Overview of eDNA and Applications for Research and Monitoring of Lampreys

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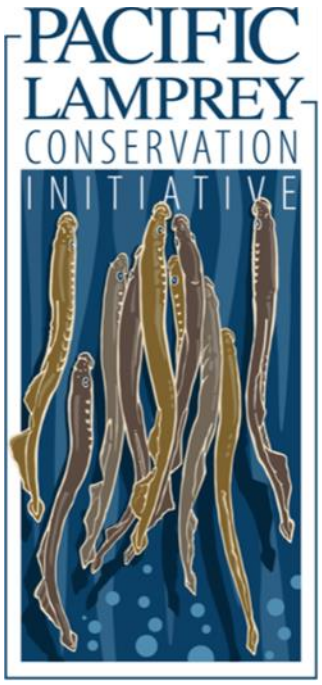

Lamprey Technical Workgroup

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1 Introduction

This document was developed by the Genetics Subgroup of the Lamprey Technical Workgroup at the request of the Conservation Team of the Pacific Lamprey Conservation Initiative (https://www.fws.gov/pacificlamprey/mainpage.cfm). This document is intended to provide a reference for fisheries managers, biologists, and other stakeholders interested in learning more about environmental DNA (eDNA) and applying this technique to lamprey research and monitoring projects. Section 2 provides an overview of eDNA, how it can be applied to answer questions about lampreys, field and laboratory protocols, analysis and interpretation of results, quality control requirements, assay design, and strengths and limitations compared with traditional lamprey survey approaches. Section 3 includes an up-to-date list of completed and ongoing applications of eDNA to study and monitor lamprey species.

The techniques and technologies for eDNA analysis are rapidly evolving. As a result, this document may not contain all current considerations for the use of this tool. Considerations here are generally oriented towards collecting samples for detection of lamprey in streams and rivers. This paper does not thoroughly discuss considerations for sampling in lentic or marine systems. Similarly, this paper does not discuss how eDNA sampling strategies may be adapted for non-lamprey species.

2 Environmental DNA Overview

2.1 What is eDNA and How Does it Work?

2.1.1 Overview

Environmental DNA refers to genetic material that is shed from organisms into their surrounding environment. Consequently, eDNA may be composed of waste product (feces and urine), mucus, skin cells, tissues, and gametes. Molecular genetic methods can be used to detect eDNA collected from water and sediment samples. Environmental DNA-based methods provide a supplement or alternative to traditional field sampling to detect and monitor a wide range of aquatic species, including fishes (Jerde et al. 2011; Takahara et al. 2013; Laramie et al. 2015a), amphibians (Ficetola et al. 2008; Pilliod et al. 2013), insects (Thomsen et al. 2012a), mollusks (Goldberg et al. 2013), and mammals (Padget-Stewart et al. 2016; Ushio et al. 2017). Several studies have demonstrated that eDNA surveys are often more sensitive, accurate, cost effective and time-efficient than traditional field sampling methods, particularly when the target organism is present at low abundance (Jerde et al. 2011; Dejean et al. 2012; Thomsen et al 2012b; Pilliod et al. 2013; Schmelzle and Kinziger 2016; Wilcox et al. 2016; Hinlo et al. 2017; Ostberg et al. 2019).

Three major processes contribute to the availability of eDNA in aquatic environments: production by source organisms, transport, and degradation (Goldberg et al. 2015). Production refers to the shedding of eDNA into the environment by organisms. The quantity shed into the environment is largely dependent on biomass, ecology, metabolism, and life history events. For example, a single spawning Pacific salmon (*Oncorhynchus* spp.) may produce more eDNA than a single filter-feeding larval lamprey. Once shed, eDNA is subject to transportation and degradation. Transport away from the source organism is facilitated by water movement, settling of eDNA from the water column into sediments, and re-suspension of genetic material from sediments into the water column. Microbial organisms (Lance et al. 2017) and abiotic factors such as ultraviolet light and water temperature (Pilliod et al. 2014; Strickler et al. 2015) promote degradation. As a result, eDNA samples are a composite of DNA from organisms upstream of a sampling point but may not necessarily represent DNA of all upstream organisms.

As with any sampling method, eDNA is not a perfect tool for accurately detecting organisms 100 percent of the time in every location. For example, the highly sensitive nature of eDNA detection leaves it prone to contamination that may result in false negative detections. However proper field and laboratory protocols can minimize this limitation. When properly applied, eDNA applications have great potential for contributing to the understanding of organism presence/absence and distribution, which are critical information needs of resource managers (Rees et al. 2014b; Thomsen and Willerslev 2015; Barnes and Turner 2016).

2.1.2 Environmental DNA Applications

Environmental DNA surveys have broad application to conservation and management. Environmental DNA methods are particularly useful for detecting organisms at low abundance, such as those that are rare, in decline, or at the edge of their distribution (Jerde et al. 2011; Dejean et al. 2012; Pilliod et al. 2013; Sigsgaard et al. 2015; McKelvey et al. 2016; Wilcox et al. 2016). Sampling aquatic eDNA is non-invasive and non-destructive; therefore, it is well suited for monitoring imperiled species (Laramie et al. 2015a; Spear et al. 2015; de Souza et al. 2016; McKelvey et al. 2016; Ostberg et al. 2018). These attributes can also enable samplers to bypass species take permits.

Environmental DNA can be used to monitor lamprey translocation and re-introduction programs (Grote and Carim 2017), identify barriers to lamprey migration (Halvorsen et al. 2020), and track recolonization following barrier removal or habitat improvement projects (Duda et al. 2021). A recent study suggests eDNA could even be used to identify and differentiate redds among species (Strobel et al. 2017).

Environmental DNA surveys are also useful for detecting aquatic invasive species (Dejean et al. 2012; Goldberg et al. 2013; Takahara et al. 2013; Díaz-Ferguson et al. 2014; Wilcox et al. 2016; Hinlo et al. 2017). Several studies are using this tool to monitor new and expanding invasions (Carim et al. 2019; USFWS 2019). Others have used eDNA methods to evaluate the success of eradication efforts (Dunker et al. 2016; Carim et al. 2020).

Because eDNA samples are collected from the environment, they can potentially contain DNA from all organisms present in a given area. As a result, eDNA samples may be

used for biodiversity assessments (Thomsen et al. 2012a; Hauck et al. 2019; Lecaudy et al. 2019) and can be reanalyzed for other target species at a later date as new objectives emerge (e.g., Dysthe et al. 2018).

Several studies conducted on diverse species across a wide range of habitats have found a positive relationship between eDNA concentration and biomass or abundance of target species (Takahara et al. 2012; Thomsen et al. 2012b; Goldberg et al. 2013; Pilliod et al. 2013; Klymus et al. 2015; Lacoursiere-Roussel, et al. 2016; Wilcox et al. 2016; Baldigo et al. 2017; Doi et al. 2017; Schloesser et al. 2018; Levi et al. 2019), suggesting that eDNA could provide a quantitative signal of relative biomass. However, the relationship between eDNA concentration and biomass or abundance for lamprey is not well-established. Schloesser et al. (2018) conducted a lab study using Sea Lamprey (*Petromyzon marinus*) and found a positive relationship between eDNA concentration and the number of adults, but no such relationship was apparent in larvae. However, they did find that eDNA detection probability increased with increasing numbers of larvae (Schloesser et al. 2018). Although precise estimates of biomass based on eDNA concentration is presently untenable, these studies suggest that eDNA concentration estimates could be informative regarding relative biomass over comparable environmental conditions. However, caution must be exercised when considering use of eDNA concentration to infer relative biomass of lampreys. The fate of detectable eDNA in aquatic environments is influenced by a suite of environmental, demographic, and biological factors with effects that are difficult to predict and control across various habitats.

2.1.3 Sampling Design

Field sampling for eDNA can be more sensitive and efficient than traditional survey methods, but it requires careful consideration and preparation when developing a sampling design. The complexity of a sample design depends on the research and monitoring objective. Single-grab samples within a sample site can provide detection/non-detection data for a point in time and adding spatial and temporal sampling can inform seasonal differences in distribution and detection. Several studies have demonstrated seasonal differences in eDNA abundance of lampreys and other species (Laramie et al. 2015a; Spear et al. 2015; de Souza et al. 2016; Furlan et al. 2016; Buxton et al. 2017; Ostberg et al. 2018; Wacker et al. 2019), indicating that detection of a given species at a particular location can be influenced by seasonal changes in a species' distribution and activity. Furthermore, target eDNA in aquatic environments may be heterogeneously distributed based on species ecology. For example, Carim et al. (2016) found higher concentrations of Opossum Shrimp (*Mysis diluviana*) eDNA in samples collected in the benthic zone of reservoirs (where the species resides during daylight hours) relative to samples collected at the water surface. Given this variation, an effective sampling design must consider both the objectives of the study and the ecology of the target species.

Although eDNA methods are more sensitive at detecting species relative to traditional methods, they may still fail to detect an organism when it is present. Incorporating replicate samples through space and time improves detection probability in addition to allowing for occupancy modeling, identification of sampling variance, and providing greater confidence in results.

2.1.4 Field Methods

Environmental DNA from aquatic species is typically collected from water samples that are either filtered or centrifuged to concentrate the genetic material. Water may be filtered directly in the field with a portable pump, or water samples may be stored on ice in a dark environment and filtered or centrifuged at a laboratory within 24 hours after sampling. Decay of DNA will occur until samples are filtered or centrifuged and the sample is placed in a preserving agent (see below); therefore, filtering samples in the field is generally preferred.

Sample collection volumes range across studies, but 0.25 – 5 L water sample volumes are typical (Pilliod et al. 2013; Laramie et al. 2015a; Gingera et al. 2016; McKelvey et al. 2016; Wilcox et al. 2016; Doi et al. 2017; Ostberg et al. 2018; Tillotson et al. 2018; USFWS 2019). Larger volumes will yield more DNA (Sepulveda et al. 2019). Collection volume, GPS coordinates of the sampling site, and the sampling date should be recorded for each water sample. Environmental covariates of interest (such as discharge, water temperature, turbidity, etc.) should also be recorded to inform the interpretation of results and elucidate associations between environmental parameters and eDNA detection.

For filtration, several different filter composition types and pore diameters are available. Studies with water samples collected from Common Carp (*Cyprinus carpio*) (Turner et al. 2014) and Brook Trout (*Salvelinus fontinalis*) (Wilcox et al. 2015b) suggest that most genetic material can be collected using filters with 1 – 10 µm pore diameter. Consequently, filters with pores ~1 µm in diameter have become popular for use with eDNA sampling. However, the choice of filter composition and pore size will depend on turbidity, filtration volume, and filter strength. Stream or lake sediments may be directly sampled for eDNA (Eichmiller et al. 2014; Turner et al. 2015). Although it has not been formally evaluated, sediment sampling may potentially be informative for identifying localized habitat patches that are occupied by larval lampreys.

Filter samples must be preserved to prevent degradation of eDNA. Three common methods of filter preservation are easy to apply in the field; placement of filters in (1) a container with silica desiccant beads (Carim et al. 2016), (2) 200-proof molecular grade ethanol (Laramie et al. 2015b), or (3) lysis buffer (Renshaw et al. 2015). Samples should be preserved immediately after filtration. Once filters have been preserved, they are relatively stable at ambient temperatures for a short period of time (days to weeks; Renshaw et al. 2015). However, degradation of DNA in the sample will be minimized if samples are stored between -20^{o}C and -80^{o}C and away from ultraviolet light, and this is recommended for long-term storage.

2.1.5 Laboratory Methods

Species identification from eDNA sampling is accomplished via targeted-species detection methods or through metabarcoding (see below). The choice of method will ultimately depend on the research or monitoring question being addressed. Targeted-species detection involves PCR amplification (conventional [cPCR]; quantitative [qPCR]; or droplet digital [ddPCR]) of the DNA extracted from a sample using species-specific genetic markers. Species-specific markers paired with qPCR and ddPCR technology are highly sensitive and effective at detecting target species that are present at low densities. Of the targeted-species detection methods, qPCR is most commonly applied.

Metabarcoding methods target multiple species in the pool of eDNA and typically involve sequencing of one or several gene regions across a wide range of taxa. This method has applications to biodiversity assessments and community structure information; however, a disadvantage is that this method does not necessarily resolve taxa to genus or species level. It may result in false negatives from lack of sensitivity and can also result in a false positive from sequencing error.

In the lab, eDNA is typically extracted from filters, centrifuged material, or sediment samples by using commercially available DNA extraction kits. After extraction, PCR can be used to test for the presence of a species using the targeted-species detection method. Presently, eDNA assays have been established for Pacific Lamprey (*Entosphenus tridentatus;* Carim et al. 2017; Ostberg et al. 2018), *Lampetra* spp. (Ostberg et al. 2018), Sea Lamprey (Gustavson et al. 2015; Gingera et al. 2016), Chestnut Lamprey *Ichthyomyzon castaneus* (Gingera et al. 2016), Silver/Northern Brook lampreys (*I. unicuspis* and *I. fossor*; Gingera et al. 2016), American Brook Lamprey *Lethenteron appendix* (Gingera et al. 2016), and Pouched Lamprey (*Geotria australis*) from Argentina (Nardi et al. 2020). Rigorous and methodical preparation and testing during the development of each target assay can ensure specificity and sensitivity of an assay to the target species. However, closely related species and intra- and inter-specific polymorphisms can present challenges to assay development and implementation of assays in natural habitats (Wilcox et al. 2015a; Ostberg et al. 2018). For example, the eDNA assay for *Lampetra* spp. developed by Ostberg et al. (2018) may have limited capability of detecting *Lampetra* in some locations south of the Columbia River Basin. Consequently, it is advisable that testing of eDNA assays on *Lampetra* tissue samples be performed prior to performing eDNA studies at locations that were not represented in Ostberg et al. (2018).

A typical qPCR run consists of DNA extracts, a suite of negative controls (see Section 2.1.7), and a serial dilution of a DNA standard that is a replica of the DNA fragment targeted in the species-specific assay. A standard curve can be generated from the serial dilution of standards, which provides information on amplification efficiency and allows the number of DNA copies in eDNA samples to be estimated through regression analysis. Replicate PCRs are performed on each DNA extract to improve detection

probability because eDNA often occurs at low concentrations in the environment which may result in inconsistent detections among PCR replicates.

2.1.6 Data Analysis and Interpretation

The analysis and interpretation of eDNA results depend on the objective and study design. For some objectives, such as roughly outlining the upstream extent of a species in a stream, the detection or non-detection of a species may be sufficient. For other objectives, such as understanding the number of samples required to maximize detection at a given location, statistical analysis and modeling may be required.

Environmental DNA often occurs at low concentrations in environmental samples; therefore, it is important to describe eDNA assay parameters around detection at low DNA concentrations. The reliability of an eDNA assay to detect DNA at low concentrations can be described by the Limit of Detection (LOD), which is the lowest concentration of standard that can be reliably detected with high confidence, for example a 95% detection probability (CLCI 2004; Bustin et al. 2009). Although eDNA samples in a study may have concentrations below an established LOD, and therefore below a desired confidence level, such samples still have application to detection/non-detection analyses. The precision of measuring eDNA at low concentrations can be described by the Limit of Quantification (LOQ), which is the lowest concentration of standard that can be reliably quantified with a defined precision, for example within a pre-defined coefficient of variation or standard deviation (CLCI 2004; Armbruster and Pry 2008; Klymus et al. 2019). Environmental DNA concentrations that exceed an established LOQ are acceptable for quantitative analysis whereas DNA concentrations that fall below the LOQ are acceptable for qualitative analysis (i.e., detection/non-detection).

Presently, there are no data interpretation criteria regarding the minimum number of positive PCRs that are required for inferring species presence. Environmental DNA studies have typically required a minimum of one positive (Laramie et al. 2015a; McKelvey et al. 2016) or more than one positive (Rees et al. 2014a; Ostberg et al. 2018) out of several PCRs performed on a sample. It is recommended that caution be used when inferring species presence at sample sites where only one of several PCR replicates tests positive for the target species and where the results were not replicated through repeat analysis of samples or repeated site visits (Goldberg et al. 2016).

A common objective to many studies is to determine whether DNA from a target species is detected at sample sites. Although detection implies the presence of target species DNA, non-detection does not necessarily imply the absence of the target species. Detection and non-detection results are qualitative data and can be applied to a range of analyses including relatively simple approaches such as generating maps that delineate species distributions or estimating naïve detection probabilities (which do not account for imperfect detections) to more complex approaches such as occupancy modeling. Occupancy modeling with eDNA has been applied across diverse taxa (Schmidt et al. 2013; Hunter et al. 2015; Schmelzle and Kinziger 2016; Sutter and Kinziger 2019), including Pacific Lamprey (Ostberg et al. 2019). Environmental DNA occupancy modeling methods account for imperfect detections across three nested, hierarchical sampling levels to estimate occupancy and detection probabilities: sample locations; water samples within location; and PCR replicates within water sample. Further, the testing of covariate effects can be performed at each of these different levels. The

occupancy models can also be used to estimate the probability of collecting eDNA in a water sample, given that the target species is present, and thereby estimate the number of water samples that may need to be collected at a location to achieve a specified probability of eDNA detection. An occupancy modeling package that is specific to eDNA application has been developed in R (Dorazio and Erickson 2018).

For some objectives, understanding the amount of target species' DNA in a sample may be useful. For example, although direct estimates of abundance and biomass are difficult to infer from eDNA results, areas with higher quantities of DNA are generally associated with areas of higher density or biomass. This information may be used to generally identify areas where a species is present at a population level density, or areas that are used by few individuals intermittently. Environmental DNA concentrations represent quantitative data and are typically estimated as DNA copy number. The precision at which the eDNA copy number can be estimated at low concentrations will depend on the LOQ. Hence, the accuracy of eDNA concentration estimates that fall below an established LOQ may be questionable.

2.1.7 Quality Control

Contamination is a major concern for eDNA studies because it can lead to false positive results; therefore, extreme care must be taken to prevent and identify contamination when it occurs. In the field, contamination can be prevented by sterilizing reusable field sampling equipment and supplies with bleach, pre-packaging collection supplies into individual "kits" for each sample location and avoiding introducing equipment or personal items from a previously sampled site. In the laboratory, contamination can be prevented by using clean practices: 1) separating the work flow of eDNA sample preparation into designated work rooms, 2) using equipment dedicated to processing eDNA samples that does not leave a designated work room, and 3) decontaminating work stations with ultraviolet light and/or bleach before and after each use.

Controls are integral to quality assurance and quality control and are used to identify contamination. Negative controls represent "blanks" containing no DNA that are included in the workflow, such as field controls, negative DNA extraction controls, and non-template controls (clean water added in place of extracted DNA in PCRs). The inclusion of "blanks" provides a means to assess the quality of the results. Positive DNA controls, such as standards or known DNA samples in cases where standards are not run, are included to confirm that the PCR amplification proceeded as expected. Other quality control processes include efficacious assay design, rigorous assay testing, replication of PCRs and water samples, and evaluating samples for PCR inhibition.

Compounds that inhibit the PCR process may be co-purified with DNA from environmental samples, resulting in delayed amplification or false negative results (see Section 2.2.2.2; Uchii et al. 2019; Lance and Guan 2020). Therefore, it is important to identify inhibited samples prior to testing for target species. Testing for inhibition is performed using internal positive controls (Goldberg et al. 2016) that are available as commercial kits. Once inhibited samples are identified, the inhibitory effects can be

alleviated by using commercial DNA clean-up kits or by diluting inhibited samples (McKee et al. 2015). In addition, some PCR mastermixes appear to be more resistant to inhibition effects than others (Uchii et al. 2019).

2.2 Strengths and Limitations Compared with Traditional Survey Approaches

2.2.1 Strengths

Environmental DNA offers a promising and economical alternative or supplement to traditional lamprey species survey approaches. Below we highlight the strengths and advantages of eDNA methods for surveying lamprey over traditional survey methods. These strengths include efficiency and sensitivity, sampling that is non-invasive, specificity of results, rapid sampling, diversity of information collected, and appropriateness of sampling for volunteers.

2.2.1.1 Efficiency and Sensitivity

Environmental DNA methods are time and cost efficient, allowing for rapid assessment of species presence and distribution at the watershed or subbasin level. McKelvey et al. (2016) found that eDNA surveys for Bull Trout (*Salvelinus confluentus*) in western Montana were fast (124 water samples were collected across five basins by a single crew in approximately 8 days) and reliable (i.e., largely consistent with past electrofishing). Collection of eDNA also results in decreased sampling delays, cost, and sampling restrictions related to State and Federal collection permits required for other sampling approaches.

Environmental DNA allows for more accurate detection of lampreys when they are present at low abundance/density (i.e., increased sensitivity). McKelvey et al. (2016) found that eDNA surveys for Bull Trout were sensitive (where Bull Trout were known to be scarce, eDNA samples were more sensitive than electrofishing). Wilcox et al. (2016) likewise found that eDNA assays were more sensitive than traditional electrofishing for determining the presence of Bull Trout, particularly at low fish densities.

2.2.1.2 Noninvasive Sampling

Sampling methods are noninvasive, making them ideal for surveying imperiled populations. Sigsgaard et al. (2015) found eDNA monitoring to be very effective at non-invasively sampling the critically endangered Danish Weather Loach (*Misgurnus fossilis*). The Danish population is thought to consist of fewer than 50 individuals. Environmental DNA methods detected this species at all sites where it had been previously detected using traditional fishing techniques, as well as in additional localities. These new occurrences detected with eDNA were later confirmed when live specimens were caught. The eDNA survey required less effort and lower costs than the traditional fishing survey and, given its sensitivity, helped indicate where more intensive traditional surveys should be focused.

2.2.1.3 Specificity of Results

Because detection is based on the presence of an organism's DNA, species or genus level identification can be made conclusively. This may be particularly valuable when surveying organisms that are difficult to identify non-lethally or in a field setting. For example, Gingera et al. (2016) designed assays that differentiated Chestnut Lamprey from Silver and Northern Brook lampreys. These species are difficult to distinguish visually as larvae, particularly below lengths of 80–110 mm (Neave et al. 2007); however, see Section 2.2.2.4 regarding lamprey species that are not genetically distinguishable.

2.2.1.4 Distribution

Surveys of eDNA are less labor intensive than traditional survey methods, allowing for a broader range of locations to be sampled more rapidly when identifying species distributions. Further, the field equipment used for eDNA surveys is light weight and less burdensome relative to traditional survey equipment, allowing areas with moderate to difficult access, such as backcountry, to be accessed more easily and, consequently, more likely to be included in species distribution surveys.

2.2.1.5 Diversity

Information from many species may be collected in a single sample. Environmental DNA samples contain DNA of all organisms present in a given area. As a result, these samples may be useful for looking at biodiversity at a given site, or for monitoring the presence of invasive species or disease that may impact lamprey populations.

2.2.1.6 Appropriate for Volunteers

The ease of sample collection encourages crowd sourced or citizen science sampling programs. Biggs et al. (2015) used eDNA to develop a national citizen science-based monitoring program for the Great Crested Newt (*Triturus cristatus*) in the United Kingdom. The eDNA approach, based on water samples collected by volunteers, was significantly more effective than traditional sampling (99.3 percent versus 44–76 percent for the different traditional sampling methods). Volunteers should attend appropriate training, and additional field controls may be advisable to confirm volunteer collections.

2.2.2 Weaknesses and Limitations

Environmental DNA has a number of limitations that should also be considered. The various assumptions and limitations of the method are still being studied and need to be more fully understood. Some of these weaknesses and limitations are discussed below, including false positive and false negative results, limited ability to determine relative biomass, and limits associated with being an indirect method of detection.

2.2.2.1 False Positives

False positives (when eDNA tests are positive but the species is not present) can be caused by methodological errors such as: contamination (where DNA detected in the assay came from a source outside of the system - e.g., when equipment or reagents came in contact with the organism or its DNA) or non-specific amplification (where the assay erroneously amplified DNA from one or more non-target species). Another type of false positive or false detection can result when DNA of the species of interest is present and correctly identified, but the species itself isn't present in the system (e.g., when DNA is transported into the system from another source such as water flow, boat movement, or a predator's feces; Mahon et al. 2013; Merkes et al. 2014).

False positives caused by methodological errors can be eliminated or reduced through the development and adherence to strict "clean" procedures (e.g., ensuring that filtration equipment and collected samples do not come into contact with the organism or its DNA), and monitored with the use of controls (i.e., blanks) inserted at multiple steps in the procedure (see Section 2.1). Non-specific amplification can be prevented by careful development and testing of the assay to ensure that DNA from only the target species is amplified (Wilcox et al. 2013). False positives or false detections caused by transport of the species' DNA into the system from other sources are harder to detect; follow-up sampling is then required to assess reproducibility of the results and management actions.

2.2.2.2 False Negatives

False negatives (failure of the assay to detect the DNA of the species when it is present) can result from the sensitivity of the assay not being sufficient for detection of low-quantity, low-quality (e.g., degraded) DNA, from conditions that result in dilution of eDNA in the water sample (e.g., low species' abundance, high flow rates), or the presence of inhibitors (e.g., tannic or humic acids) in the water sample that interfere with the PCR. Solutions to overcome these problems include improved amplification methods (e.g., probe-based qPCR or ddPCR; see Section 2.1), increased sampling effort (e.g., increasing the volume of water collected per sample, the total number of water samples, and including repeat sampling events to establish trend over time), and use of established and new methods that reduce or detect the effect of inhibitors on the PCR reaction (e.g., Dingle et al. 2004; McKee et al. 2015).

2.2.2.3 Relative Biomass

The quantity of eDNA in a sample may be used to understand changes in relative biomass across various locations, or at one location sampled over time. Spawning has been associated with higher release of eDNA. Monitoring eDNA quantity at a site over time may provide insights into spawn times. Some studies show that eDNA assays may be able to quantify relative biomass of a species (Lodge et al. 2012; Lacoursière-Roussel et al. 2016; Schloesser et al. 2018) or detect important life history events such as spawning (Erickson et al. 2016; Gingera et al. 2016); however, the relationship between species abundance and eDNA detectability under different environmental conditions is still poorly understood, as is the relationship between eDNA concentration and biomass.

2.2.2.4 Indirect Method

Because it is an indirect method of detection, eDNA does not provide information on the size, stage, or physical condition of the organisms. Due to variability in DNA production from across life stages, and variation in DNA transport and degradation across systems, eDNA methods cannot be used to estimate fish densities or biomass at a given site. However, eDNA surveys may provide guidance for areas where populations are present and surveys could benefit from more targeted sampling.

Environmental DNA assays can only distinguish among taxonomic groups that are genetically distinct. For example, Silver and Northern Brook lampreys and Western River and Brook lampreys (*L. ayresii* and *L. richardsoni*, respectively) cannot be distinguished genetically (Docker 2009; Docker et al. 2016; Gingera et al. 2016). However, direct capture of larvae would also not permit species-level identification, because they are also not visually distinguishable during this stage. Only the collection of post-metamorphic individuals would provide greater resolution than eDNA assays. However, identification to at least genus (e.g., distinguishing the frequently co-occurring Pacific Lamprey from the Western Brook Lamprey) is achievable with eDNA assays (Carim et al. 2017).

Environmental DNA does not provide direct information on the size or stage of the organisms (e.g., larvae or adults), nor does it confirm that the organism is alive. For example, eDNA assays were investigated as a possible cost-effective alternative or supplement to electrofishing for larval Sea Lamprey in the Great Lakes Basin (Gingera et al. 2016). However, detection of eDNA does not provide information on the size structure of the larvae, nor does it distinguish between DNA shed by the larvae versus DNA shed by spawning adults. In fact, given that the large-bodied, free-swimming adult lamprey, their gametes, and later their carcasses are expected to shed considerably more DNA than the small-bodied burrowing larvae, the eDNA signal from the spawning adults would be expected to overwhelm the larval signal for some time after spawning. Gingera et al. (2016) found that eDNA detection remained high until spawning ceased at the end of June, but decreased thereafter so that eDNA signals detected from mid-August onward were interpreted to be largely or exclusively from larvae.

Although eDNA does not provide direct life history information, with more studies correlating eDNA signal to various life history events, it appears that some inferences can be made. In the example above, spikes in eDNA corresponded to Sea Lamprey spawning; similarly, Erickson et al. (2016) found that eDNA concentration was correlated with pre-spawning movement in Bighead Carp (*Hypophthalmichthys nobilis*) and suggested that mass movement could be used as a proxy for predicting spawning.

Environmental DNA methods have been widely validated for use in detecting presence/absence of an organism but use in estimating relative biomass across sites or waterbodies is still unclear. Research to date suggests that eDNA may be able to quantify relative abundance, at least over comparable environmental conditions (e.g., Lodge et al. 2012; Lacoursière-Roussel et al. 2016). However, the effects of different environmental variables (e.g., flow rate, temperature, DNA degradation) have yet to be

fully elucidated (see Section 2.1) and should be explicitly addressed in all study designs looking to relate eDNA to abundance or biomass.

3 Application of eDNA to Lamprey Research and Monitoring

As described above, eDNA methods have the potential to revolutionize monitoring of aquatic species, particularly cryptic organisms like burrowed larval lampreys. Environmental DNA assays have been developed for at least seven lamprey species or species complexes (i.e., *Lampetra* spp. and Northern Brook/Silver lampreys, where closely related species cannot be distinguished). Monitoring with eDNA can reduce the level of effort required to detect species presence, especially across large spatial scales or in locations that are difficult or dangerous to sample using electrofishing. Furthermore, eDNA may also help provide estimates of relative abundance, at least under comparable environmental conditions, and help delineate the time and location of lamprey spawning. Given this potential, a number of projects applying eDNA methods to lamprey research or monitoring have been conducted and even more are now in progress (Table 1). Contact information for genetics labs that have provided eDNA services for lamprey applications is provided in Appendix A.

Table 1. Completed or ongoing applications of eDNA sampling to lamprey research and monitoring as of March 2021. Completed projects are listed chronologically; ongoing applications are listed alphabetically by lead organization.

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
Completed					
University College Dublin	Jens Carlson	Mulkear River and Annagh River, Ireland	An eDNA assay for Irish *Petromyzon marinus* and *Salmo trutta* and field validation in running water	Jens Carlson (University College Dublin)	Gustavson et al. (2015)
University of Manitoba	Margaret Docker and Timothy Gingera	Little Thessalon River, ON, Canada and lab	Detection and identification of lampreys in Great Lakes streams using environmental DNA	Margaret Docker (University of Manitoba)	Gingera et al. (2016)
USFWS – Mid-Columbia Fish and Wildlife Conservation Office	Ann Grote	Wenatchee River, WA	Using eDNA sampling to detect Pacific Lamprey in a large river: 2016 Wenatchee River Pilot Study.	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	Grote and Carim (2017)
USFS – National Genomics Center for Wildlife and Fish Conservation	Kellie Carim	Columbia River Basin	A Noninvasive Tool to Assess the Distribution of Pacific Lamprey (*Entosphenus tridentatus*) in the Columbia River Basin	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	Carim et al. (2017)
USGS – Western Fisheries Research Center	Carl Ostberg	Puget Sound watersheds	Distribution and seasonal differences in Pacific Lamprey and *Lampetra* spp eDNA across 18 Puget Sound watersheds	Carl Ostberg (USGS – Western Fisheries Research Center)	Ostberg et al. (2018)
USGS – Upper Midwest Environmental Sciences Center	Nick Schloesser, Chris Merkes, and Jon Amberg	Laboratory tanks	Correlating Sea Lamprey density with environmental DNA detections in the lab	Jon Amberg (USGS – Upper Midwest Environmental Sciences Center)	Schloesser et al. (2018)

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
University College Dublin	Fiona S. A. Bracken, Jens Carlsson	Mulkear Catchment	Identifying spawning sites and other critical habitat in lotic systems using eDNA "snapshots": A case study using the Sea Lamprey *Petromyzon marinus* L.	Jens Carlson (University College Dublin)	Bracken et al. (2018)
University of Alaska Fairbanks	Shink, K. G., T. M. Sutton, J. M. Murphy, and J. A. Lopez.	Bering Sea	Utilizing DNA metabarcoding to characterize the diet of marine-phase Arctic Lamprey (*Lethenteron camtschaticum*) in the eastern Bering Sea.		Shink et al. (2019)
USGS – Western Fisheries Research Center	Carl Ostberg, Jeff Jolley	Chehalis Basin	Evaluation of environmental DNA surveys for identifying occupancy and spatial distribution of Pacific Lamprey (*Entosphenus tridentatus*) and *Lampetra* spp. in a Washington coast watershed	Carl Ostberg (USGS – Western Fisheries Research Center)	Ostberg et al. (2019)
Yakama Nation Fisheries / Douglas County PUD / USFS – National Genomics Center for Wildlife and Fish Conservation	R. Lampman, Andrew Gingerich, Chas Kyger, and Kellie Carim	Columbia River Basin (Wells, Rocky Reach, McNary, and Bonneville dams, & mainstem & tributaries upstream of Wells Dam	Wells Project Pilot eDNA Sampling; experimental use of eDNA to monitor and track changes in eDNA signature over time in association with translocation activities in Upper Columbia Wells Project area (including Lower and Mid-Columbia region for comparison)	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	Lampman and Lumley (2020)
Yakama Nation Fisheries	Ralph Lampman	Subbasins in middle and upper Columbia River Basin	Coarse-scale eDNA samples were collected in nine subbasins within the Yakama Nation Ceded Lands. Pacific Lamprey eDNA was confirmed in 40 sites within the Klickitat, Lower Yakima, Upper Yakima, Crab, Wenatchee, Entiat, and Methow subbasins, while none was found within the White Salmon and Naches subbasins.	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	Lumley et al. (2020)

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
Laboratorio de Ecología, Fisiología y Evolución de Organismos Acuáticos, Ushuaia, Argentina	Cristina Fernanda Nardi, Tomás Chalde, et al.	Patagonia (Argentina)	Detection of lamprey in Southernmost South America by environmental DNA (eDNA) and molecular evidence for a new species		Nardi et al. (2020)
USGS – Western Fisheries Research Center	Carl Ostberg, Jeff Duda	Elwha Basin	Environmental DNA is an effective tool to track recolonizing migratory fish following large-scale dam removal	Carl Ostberg (USGS – Western Fisheries Research Center)	Duda et al. (2021)
Ongoing					
Cow Creek Band of Umpqua Tribe / USGS	Kelly Coates, Travis Mackie	North Umpqua Basin	Establishing baseline data on distribution of lampreys, other native fishes, and habitat requirements to help inform management decisions and restoration activities. Surveys will be conducted in the North Umpqua River Basin using electrofishing and eDNA collection to verify results.	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process
South Slough National Estuarine Research Reserve	Shon Schooler, Jenni Schmitt Alison Watts	Coos Bay	Lamprey distribution in tributaries to the South Slough in Coos Bay. Part of a larger project applying eDNA methods in estuaries, both in Oregon and New England.	Alison W. Watts (University of New Hampshire) and Kellie Carim (USFS National Genomics Center for Wildlife and Fish Conservation	In process
USFWS – Mid-Columbia Fish and Wildlife Conservation Office	Ann Grote	Icicle Creek	Monitoring translocation and possible recolonization of Icicle Creek in the Wenatchee River	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
USFWS – Mid-Columbia Fish and Wildlife Conservation Office	Ann Grote	Wenatchee River, WA	Monitoring translocation and distribution in the Upper Wenatchee River basin	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process
USFWS – Mid-Columbia Fish and Wildlife Conservation Office	Ann Grote	Okanogan River	Monitoring changing distribution with eDNA and electrofishing occupancy comparison	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process
USFS – National Genomics Center for Wildlife and Fish Conservation	Kellie Carim/Ann Grote/ H. McClellen	*Lampetra* historic/known range (focus on WA and OR)	Development and validation of a region-wide *Lampetra* eDNA marker	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In preparation
USFS – National Genomics Center for Wildlife and Fish Conservation	Kellie Carim, Michael Young and Dan Isaak	Historic range of Pacific lamprey in the U.S.	eDNA Basin-wide Lamprey Inventory and Monitoring Project (eBLIMP): using eDNA to identify and monitor the distribution of Pacific Lamprey.	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process
USFS – National Genomics Center for Wildlife and Fish Conservation	Kellie Carim, Michael Young and Dan Isaak	Streams in Oregon and Idaho	Comparison of eDNA detections and density of juvenile Pacific Lamprey in stream habitat	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process
USFS and Cow Creek Band of Umpqua Tribes	Casey Baldwin, Steve Burns, Kelly Coates	SW Oregon	SW Oregon eDNA Presence Survey Sampling for Pacific Lamprey and Associated Species	Kellie Carim (USFS – National Genomics Center for Wildlife and Fish Conservation)	In process

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
USGS – Western Fisheries Research Center	Marty Liedtke and Carl Ostberg	Laboratory tanks	Establish relationship between eDNA and biomass. Investigate persistence of eDNA following removal of the source and the distance from the source at eDNA can be detected. Analyzed both water and sediment samples for these tests.	Carl Ostberg (USGS – Western Fisheries Research Center)	In process
University of Alaska Fairbanks	Andres Lopez and Trent Sutton	Susitna River Basin, Alaska	This study will yield the following information: (1) relative abundance, size-frequency distributions and relationships, and habitat attributes by life stage for each species; (2) a complete list of reagents and conditions for eDNA assays; (3) raw sequence data from ddRAD-seq libraries from each tissue sample examined; (4) genotype datasets for the set of individuals sequenced; and (5) a population genomics report summarizing conclusions supported by the genotype data regarding the extent and distribution of genetic diversity in the three focal lamprey aggregations.	Andres Lopez (University of Alaska Fairbanks)	In process
University of Manitoba	Margaret Docker	Manitoba	Using environmental DNA to survey native lamprey distribution (*Ichthyomyzon* spp.) in Manitoba	Margaret Docker (University of Manitoba)	In process

Lead Organization	Principal Investigator(s)	Study System	Study Title or Objectives	Genetics Lab	Publication or Report
University of Manitoba / University of Guelph / Fisheries and Oceans Canada / US Fish and Wildlife Service	Margaret Docker, Bob Hanner, Mike Steeves & Rebecca Philipps	Great Lakes	Field-ready environmental DNA (eDNA) protocols and tools for Sea Lamprey assessment	Margaret Docker (University of Manitoba) & Bob Hanner (University of Guelph)	In process
Washington State Department of Natural Resources	Joy Polston-Barnes & Jessica Olmstead	Nisqually River	Using environmental DNA sediment sampling to evaluate a detection method for Pacific Lamprey larvae. Research questions: 1) Does eDNA analysis of riverbed sediment accurately identify presence and abundance of lamprey larvae? 2) How does eDNA sediment analysis compare to other methodologies used to identify the presence of lamprey larvae, including eDNA water analysis and electrofishing?	(WDFW) Molecular Genetics Laboratory	In process
Yakama Nation Fisheries / USGS – Western Fisheries Research Center / PNNL	Ralph Lampman, Carl Ostberg, and Bob Mueller	Yakima	Monitoring of temporal relative changes in 1) eDNA signatures and 2) lamprey species relative abundance using deep water shocking and electrofishing in mainstem Yakima River and Sunnyside and Wapato irrigation diversions during the irrigation season.	Carl Ostberg (USGS – Western Fisheries Research Center)	In process

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Appendix A

Appendix Table A-1. Genetics labs that have provided eDNA services for lamprey applications.

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