Writing Sample:

Introduction from undergraduate thesis entitled "Description, Comparison, and Prediction of Pacific Lamprey and Western Brook Lamprey Habitats in the Southern Oregon Coastal Watershed"

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Lampreys are jawless fish that belong to one of the most ancient extant lineages, evolving into their current form about 340 million years ago (Wyodski & Whitney 2003). Oregon constitutes crucial lamprey habitat and hosts 10 of the roughly 40 lamprey species distributed globally (Clemens & Wade 2023). Five of the ten species inhabiting Oregon are listed by the state as Sensitive Species, including the Pacific lamprey (*Entosphenus tridentatus*) and western brook lamprey (*Lampetra ayresii*) (Clemens et al. 2020).

The western brook lamprey has historically been referred to as *Lampetra richardsoni* due to inconsistencies between its resident lifestyle and the anadromous tendencies of its satellite species, the western river lamprey (*Lampetra ayresii*). Carim et al. 2023 proposes the consolidation of these species on the basis of genetic similarity and classification under *Lampetra ayresii* due to its precedence. The present study uses *Lampetra ayresii* in agreement with Carim et al. 2023.

The Pacific lamprey and western brook lamprey coexist in southern Oregon coastal watersheds and exhibit significant habitat overlap. This habitat overlap may be explained by the species similarities in environmental requirements during reproduction and early life stages. Both species require deep pockets of sediment to build their redds, or nests, to shelter their eggs from the water column. After hatching, ammocoetes, or larvae, remain nearby for several years (*E.*

tridentatus for 5-7 yrs, *L. ayresii* for 4 yrs) before metamorphosing (McPhail 2007). During this time, ammocoetes bury themselves in pockets of sediment during the day and extend their bodies into the water column at night to filter feed.

Following metamorphosis, there are few similarities between these species. The western brook lamprey is a resident species living the duration of its life in freshwater streams. This species obtains nutrients solely from filter feeding until they are adults, when feeding ceases entirely. In contrast, the Pacific lamprey is anadromous and migrates to the Pacific Ocean as a juvenile after living in freshwater for 3-7 years (Ostberg et al. 2019). Pacific lampreys live in the ocean for 20-40 months where they become parasitic on marine fish and mammals until they are ready to reenter freshwater streams and spawn (Wyodski & Whitney 2003; Ostberg et al. 2019; Close 2002). Both species die within several months of spawning (Wyodski & Whitney 2003). There is also a notable difference in the species' maximum size, as the Pacific lamprey can reach up to 30 in (850 mm) in length while western brook lampreys are rarely longer than 7 in (180 mm) (Wyodski & Whitney 2003). Marked differences in the sizes and life histories of these two species suggest that attention to variables associated with these differences should allow for distinction between their preferred habitats.

Despite the negative connotation lampreys hold for many Americans as a result of sea lamprey invasions in the Great Lakes, they typically balance ecosystems and serve many invaluable ecological and cultural functions (Close 2002). Lampreys serve as prey for other animals throughout all stages of their lives. Freshwater fish, namely rainbow trout and speckled dace, have been observed consuming lamprey eggs that overflow from redds (Close 2002). Coho salmon are known to prey on ammocoetes as they first emerge from their eggs (Close 2002).

Ammocoetes and adults are known prey items of many freshwater fish, birds (including great blue herons), terrestrial mammals, and pinnipeds (Dunkle et al. 2020; Close 2002).

Many of the species that prey on lampreys also hunt salmonids. In comparison with salmon, lampreys are easier to capture, have higher caloric value, and often migrate in dense schools (Close 2002). Collis et al. 2001 found that 71% by volume of four Columbia River larids' (gulls) diets in May was composed of lampreys. Furthermore, Pacific lampreys have been identified as the most common prey item of seals and sea lions in the Pacific Northwest (Roffe & Mate 1984). Because seabirds and pinnipeds are primary predators of salmon, their consumption of lampreys can act as a buffer against salmonid predation and relieve pressure on these populations. Declining lamprey populations and neglected conservation can be equated with increased predation of salmonids (Kalan et al. 2023; Wyodski & Whitney 2003).

Lampreys continue to feed their ecosystems after death, acting as sustenance for many of the same predators listed above, but also for smaller fauna not capable of hunting live lamprey. The migratory lifestyle of Pacific lampreys aids in the distribution of marine nutrients, an important service considering the disparity between the eutrophic NE Pacific and oligotrophic freshwater and riparian ecosystems. Influx of subsidies in lotic ecosystems has proven to be an important contributor to primary and secondary production (Dunkle et al. 2020). Lampreys only absorb 30-40% of the nutrients from food they ingest, meaning they also support the diets of smaller animals and meiofauna. Undigested food is passed in a form finer than when it was originally ingested, facilitating the uptake of nutrients by smaller suspension and deposit feeders (Close 2002).

The behavior associated with lamprey redd construction establishes them as ecosystem engineers. Physical and chemical properties of the stream bed determine the types of species and communities that may thrive there. Redd construction requires lampreys to disturb the substrate, facilitating the cycling of nutrients, energy, and oxygen between interstitial habitat and the water column (Boeker & Geist 2016). These services, in conjunction with the microhabitats formed during burrowing, aid in benthic macroinvertebrate survival and reproduction and are important in meiofaunal community composition.

Kalan et al. (2023) reported on Pacific lampreys' contributions to maintaining and improving water quality. Suspended coliforms, notably Echerichia coli (*E. coli*), can enter streams from agricultural practices, sewage, and defecation by animals and people, and runoff. High concentrations of E. coli threaten water quality, biodiversity, and human and ecological health. Kalan et al. (2023) showed that the filter feeding behavior of lampreys, specifically ammocoetes, significantly decreased concentrations of E. coli in the water column. With this behavior, lampreys not only support the health of other native species but make bodies of freshwater safe for human uses including water consumption, recreation, and fishing (Kalan et al. 2023).

Lampreys also have significance among humans due to their viability as bait for fishing, feed for salmonid cultures, and uses among indigenous tribes. Since time immemorial, indigenous groups have captured and processed lampreys for food by smoking, sun-drying, and salting the fish. It has been reported that they valued lampreys as highly as salmon and that the lampreys also had ceremonial and medicinal purposes for tribes (Wyodski & Whitney 2003). In addition to acting as a food source, lampreys were also valued for their rich oil which was often applied to the body and hair. The channeling and damming of lotic systems has significantly decreased the amount of suitable lamprey habitat accessible for fishing. The decline of lamprey is associated with a loss of fishing opportunities and thus a loss of culture, as many young indigenous people are no longer familiar with the capture, preparation, and use of lampreys (Close 2002).

Flow regulation of rivers not only changes habitats and the amount of flowing water to distribute nutrients to suspension feeders, but also establishes barriers to migration. While the Pacific lamprey's larger body size allows it to overcome some barriers by suctioning to flat surfaces, doing so is nearly impossible for smaller lampreys like the western brook lamprey (Clemens & Wade 2023; Wyodski & Whitney 2003). Between 1966 and 2001, the number of Pacific lampreys recorded at Winchester Dam in the Umpqua River dropped from 46,785 to 34 (Close 2002). Lampreys' reliance on benthic habitat also makes them incredibly vulnerable to any changes in hydrogeomorphic features and general habitat degradation (Boeker & Geist 2016). Regardless of population declines and loss of habitat, lamprey conservation has historically received little attention and the western brook and Pacific lampreys were both denied for inclusion in the Endangered Species Act in 2004 (Carim et al. 2023).

One of the most common methods used in lamprey studies is electrofishing which requires researchers to use equipment to influence lampreys to leave the sediment where they burrow, capture and identify them, and record their metrics. Although this was the standard study method in the past, its requirement for specialized equipment, researchers trained in identification, and disturbing the species and their environments makes it impractical. Even with specialized equipment and team members, errors in taxonomic identification are possible and habitats can be hard to reach (Balasingham et al. 2017). These issues are particularly relevant to this project because aside from their adult size, the Pacific lamprey and western brook lamprey can be exceedingly difficult to identify due to the morphological simplicity of family Petromyzontidae. This becomes an issue when attempting to distinguish between the species' ammocoetes, as they are of similar size and only differ phenotypically by the pigmentation in their caudal fins.

The sampling effort that provided the data used in this analysis tested a genetic barcoding method that uses environmental DNA (eDNA) collected from streams to determine species presence (Schooler et al. 2022). This procedure stands to resolve the issues presented by common study methods such as electrofishing. eDNA sampling is a new method of species detection that has been rapidly growing in the field of macrobial aquatic species observation over the last decade (Carim et al. 2016). The process of eDNA sampling requires researchers to pass stream water through filters, then test for the presence of DNA from the target species. eDNA can come from many sources including shed skin cells or exoskeletons, urine, feces, sperm, eggs, mucus, and decaying tissues (Balasingham et al. 2017; Seymour et al. 2018). In addition to resolving the issues of false identification, habitat accessibility, and the complications of capturing individuals, eDNA methods are favorable because of their sensitivity, reliability, and efficiency. Each sample requires 15 minutes or less to collect, significantly decreasing sampling time and cost (Carim et al. 2016; Balasingham et al. 2017). eDNA is detectable for hours to days after the source is removed and can be detected in instances where other methods may incorrectly deduce absence. This short persistence time frame offers an additional positive of the method: near real-time monitoring (Seymour et al. 2018).

While results from eDNA can be quantitative to give some indication of population density or distance from the sample site, the eDNA results used in this analysis simply indicate presence or absence (Balasingham 2017; Seymour et al. 2018). It is also worth noting that most past eDNA studies have been undertaken in lentic ecosystems (Seymour et al. 2018). There are many factors to consider when trying to understand spatial patterns via eDNA sampling in lotic ecosystems. As lentic ecosystems experience very low levels of flow, their conditions are more static and have a fairly low rate of change. The flow rate, pH, and temperature of lotic ecosystems, on the other hand, can change drastically day by day depending on the season, weather, and organic input (Seymour et al. 2018). These factors all influence eDNA persistence and detection and thus, understanding the meaning of eDNA results from these systems can be difficult. It is also important to realize that sampling a flowing system entails sampling upstream habitat as well as the sample location. For example, in a study of a river in eastern Canada, Balasingham et al. (2017) found that residual eDNA was still detected 11.5 hours after the source was removed at the source site and the residual eDNA signal strength decreased as sample distance increased downstream. In a meta-analysis of eDNA downstream transport Jo and Yamanaka (2022) found that most eDNA particles travel less than 2km under ordinary hydrogeographic conditions.

Due to the difficulties associated with elucidating the extent of upstream habitat that is included in an eDNA sample, especially when using presence/absence rather than quantitative data, the GIS analysis used in this study treats each sample as a discrete sample site. It is acknowledged that the specific sample site identified as suitable based on a positive PCR may not actually contain lampreys, but rather be located downstream from the detected suitable habitat. Therefore, this caveat should be considered when assessing the results of the analysis. While this may produce misleading results for variables like aspect, general trends in elevation, for example, should still be accurate. For some variables, such as elevation limits, the properties of eDNA sampling are a benefit because the sample integrates over a much longer stream distance than a discrete sample would, so determinations of absence above the sample location are more robust.

This study aimed to analyze presence data collected using eDNA methods to elucidate the specific environmental variables that define Pacific lamprey and western brook lamprey habitats. Lampreys face many threats, many of which (e.g. habitat degradation and simplification, loss of riparian cover, artificial barriers) are a direct result of human activities (Clemens & Wade 2023). Lampreys serve countless ecological and cultural functions, so proper assessment of their presence and threats is crucial to informing management and restoration practices, as well as construction projects (Schooler et al. 2022). Understanding defining habitat characteristics would allow for quick assessment of risk to lampreys before undergoing invasive projects.

The products of this analysis should be useful in understanding the likelihood of lamprey presence in a given stream based on variables that prove significant. While the predictions made by the culminating/culminant/cumulative models cannot stand on their own due to exclusion of temporally variable factors like flow and temperature, they should be informative preliminary tools for determining whether presence of these species is likely or at all possible.