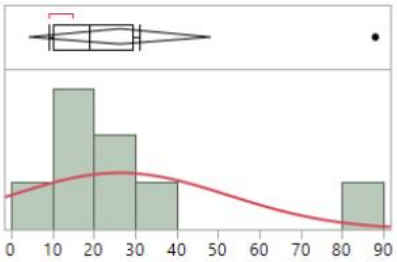
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.896421	0.2682

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Missed



Normal(26.25,26.1957)

Quantiles

100.0%	maximum	88
99.5%		88
97.5%		88
90.0%		88
75.0%	quartile	29.25
50.0%	median	19
25.0%	quartile	10
10.0%		9
2.5%		9
0.5%		9
0.0%	minimum	9

Summary Statistics

Mean	26.25
Std Dev	26.195692
Std Err Mean	9.2615758
Upper 95% Mean	48.150147
Lower 95% Mean	4.3498533
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	26.25	4.3498533	48.150147
Dispersion	σ	26.195692	17.319914	53.315361

Measure	
-2*LogLikelihood	73.952536
AICc	80.352536
BIC	78.111419

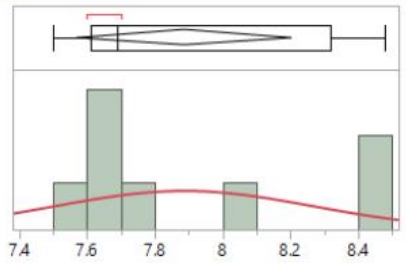
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.684608	0.0015*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

pH



Normal(7.88625,0.38139)

Quantiles

100.0%	maximum	8.48
99.5%		8.48
97.5%		8.48
90.0%		8.48
75.0%	quartile	8.32
50.0%	median	7.69
25.0%	quartile	7.6125
10.0%		7.5
2.5%		7.5
0.5%		7.5
0.0%	minimum	7.5

Summary Statistics

Mean	7.88625
Std Dev	0.3813861
Std Err Mean	0.1348403
Upper 95% Mean	8.2050968
Lower 95% Mean	7.5674032
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	7.88625	7.5674032	8.2050968
Dispersion	σ	0.3813861	0.2521626	0.7762245

Measure	
-2*LogLikelihood	6.279928
AICc	12.679928
BIC	10.438811

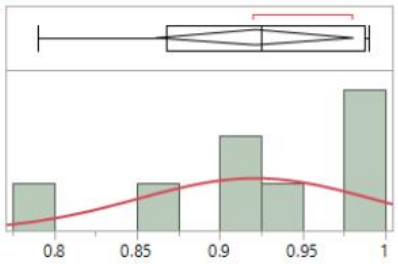
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.835676	0.0680

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Sand



Normal(0.92125,0.071)

Quantiles

100.0%	maximum	0.99
99.5%		0.99
97.5%		0.99
90.0%		0.99
75.0%	quartile	0.9875
50.0%	median	0.925
25.0%	quartile	0.8675
10.0%		0.79
2.5%		0.79
0.5%		0.79
0.0%	minimum	0.79

Summary Statistics

Mean	0.92125
Std Dev	0.0710005
Std Err Mean	0.0251025
Upper 95% Mean	0.9806079
Lower 95% Mean	0.8618921
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.92125	0.8618921	0.9806079
Dispersion	σ	0.0710005	0.0469437	0.1445053

Measure	
-2*LogLikelihood	-20.61808
AICc	-14.21808
BIC	-16.45919

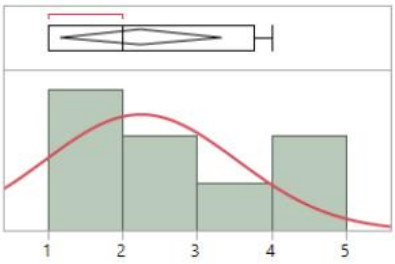
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.881472	0.1945

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

ShorelineType



Normal(2.25,1.28174)

Quantiles

100.0%	maximum	4
99.5%		4
97.5%		4
90.0%		4
75.0%	quartile	3.75
50.0%	median	2
25.0%	quartile	1
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Summary Statistics

Mean	2.25
Std Dev	1.2817399
Std Err Mean	0.4531635
Upper 95% Mean	3.3215614
Lower 95% Mean	1.1784386
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	2.25	1.1784386	3.3215614
Dispersion	σ	1.2817399	0.8474533	2.6086894

Measure	
-2*LogLikelihood	25.674512
AICc	32.074512
BIC	29.833395

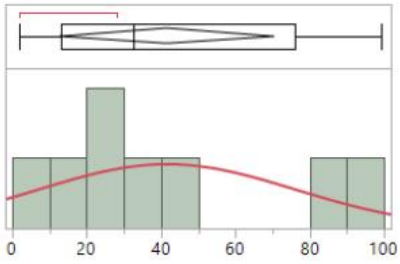
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.843385	0.0816

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Small



Normal(41.625,34.5416)

Quantiles

100.0%	maximum	99
99.5%		99
97.5%		99
90.0%		99
75.0%	quartile	76
50.0%	median	32.5
25.0%	quartile	13.25
10.0%		2
2.5%		2
0.5%		2
0.0%	minimum	2

Summary Statistics

Mean	41.625
Std Dev	34.541642
Std Err Mean	12.212314
Upper 95% Mean	70.502535
Lower 95% Mean	12.747465
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	41.625	12.747465	70.502535
Dispersion	σ	34.541642	22.83804	70.301639

Measure	
-2*LogLikelihood	78.377666
AICc	84.777666
BIC	82.536549

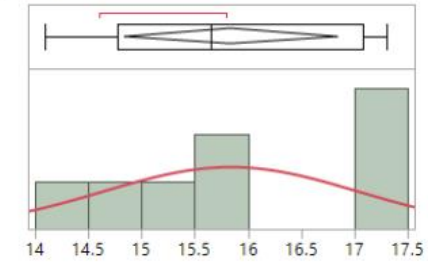
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.916183	0.3997

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Temp



Normal(15.8375,1.19754)

Quantiles

100.0%	maximum	17.3
99.5%		17.3
97.5%		17.3
90.0%		17.3
75.0%	quartile	17.075
50.0%	median	15.65
25.0%	quartile	14.775
10.0%		14.1
2.5%		14.1
0.5%		14.1
0.0%	minimum	14.1

Summary Statistics

Mean	15.8375
Std Dev	1.1975421
Std Err Mean	0.4233951
Upper 95% Mean	16.83867
Lower 95% Mean	14.83633
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	15.8375	14.83633	16.83867
Dispersion	σ	1.1975421	0.7917839	2.4373241

Measure	
-2*LogLikelihood	24.587356
AICc	30.987356
BIC	28.746239

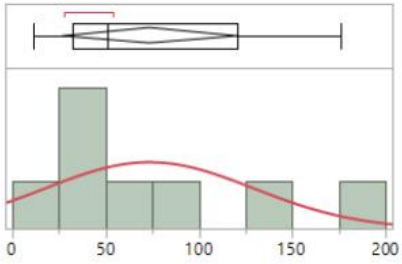
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.921552	0.4426

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Total Efish



Normal(73.5,55.8774)

Quantiles

100.0%	maximum	176
99.5%		176
97.5%		176
90.0%		176
75.0%	quartile	120.75
50.0%	median	51
25.0%	quartile	32.5
10.0%		11
2.5%		11
0.5%		11
0.0%	minimum	11

Summary Statistics

Mean	73.5
Std Dev	55.877417
Std Err Mean	19.75565
Upper 95% Mean	120.21469
Lower 95% Mean	26.78531
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	73.5	26.78531	120.21469
Dispersion	σ	55.877417	36.944704	113.72575

Measure	
-2*LogLikelihood	86.073581
AICc	92.473581
BIC	90.232465

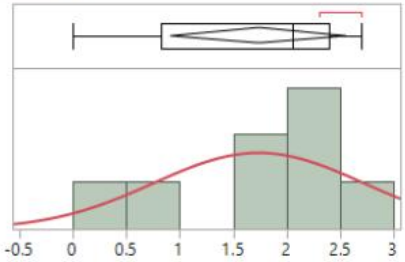
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.908037	0.3404

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Turbidity



Normal(1.7375,0.97678)

Quantiles

100.0%	maximum	2.7
99.5%		2.7
97.5%		2.7
90.0%		2.7
75.0%	quartile	2.4
50.0%	median	2.05
25.0%	quartile	0.825
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	1.7375
Std Dev	0.9767841
Std Err Mean	0.3453453
Upper 95% Mean	2.5541119
Lower 95% Mean	0.9208881
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.7375	0.9208881	2.5541119
Dispersion	σ	0.9767841	0.6458244	1.9880214

Measure	
-2*LogLikelihood	21.327182
AICc	27.727182
BIC	25.486065

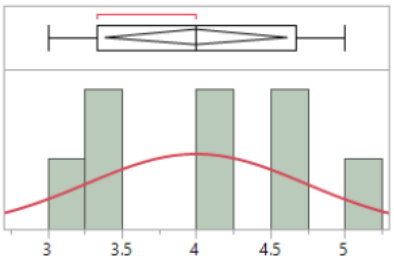
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.839555	0.0745

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Water Clarity



Normal(4,0.73636)

Quantiles

100.0%	maximum	5
99.5%		5
97.5%		5
90.0%		5
75.0%	quartile	4.67
50.0%	median	4
25.0%	quartile	3.33
10.0%		3
2.5%		3
0.5%		3
0.0%	minimum	3

Summary Statistics

Mean	4
Std Dev	0.7363617
Std Err Mean	0.2603432
Upper 95% Mean	4.6156138
Lower 95% Mean	3.3843862
N	8

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	4	3.3843862	4.6156138
Dispersion	σ	0.7363617	0.4868633	1.4986964

Measure	
-2*LogLikelihood	16.806475
AICc	23.206475
BIC	20.965358

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.919939	0.4294

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

2. Site-level Statistics: Larval Detection Method Multivariate Analysis

Multivariate

Correlations

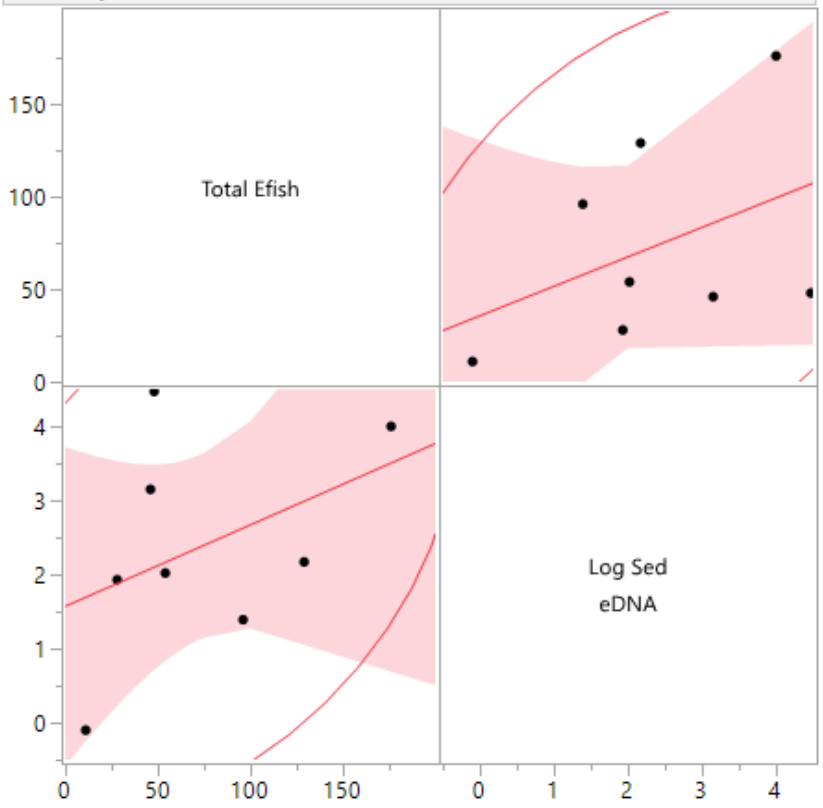
	Total Efish	Log Sed eDNA
Total Efish	1.0000	0.4181
Log Sed eDNA	0.4181	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Log Sed eDNA
Total Efish	<.0001	0.3026
Log Sed eDNA	0.3026	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Log Sed eDNA	Total Efish	0.4286	0.2894	

Warning: sample size of 8 is too small, P value suspect.

3. Site-level Statistics: Multivariate Analysis with Total Electrofishing Detections

Multivariate

Correlations

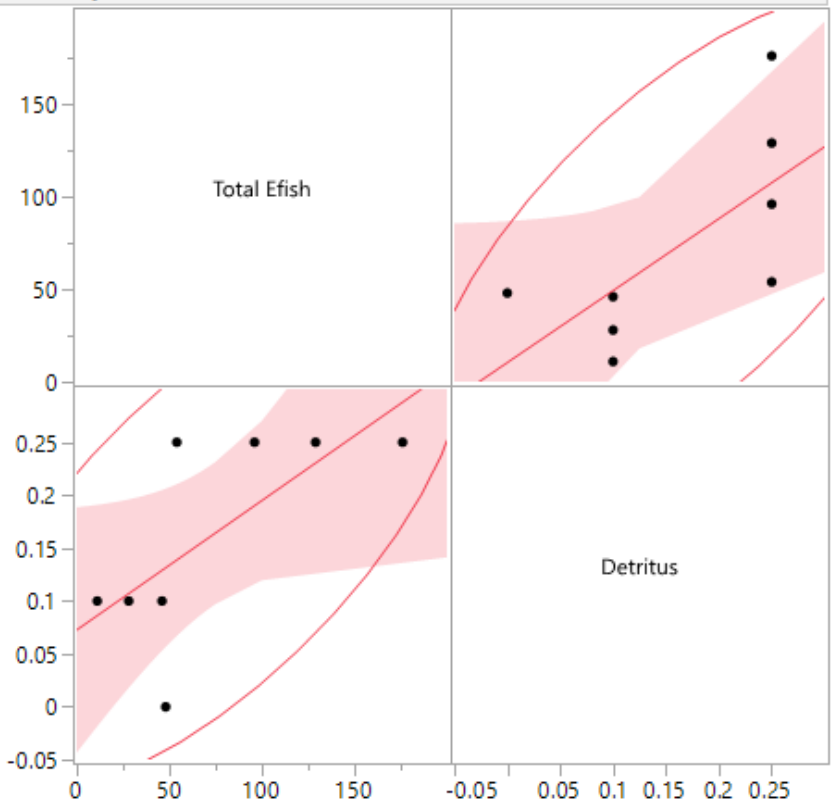
	Total Efish	Detritus
Total Efish	1.0000	0.6888
Detritus	0.6888	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Detritus
Total Efish	<.0001	0.0589
Detritus	0.0589	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Detritus	Total Efish	0.7564	0.0299*	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

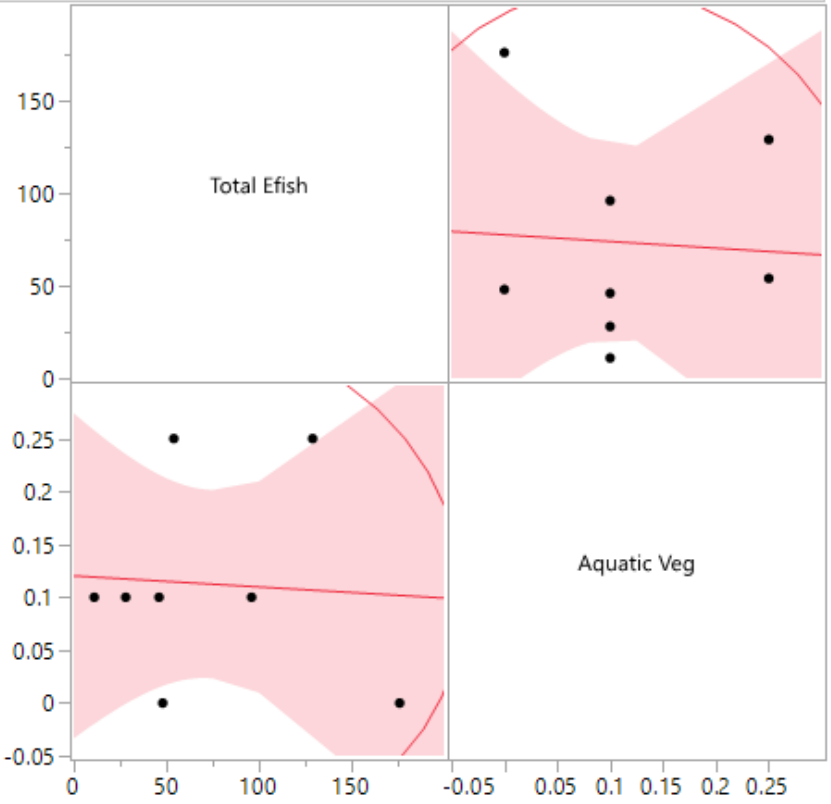
	Total Efish	Aquatic Veg
Total Efish	1.0000	-0.0616
Aquatic Veg	-0.0616	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Aquatic Veg
Total Efish	<.0001	0.8848
Aquatic Veg	0.8848	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Aquatic Veg	Total Efish	0.0000	1.0000	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

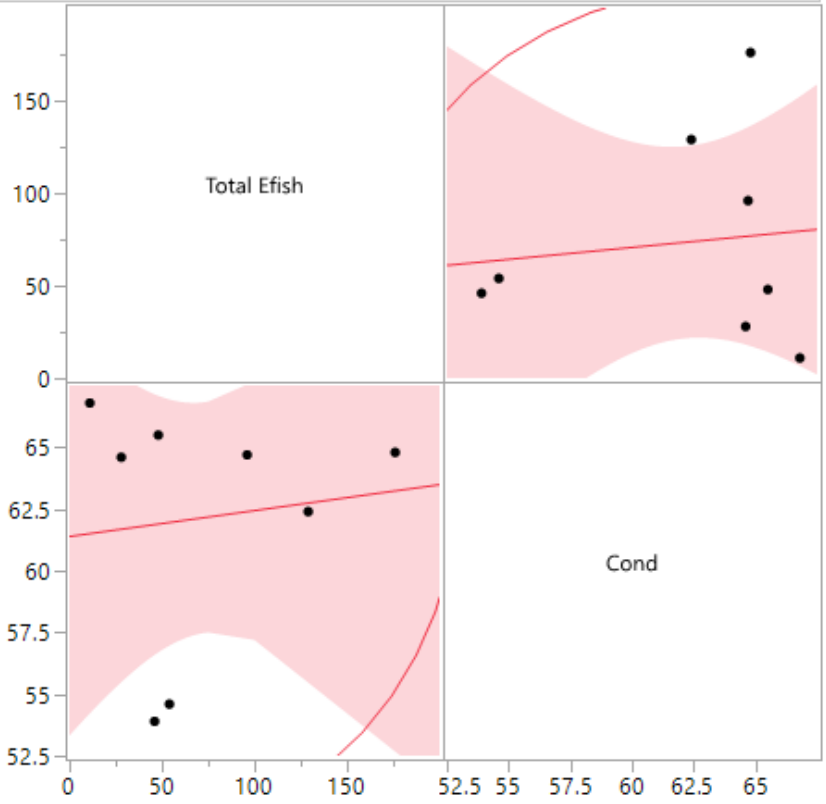
	Total Efish	Cond
Total Efish	1.0000	0.1165
Cond	0.1165	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Cond
Total Efish	<.0001	0.7835
Cond	0.7835	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Cond	Total Efish	-0.1429	0.7358	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

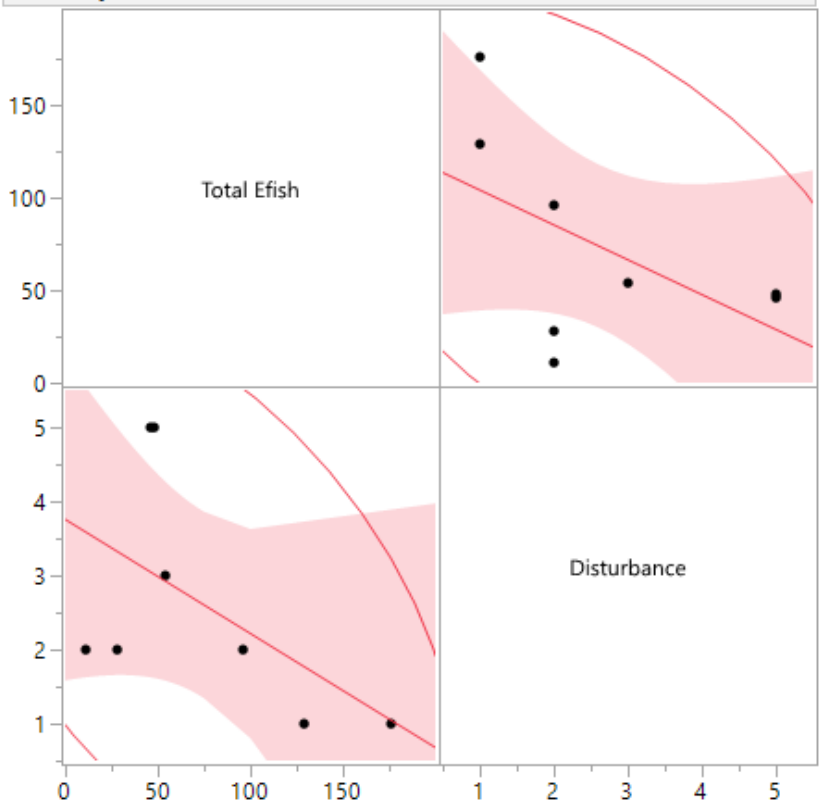
	Total Efish	Disturbance
Total Efish	1.0000	-0.5384
Disturbance	-0.5384	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Disturbance
Total Efish	<.0001	0.1687
Disturbance	0.1687	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Disturbance	Total Efish	-0.5189	0.1876	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

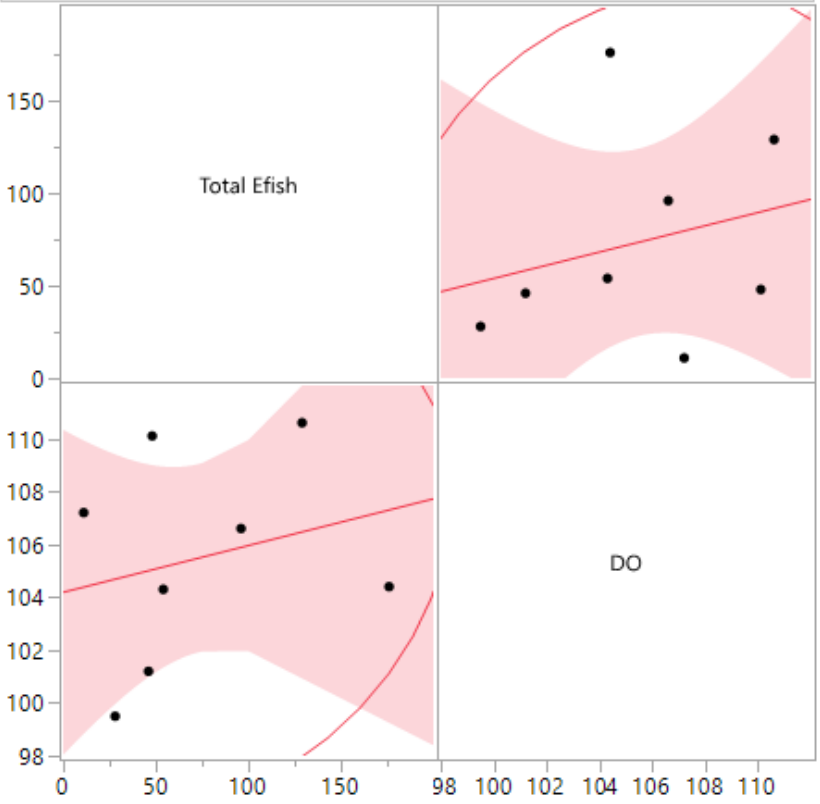
	Total Efish	DO
Total Efish	1.0000	0.2509
DO	0.2509	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	DO
Total Efish	<.0001	0.5490
DO	0.5490	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
DO	Total Efish	0.3095	0.4556	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

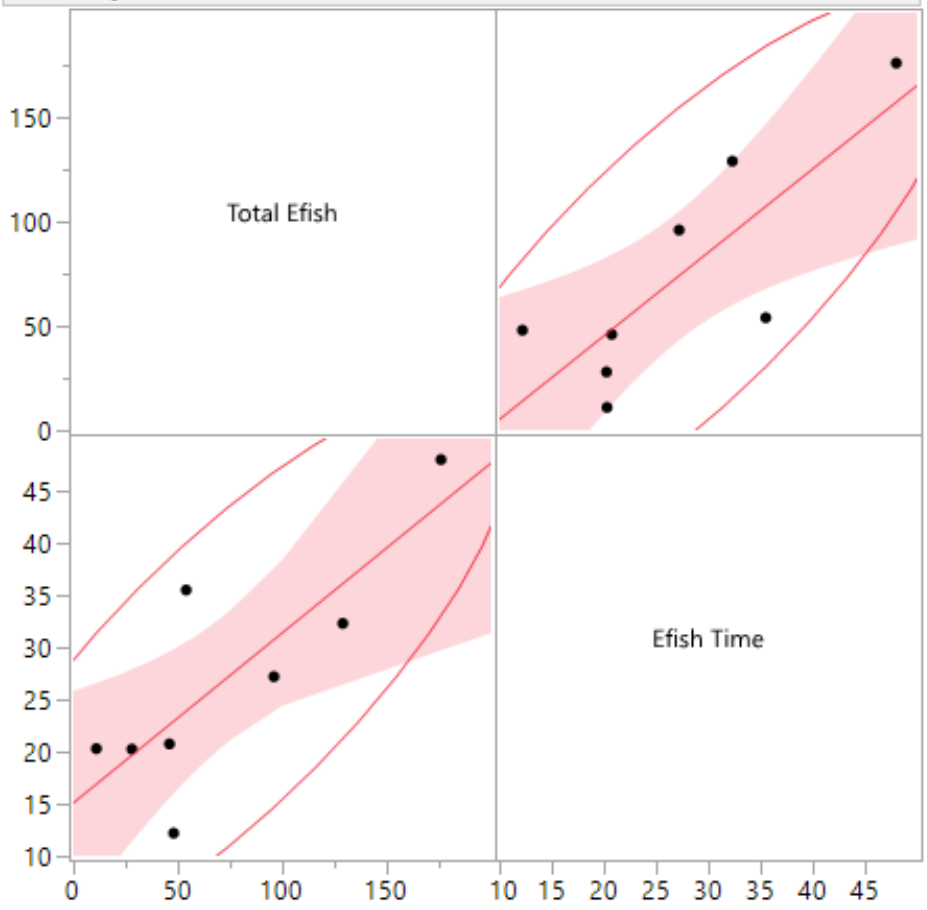
	Total Efish	Efish Time
Total Efish	1.0000	0.8073
Efish Time	0.8073	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Efish Time
Total Efish	<.0001	0.0154
Efish Time	0.0154	<.0001

Scatterplot Matrix



Multivariate

Correlations

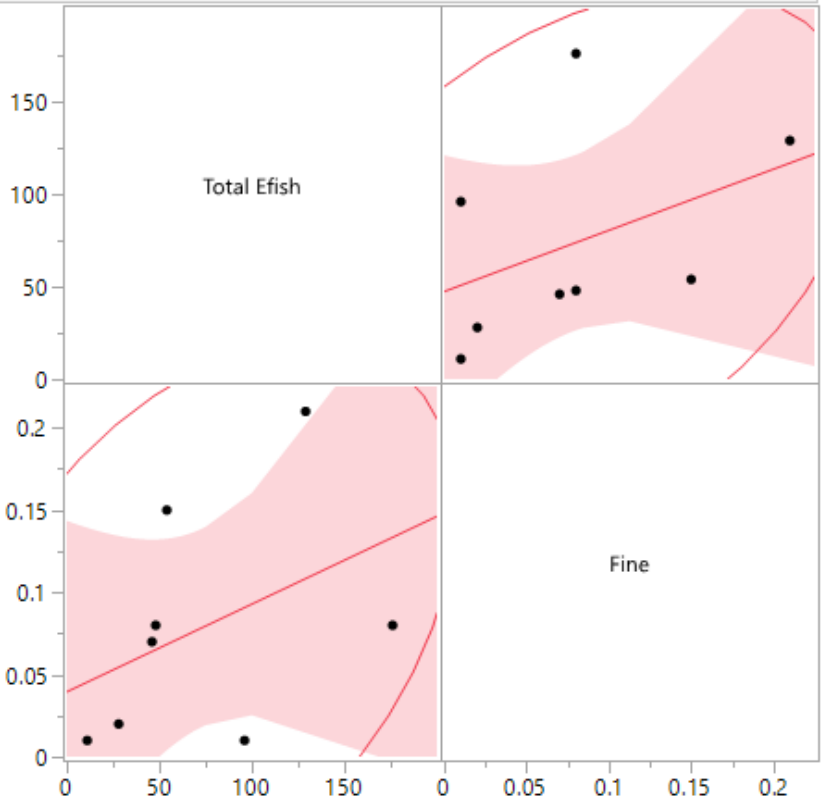
	Total Efish	Fine
Total Efish	1.0000	0.4197
Fine	0.4197	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Fine
Total Efish	<.0001	0.3006
Fine	0.3006	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Total Efish	0.5663	0.1434	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

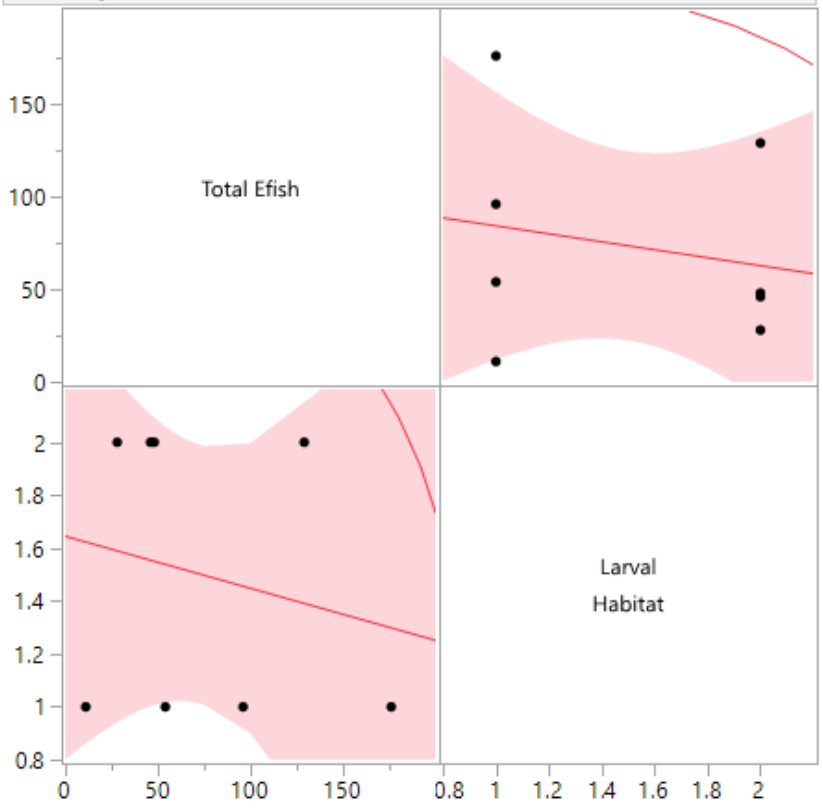
	Total Efish	Larval Habitat
Total Efish	1.0000	-0.2057
Larval Habitat	-0.2057	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Larval Habitat
Total Efish	<.0001	0.6251
Larval Habitat	0.6251	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Larval Habitat	Total Efish	-0.2182	0.6036	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

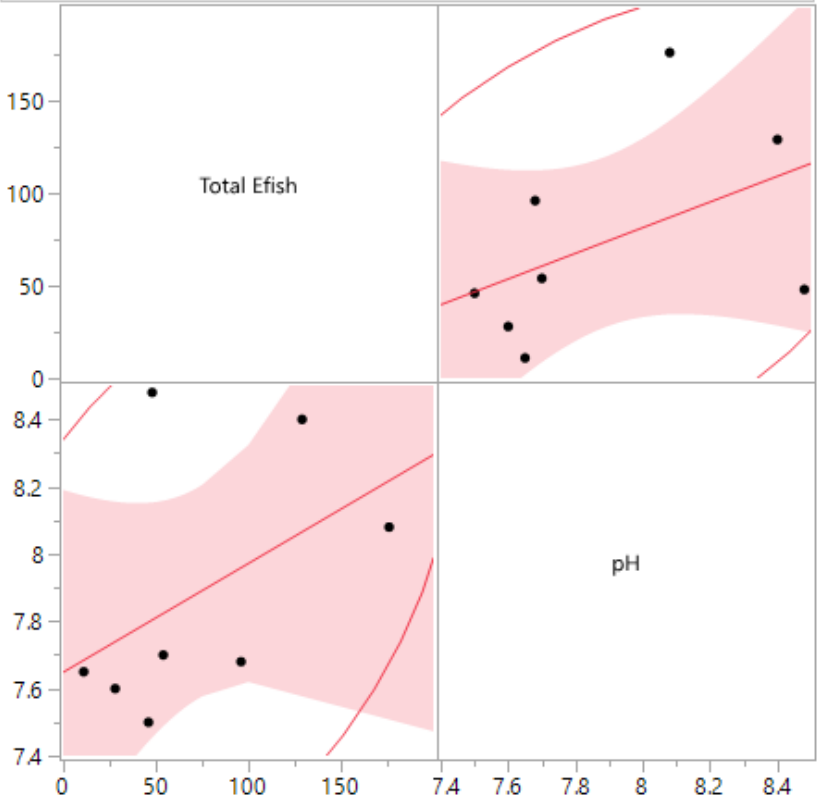
	Total Efish	pH
Total Efish	1.0000	0.4735
pH	0.4735	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	pH
Total Efish	<.0001	0.2360
pH	0.2360	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
pH	Total Efish	0.6190	0.1017	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

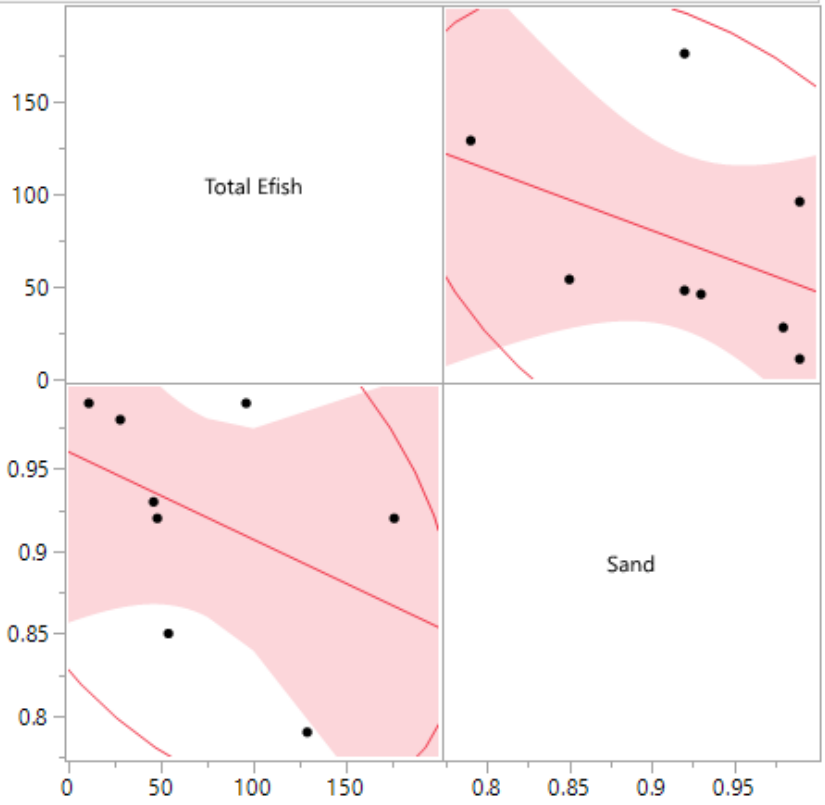
	Total Efish	Sand
Total Efish	1.0000	-0.4197
Sand	-0.4197	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Sand
Total Efish	<.0001	0.3006
Sand	0.3006	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Total Efish	-0.5663	0.1434	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

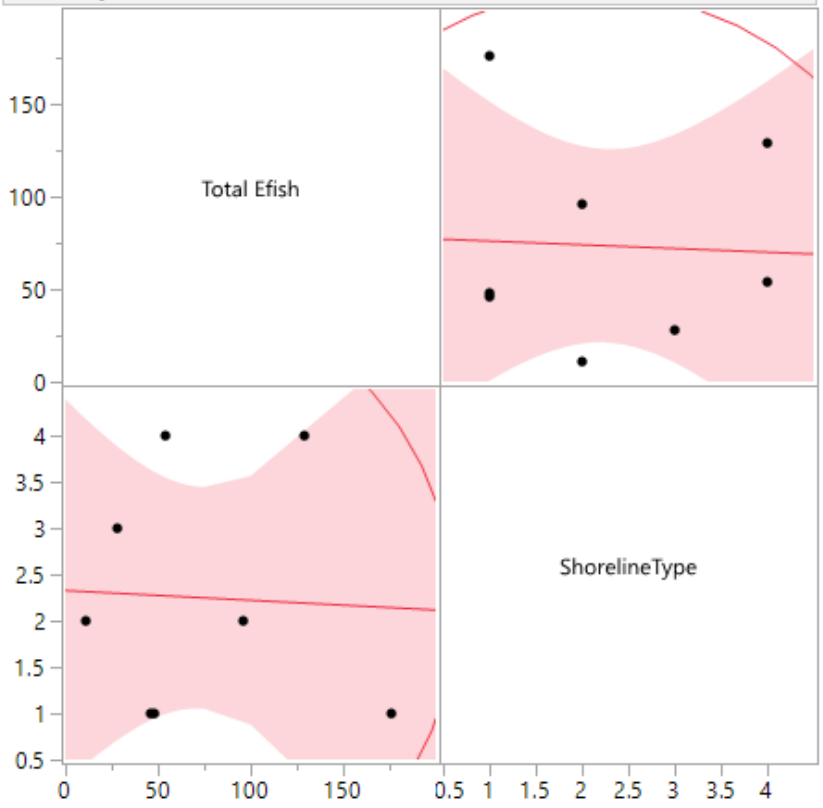
	Total Efish	ShorelineType
Total Efish	1.0000	-0.0459
ShorelineType	-0.0459	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	ShorelineType
Total Efish	<.0001	0.9141
ShorelineType	0.9141	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
ShorelineType	Total Efish	0.0371	0.9306	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

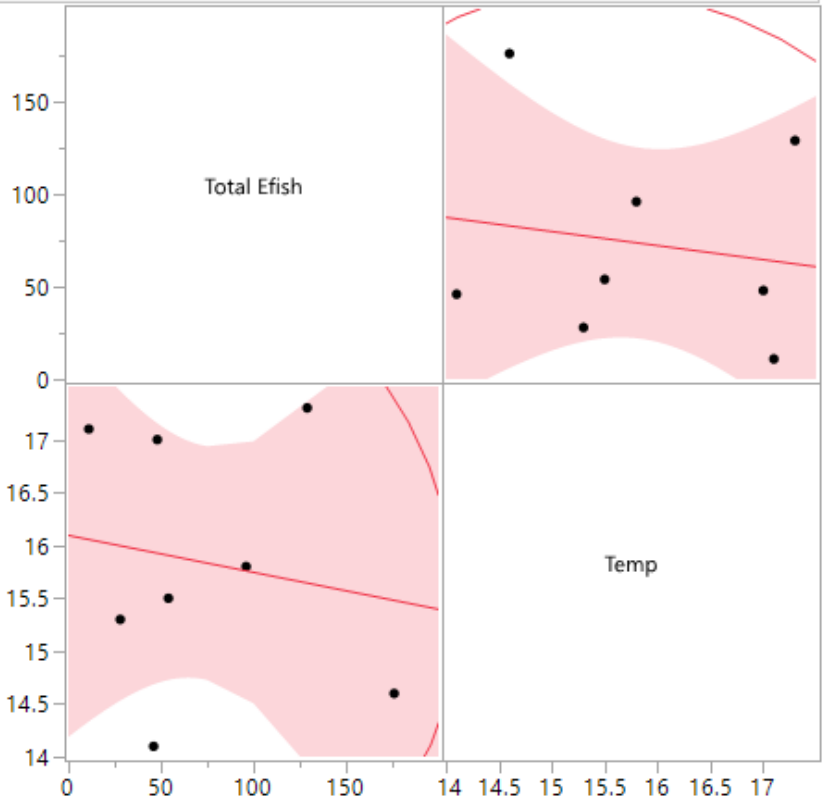
	Total Efish	Temp
Total Efish	1.0000	-0.1628
Temp	-0.1628	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Temp
Total Efish	<.0001	0.7001
Temp	0.7001	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Temp	Total Efish	0.0000	1.0000	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

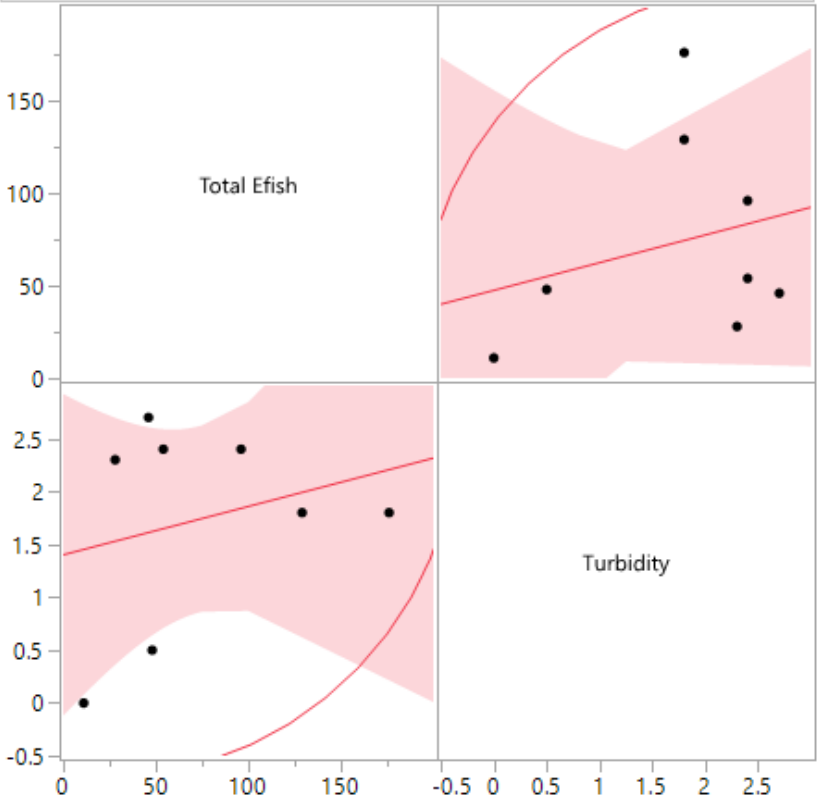
	Total Efish	Turbidity
Total Efish	1.0000	0.2616
Turbidity	0.2616	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Turbidity
Total Efish	<.0001	0.5314
Turbidity	0.5314	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Turbidity	Total Efish	0.1205	0.7763	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

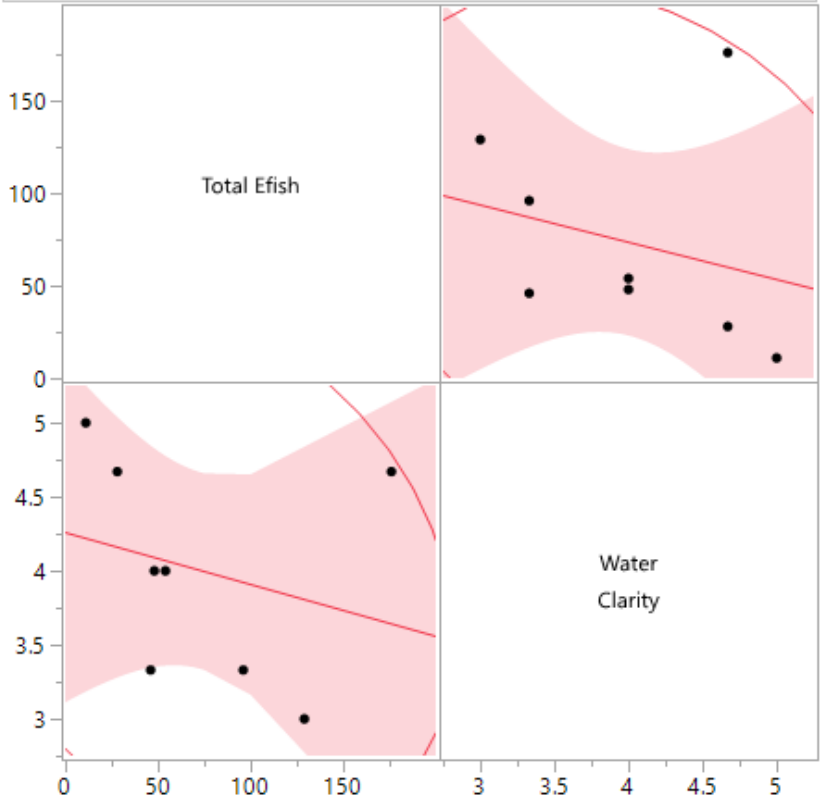
	Total Efish	Water Clarity
Total Efish	1.0000	-0.2655
Water Clarity	-0.2655	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Water Clarity
Total Efish	<.0001	0.5251
Water Clarity	0.5251	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Water Clarity	Total Efish	-0.4607	0.2507	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

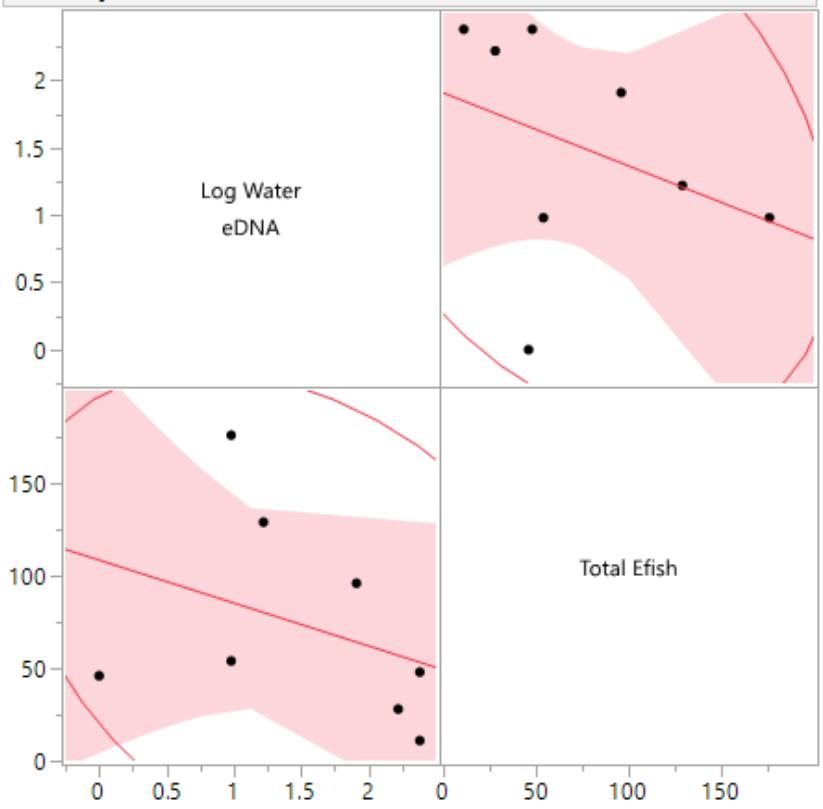
	Log Water eDNA	Total Efish
Log Water eDNA	1.0000	-0.3546
Total Efish	-0.3546	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Water eDNA	Total Efish
Log Water eDNA	<.0001	0.3888
Total Efish	0.3888	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Total Efish	Log Water eDNA	-0.4579	0.2539	

Warning: sample size of 8 is too small, P value suspect.

4. Site-level Statistics: Multivariate Analysis with LOG Sediment eDNA Concentrations

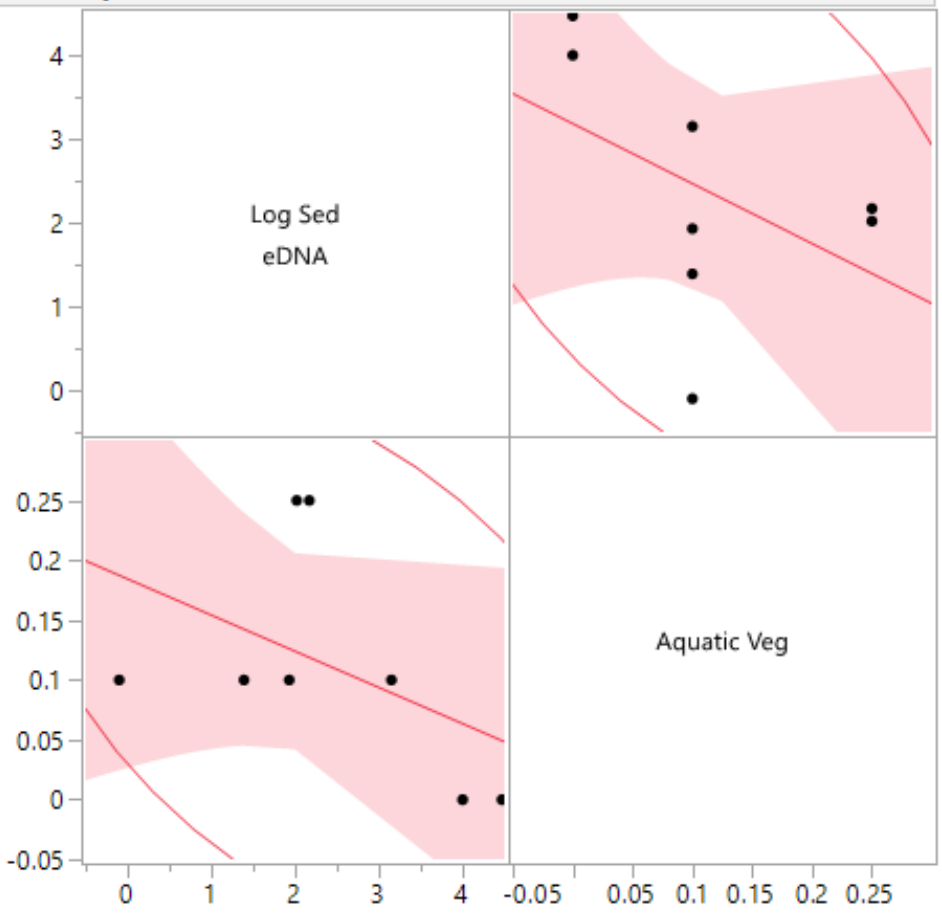
Multivariate

Correlations

	Log Sed eDNA	Aquatic Veg
Log Sed eDNA	1.0000	-0.4653
Aquatic Veg	-0.4653	1.0000

The correlations are estimated by Row-wise method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Aquatic Veg	Log Sed eDNA	-0.4629	0.2481	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

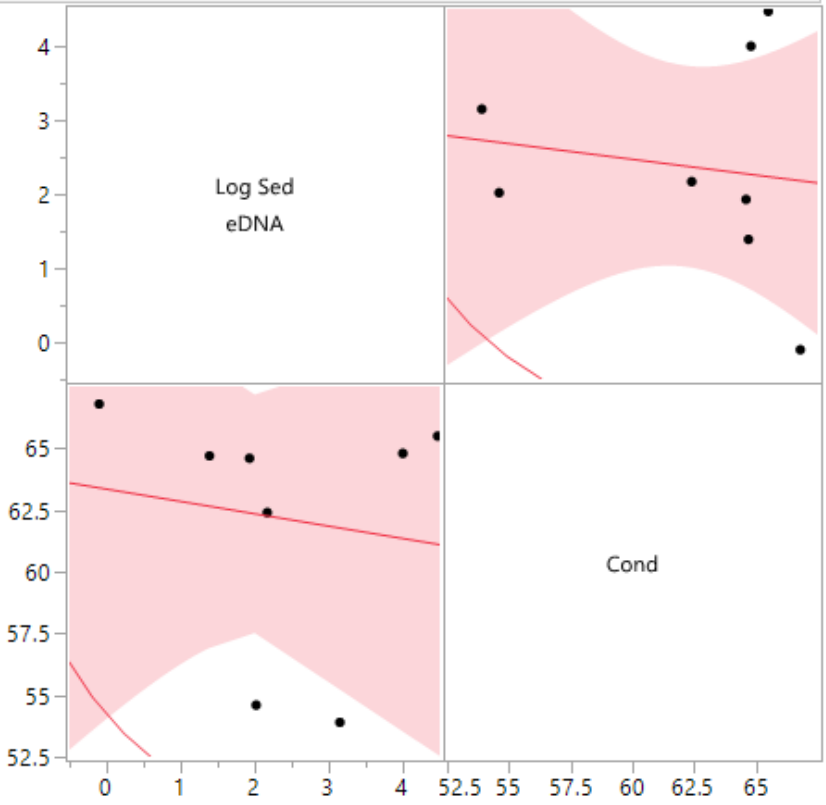
	Log Sed eDNA	Cond
Log Sed eDNA	1.0000	-0.1459
Cond	-0.1459	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Cond
Log Sed eDNA	<.0001	0.7303
Cond	0.7303	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Cond	Log Sed eDNA	-0.1190	0.7789	

Warning: sample size of 8 is too small, P value suspect.

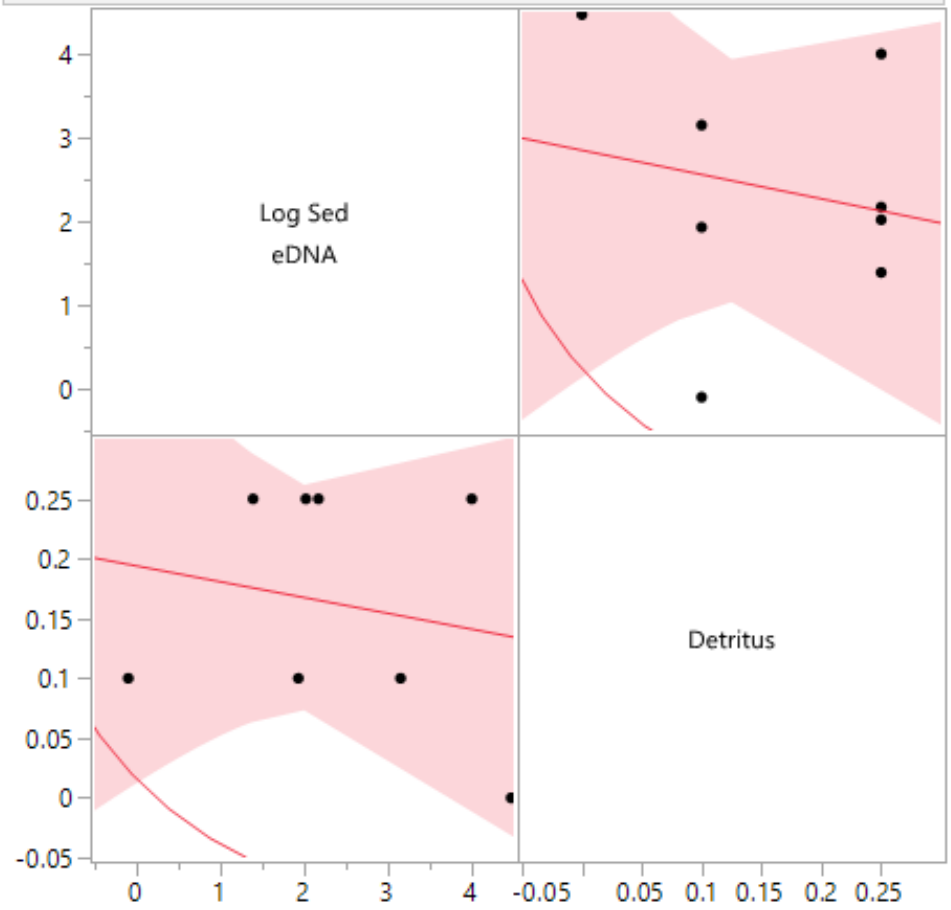
Multivariate

Correlations

	Log Sed eDNA	Detritus
Log Sed eDNA	1.0000	-0.1957
Detritus	-0.1957	1.0000

The correlations are estimated by Row-wise method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Detritus	Log Sed eDNA	-0.1826	0.6652	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

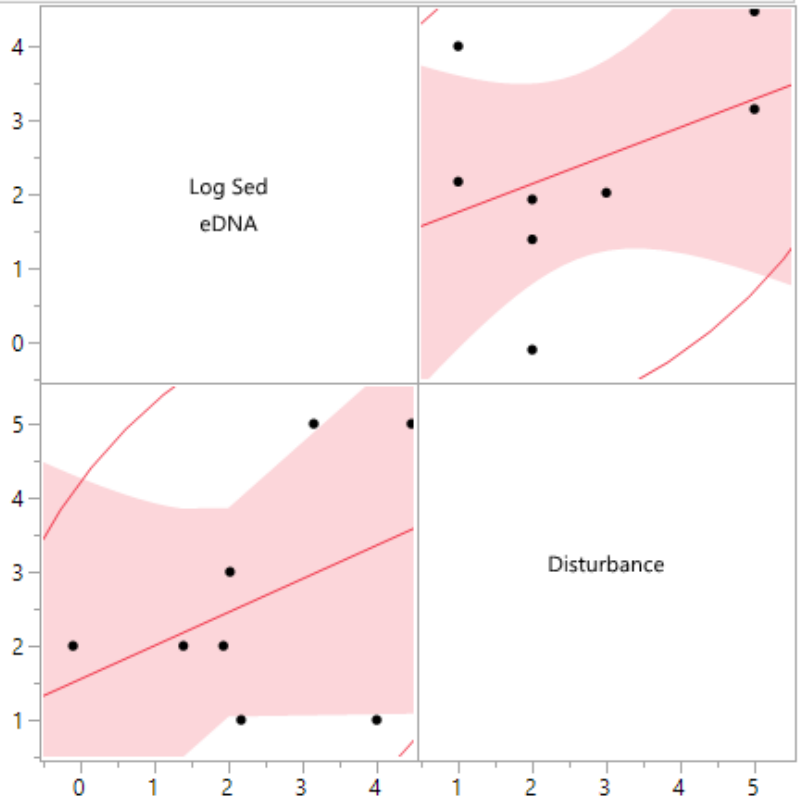
	Log Sed eDNA	Disturbance
Log Sed eDNA	1.0000	0.4150
Disturbance	0.4150	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Disturbance
Log Sed eDNA	<.0001	0.3066
Disturbance	0.3066	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Disturbance	Log Sed eDNA	0.2224	0.5966	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

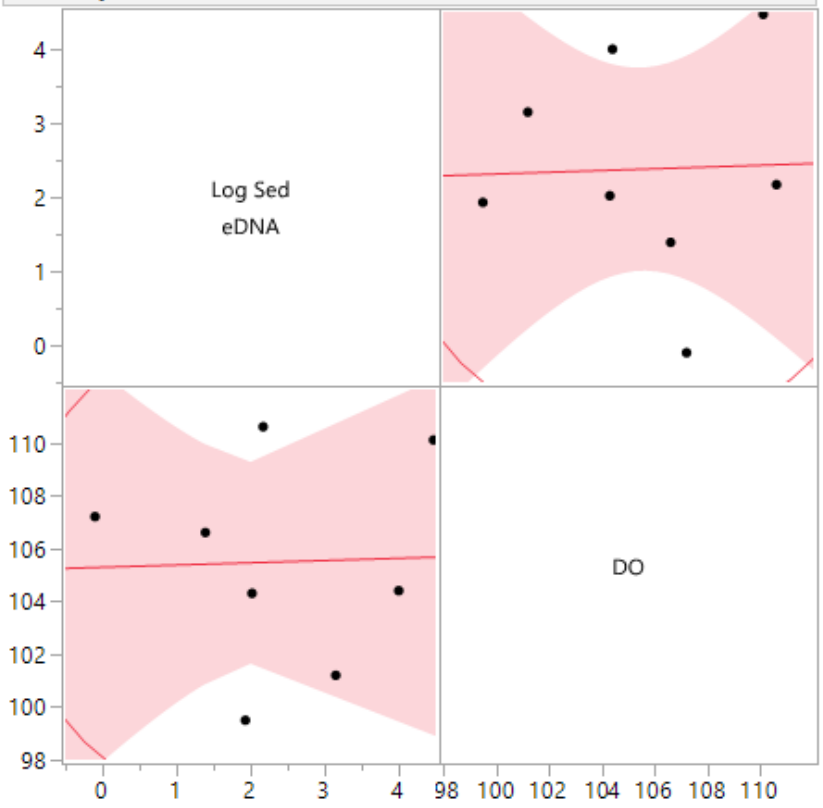
	Log Sed eDNA	DO
Log Sed eDNA	1.0000	0.0316
DO	0.0316	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	DO
Log Sed eDNA	<.0001	0.9409
DO	0.9409	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
DO	Log Sed eDNA	0.1190	0.7789	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

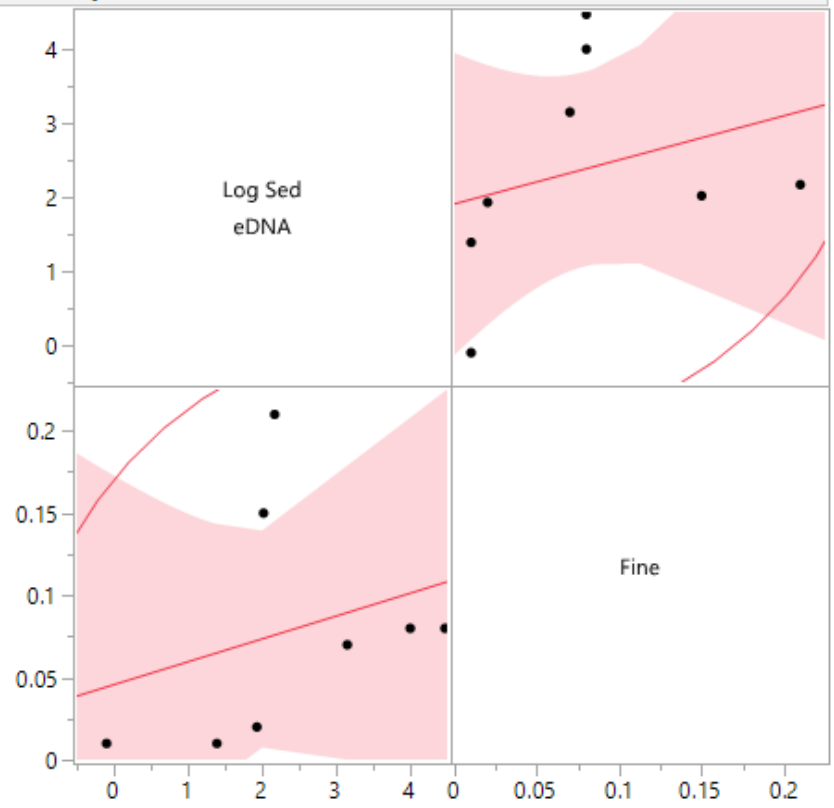
	Log Sed eDNA	Fine
Log Sed eDNA	1.0000	0.2873
Fine	0.2873	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Fine
Log Sed eDNA	<.0001	0.4902
Fine	0.4902	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Log Sed eDNA	0.6266	0.0965	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

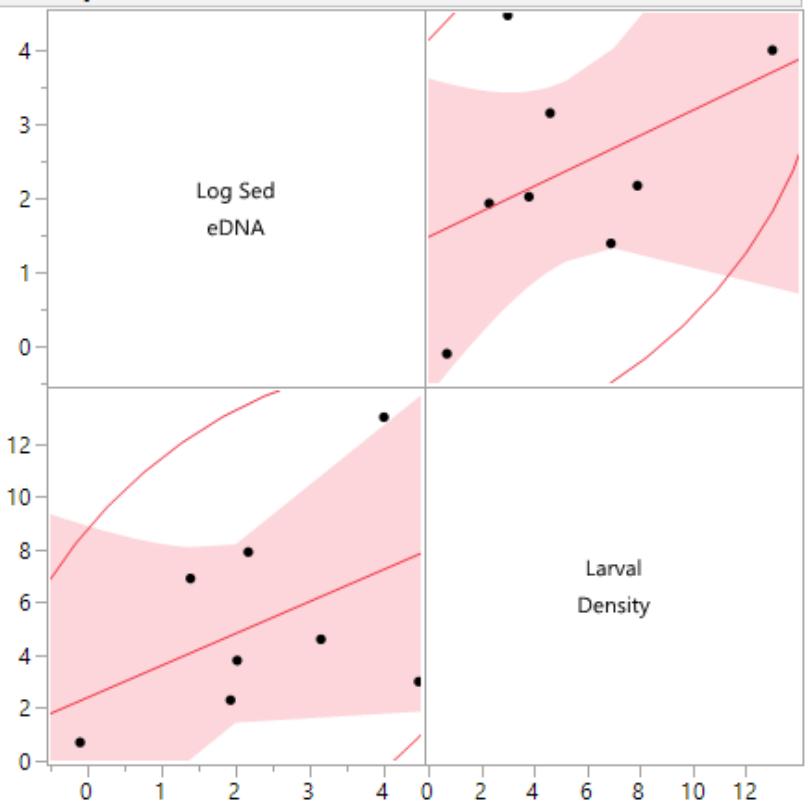
	Log Sed eDNA	Larval Density
Log Sed eDNA	1.0000	0.4557
Larval Density	0.4557	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Larval Density
Log Sed eDNA	<.0001	0.2565
Larval Density	0.2565	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Larval Density	Log Sed eDNA	0.4286	0.2894	

Warning: sample size of 8 is too small, P value suspect.

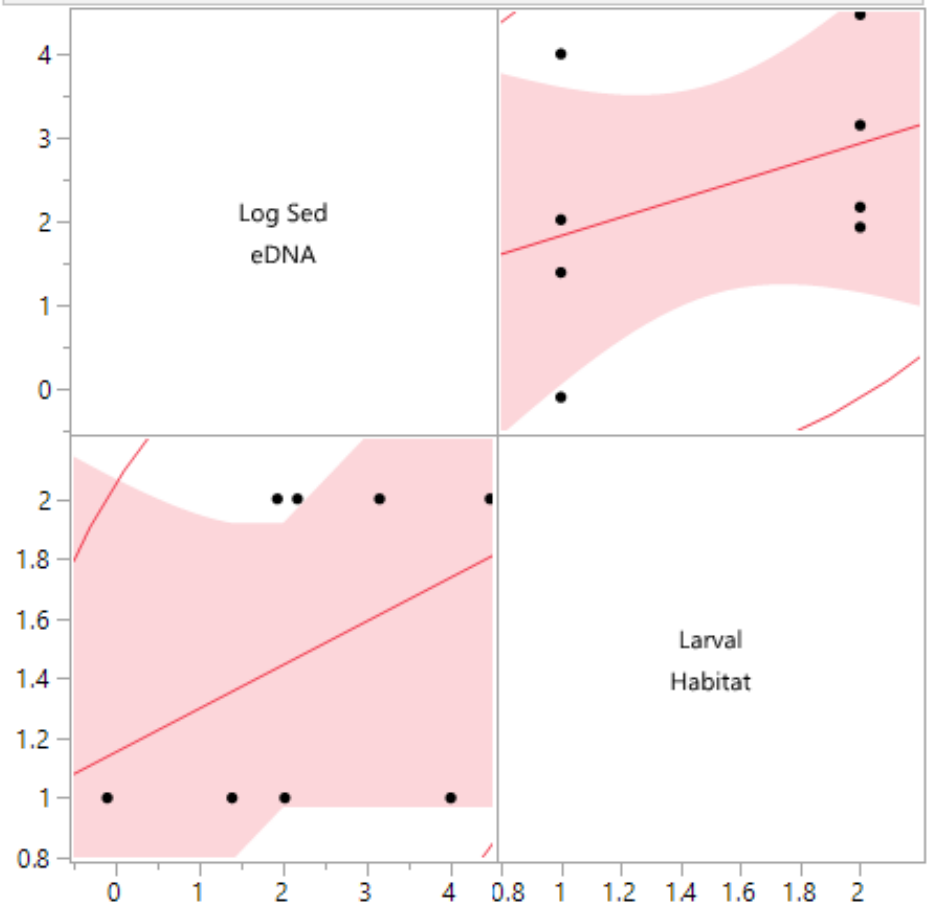
Multivariate

Correlations

	Log Sed eDNA	Larval Habitat
Log Sed eDNA	1.0000	0.4013
Larval Habitat	0.4013	1.0000

The correlations are estimated by Row-wise method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Larval Habitat	Log Sed eDNA	0.4364	0.2797	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

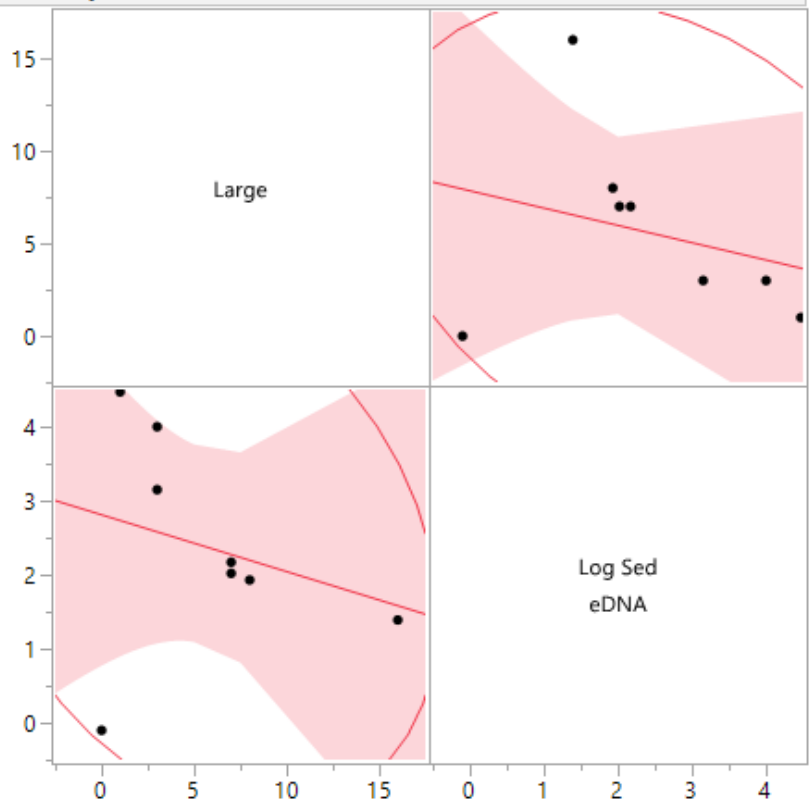
	Large	Log Sed eDNA
Large	1.0000	-0.2679
Log Sed eDNA	-0.2679	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Large	Log Sed eDNA
Large	<.0001	0.5212
Log Sed eDNA	0.5212	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Log Sed eDNA	Large	-0.3133	0.4499	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

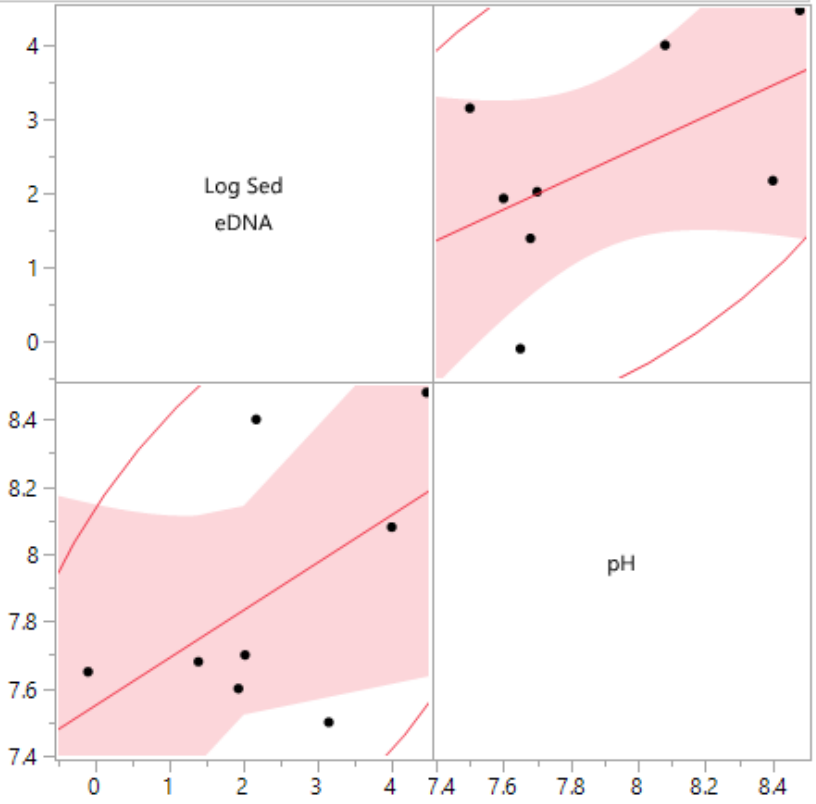
	Log Sed eDNA	pH
Log Sed eDNA	1.0000	0.5448
pH	0.5448	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	pH
Log Sed eDNA	<.0001	0.1627
pH	0.1627	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
pH	Log Sed eDNA	0.5238	0.1827	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

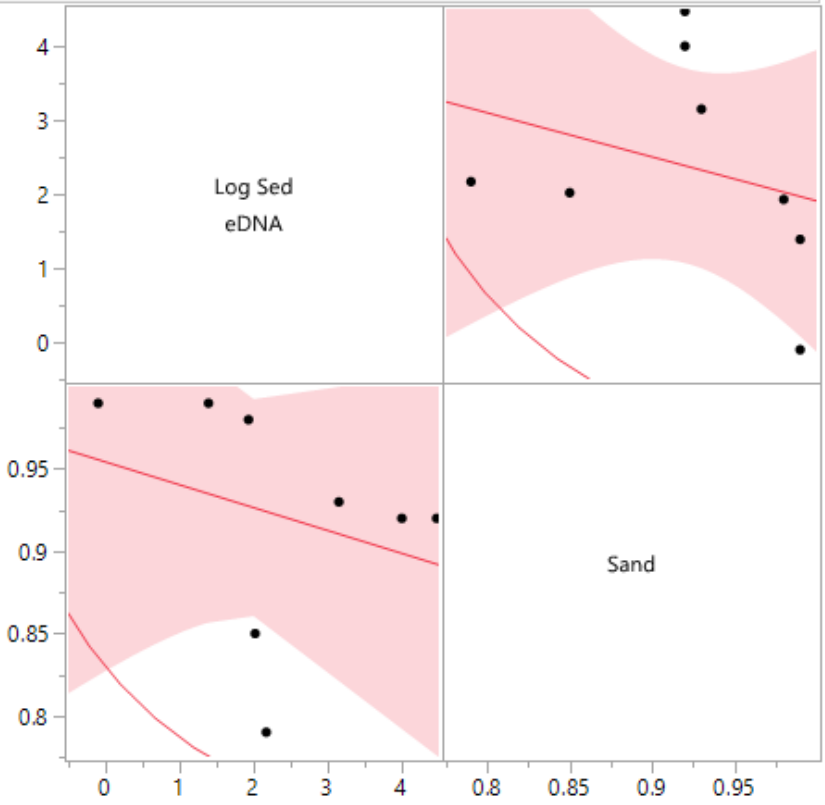
	Log Sed eDNA	Sand
Log Sed eDNA	1.0000	-0.2873
Sand	-0.2873	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Sand
Log Sed eDNA	<.0001	0.4902
Sand	0.4902	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Log Sed eDNA	-0.6266	0.0965	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

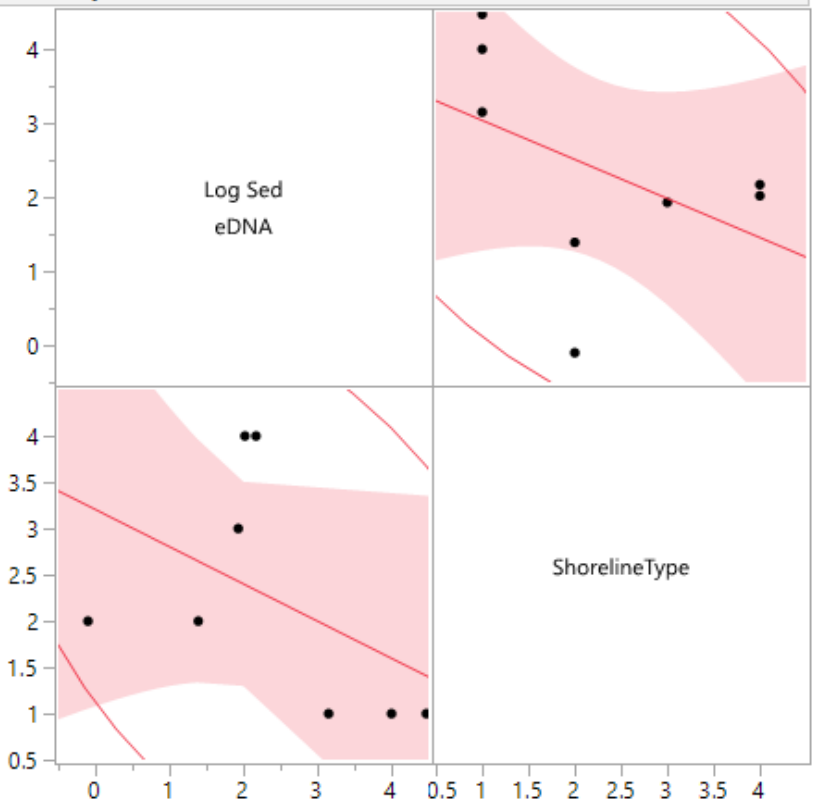
	Log Sed eDNA	ShorelineType
Log Sed eDNA	1.0000	-0.4606
ShorelineType	-0.4606	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	ShorelineType
Log Sed eDNA	<.0001	0.2508
ShorelineType	0.2508	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
ShorelineType	Log Sed eDNA	-0.5189	0.1876	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

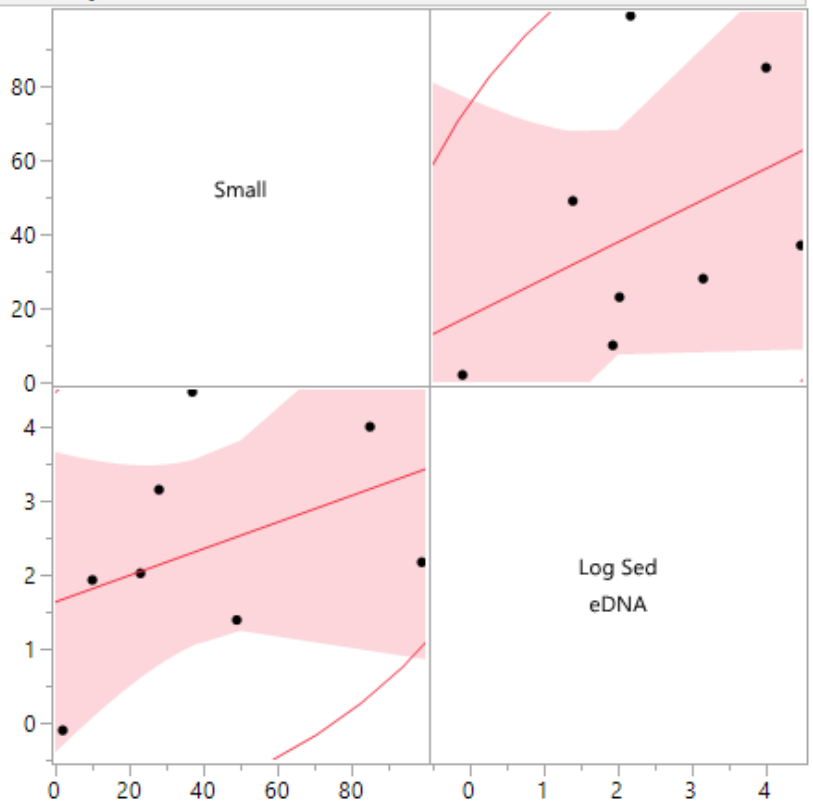
	Small	Log Sed eDNA
Small	1.0000	0.4224
Log Sed eDNA	0.4224	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Small	Log Sed eDNA
Small	<.0001	0.2972
Log Sed eDNA	0.2972	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Log Sed eDNA	Small	0.5238	0.1827	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

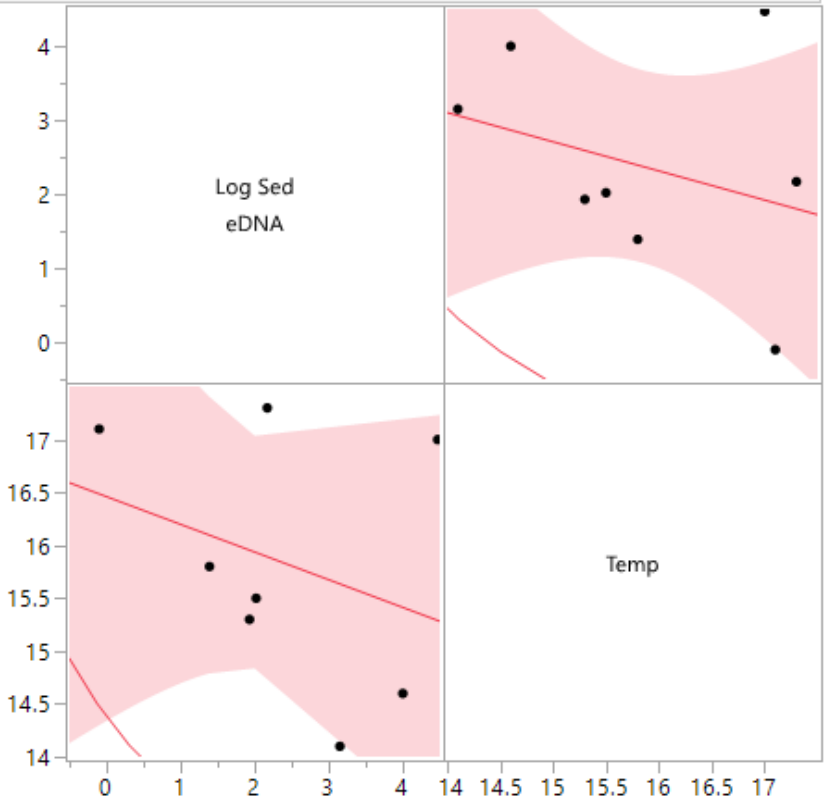
	Log Sed eDNA	Temp
Log Sed eDNA	1.0000	-0.3210
Temp	-0.3210	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Temp
Log Sed eDNA	<.0001	0.4383
Temp	0.4383	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Temp	Log Sed eDNA	-0.2857	0.4927	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

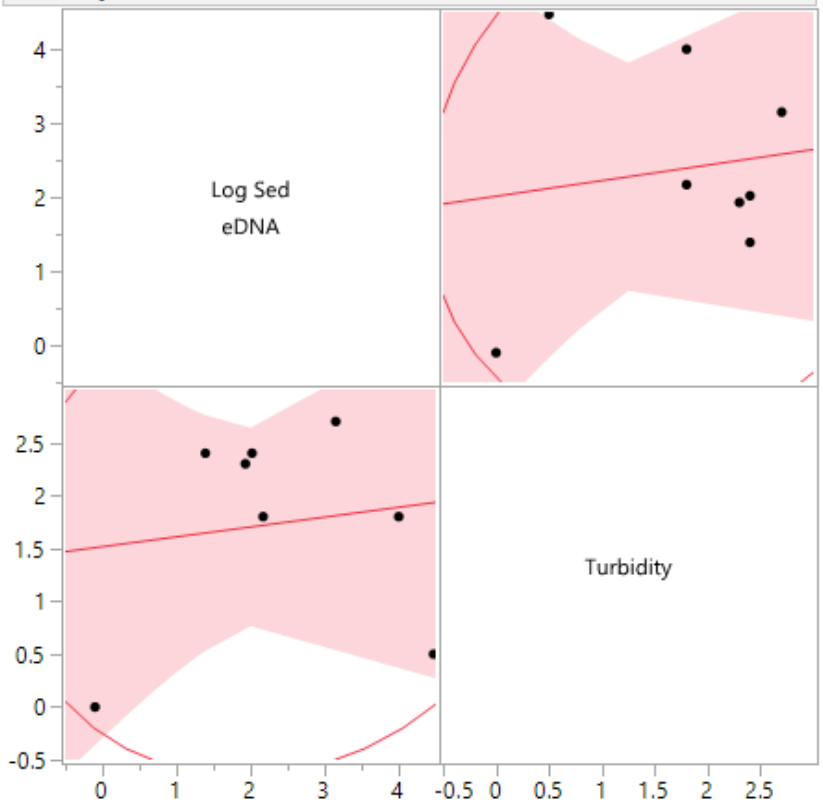
	Log Sed eDNA	Turbidity
Log Sed eDNA	1.0000	0.1399
Turbidity	0.1399	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Turbidity
Log Sed eDNA	<.0001	0.7411
Turbidity	0.7411	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Turbidity	Log Sed eDNA	-0.0241	0.9548	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

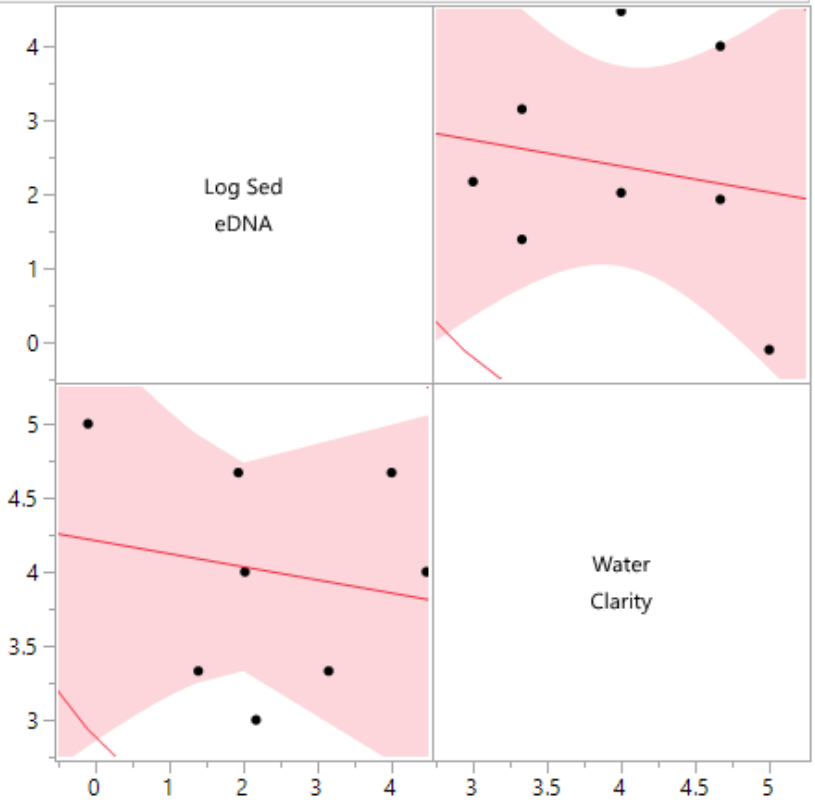
	Log Sed eDNA	Water Clarity
Log Sed eDNA	1.0000	-0.1769
Water Clarity	-0.1769	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Log Sed eDNA	Water Clarity
Log Sed eDNA	<.0001	0.6752
Water Clarity	0.6752	<.0001

Scatterplot Matrix



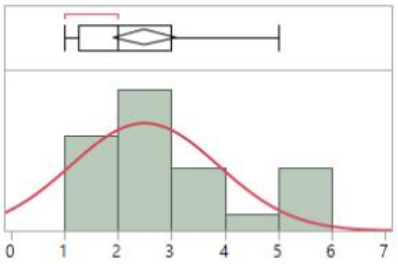
Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Water Clarity	Log Sed eDNA	-0.2425	0.5629	

Warning: sample size of 8 is too small, P value suspect.

5. Grid-level Statistics: Normal distribution & Goodness-of-fit tests

Disturbance



Normal(2.5,1.38313)

Quantiles

100.0%	maximum	5
99.5%		5
97.5%		5
90.0%		5
75.0%	quartile	3
50.0%	median	2
25.0%	quartile	1.25
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Summary Statistics

Mean	2.5
Std Dev	1.3831281
Std Err Mean	0.2823299
Upper 95% Mean	3.0840438
Lower 95% Mean	1.9159562
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	2.5	1.9159562	3.0840438
Dispersion	σ	1.3831281	1.074986	1.9401977

Measure	
-2*LogLikelihood	82.67774
AICc	87.249168
BIC	89.033847

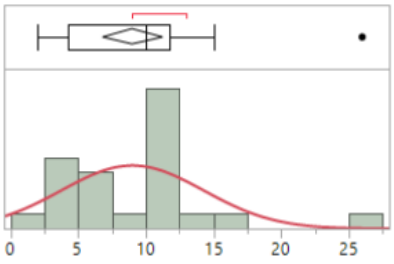
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.835765	0.0012*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

EfishTime



Normal(9,5.25853)

Quantiles

100.0%	maximum	26
99.5%		26
97.5%		26
90.0%		14
75.0%	quartile	11.75
50.0%	median	10
25.0%	quartile	4.25
10.0%		3
2.5%		2
0.5%		2
0.0%	minimum	2

Summary Statistics

Mean	9
Std Dev	5.2585334
Std Err Mean	1.0733936
Upper 95% Mean	11.220484
Lower 95% Mean	6.7795161
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	9	6.7795161	11.220484
Dispersion	σ	5.2585334	4.0870036	7.3764636

Measure	
-2*LogLikelihood	146.78195
AICc	151.35338
BIC	153.13806

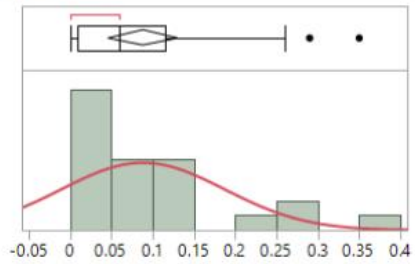
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.866551	0.0045*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Fine



Normal(0.08833,0.09876)

Quantiles

100.0%	maximum	0.35
99.5%		0.35
97.5%		0.35
90.0%		0.275
75.0%	quartile	0.115
50.0%	median	0.06
25.0%	quartile	0.01
10.0%		0.005
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	0.0883333
Std Dev	0.0987604
Std Err Mean	0.0201594
Upper 95% Mean	0.1300362
Lower 95% Mean	0.0466305
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.0883333	0.0466305	0.1300362
Dispersion	σ	0.0987604	0.076758	0.1385372

Measure	
-2*LogLikelihood	-44.01375
AICc	-39.44232
BIC	-37.65764

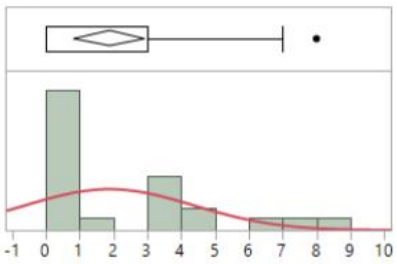
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.803822	0.0003*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Large



Normal(1.875,2.4902)

Quantiles

100.0%	maximum	8
99.5%		8
97.5%		8
90.0%		6.5
75.0%	quartile	3
50.0%	median	0
25.0%	quartile	0
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	1.875
Std Dev	2.4901982
Std Err Mean	0.5083096
Upper 95% Mean	2.9265185
Lower 95% Mean	0.8234815
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.875	0.8234815	2.9265185
Dispersion	σ	2.4901982	1.9354159	3.4931519

Measure	
-2*LogLikelihood	110.90244
AICc	115.47387
BIC	117.25855

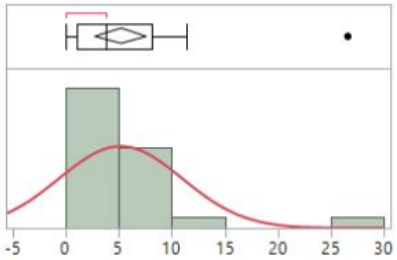
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.762741	<.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Larval Density



Normal(5.17917,5.80472)

Quantiles

100.0%	maximum	26.6
99.5%		26.6
97.5%		26.6
90.0%		10.5
75.0%	quartile	8.1
50.0%	median	3.8
25.0%	quartile	1.025
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	5.1791667
Std Dev	5.8047192
Std Err Mean	1.1848833
Upper 95% Mean	7.6302846
Lower 95% Mean	2.7280487
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	5.1791667	2.7280487	7.6302846
Dispersion	σ	5.8047192	4.5115066	8.1426314

Measure	
-2*LogLikelihood	151.52527
AICc	156.0967
BIC	157.88138

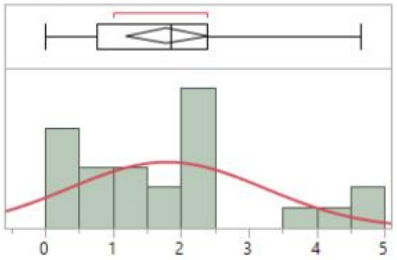
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.772183	0.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

LOG Sed eDNA



Normal(1.79338,1.43965)

Quantiles

100.0%	maximum	4.642542867
99.5%		4.642542867
97.5%		4.642542867
90.0%		4.5562977515
75.0%	quartile	2.377725755
50.0%	median	1.8582667215
25.0%	quartile	0.7587817193
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	1.7933807
Std Dev	1.4396493
Std Err Mean	0.2938672
Upper 95% Mean	2.4012913
Lower 95% Mean	1.1854701
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	1.7933807	1.1854701	2.4012913
Dispersion	σ	1.4396493	1.118915	2.0194833

Measure	
-2*LogLikelihood	84.600226
AICc	89.171655
BIC	90.956334

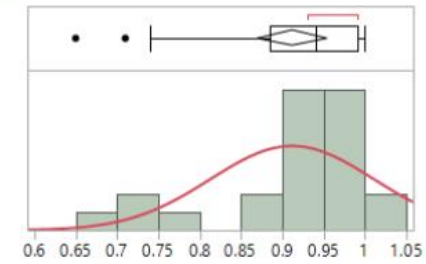
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.904322	0.0266*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Sand



Normal(0.91167,0.09876)

Quantiles

100.0%	maximum	1
99.5%		1
97.5%		1
90.0%		0.995
75.0%	quartile	0.99
50.0%	median	0.94
25.0%	quartile	0.885
10.0%		0.725
2.5%		0.65
0.5%		0.65
0.0%	minimum	0.65

Summary Statistics

Mean	0.9116667
Std Dev	0.0987604
Std Err Mean	0.0201594
Upper 95% Mean	0.9533695
Lower 95% Mean	0.8699638
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.9116667	0.8699638	0.9533695
Dispersion	σ	0.0987604	0.076758	0.1385372

Measure	
-2*LogLikelihood	-44.01375
AICc	-39.44232
BIC	-37.65764

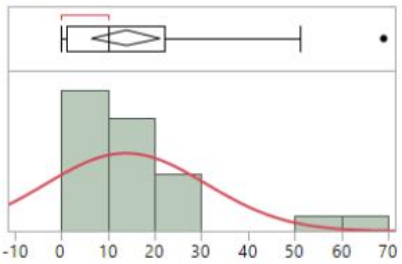
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.803822	0.0003*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Small



Normal(13.875,17.1498)

Quantiles

100.0%	maximum	69
99.5%		69
97.5%		69
90.0%		40
75.0%	quartile	22
50.0%	median	10
25.0%	quartile	1
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	13.875
Std Dev	17.149756
Std Err Mean	3.5006793
Upper 95% Mean	21.116707
Lower 95% Mean	6.6332932
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	13.875	6.6332932	21.116707
Dispersion	σ	17.149756	13.329023	24.057002

Measure	
-2*LogLikelihood	203.52428
AICc	208.09571
BIC	209.88039

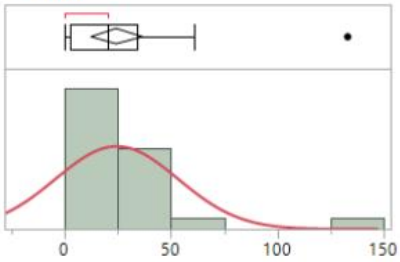
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.778047	0.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Total Efish



Normal(24.5,28.7795)

Quantiles

100.0%	maximum	133
99.5%		133
97.5%		133
90.0%		53.5
75.0%	quartile	33.75
50.0%	median	20.5
25.0%	quartile	2.75
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	24.5
Std Dev	28.779522
Std Err Mean	5.8745953
Upper 95% Mean	36.652526
Lower 95% Mean	12.347474
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	24.5	12.347474	36.652526
Dispersion	σ	28.779522	22.367835	40.370779

Measure	
-2*LogLikelihood	228.37293
AICc	232.94435
BIC	234.72903

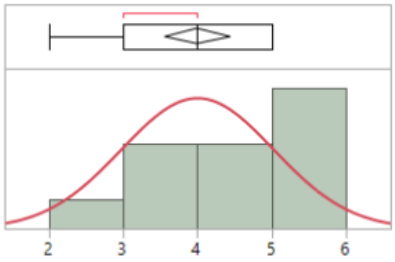
Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.750595	<.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Water Clarity



Normal(4,1.02151)

Quantiles

100.0%	maximum	5
99.5%		5
97.5%		5
90.0%		5
75.0%	quartile	5
50.0%	median	4
25.0%	quartile	3
10.0%		2.5
2.5%		2
0.5%		2
0.0%	minimum	2

Summary Statistics

Mean	4
Std Dev	1.0215078
Std Err Mean	0.2085144
Upper 95% Mean	4.4313449
Lower 95% Mean	3.5686551
N	24

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	4	3.5686551	4.4313449
Dispersion	σ	1.0215078	0.7939298	1.432931

Measure	
-2*LogLikelihood	68.13048
AICc	72.701909
BIC	74.486588

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.829721	0.0009*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

6. Grid-level Statistics: Larval Detection Method Multivariate Analysis

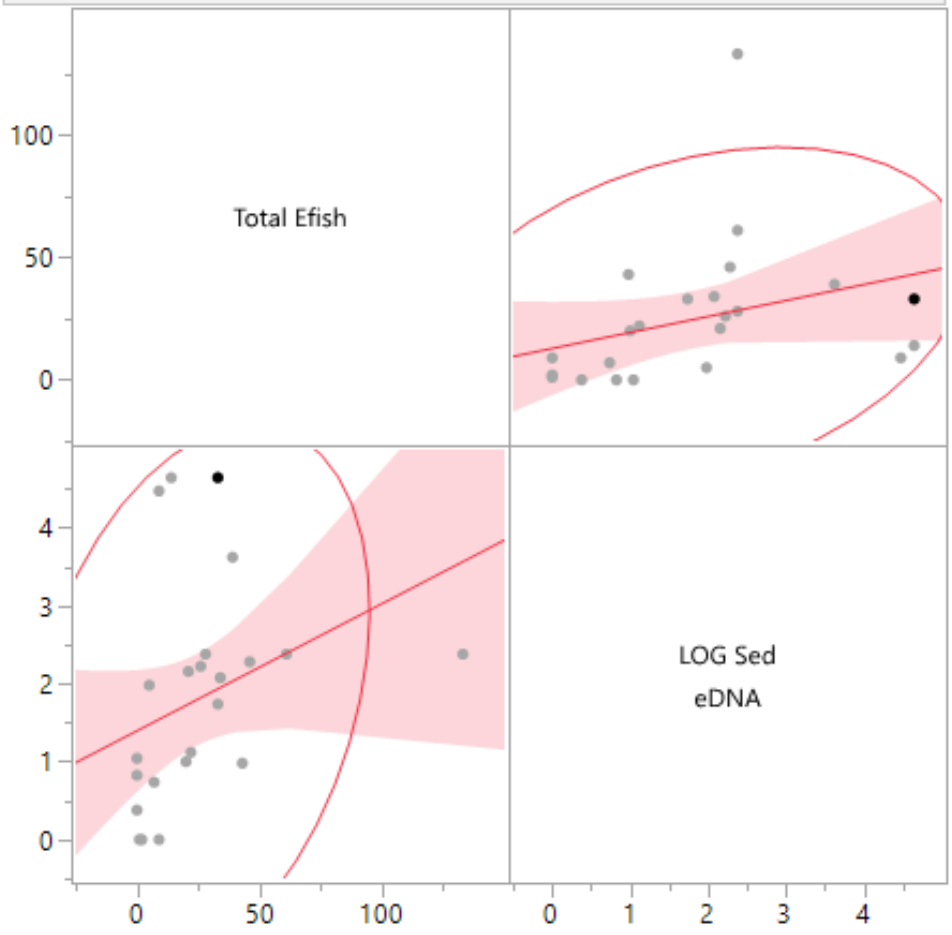
Multivariate

Correlations

	Total Efish	LOG Sed eDNA
Total Efish	1.0000	0.3260
LOG Sed eDNA	0.3260	1.0000

The correlations are estimated by Row-wise method.

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
LOG Sed eDNA	Total Efish	0.6187	0.0013*	

7. Grid-level Statistics: Multivariate Analysis with Total Electrofishing Detections

Multivariate

Correlations

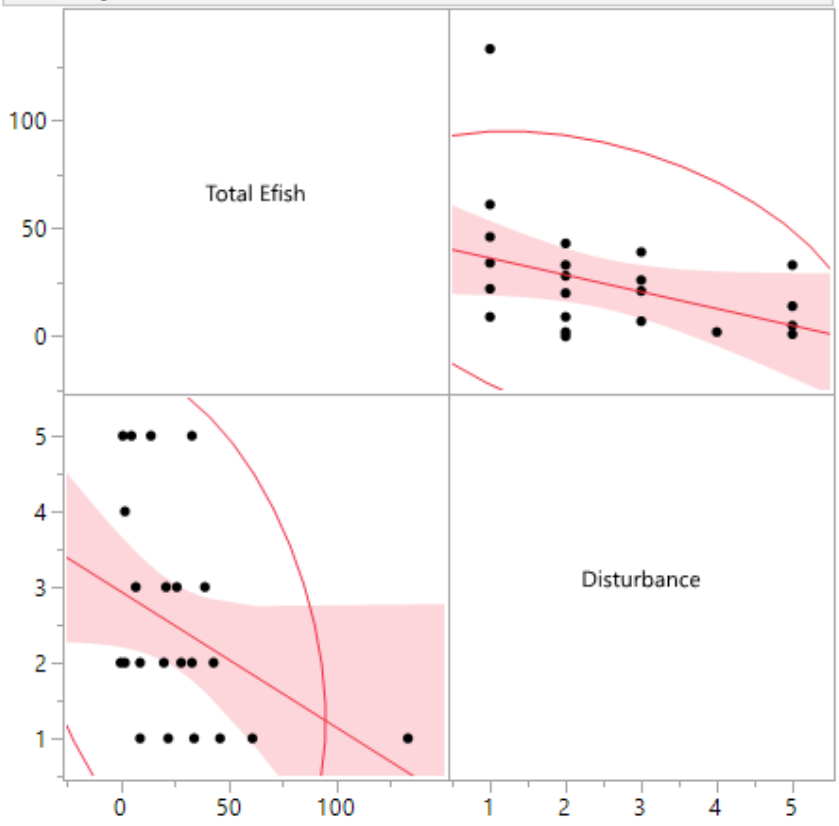
	Total Efish	Disturbance
Total Efish	1.0000	-0.3746
Disturbance	-0.3746	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Disturbance
Total Efish	<.0001	0.0713
Disturbance	0.0713	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Disturbance	Total Efish	-0.3792	0.0676	

Multivariate

Correlations

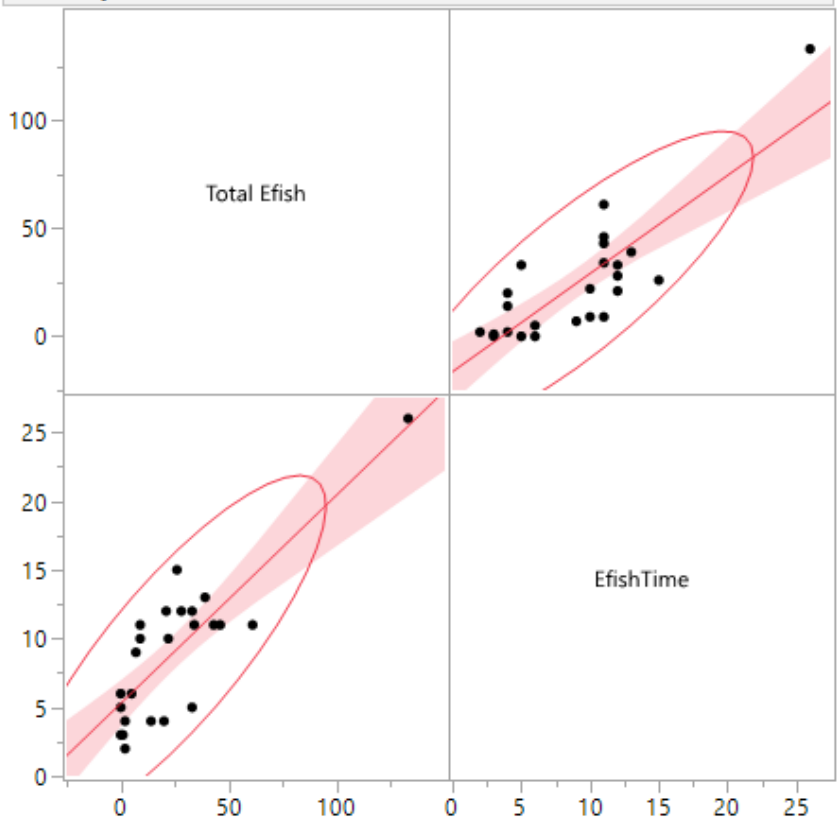
	Total Efish	EfishTime
Total Efish	1.0000	0.8300
EfishTime	0.8300	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	EfishTime
Total Efish	<.0001	<.0001
EfishTime	<.0001	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
EfishTime	Total Efish	0.7275	<.0001*	

Multivariate

Correlations

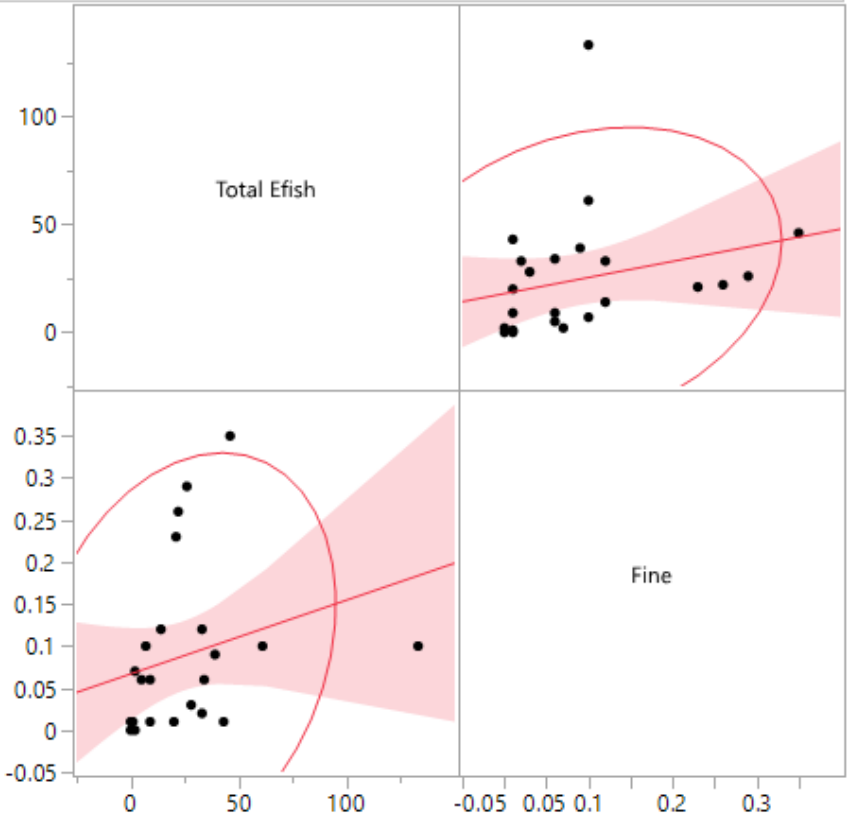
	Total Efish	Fine
Total Efish	1.0000	0.2562
Fine	0.2562	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Fine
Total Efish	<.0001	0.2268
Fine	0.2268	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Total Efish	0.5315	0.0075*	

Multivariate

Correlations

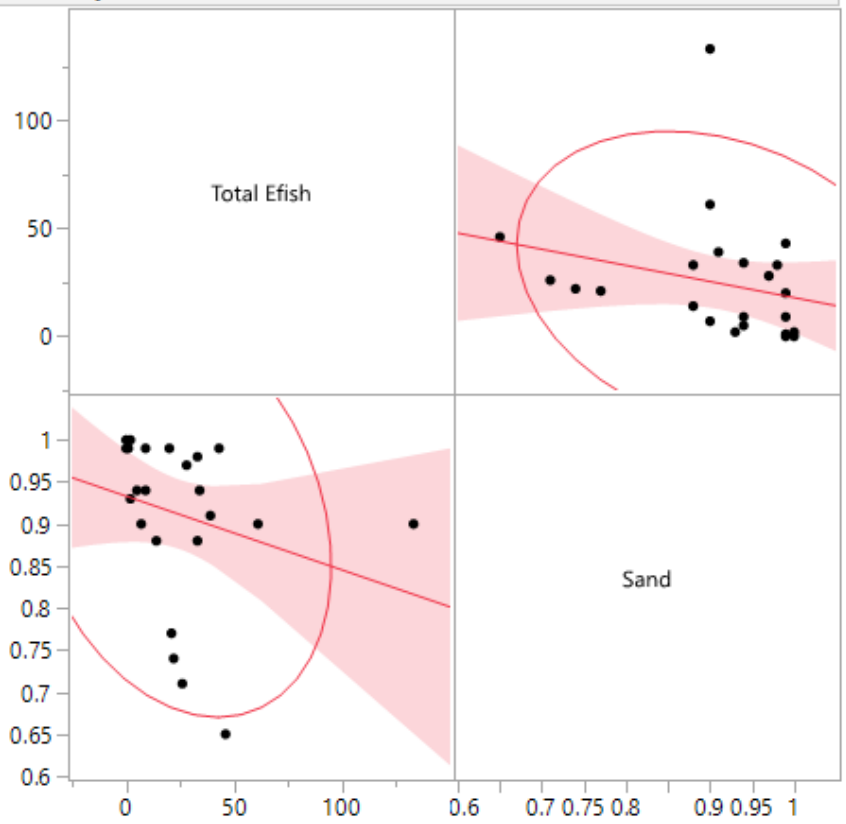
	Total Efish	Sand
Total Efish	1.0000	-0.2562
Sand	-0.2562	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Sand
Total Efish	<.0001	0.2268
Sand	0.2268	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Total Efish	-0.5315	0.0075*	

Multivariate

Correlations

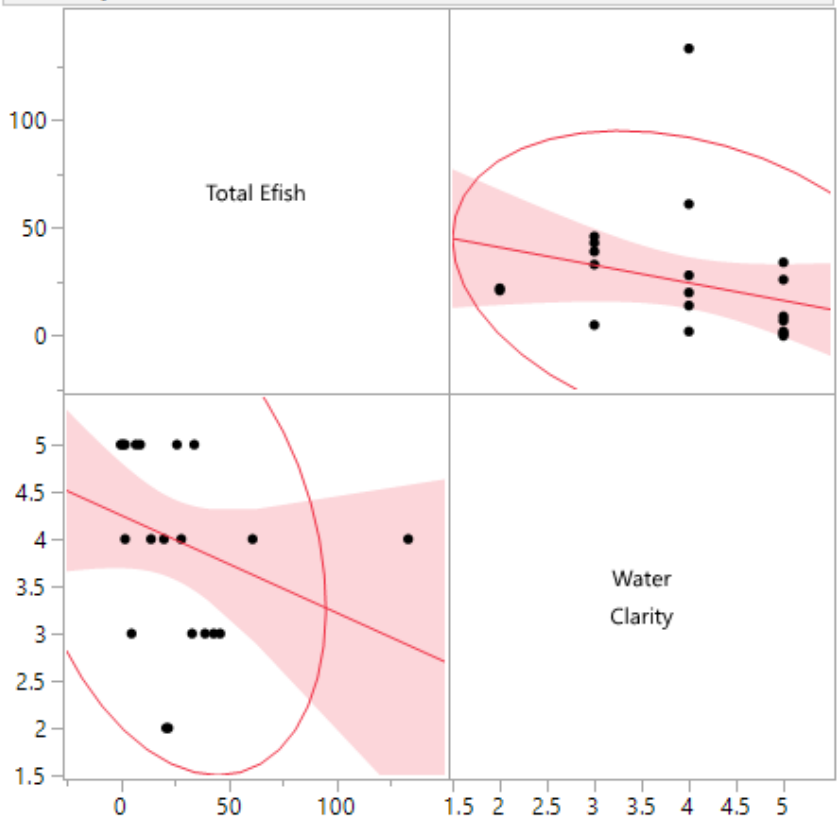
	Total Efish	Water Clarity
Total Efish	1.0000	-0.2913
Water Clarity	-0.2913	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Total Efish	Water Clarity
Total Efish	<.0001	0.1672
Water Clarity	0.1672	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Water Clarity	Total Efish	-0.5406	0.0064*	

8. Grid-level Statistics: Multivariate Analysis with LOG Sediment eDNA Concentrations

Multivariate

Correlations

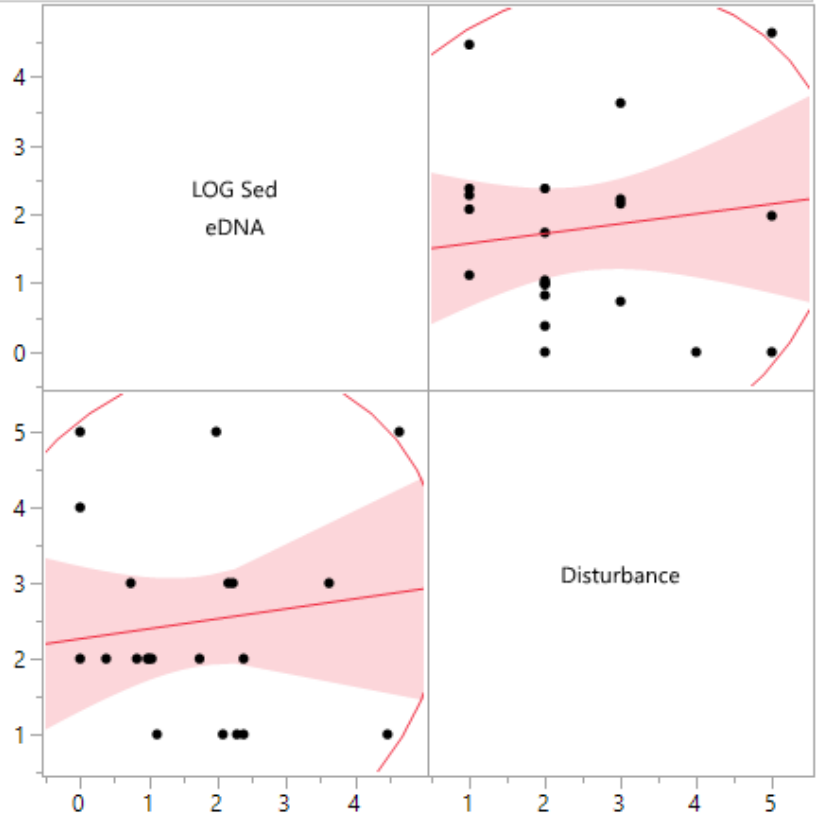
	LOG Sed eDNA	Disturbance
LOG Sed eDNA	1.0000	0.1377
Disturbance	0.1377	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Disturbance
LOG Sed eDNA	<.0001	0.5211
Disturbance	0.5211	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Disturbance	LOG Sed eDNA	-0.0878	0.6833	

Multivariate

Correlations

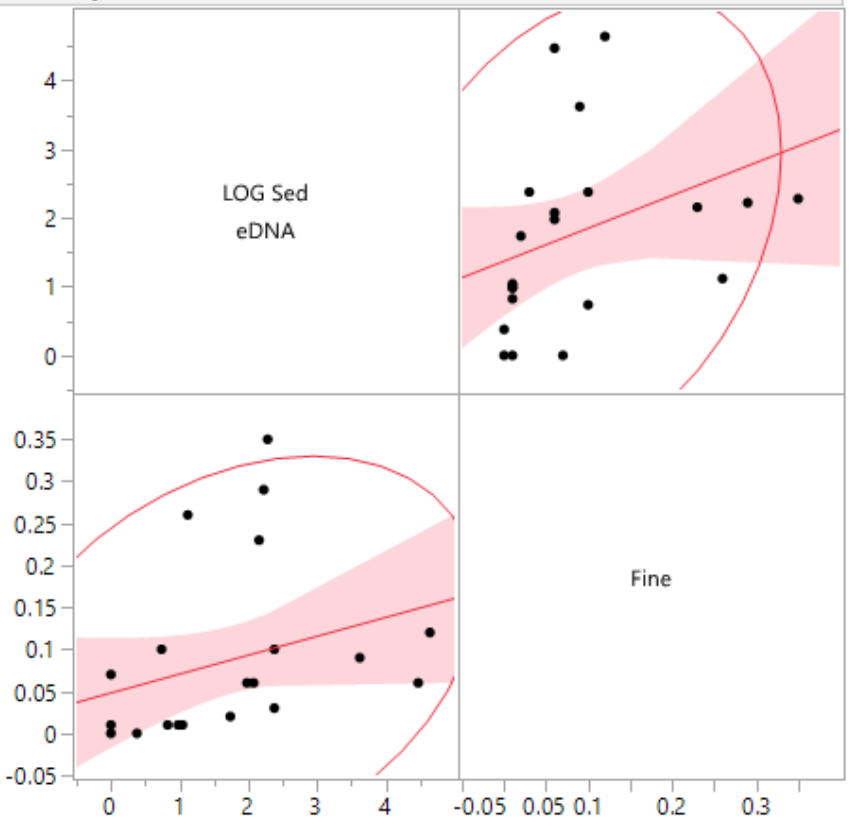
	LOG Sed eDNA	Fine
LOG Sed eDNA	1.0000	0.3281
Fine	0.3281	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Fine
LOG Sed eDNA	<.0001	0.1176
Fine	0.1176	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	LOG Sed eDNA	0.6248	0.0011*	

Multivariate

Correlations

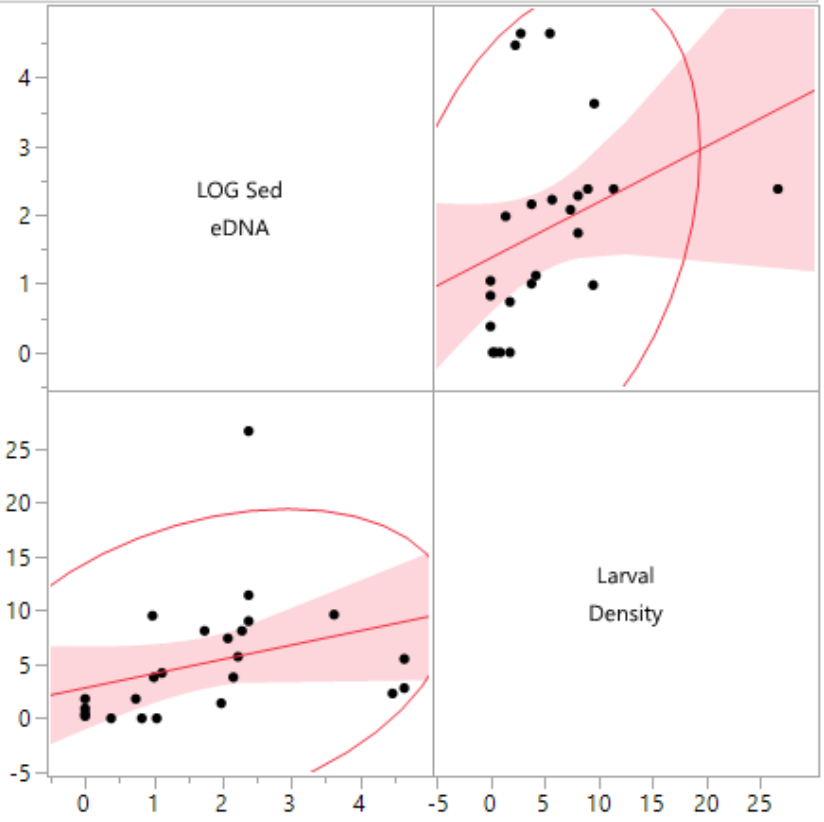
	LOG Sed eDNA	Larval Density
LOG Sed eDNA	1.0000	0.3282
Larval Density	0.3282	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Larval Density
LOG Sed eDNA	<.0001	0.1174
Larval Density	0.1174	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Larval Density	LOG Sed eDNA	0.6248	0.0011*	

Multivariate

Correlations

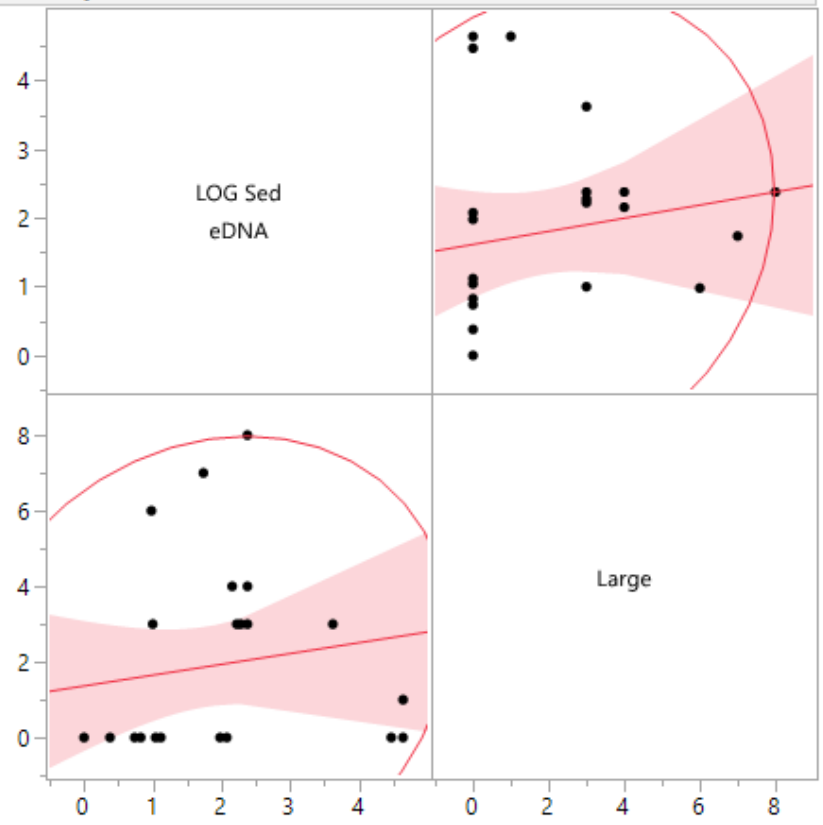
	LOG Sed eDNA	Large
LOG Sed eDNA	1.0000	0.1654
Large	0.1654	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Large
LOG Sed eDNA	<.0001	0.4399
Large	0.4399	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Large	LOG Sed eDNA	0.4129	0.0449*	

Multivariate

Correlations

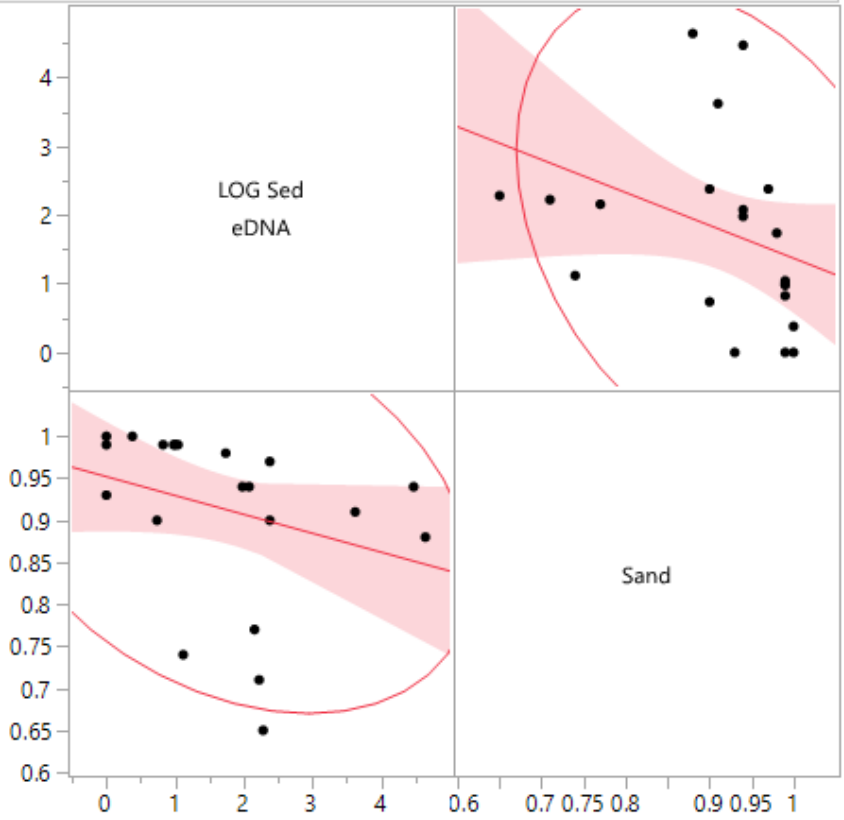
	LOG Sed eDNA	Sand
LOG Sed eDNA	1.0000	-0.3281
Sand	-0.3281	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Sand
LOG Sed eDNA	<.0001	0.1176
Sand	0.1176	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	LOG Sed eDNA	-0.6248	0.0011*	

Multivariate

Correlations

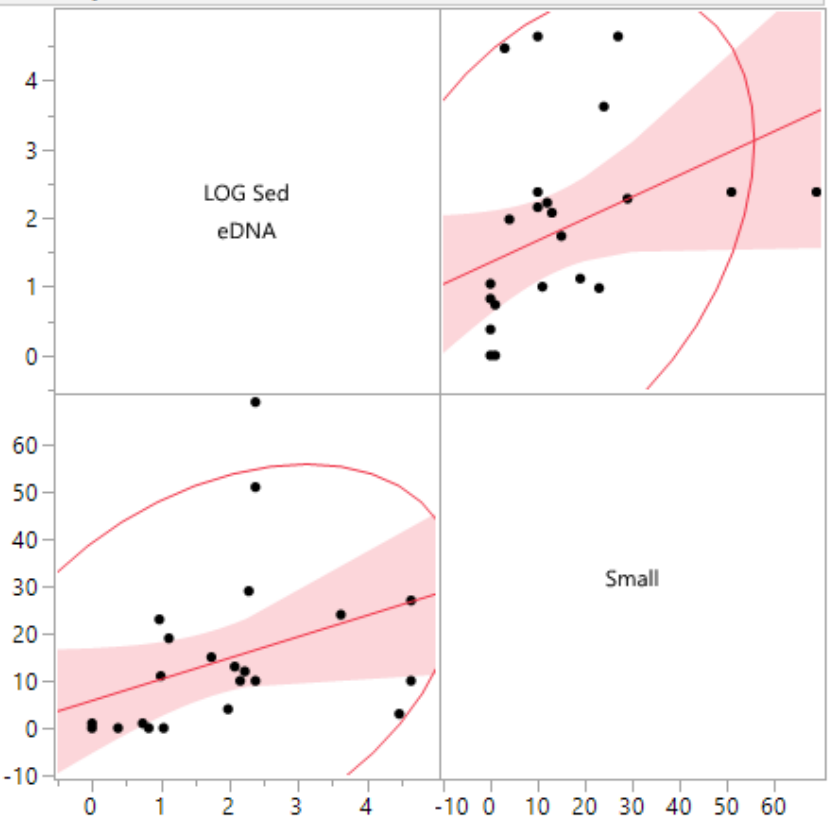
	LOG Sed eDNA	Small
LOG Sed eDNA	1.0000	0.3783
Small	0.3783	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Small
LOG Sed eDNA	<.0001	0.0683
Small	0.0683	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Small	LOG Sed eDNA	0.6626	0.0004*	

Multivariate

Correlations

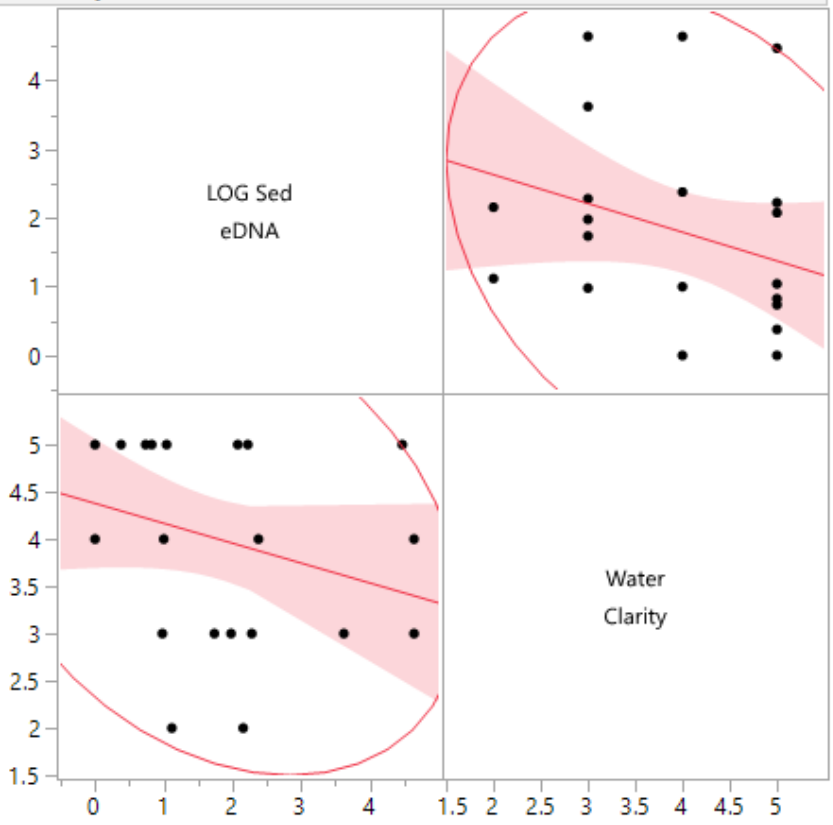
	LOG Sed eDNA	Water Clarity
LOG Sed eDNA	1.0000	-0.2967
Water Clarity	-0.2967	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	LOG Sed eDNA	Water Clarity
LOG Sed eDNA	<.0001	0.1591
Water Clarity	0.1591	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Water Clarity	LOG Sed eDNA	-0.3959	0.0555	

9. Grid-level Statistics: Multivariate Analysis with Larval and Sediment Size

Multivariate

Correlations

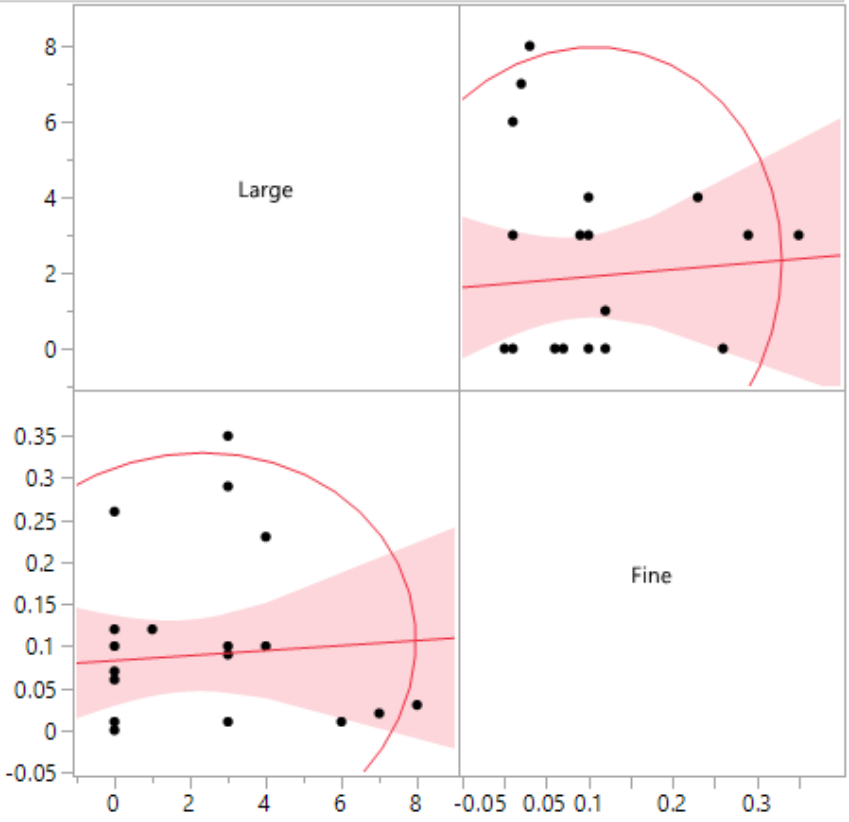
	Large	Fine
Large	1.0000	0.0751
Fine	0.0751	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Large	Fine
Large	<.0001	0.7271
Fine	0.7271	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Large	0.2319	0.2754	

Multivariate

Correlations

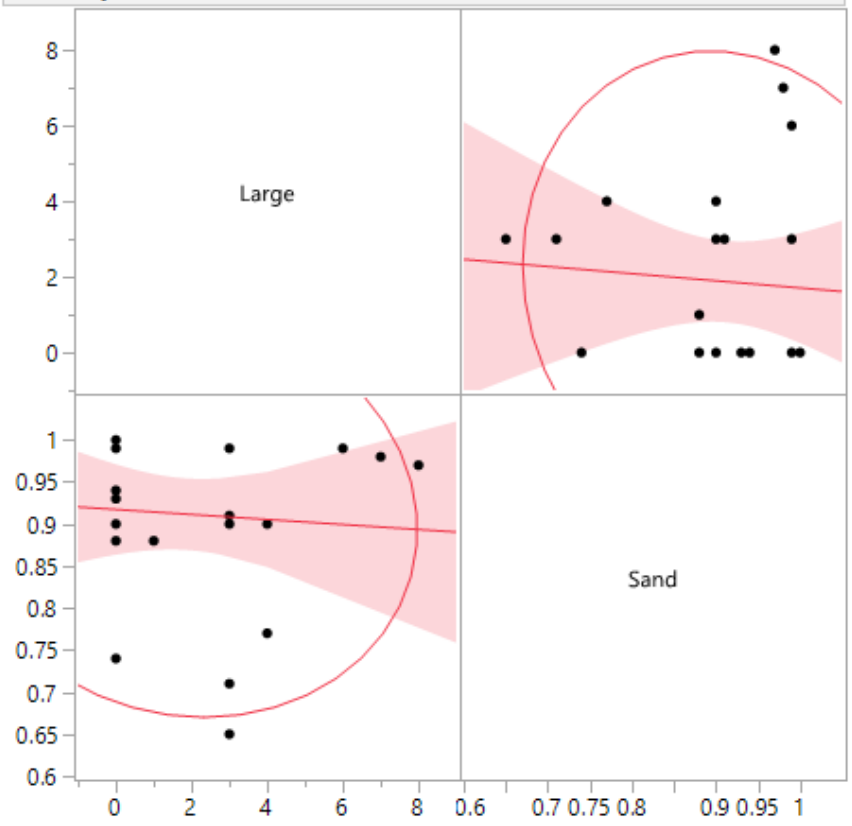
	Large	Sand
Large	1.0000	-0.0751
Sand	-0.0751	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Large	Sand
Large	<.0001	0.7271
Sand	0.7271	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Large	-0.2319	0.2754	

Multivariate

Correlations

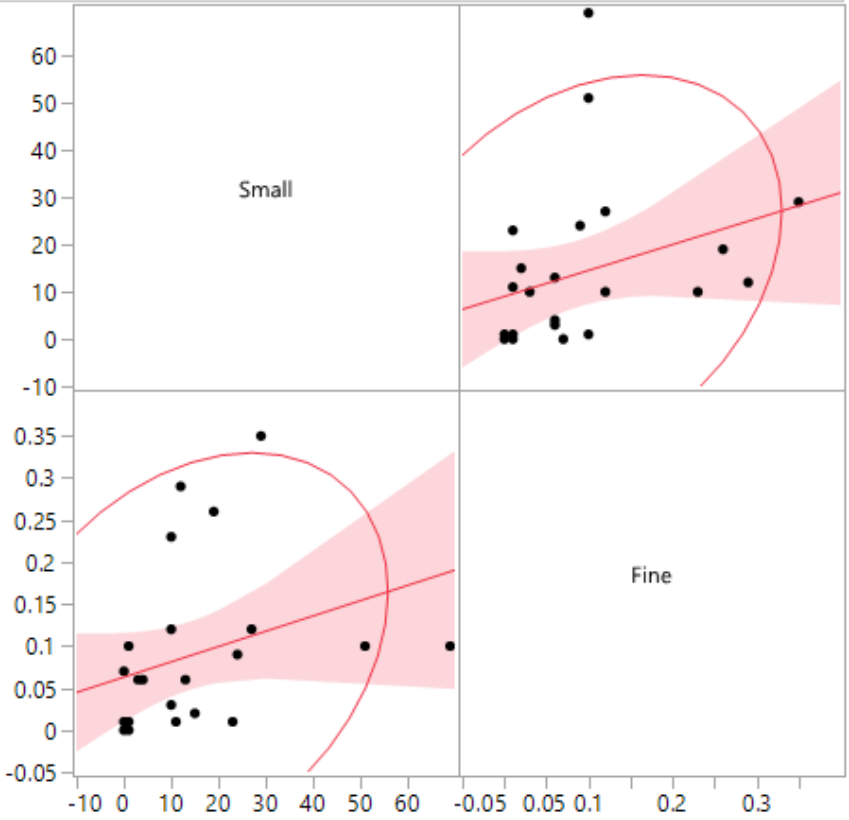
	Small	Fine
Small	1.0000	0.3159
Fine	0.3159	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Small	Fine
Small	<.0001	0.1327
Fine	0.1327	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Small	0.5761	0.0032*	

Multivariate

Correlations

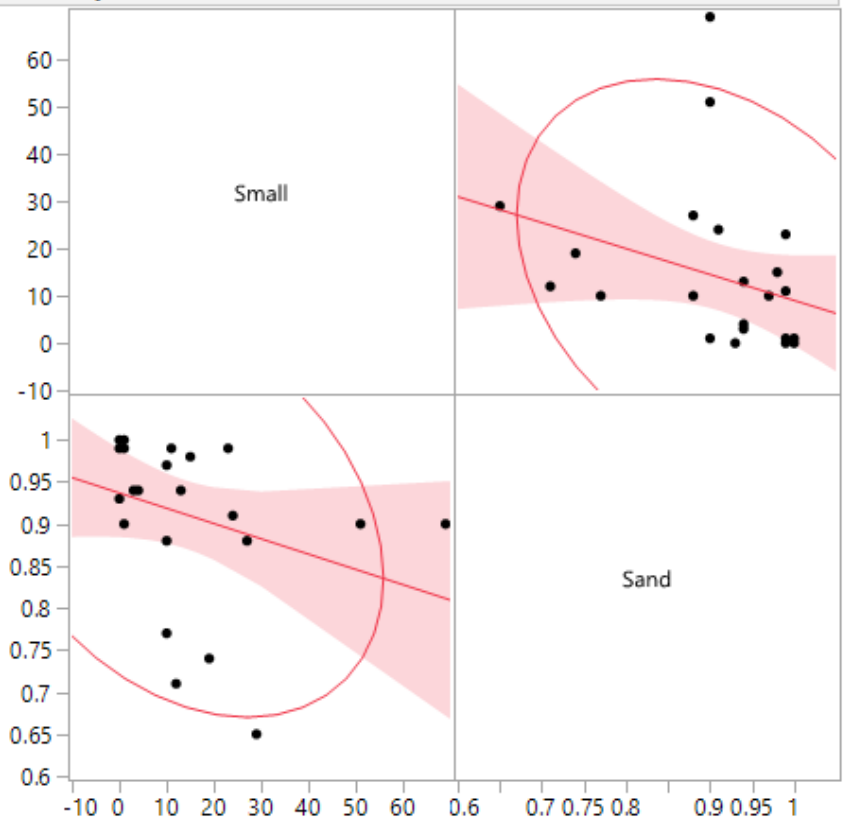
	Small	Sand
Small	1.0000	-0.3159
Sand	-0.3159	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Small	Sand
Small	<.0001	0.1327
Sand	0.1327	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Small	-0.5761	0.0032*	

Multivariate

Correlations

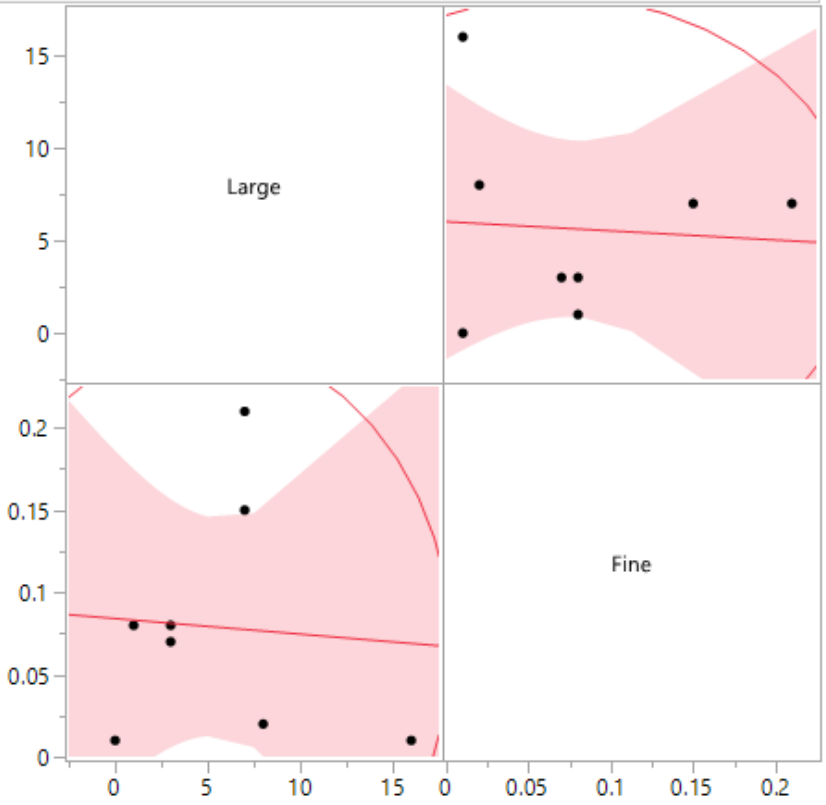
	Large	Fine
Large	1.0000	-0.0682
Fine	-0.0682	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Large	Fine
Large	<.0001	0.8725
Fine	0.8725	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Large	-0.0183	0.9657	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

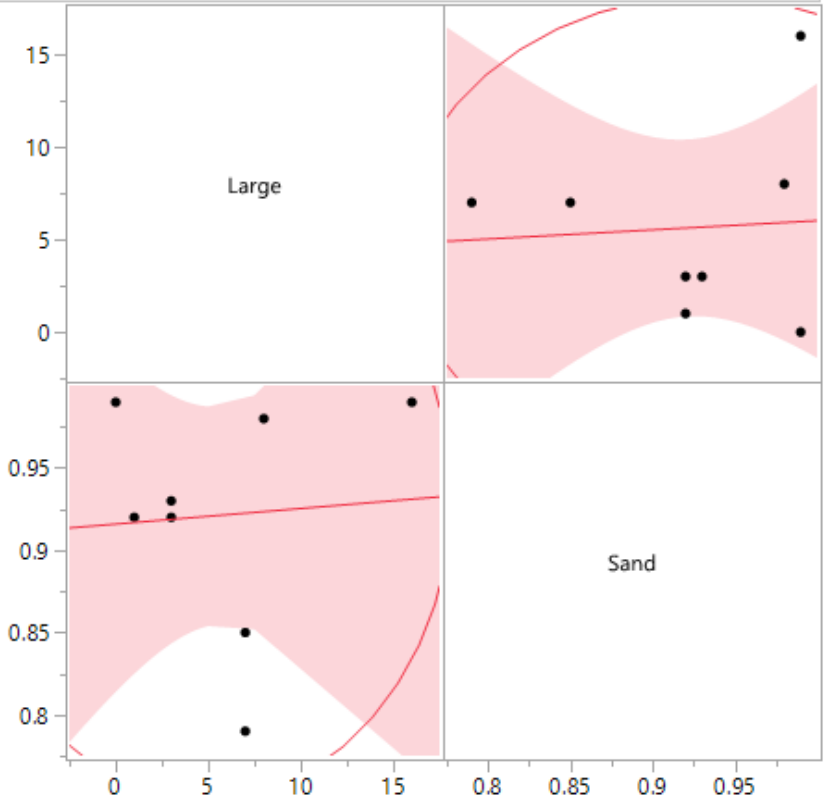
	Large	Sand
Large	1.0000	0.0682
Sand	0.0682	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Large	Sand
Large	<.0001	0.8725
Sand	0.8725	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Large	0.0183	0.9657	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

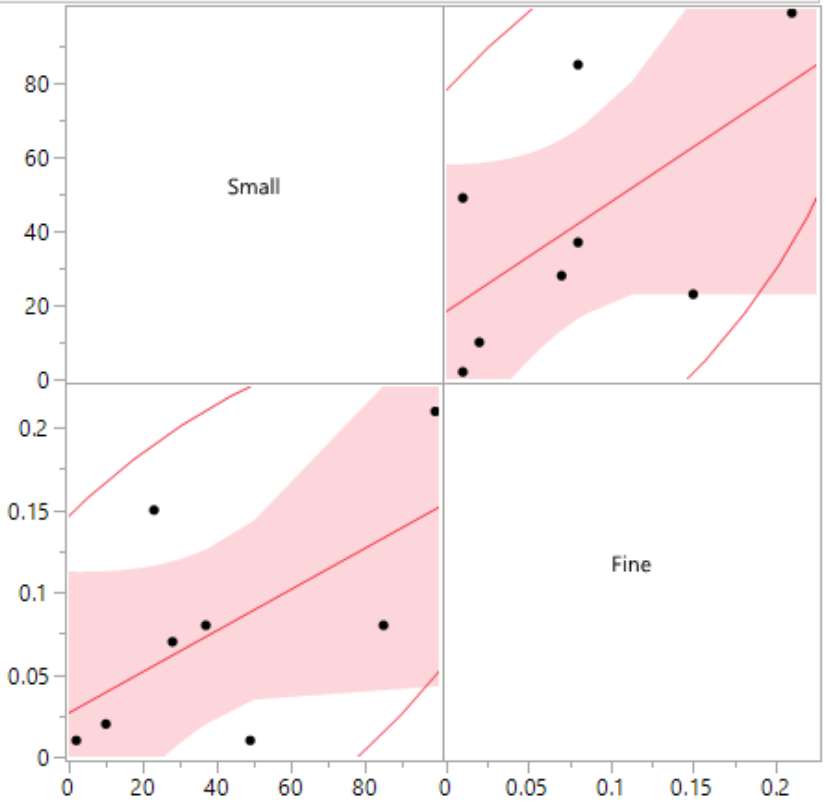
	Small	Fine
Small	1.0000	0.6085
Fine	0.6085	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Small	Fine
Small	<.0001	0.1094
Fine	0.1094	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Fine	Small	0.5181	0.1884	

Warning: sample size of 8 is too small, P value suspect.

Multivariate

Correlations

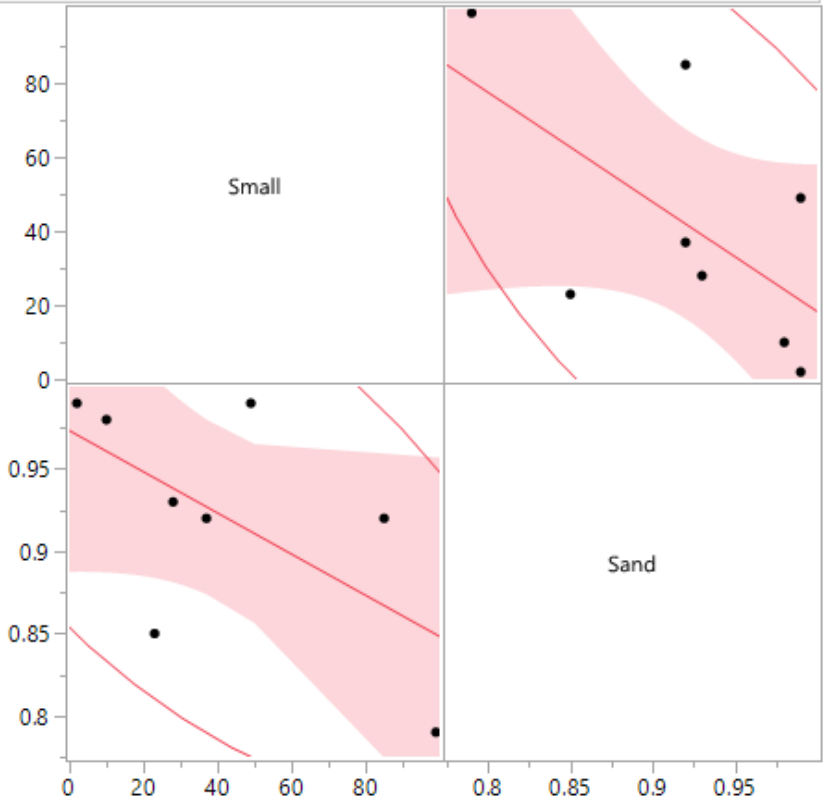
	Small	Sand
Small	1.0000	-0.6085
Sand	-0.6085	1.0000

The correlations are estimated by Row-wise method.

Correlation Probability

	Small	Sand
Small	<.0001	0.1094
Sand	0.1094	<.0001

Scatterplot Matrix



Nonparametric: Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|	-.8 -.6 -.4 -.2 0 .2 .4 .6 .8
Sand	Small	-0.5181	0.1884	

Warning: sample size of 8 is too small, P value suspect.

Appendix E. Sample of R Script for eDNA Occupancy Model

From Dorazio and Erickson (2018): EDNAOCCUPANCY may be downloaded from the following repository: https://doi.org/10.5066/f7q23z67. This repository includes instructions for installing the package. A vignette is included to provide guidance for new users.

Lampetra spp. eDNA Detections in Sediment

Open R Project in RStudio®:

```
> install.packages("mvtnorm")

> install.packages("pROC")
```

Open R Script: COVARIATES_Lamprey_SEDIMENT_eDNA_Occupancy.R

```
> #Download and load Libraries
> install.packages("eDNAoccupancy_0.2.2.tar.gz", repos=NULL,
type="source")
> library(mvtnorm)
> library(pROC)
> library(eDNAoccupancy)

> ####################################

> ##Lampetra spp. estimates
>
> #Read in the detection data
> LampetraDetections<-
read.csv("LamptDetections_02062019_addedPCRs.csv", header =
TRUE,row.names = NULL, na.strings = "", stringsAsFactors = F)#read in
the file

> LampetraDetections
   site sample pcr1 pcr2 pcr3
1   1-S      1    1    1    1
2   1-S      2    1    1    1
3   1-S      3    1    1    1
4   1-S      4    1    1    1
5   1-S      5    1    1    1
6   1-S      6    1    1    1
7   1-S      7    0    0    0
8   1-S      8    0    0    0
9   1-S      9    0    0    0
10  2-S      1    0    0    0
11  2-S      2    1    0    0
12  2-S      3    0    0    0
13  2-S      4    0    0    0
14  2-S      5    0    0    0
15  2-S      6    0    0    0
16  2-S      7    0    0    0
17  2-S      8    0    0    0
18  2-S      9    0    0    0
19  3-S      1    1    1    1
```

20	3-S	2	1	1	1
21	3-S	3	1	1	1
22	3-S	4	1	0	1
23	3-S	5	1	1	1
24	3-S	6	1	1	0
25	3-S	7	1	1	0
26	3-S	8	1	0	0
27	3-S	9	0	0	0
28	4-S	1	1	0	0
29	4-S	2	1	0	0
30	4-S	3	1	1	0
31	4-S	4	1	1	1
32	4-S	5	1	1	1
33	4-S	6	1	1	1
34	4-S	7	1	1	1
35	4-S	8	1	1	1
36	4-S	9	1	1	1
37	5-S	1	1	1	1
38	5-S	2	1	1	1
39	5-S	3	1	1	1
40	5-S	4	1	1	1
41	5-S	5	1	1	1
42	5-S	6	1	1	1
43	5-S	7	1	1	0
44	5-S	8	1	1	1
45	5-S	9	1	1	0
46	6-S	1	1	1	1
47	6-S	2	1	1	1
48	6-S	3	1	1	1
49	6-S	4	0	0	0
50	6-S	5	0	0	0
51	6-S	6	0	0	0
52	6-S	7	1	1	1
53	6-S	8	1	1	1
54	6-S	9	1	1	1
55	7-S	1	0	0	0
56	7-S	2	0	0	0
57	7-S	3	1	1	0
58	7-S	4	1	1	1
59	7-S	5	1	1	1
60	7-S	6	1	1	1
61	7-S	7	1	1	1
62	7-S	8	1	1	1
63	7-S	9	1	1	1
64	8-S	1	1	1	1
65	8-S	2	1	1	1
66	8-S	3	1	1	1
67	8-S	4	1	1	1
68	8-S	5	1	1	1
69	8-S	6	1	1	1
70	8-S	7	1	1	1
71	8-S	8	1	1	1
72	8-S	9	1	1	1

```
> head(Lampetra Detections)
  site sample pcr1 pcr2 pcr3
1  1-S      1    1    1    1
2  1-S      2    1    1    1
3  1-S      3    1    1    1
4  1-S      4    1    1    1
5  1-S      5    1    1    1
6  1-S      6    1    1    1

> #Read in the detection data into proper format
> Lampt_Detections = occData(Lampetra Detections, siteColName = 'site',
sampleColName = 'sample')

> # Number of detections per sample
> head(Lampt_Detections$y)
    [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9]
1-S    3    3    3    3    3    3    0    0    0
2-S    0    1    0    0    0    0    0    0    0
3-S    3    3    3    2    3    2    2    1    0
4-S    1    1    2    3    3    3    3    3    3
5-S    3    3    3    3    3    3    2    3    2
6-S    3    3    3    0    0    0    3    3    3

> # Number of PCR replicated per sample
> head(Lampt_Detections$K)
    [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9]
1-S    3    3    3    3    3    3    3    3    3
2-S    3    3    3    3    3    3    3    3    3
3-S    3    3    3    3    3    3    3    3    3
4-S    3    3    3    3    3    3    3    3    3
5-S    3    3    3    3    3    3    3    3    3
6-S    3    3    3    3    3    3    3    3    3

> #set seed for model
> set.seed(3434)

> #Read in covariate data
> LampSurveyData<-
read.csv("Lamprey_Site_Data_covariates_03112019.csv", header =
TRUE,row.names = NULL, na.strings = "", stringsAsFactors = F)#read in
the file
> LampSurveyData.sc = scaleData(LampSurveyData)
> LampSurveyData.sc
  site      Small      Large    Captured  ElectroTotal  Larvae_m2 Temp_C
1 1-S -0.1338964 -0.9024007 -0.2582932 -0.4563561 -0.49979742 0.9707382
2 2-S -1.1471661 -1.0975143 -1.2635426 -1.1185199 -1.33462388 1.0542429
3 3-S -0.9155616 0.4633949 -0.8167651 -0.8142825 -0.93917977 -0.4488360
4 4-S  0.2135104 2.0243042  0.4956438  0.4026671  0.81834962 -0.0313141
5 5-S -0.5392043 0.2682813 -0.4816820 -0.3489782 -0.49979742 -0.2818274
6 6-S -0.3944514-0.5121734 -0.4537584 -0.4921487 -0.06041507 -1.4508883
7 7-S  1.6610386 0.2682813  1.6405111  0.9932456  1.25773197  1.2212513
8 8-S 1.2557307 -0.5121734  1.1378865  1.8343726  1.25773197 -1.0333667
     DO_.    spConductivity_µS.cm  spCondTemp_C       pH   Turbidity_NTU
1  1.1720691           0.66278496   0.97073829  1.5568217   -1.13679712
2  0.4351585           0.92094839   1.05424266 -0.6194508   -1.89466187
3 -1.5214664           0.48405643  -0.44883599 -0.7505516    0.56839856
```

```
4  0.2826942           0.50391516  -0.03131414 -0.5407909    0.66313165
5 -0.3017522          -1.50181611  -0.28182725 -0.4883506    0.66313165
6 -1.0894843          -1.64082719  -1.45088842 -1.0127531    0.94733093
7  1.2991227           0.04716447   1.22125140  1.3470595    0.09473309
8 -0.2763415           0.52377388  -1.03336657  0.5080157    0.09473309
Streamflow_cfs     Fine      Sand   larvalHabDesc larvalHabType AquaVeg
1   0.1443376 0.01760551 -0.01760551  0.9354143  -0.9752369 -1.1788579
2   0.4330127 -0.96830300  0.96830300 -0.9354143  -0.1950474 -0.1309842
3   0.4330127 -0.82745893  0.82745893  0.9354143   0.5851421 -0.1309842
4   0.4330127 -0.96830300  0.96830300 -0.9354143  -0.1950474 -0.1309842
5   0.4330127  1.00351402 -1.00351402 -0.9354143   1.3653316  1.4408263
6   0.4330127 -0.12323856  0.12323856  0.9354143  -0.9752369 -0.1309842
7   0.1443376  1.84857845 -1.84857845  0.9354143   1.3653316  1.4408263
8  -2.4537386  0.01760551 -0.01760551 -0.9354143  -0.9752369 -1.1788579
    Detritus
1 -1.6397062
2 -0.6306562
3 -0.6306562
4  0.8829187
5  0.8829187
6 -0.6306562
7  0.8829187
8  0.8829187
```

Fit the Occupancy Model with Covariates:

ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large+Per_Fine):

```
> #Fit the occ model with covariates
> fit = occModel(formulaSite = ~ 1,
+                formulaSiteAndSample = ~ Lamprey+AquaticVeg,
+                formulaReplicate = ~ Lamprey+Large+Per_Fine,
+                detectionMats = Lampt_Detections,
+                siteData = LampSurveyData.sc,
+                niter = 11000,niterInterval = 2000,
+                siteColName = 'site')
Begin MCMC sampling:

..... drawing sample # 2000  after  0.9692745  minutes
..... drawing sample # 4000  after  1.922542  minutes
..... drawing sample # 6000  after  2.958568  minutes
..... drawing sample # 8000  after  3.965242  minutes
..... drawing sample # 10000  after  4.925806  minutes
Completed  11000  draws of MCMC algorithm
>
> posteriorSummary(fit, burnin=1000, mcError=TRUE)
Bayesian estimates of model parameters
                    Mean    50%   2.5% 97.5%
beta..Intercept.   1.478  1.437  0.401 2.751
alpha..Intercept.  0.883  0.879  0.513 1.288
alpha.Lamprey      0.741  0.724  0.299 1.267
alpha.AquaticVeg   0.055  0.057 -0.360 0.453
delta..Intercept.  1.542  1.532  1.181 1.949
delta.Lamprey      0.547  0.522  0.052 1.148
delta.Large       -0.353 -0.349 -0.768 0.021
delta.Per_Fine     0.150  0.146 -0.248 0.571
```

```
Monte Carlo SE of Bayesian estimates
                     Mean    50%   2.5%  97.5%
beta..Intercept.  0.0058 0.0069 0.0118 0.0174
alpha..Intercept. 0.0023 0.0029 0.0049 0.0062
alpha.Lamprey     0.0029 0.0037 0.0054 0.0086
alpha.AquaticVeg  0.0024 0.0032 0.0070 0.0054
delta..Intercept. 0.0024 0.0032 0.0043 0.0058
delta.Lamprey     0.0034 0.0047 0.0061 0.0085
delta.Large       0.0026 0.0032 0.0054 0.0049
delta.Per_Fine    0.0029 0.0035 0.0052 0.0076
NULL
> #assess how the run went
> plotTrace(fit, c('beta..Intercept.', 'alpha..Intercept.',
'delta..Intercept.'), burnin=1000)
> plotACF(fit, c('beta..Intercept.', 'alpha..Intercept.',
'delta..Intercept.'), burnin=1000)
>
> #get estimates of psi, theta and p
> psi = posteriorSummaryOfSiteOccupancy(fit, burnin = 1000)
>
> theta = posteriorSummaryOfSampleOccupancy(fit, burnin = 1000)
>
> p = posteriorSummaryOfDetection(fit, burnin=1000)
>
> #obtain median, lower and upper limits of estimates
> postMedian = cbind(psi=psi$median, theta=theta$median[,1],
p=p$median[,1])
>
> postLowerLimit =  cbind(psi=psi$lower, theta=theta$lower[,1],
p=p$lower[,1])
>
> postUpperLimit =  cbind(psi=psi$upper, theta=theta$upper[,1],
p=p$upper[,1])
>
> #print the 95% confidence intervals and median
> CR='\n'
> cat(CR, 'Estimates of posterior median:', CR)

 Estimates of posterior median:
> print(postMedian)
          psi     theta         p
1-S 0.9246914 0.6808308 0.9455543
2-S 0.9246914 0.5193100 0.8800006
3-S 0.9246914 0.6068831 0.7896136
4-S 0.9246914 0.8766100 0.8148954
5-S 0.9246914 0.7576561 0.9182619
6-S 0.9246914 0.6947710 0.9233771
7-S 0.9246914 0.9541074 0.9877232
8-S 0.9246914 0.9829465 0.9960782
>
> cat(CR, 'Estimates of posterior 2.5% quantile:', CR)

 Estimates of posterior 2.5% quantile:
> print(postLowerLimit)
          psi     theta         p
1-S 0.6558736 0.4643877 0.8621794
2-S 0.6558736 0.3236835 0.6702580
3-S 0.6558736 0.4437123 0.6480936
4-S 0.6558736 0.7562645 0.6584472
5-S 0.6558736 0.5154885 0.8079381
6-S 0.6558736 0.5629127 0.8419528
7-S 0.6558736 0.8180100 0.9271268
8-S 0.6558736 0.8418455 0.9358949
>
```

```
> cat(CR, 'Estimates of posterior 97.5% quantile:', CR)
 Estimates of posterior 97.5% quantile:
> print(postUpperLimit)
          psi     theta         p
1-S 0.9970311 0.8600107 0.9849394
2-S 0.9970311 0.7071051 0.9721785
3-S 0.9970311 0.7534867 0.8915757
4-S 0.9970311 0.9582974 0.9250174
5-S 0.9970311 0.9180223 0.9780160
6-S 0.9970311 0.8087270 0.9702848
7-S 0.9970311 0.9949035 0.9992156
8-S 0.9970311 0.9998655 0.9999901
>
> #get estimates on model success (the lower the number, the better)
> posteriorPredictiveLoss(fit,burnin=1000)
$criterion
[1] 30.6048

$lackOfFit
[1] 15.607

$predVariance
[1] 14.9978

>
> WAIC(fit, burnin = 1000)
$criterion
[1] 0.5390554

$lackOfFit
[1] 0.4368915

$predVariance
[1] 0.102164
```

ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large):

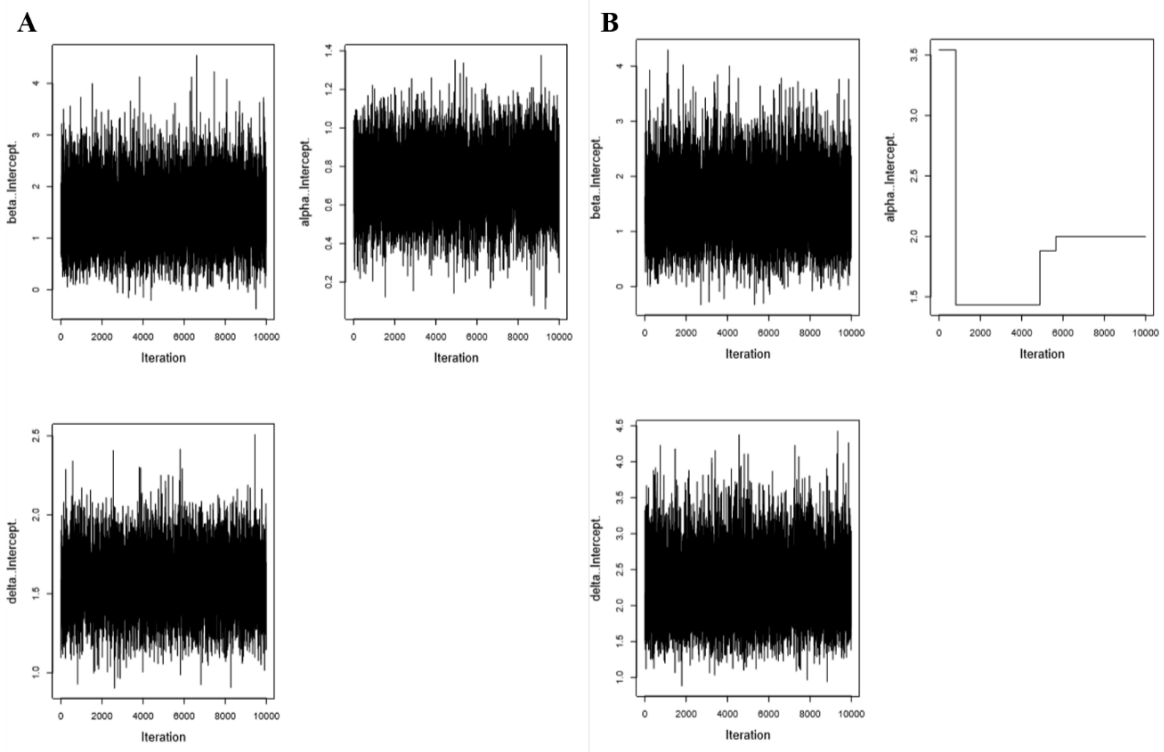
```
> #Fit the occ model with covariates
> fit = occModel(formulaSite = ~ 1,
+                formulaSiteAndSample = ~ Lamprey+AquaticVeg,
+                formulaReplicate = ~ Lamprey+Large,
+                detectionMats = Lampt_Detections,
+                siteData = LampSurveyData.sc,
+                niter = 11000,niterInterval = 2000,
+                siteColName = 'site')
Begin MCMC sampling:

..... drawing sample # 2000  after  0.7219359  minutes
..... drawing sample # 4000  after  1.448393  minutes
..... drawing sample # 6000  after  2.17864  minutes
..... drawing sample # 8000  after  3.067796  minutes
..... drawing sample # 10000  after  3.917507  minutes
Completed  11000  draws of MCMC algorithm
>
> posteriorSummary(fit, burnin=1000, mcError=TRUE)
Bayesian estimates of model parameters
                    Mean    50%   2.5%  97.5%
beta..Intercept.   1.488  1.446  0.420  2.770
alpha..Intercept.  0.885  0.879  0.502  1.285
alpha.Lamprey      0.746  0.733  0.285  1.284
alpha.AquaticVeg   0.063  0.062 -0.346  0.467
```

```
delta..Intercept.  1.557  1.547  1.197  1.973
delta.Lamprey      0.621  0.603  0.168  1.168
delta.Large       -0.430 -0.429 -0.772 -0.104

Monte Carlo SE of Bayesian estimates
                    Mean    50%   2.5%  97.5%
beta..Intercept.  0.0061 0.0075 0.0117 0.0179
alpha..Intercept. 0.0024 0.0026 0.0052 0.0115
alpha.Lamprey     0.0032 0.0035 0.0048 0.0123
alpha.AquaticVeg  0.0023 0.0030 0.0070 0.0067
delta..Intercept. 0.0030 0.0034 0.0061 0.0063
delta.Lamprey     0.0036 0.0049 0.0052 0.0082
delta.Large       0.0027 0.0030 0.0049 0.0062
NULL
> #assess how the run went
> plotTrace(fit, c('beta..Intercept.', 'alpha..Intercept.',
'delta..Intercept.'), burnin=1000)
> plotACF(fit, c('beta..Intercept.', 'alpha..Intercept.',
'delta..Intercept.'), burnin=1000)
>
> #get estimates of psi, theta and p
> psi = posteriorSummaryOfSiteOccupancy(fit, burnin = 1000)
>
> theta = posteriorSummaryOfSampleOccupancy(fit, burnin = 1000)
>
> p = posteriorSummaryOfDetection(fit, burnin=1000)
>
> #obtain median, lower and upper limits of estimates
> postMedian = cbind(psi=psi$median, theta=theta$median[,1],
p=p$median[,1])
>
> postLowerLimit =  cbind(psi=psi$lower, theta=theta$lower[,1],
p=p$lower[,1])
>
> postUpperLimit =  cbind(psi=psi$upper, theta=theta$upper[,1],
p=p$upper[,1])
>
> #print the 95% confidence intervals and median
> CR='\n'
> cat(CR, 'Estimates of posterior median:', CR)

 Estimates of posterior median:
> print(postMedian)
         psi     theta         p
1-S 0.925888 0.6790916 0.9512816
2-S 0.925888 0.5179916 0.9092979
3-S 0.925888 0.6068984 0.8036426
4-S 0.925888 0.8782818 0.8253823
5-S 0.925888 0.7607119 0.8892366
6-S 0.925888 0.6939586 0.9291897
7-S 0.925888 0.9549634 0.9786438
8-S 0.925888 0.9836679 0.9979102
>
> cat(CR, 'Estimates of posterior 2.5% quantile:', CR)

 Estimates of posterior 2.5% quantile:
> print(postLowerLimit)
          psi     theta         p
1-S 0.6629187 0.4529927 0.8749273
2-S 0.6629187 0.3159179 0.7691216
3-S 0.6629187 0.4431841 0.6669353
4-S 0.6629187 0.7538573 0.6679366
5-S 0.6629187 0.5174987 0.8210535
6-S 0.6629187 0.5580615 0.8533066
```

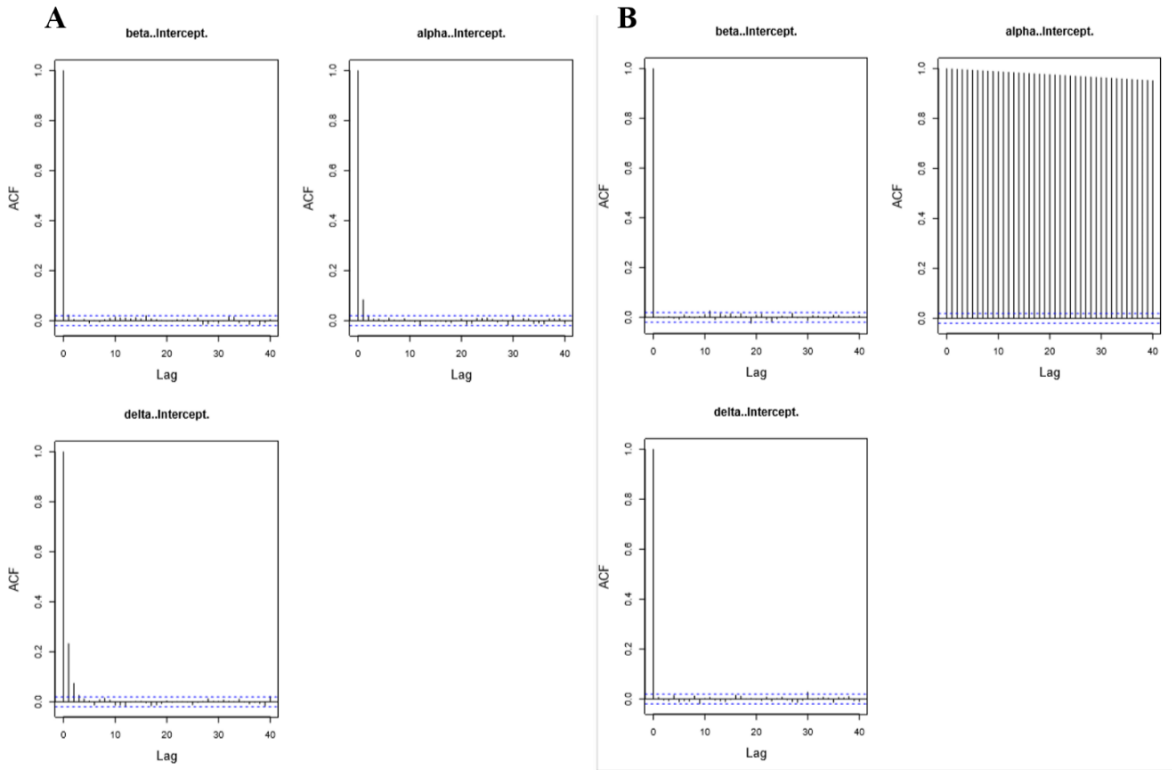
```
7-S 0.6629187 0.8174351 0.9239362
8-S 0.6629187 0.8351971 0.9644929
>
> cat(CR, 'Estimates of posterior 97.5% quantile:', CR)

 Estimates of posterior 97.5% quantile:
> print(postUpperLimit)
          psi     theta         p
1-S 0.9971933 0.8585419 0.9862908
2-S 0.9971933 0.7066336 0.9735073
3-S 0.9971933 0.7517664 0.8994177
4-S 0.9971933 0.9584148 0.9289699
5-S 0.9971933 0.9207391 0.9379206
6-S 0.9971933 0.8056288 0.9719735
7-S 0.9971933 0.9951601 0.9979693
8-S 0.9971933 0.9998471 0.9999932
>
> #get estimates on model success (the lower the number, the better)
> posteriorPredictiveLoss(fit,burnin=1000)
$criterion
[1] 30.6332

$lackOfFit
[1] 16.01374

$predVariance
[1] 14.61946

>
> WAIC(fit, burnin = 1000)
$criterion
[1] 0.5232673

$lackOfFit
[1] 0.4390616

$predVariance
[1] 0.08420576
```

Example of good (A) and bad (B) fit trace and autocorrelation plots:

Trace Plots



Autocorrelation plots



Appendix F. eDNA qPCR data from WDFW

Table 1. Lampetra spp. qPCR detections organized by sample name (site, 1-8; grid, Upper, Middle, Lower; sample type, sediment or water; and replicate, 1-3), Cycling Threshold (C_T) value, and DNA concentration.

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S1-U-S-R1	34.033	Y	238.63	Medium
S1-U-S-R1	35.223	Y	238.63	Medium
S1-U-S-R1	35.254	Y	238.63	Medium
S1-U-S-R2	34.783	Y	238.63	Medium
S1-U-S-R2	35.196	Y	238.63	Medium
S1-U-S-R2	35.817	Y	238.63	Medium
S1-U-S-R3	32.135	Y	131,246.50	High
S1-U-S-R3	32.225	Y	131,246.50	High
S1-U-S-R3	32.256	Y	131,246.50	High
S1-M-S-R1	28.103	Y	131,246.50	High
S1-M-S-R1	28.194	Y	131,246.50	High
S1-M-S-R1	28.416	Y	131,246.50	High
S1-M-S-R2	36.015	Y	23.86	Medium
S1-M-S-R2	36.412	Y	23.86	Medium
S1-M-S-R2	35.393	Y	238.63	Medium
S1-M-S-R3	36.205	Y	23.86	Medium
S1-M-S-R3	35.361	Y	238.63	Medium
S1-M-S-R3	35.994	Y	238.63	Medium
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-W1	33.143	Y	238.63	Medium
S1-W1	33.270	Y	238.63	Medium
S1-W1	35.129	Y	238.63	Medium
S2-U-S-R1	UNDETM	N	NA	NA
S2-U-S-R1	UNDETM	N	NA	NA

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S2-U-S-R1	UNDETM	N	NA	NA
S2-U-S-R2	40.413	Y	2.39	Low
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-W2	34.620	Y	238.63	Medium
S2-W2	34.906	Y	238.63	Medium
S2-W2	35.456	Y	238.63	Medium
S2-W2	NA	NA	NA	NA
S2-W2	NA	NA	NA	NA
S2-W2	NA	NA	NA	NA
S3-U-S-R1	33.801	Y	238.63	Medium
S3-U-S-R1	33.956	Y	238.63	Medium
S3-U-S-R1	34.093	Y	238.63	Medium

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S3-U-S-R2	33.804	Y	238.63	Medium
S3-U-S-R2	34.203	Y	238.63	Medium
S3-U-S-R2	35.077	Y	238.63	Medium
S3-U-S-R3	35.625	Y	238.63	Medium
S3-U-S-R3	35.700	Y	238.63	Medium
S3-U-S-R3	35.992	Y	238.63	Medium
S3-M-S-R1	39.721	Y	2.39	Low
S3-M-S-R1	UNDETM	N	NA	NA
S3-M-S-R1	UNDETM	N	NA	NA
S3-M-S-R2	39.306	Y	2.39	Low
S3-M-S-R2	37.746	Y	23.86	Medium
S3-M-S-R2	UNDETM	N	NA	NA
S3-M-S-R3	38.452	Y	2.39	Low
S3-M-S-R3	38.829	Y	2.39	Low
S3-M-S-R3	UNDETM	N	NA	NA
S3-L-S-R1	38.249	Y	2.39	Low
S3-L-S-R1	39.309	Y	2.39	Low
S3-L-S-R1	UNDETM	N	NA	NA
S3-L-S-R2	39.175	Y	2.39	Low
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	36.728	Y	23.86	Medium
S3-L-S-R2	36.940	Y	23.86	Medium
S3-L-S-R3	UNDETM	N	NA	NA
S3-L-S-R3	UNDETM	N	NA	NA
S3-L-S-R3	UNDETM	N	NA	NA
S3-W3	35.437	Y	238.63	Medium
S3-W3	36.451	Y	23.86	Medium
S3-W3	35.559	Y	238.63	Medium
S3-W3	NA	NA	NA	NA
S3-W3	NA	NA	NA	NA
S3-W3	NA	NA	NA	NA
S4-U-S-R1	39.382	Y	2.39	Low
S4-U-S-R1	UNDETM	N	NA	NA
S4-U-S-R1	UNDETM	N	NA	NA
S4-U-S-R2	38.826	Y	2.39	Low

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R2	36.839	Y	23.86	Medium
S4-U-S-R2	36.634	Y	23.86	Medium
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R3	38.604	Y	2.39	Low
S4-U-S-R3	39.430	Y	2.39	Low
S4-U-S-R3	UNDETM	N	NA	NA
S4-M-S-R1	36.672	Y	23.86	Medium
S4-M-S-R1	37.929	Y	23.86	Medium
S4-M-S-R1	35.549	Y	238.63	Medium
S4-M-S-R2	37.699	Y	23.86	Medium
S4-M-S-R2	UNDETM	N	NA	NA
S4-M-S-R2	UNDETM	N	NA	NA
S4-M-S-R3	36.276	Y	23.86	Medium
S4-M-S-R3	36.627	Y	23.86	Medium
S4-M-S-R3	37.378	Y	23.86	Medium
S4-L-S-R1	38.211	Y	2.39	Low
S4-L-S-R1	40.419	Y	2.39	Low
S4-L-S-R1	37.935	Y	23.86	Medium
S4-L-S-R2	38.923	Y	2.39	Low
S4-L-S-R2	40.109	Y	2.39	Low
S4-L-S-R2	40.186	Y	2.39	Low
S4-L-S-R3	38.179	Y	2.39	Low
S4-L-S-R3	36.810	Y	23.86	Medium
S4-L-S-R3	37.548	Y	23.86	Medium
S4-W4	39.467	Y	2.39	Low
S4-W4	39.669	Y	2.39	Low
S4-W4	35.970	Y	238.63	Medium
S5-U-S-R1	36.713	Y	23.86	Medium
S5-U-S-R1	34.953	Y	238.63	Medium
S5-U-S-R1	35.084	Y	238.63	Medium
S5-U-S-R2	36.013	Y	23.86	Medium
S5-U-S-R2	36.028	Y	23.86	Medium
S5-U-S-R2	35.960	Y	238.63	Medium
S5-U-S-R3	36.092	Y	23.86	Medium
S5-U-S-R3	35.014	Y	238.63	Medium

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S5-U-S-R3	35.257	Y	238.63	Medium
S5-M-S-R1	34.197	Y	238.63	Medium
S5-M-S-R1	34.390	Y	238.63	Medium
S5-M-S-R1	35.033	Y	238.63	Medium
S5-M-S-R2	33.976	Y	238.63	Medium
S5-M-S-R2	34.230	Y	238.63	Medium
S5-M-S-R2	35.515	Y	238.63	Medium
S5-M-S-R3	37.456	Y	23.86	Medium
S5-M-S-R3	37.485	Y	23.86	Medium
S5-M-S-R3	37.578	Y	23.86	Medium
S5-L-S-R1	39.128	Y	2.39	Low
S5-L-S-R1	39.461	Y	2.39	Low
S5-L-S-R1	UNDETM	N	NA	NA
S5-L-S-R2	38.878	Y	2.39	Low
S5-L-S-R2	40.117	Y	2.39	Low
S5-L-S-R2	37.624	Y	23.86	Medium
S5-L-S-R3	38.955	Y	2.39	Low
S5-L-S-R3	40.040	Y	2.39	Low
S5-L-S-R3	UNDETM	N	NA	NA
S5-W5	38.962	Y	2.39	Low
S5-W5	43.391	Y	2.39	Low
S5-W5	37.010	Y	23.86	Medium
S6-U-S-R1	36.759	Y	23.86	Medium
S6-U-S-R1	37.516	Y	23.86	Medium
S6-U-S-R1	35.693	Y	238.63	Medium
S6-U-S-R2	36.036	Y	23.86	Medium
S6-U-S-R2	36.803	Y	23.86	Medium
S6-U-S-R2	36.844	Y	23.86	Medium
S6-U-S-R3	36.565	Y	23.86	Medium
S6-U-S-R3	35.194	Y	238.63	Medium
S6-U-S-R3	35.736	Y	238.63	Medium
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S6-M-S-R3	UNDETM	N	NA	NA
S6-M-S-R3	UNDETM	N	NA	NA
S6-M-S-R3	UNDETM	N	NA	NA
S6-L-S-R1	36.235	Y	23.86	Medium
S6-L-S-R1	36.816	Y	23.86	Medium
S6-L-S-R1	37.527	Y	23.86	Medium
S6-L-S-R1	NA	NA	NA	NA
S6-L-S-R1	NA	NA	NA	NA
S6-L-S-R1	NA	NA	NA	NA
S6-L-S-R2	35.359	Y	238.63	Medium
S6-L-S-R2	36.292	Y	23.86	Medium
S6-L-S-R2	35.707	Y	238.63	Medium
S6-L-S-R2	NA	NA	NA	NA
S6-L-S-R2	NA	NA	NA	NA
S6-L-S-R2	NA	NA	NA	NA
S6-L-S-R3	35.121	Y	238.63	Medium
S6-L-S-R3	35.401	Y	238.63	Medium
S6-L-S-R3	35.872	Y	238.63	Medium
S6-W6	UNDETM	N	NA	NA
S6-W6	UNDETM	N	NA	NA
S6-W6	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R3	38.801	Y	2.39	Low
S7-U-S-R3	37.606	Y	23.86	Medium
S7-U-S-R3	UNDETM	N	NA	NA
S7-M-S-R1	35.357	Y	238.63	Medium
S7-M-S-R1	35.809	Y	238.63	Medium
S7-M-S-R1	35.857	Y	238.63	Medium
S7-M-S-R2	36.598	Y	23.86	Medium
S7-M-S-R2	37.811	Y	23.86	Medium
S7-M-S-R2	35.835	Y	238.63	Medium
S7-M-S-R3	35.058	Y	238.63	Medium

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S7-M-S-R3	35.703	Y	238.63	Medium
S7-M-S-R3	35.829	Y	238.63	Medium
S7-L-S-R1	34.111	Y	238.63	Medium
S7-L-S-R1	34.580	Y	238.63	Medium
S7-L-S-R1	35.346	Y	238.63	Medium
S7-L-S-R2	33.765	Y	238.63	Medium
S7-L-S-R2	33.782	Y	238.63	Medium
S7-L-S-R2	34.521	Y	238.63	Medium
S7-L-S-R3	33.677	Y	238.63	Medium
S7-L-S-R3	33.888	Y	238.63	Medium
S7-L-S-R3	33.961	Y	238.63	Medium
S7-W7	39.939	Y	2.39	Low
S7-W7	36.964	Y	23.86	Medium
S7-W7	37.731	Y	23.86	Medium
S8-U-S-R1	34.138	Y	238.63	Medium
S8-U-S-R1	34.879	Y	238.63	Medium
S8-U-S-R1	35.329	Y	238.63	Medium
S8-U-S-R2	33.375	Y	238.63	Medium
S8-U-S-R2	33.815	Y	238.63	Medium
S8-U-S-R2	33.952	Y	238.63	Medium
S8-U-S-R3	34.335	Y	238.63	Medium
S8-U-S-R3	34.470	Y	238.63	Medium
S8-U-S-R3	34.532	Y	238.63	Medium
S8-M-S-R1	36.207	Y	23.86	Medium
S8-M-S-R1	36.656	Y	23.86	Medium
S8-M-S-R1	36.667	Y	23.86	Medium
S8-M-S-R2	36.603	Y	23.86	Medium
S8-M-S-R2	36.789	Y	23.86	Medium
S8-M-S-R2	35.917	Y	238.63	Medium
S8-M-S-R3	35.042	Y	238.63	Medium
S8-M-S-R3	35.633	Y	238.63	Medium
S8-M-S-R3	35.896	Y	238.63	Medium
S8-L-S-R1	31.939	Y	2386.30	High
S8-L-S-R1	32.230	Y	131,246.50	High
S8-L-S-R1	32.288	Y	131,246.50	High
S8-L-S-R2	34.931	Y	238.63	Medium
S8-L-S-R2	35.235	Y	238.63	Medium

Sample Name	C_T: *Lampetra*	*Lampetra* Detected?	*Lampetra* DNA Concentration (copies/µl)	DNA Concentration Category
S8-L-S-R2	35.606	Y	238.63	Medium
S8-L-S-R3	38.868	Y	2.39	Low
S8-L-S-R3	34.612	Y	238.63	Medium
S8-L-S-R3	35.990	Y	238.63	Medium
S8-W8	36.177	Y	23.86	Medium
S8-W8	38.730	Y	2.39	Low
S8-W8	38.974	Y	2.39	Low
S8-W8	NA	NA	NA	NA
S8-W8	NA	NA	NA	NA
S8-W8	NA	NA	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA

Table 2. Pacific lamprey qPCR detections organized by sample name (site, 1-8; grid, Upper, Middle, Lower; sample type, sediment or water; and replicate, 1-3), Cycling Threshold (C_T) value, and DNA concentration.

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S1-U-S-R1	UNDETM	N	NA	NA
S1-U-S-R1	UNDETM	N	NA	NA
S1-U-S-R1	UNDETM	N	NA	NA
S1-U-S-R2	UNDETM	N	NA	NA
S1-U-S-R2	UNDETM	N	NA	NA
S1-U-S-R2	UNDETM	N	NA	NA
S1-U-S-R3	UNDETM	N	NA	NA
S1-U-S-R3	UNDETM	N	NA	NA
S1-U-S-R3	UNDETM	N	NA	NA
S1-M-S-R1	UNDETM	N	NA	NA
S1-M-S-R1	UNDETM	N	NA	NA
S1-M-S-R1	UNDETM	N	NA	NA
S1-M-S-R2	UNDETM	N	NA	NA
S1-M-S-R2	UNDETM	N	NA	NA
S1-M-S-R2	UNDETM	N	NA	NA
S1-M-S-R3	UNDETM	N	NA	NA
S1-M-S-R3	UNDETM	N	NA	NA
S1-M-S-R3	UNDETM	N	NA	NA
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R1	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R2	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-L-S-R3	UNDETM	N	NA	NA
S1-W1	UNDETM	N	NA	NA
S1-W1	UNDETM	N	NA	NA
S1-W1	UNDETM	N	NA	NA
S2-U-S-R1	UNDETM	N	NA	NA
S2-U-S-R1	UNDETM	N	NA	NA
S2-U-S-R1	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S2-U-S-R2	UNDETM	N	NA	NA
S2-U-S-R2	NA	NA	NA	NA
S2-U-S-R2	NA	NA	NA	NA
S2-U-S-R2	NA	NA	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-U-S-R3	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R1	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R2	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-M-S-R3	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R1	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R2	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-L-S-R3	UNDETM	N	NA	NA
S2-W2	38.204	Y	8.85	Low
S2-W2	UNDETM	N	NA	NA
S2-W2	UNDETM	N	NA	NA
S2-W2	UNDETM	N	NA	NA
S2-W2	UNDETM	N	NA	NA
S2-W2	UNDETM	N	NA	NA
S3-U-S-R1	UNDETM	N	NA	NA
S3-U-S-R1	UNDETM	N	NA	NA
S3-U-S-R1	UNDETM	N	NA	NA
S3-U-S-R2	UNDETM	N	NA	NA
S3-U-S-R2	UNDETM	N	NA	NA
S3-U-S-R2	UNDETM	N	NA	NA
S3-U-S-R3	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S3-U-S-R3	UNDETM	N	NA	NA
S3-U-S-R3	UNDETM	N	NA	NA
S3-M-S-R1	UNDETM	N	NA	NA
S3-M-S-R1	UNDETM	N	NA	NA
S3-M-S-R1	UNDETM	N	NA	NA
S3-M-S-R2	UNDETM	N	NA	NA
S3-M-S-R2	UNDETM	N	NA	NA
S3-M-S-R2	UNDETM	N	NA	NA
S3-M-S-R3	UNDETM	N	NA	NA
S3-M-S-R3	UNDETM	N	NA	NA
S3-M-S-R3	UNDETM	N	NA	NA
S3-L-S-R1	UNDETM	N	NA	NA
S3-L-S-R1	UNDETM	N	NA	NA
S3-L-S-R1	UNDETM	N	NA	NA
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	UNDETM	N	NA	NA
S3-L-S-R2	NA	NA	NA	NA
S3-L-S-R2	NA	NA	NA	NA
S3-L-S-R2	NA	NA	NA	NA
S3-L-S-R3	UNDETM	N	NA	NA
S3-L-S-R3	UNDETM	N	NA	NA
S3-L-S-R3	UNDETM	N	NA	NA
S3-W3	39.333	Y	8.85	Low
S3-W3	UNDETM	N	NA	NA
S3-W3	UNDETM	N	NA	NA
S3-W3	UNDETM	N	NA	NA
S3-W3	UNDETM	N	NA	NA
S3-W3	UNDETM	N	NA	NA
S4-U-S-R1	UNDETM	N	NA	NA
S4-U-S-R1	UNDETM	N	NA	NA
S4-U-S-R1	UNDETM	N	NA	NA
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R2	UNDETM	N	NA	NA
S4-U-S-R2	NA	NA	NA	NA
S4-U-S-R2	NA	NA	NA	NA
S4-U-S-R2	NA	NA	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S4-U-S-R3	UNDETM	N	NA	NA
S4-U-S-R3	UNDETM	N	NA	NA
S4-U-S-R3	UNDETM	N	NA	NA
S4-M-S-R1	UNDETM	N	NA	NA
S4-M-S-R1	UNDETM	N	NA	NA
S4-M-S-R1	UNDETM	N	NA	NA
S4-M-S-R2	UNDETM	N	NA	NA
S4-M-S-R2	UNDETM	N	NA	NA
S4-M-S-R2	UNDETM	N	NA	NA
S4-M-S-R3	UNDETM	N	NA	NA
S4-M-S-R3	UNDETM	N	NA	NA
S4-M-S-R3	UNDETM	N	NA	NA
S4-L-S-R1	UNDETM	N	NA	NA
S4-L-S-R1	UNDETM	N	NA	NA
S4-L-S-R1	UNDETM	N	NA	NA
S4-L-S-R2	UNDETM	N	NA	NA
S4-L-S-R2	UNDETM	N	NA	NA
S4-L-S-R2	UNDETM	N	NA	NA
S4-L-S-R3	UNDETM	N	NA	NA
S4-L-S-R3	UNDETM	N	NA	NA
S4-L-S-R3	UNDETM	N	NA	NA
S4-W4	UNDETM	N	NA	NA
S4-W4	UNDETM	N	NA	NA
S4-W4	UNDETM	N	NA	NA
S5-U-S-R1	UNDETM	N	NA	NA
S5-U-S-R1	UNDETM	N	NA	NA
S5-U-S-R1	UNDETM	N	NA	NA
S5-U-S-R2	UNDETM	N	NA	NA
S5-U-S-R2	UNDETM	N	NA	NA
S5-U-S-R2	UNDETM	N	NA	NA
S5-U-S-R3	UNDETM	N	NA	NA
S5-U-S-R3	UNDETM	N	NA	NA
S5-U-S-R3	UNDETM	N	NA	NA
S5-M-S-R1	UNDETM	N	NA	NA
S5-M-S-R1	UNDETM	N	NA	NA
S5-M-S-R1	UNDETM	N	NA	NA
S5-M-S-R2	UNDETM	N	NA	NA
S5-M-S-R2	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S5-M-S-R2	UNDETM	N	NA	NA
S5-M-S-R3	UNDETM	N	NA	NA
S5-M-S-R3	UNDETM	N	NA	NA
S5-M-S-R3	UNDETM	N	NA	NA
S5-L-S-R1	UNDETM	N	NA	NA
S5-L-S-R1	UNDETM	N	NA	NA
S5-L-S-R1	UNDETM	N	NA	NA
S5-L-S-R2	UNDETM	N	NA	NA
S5-L-S-R2	UNDETM	N	NA	NA
S5-L-S-R2	UNDETM	N	NA	NA
S5-L-S-R3	UNDETM	N	NA	NA
S5-L-S-R3	UNDETM	N	NA	NA
S5-L-S-R3	UNDETM	N	NA	NA
S5-W5	UNDETM	N	NA	NA
S5-W5	UNDETM	N	NA	NA
S5-W5	UNDETM	N	NA	NA
S6-U-S-R1	UNDETM	N	NA	NA
S6-U-S-R1	UNDETM	N	NA	NA
S6-U-S-R1	UNDETM	N	NA	NA
S6-U-S-R2	UNDETM	N	NA	NA
S6-U-S-R2	UNDETM	N	NA	NA
S6-U-S-R2	UNDETM	N	NA	NA
S6-U-S-R3	UNDETM	N	NA	NA
S6-U-S-R3	UNDETM	N	NA	NA
S6-U-S-R3	UNDETM	N	NA	NA
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R1	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA
S6-M-S-R2	UNDETM	N	NA	NA
S6-M-S-R3	UNDETM	N	NA	NA
S6-M-S-R3	UNDETM	N	NA	NA
S6-M-S-R3	UNDETM	N	NA	NA
S6-L-S-R1	37.206	Y	16.08	Medium
S6-L-S-R1	UNDETM	N	NA	NA
S6-L-S-R1	UNDETM	N	NA	NA
S6-L-S-R1	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S6-L-S-R1	UNDETM	N	NA	NA
S6-L-S-R1	UNDETM	N	NA	NA
S6-L-S-R2	23.685	Y	16084.36	High
S6-L-S-R2	UNDETM	N	NA	NA
S6-L-S-R2	UNDETM	N	NA	NA
S6-L-S-R2	38.726	Y	16.08	Medium
S6-L-S-R2	UNDETM	N	NA	Medium
S6-L-S-R2	37.069	Y	16.08	Medium
S6-L-S-R3	UNDETM	N	NA	NA
S6-L-S-R3	UNDETM	N	NA	NA
S6-L-S-R3	UNDETM	N	NA	NA
S6-W6	UNDETM	N	NA	NA
S6-W6	UNDETM	N	NA	NA
S6-W6	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R1	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R2	UNDETM	N	NA	NA
S7-U-S-R3	UNDETM	N	NA	NA
S7-U-S-R3	UNDETM	N	NA	NA
S7-U-S-R3	UNDETM	N	NA	NA
S7-M-S-R1	UNDETM	N	NA	NA
S7-M-S-R1	UNDETM	N	NA	NA
S7-M-S-R1	UNDETM	N	NA	NA
S7-M-S-R2	UNDETM	N	NA	NA
S7-M-S-R2	UNDETM	N	NA	NA
S7-M-S-R2	UNDETM	N	NA	NA
S7-M-S-R3	UNDETM	N	NA	NA
S7-M-S-R3	UNDETM	N	NA	NA
S7-M-S-R3	UNDETM	N	NA	NA
S7-L-S-R1	UNDETM	N	NA	NA
S7-L-S-R1	UNDETM	N	NA	NA
S7-L-S-R1	UNDETM	N	NA	NA
S7-L-S-R2	UNDETM	N	NA	NA
S7-L-S-R2	UNDETM	N	NA	NA
S7-L-S-R2	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S7-L-S-R3	UNDETM	N	NA	NA
S7-L-S-R3	UNDETM	N	NA	NA
S7-L-S-R3	UNDETM	N	NA	NA
S7-W7	UNDETM	N	NA	NA
S7-W7	UNDETM	N	NA	NA
S7-W7	UNDETM	N	NA	NA
S8-U-S-R1	UNDETM	N	NA	NA
S8-U-S-R1	UNDETM	N	NA	NA
S8-U-S-R1	UNDETM	N	NA	NA
S8-U-S-R2	UNDETM	N	NA	NA
S8-U-S-R2	UNDETM	N	NA	NA
S8-U-S-R2	UNDETM	N	NA	NA
S8-U-S-R3	UNDETM	N	NA	NA
S8-U-S-R3	UNDETM	N	NA	NA
S8-U-S-R3	UNDETM	N	NA	NA
S8-M-S-R1	UNDETM	N	NA	NA
S8-M-S-R1	UNDETM	N	NA	NA
S8-M-S-R1	UNDETM	N	NA	NA
S8-M-S-R2	UNDETM	N	NA	NA
S8-M-S-R2	UNDETM	N	NA	NA
S8-M-S-R2	UNDETM	N	NA	NA
S8-M-S-R3	UNDETM	N	NA	NA
S8-M-S-R3	UNDETM	N	NA	NA
S8-M-S-R3	UNDETM	N	NA	NA
S8-L-S-R1	UNDETM	N	NA	NA
S8-L-S-R1	UNDETM	N	NA	NA
S8-L-S-R1	UNDETM	N	NA	NA
S8-L-S-R2	UNDETM	N	NA	NA
S8-L-S-R2	UNDETM	N	NA	NA
S8-L-S-R2	UNDETM	N	NA	NA
S8-L-S-R3	UNDETM	N	NA	NA
S8-L-S-R3	UNDETM	N	NA	NA
S8-L-S-R3	UNDETM	N	NA	NA
S8-W8	38.596	Y	8.85	Low
S8-W8	UNDETM	N	NA	NA
S8-W8	UNDETM	N	NA	NA
S8-W8	UNDETM	N	NA	NA
S8-W8	UNDETM	N	NA	NA

Sample Name	CT: Pacific Lamprey	Pacific Lamprey Detected?	Pacific Lamprey Copy #	DNA Copy # Category
S8-W8	UNDETM	N	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/08/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/11/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/17/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/18/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA
Neg 10/24/18	UNDETM	N	NA	NA

Appendix G. eDNA Occupancy Model - Covariates

Top 10 out of 55 models with covariates that fit the data best. Covariates measurements were taken at each site and are potential environmental indicators for predicting probability of *Lampetra* spp. eDNA occupancy in sediment samples.

Site	Occupancy in site (ψ) (95% CI)	Occupancy in sample (θ) (95% CI)	Detection in replicate (ρ) (95% CI)	PPLC	WAIC
#1	ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large+Per_Fine)				
1	0.92 (0.66 - 1.00)	0.68 (0.46 - 0.86)	0.95 (0.86 - 0.98)	30.60	0.54
2	0.92 (0.66 - 1.00)	0.52 (0.32 - 0.71)	0.88 (0.67 - 0.97)	30.60	0.54
3	0.92 (0.66 - 1.00)	0.61 (0.44 - 0.75)	0.79 (0.65 - 0.89)	30.60	0.54
4	0.92 (0.66 - 1.00)	0.88 (0.76 - 0.96)	0.81 (0.66 - 0.93)	30.60	0.54
5	0.92 (0.66 - 1.00)	0.76 (0.52 - 0.92)	0.92 (0.81 - 0.98)	30.60	0.54
6	0.92 (0.66 - 1.00)	0.69 (0.56 - 0.81)	0.92 (0.84 - 0.97)	30.60	0.54
7	0.92 (0.66 - 1.00)	0.95 (0.82 - 0.99)	0.99 (0.93 - 1.00)	30.60	0.54
8	0.92 (0.66 - 1.00)	0.98 (0.84 - 1.00)	1.00 (0.94 - 1.00)	30.60	0.54
#2	ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large)				
1	0.93 (0.66 - 1.00)	0.68 (0.45 - 0.86)	0.95 (0.87 - 0.99)	30.63	0.52
2	0.93 (0.66 - 1.00)	0.52 (0.32 - 0.71)	0.91 (0.77 - 0.97)	30.63	0.52
3	0.93 (0.66 - 1.00)	0.61 (0.44 - 0.75)	0.80 (0.67 - 0.90)	30.63	0.52
4	0.93 (0.66 - 1.00)	0.88 (0.75 - 0.96)	0.83 (0.67 - 0.93)	30.63	0.52
5	0.93 (0.66 - 1.00)	0.76 (0.52 - 0.92)	0.89 (0.82 - 0.94)	30.63	0.52
6	0.93 (0.66 - 1.00)	0.69 (0.56 - 0.81)	0.93 (0.85 - 0.97)	30.63	0.52
7	0.93 (0.66 - 1.00)	0.95 (0.82 - 1.00)	0.98 (0.92 - 1.00)	30.63	0.52
8	0.93 (0.66 - 1.00)	0.98 (0.84 - 1.00)	1.00 (0.96 - 1.00)	30.63	0.52
#3	ψ(·), θ(Lamprey+AquaticVeg+pH), ρ(Lamprey+Large+Per_Fine)				
1	0.92 (0.66 - 1.00)	0.54 (0.26 - 0.81)	0.94 (0.86 - 0.99)	30.71	0.54
2	0.92 (0.66 - 1.00)	0.52 (0.32 - 0.71)	0.88 (0.66 - 0.97)	30.71	0.54
3	0.92 (0.66 - 1.00)	0.65 (0.48 - 0.80)	0.79 (0.65 - 0.89)	30.71	0.54
4	0.92 (0.66 - 1.00)	0.93 (0.80 - 0.99)	0.82 (0.65 - 0.93)	30.71	0.54
5	0.92 (0.66 - 1.00)	0.75 (0.50 - 0.91)	0.92 (0.80 - 1.00)	30.71	0.54
6	0.92 (0.66 - 1.00)	0.78 (0.61 - 0.91)	0.92 (0.84 - 0.97)	30.71	0.54
7	0.92 (0.66 - 1.00)	0.92 (0.72 - 1.00)	0.99 (0.93 - 1.00)	30.71	0.54
8	0.92 (0.66 - 1.00)	1.00 (0.88 - 1.00)	1.00 (0.94 - 1.00)	30.71	0.54
#4	ψ(·), θ(Lamprey+AquaticVeg+pH), ρ(Lamprey+Large)				
1	0.93 (0.67 - 1.00)	0.54 (0.26 - 0.82)	0.95 (0.88 - 0.99)	30.85	0.52
2	0.93 (0.67 - 1.00)	0.52 (0.32 - 0.71)	0.91 (0.77 - 0.97)	30.85	0.52
3	0.93 (0.67 - 1.00)	0.65 (0.48 - 0.80)	0.80 (0.67 - 0.90)	30.85	0.52
4	0.93 (0.67 - 1.00)	0.93 (0.79 - 0.99)	0.82 (0.66 - 0.93)	30.85	0.52

Site	Occupancy in site (ψ) (95% CI)	Occupancy in sample (θ) (95% CI)	Detection in replicate (ρ) (95% CI)	PPLC	WAIC
5	0.93 (0.67 - 1.00)	0.74 (0.50 - 0.91)	0.89 (0.82 - 0.94)	30.85	0.52
6	0.93 (0.67 - 1.00)	0.78 (0.61 - 0.91)	0.93 (0.86 - 0.97)	30.85	0.52
7	0.93 (0.67 - 1.00)	0.92 (0.70 - 0.99)	0.98 (0.92 - 1.00)	30.85	0.52
8	0.93 (0.67 - 1.00)	1.00 (0.88 - 1.00)	1.00 (0.96 - 1.00)	30.85	0.52
#5	ψ(·), θ(·), ρ(Lamprey+Large)				
1	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.95 (0.87 - 0.99)	30.95	0.54
2	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.91 (0.75 - 0.97)	30.95	0.54
3	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.80 (0.66 - 0.90)	30.95	0.54
4	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.83 (0.67 - 0.93)	30.95	0.54
5	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.89 (0.82 - 0.94)	30.95	0.54
6	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.93 (0.85- 0.97)	30.95	0.54
7	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.98 (0.93 - 1.00)	30.95	0.54
8	0.92 (0.67 - 1.00)	0.76 (0.65 - 0.85)	1.00 (0.96 - 1.00)	30.95	0.54
#6	ψ(·), θ(Lamprey+AquaticVeg), ρ(Lamprey+Large+Per_Fine+pH)				
1	0.93 (0.67 - 1.00)	0.68 (0.46 - 0.86)	0.95 (0.80 - 1.00)	31.10	0.55
2	0.93 (0.67 - 1.00)	0.52 (0.32 - 0.72)	0.88 (0.67 - 0.98)	31.10	0.55
3	0.93 (0.67 - 1.00)	0.61 (0.45- 0.76)	0.79 (0.65 - 0.89)	31.10	0.55
4	0.93 (0.67 - 1.00)	0.88 (0.76 - 0.93)	0.82 (0.63 - 0.93)	31.10	0.55
5	0.93 (0.67 - 1.00)	0.76 (0.52 - 0.92)	0.92 (0.80 - 0.98)	31.10	0.55
6	0.93 (0.67 - 1.00)	0.70 (0.56 - 0.81)	0.92 (0.80 - 0.98)	31.10	0.55
7	0.93 (0.67 - 1.00)	0.96 (0.82 - 1.00)	0.99 (0.93 - 1.00)	31.10	0.55
8	0.93 (0.67 - 1.00)	0.98 (0.83 - 1.00)	1.00 (0.94 - 1.00)	31.10	0.55
#7	ψ(·), θ(AquaticVeg), ρ(Lamprey+Large)				
1	0.93 (0.67 - 1.00)	0.21 (0.46 - 0.86)	0.95 (0.87 - 0.99)	31.22	0.54
2	0.93 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.90 (0.75 - 0.97)	31.22	0.54
3	0.93 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.80 (0.66 - 0.90)	31.22	0.54
4	0.93 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.83 (0.67 - 0.93)	31.22	0.54
5	0.93 (0.67 - 1.00)	0.81 (0.62 - 0.94)	0.89 (0.82 - 0.94)	31.22	0.54
6	0.93 (0.67 - 1.00)	0.76 (0.65 - 0.85)	0.93 (0.85 - 0.97)	31.22	0.54
7	0.93 (0.67 - 1.00)	0.81 (0.62 - 0.94)	0.98 (0.92 - 1.00)	31.22	0.54
8	0.93 (0.67 - 1.00)	0.71 (0.53 - 0.86)	1.00 (0.96 - 1.00)	31.22	0.54
#8	ψ(·), θ(Lamprey+AquaticVeg), ρ(·)				
1	0.93 (0.67 - 1.00)	0.68 (0.46 - 0.86)	0.92 (0.86 - 0.95)	31.51	0.56
2	0.93 (0.67 - 1.00)	0.52 (0.32 - 0.71)	0.83 (0.73 - 0.91)	31.51	0.56
3	0.93 (0.67 - 1.00)	0.61 (0.45- 0.75)	0.85 (0.76 - 0.91)	31.51	0.56
4	0.93 (0.67 - 1.00)	0.88 (0.76 - 0.96)	0.83 (0.73 - 0.91)	31.51	0.56
5	0.93 (0.67 - 1.00)	0.76 (0.53 - 0.92)	0.96 (0.85 - 0.99)	31.51	0.56

Site	Occupancy in site (ψ) (95% CI)	Occupancy in sample (θ) (95% CI)	Detection in replicate (ρ) (95% CI)	PPLC	WAIC
6	0.93 (0.67 - 1.00)	0.69 (0.56 - 0.81)	0.91 (0.85 - 0.95)	31.51	0.56
7	0.93 (0.67 - 1.00)	0.95 (0.82 - 1.00)	0.98 (0.92 - 1.00)	31.51	0.56
8	0.93 (0.67 - 1.00)	0.98 (0.84 - 1.00)	0.92 (0.86 - 0.95)	31.51	0.56
#9	ψ(·), θ(·), ρ(Large)				
1	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.95 (0.89 - 0.99)	32.27	0.55
2	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.96 (0.89 - 0.99)	32.27	0.55
3	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.90 (0.85 - 0.94)	32.27	0.55
4	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.80 (0.64 - 0.92)	32.27	0.55
5	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.91 (0.86 - 0.95)	32.27	0.55
6	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.94 (0.88- 0.98)	32.27	0.55
7	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.91 (0.86 - 0.95)	32.27	0.55
8	0.93 (0.66 - 1.00)	0.76 (0.66 - 0.85)	0.94 (0.88 - 0.98)	32.27	0.55
#10	ψ(·), θ(·), ρ(Per_Fine)				
1	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.91 (0.86 - 0.95)	32.3943	0.60
2	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.83 (0.72 - 0.91)	32.3943	0.60
3	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.84 (0.75 - 0.91)	32.3943	0.60
4	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.83 (0.72 - 0.91)	32.3943	0.60
5	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.96 (0.91 - 0.99)	32.3943	0.60
6	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.90 (0.85 - 0.95)	32.3943	0.60
7	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.98 (0.92 - 1.00)	32.3943	0.60
8	0.93 (0.67 - 1.00)	0.76 (0.66 - 0.85)	0.91 (0.86 - 0.95)	32.3943	0.60