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Evaluation of Larval Pacific Lamprey Occupancy of Habitat Restoration Sites in the Portland Harbor Superfund Area

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On the cover: *Photograph of a tributary in the Harborton Restoration project area. (Photo by M. Blanchard).*

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Abstract – Habitat restoration actions focused on the recovery of juvenile Chinook salmon *Oncorhynchus tshawytscha* are being implemented in the Portland Harbor Superfund area of the Willamette River. These actions may also have effects on co-occurring Pacific Lamprey *Entosphenus tridentatus*. Use of restored habitats by lampreys, particularly the larval life stage, has not been extensively studied. Therefore, there is a benefit from monitoring the effectiveness of these efforts relative to larval Pacific Lamprey. Determining the effects of habitat restoration actions on Pacific Lamprey requires evaluation of lamprey occurrence before and after project implementation. This study is focused on the occupancy of larval Pacific Lamprey and *Lampetra* spp. in shoreline, confluence, and tributary habitats at five restoration sites. These restoration sites have been constructed to provide compensation for injuries to natural resources as part of the Portland Harbor Natural Resource Damage Assessment (NRDA). These restoration sites include Alder Point, Harborton, Linnton, Triangle Park, and Rinearson. In addition, the study is evaluating the occupancy of lamprey at a non-NRDA site, PGE 13.1, located in a reach of the Willamette River that bisects the city of Portland. In 2023, we sampled three restoration sites, Harborton, Linnton, and Rinearson. We also evaluated whether larval Pacific Lamprey occupied corresponding habitats at five reference sites in the Portland Harbor Superfund area (McCarthy Creek, Columbia Slough, Cemetery Creek, Oswego Creek, and Multnomah Channel). A generalized random tessellation-stratified approach was used to select random, spatially-balanced sample quadrats (30 m x 30 m square) across the lower Willamette River and Multnomah Channel, or sample reaches (50-m) in wadable tributaries. In 2023, no larval lamprey were detected at Harborton south confluence, delta confluence, or tributary, however, one larval lamprey was captured at the Harborton north confluence. This was the first detection in the north confluence post restoration. No lamprey were detected in the confluence, shoreline, or tributary habitat at the Linnton restoration site. At the Rinearson Natural Area, larval lamprey were detected in one of the seven tributary reaches ($d = 0.33$), and eight larval lamprey were detected in five of the ten Rinearson confluence sites ($d = 0.50$). At the five reference sites sampled in 2023, lamprey were detected at Columbia Slough, Cemetery Creek, and Oswego Creek. A total of 10 larval lamprey were captured at restoration sites and 13 larval lamprey and one transforming Lampetra spp. were captured at reference sites. This information is being used as part of a long-term evaluation of the effects of habitat restoration on occupancy and distribution of larval lamprey in the Portland Harbor Superfund area.

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Table of Contents

Monica R. Blanchard, Joseph J. Skalicky and Timothy A. Whitesel 1
Disclaimers 4
Evaluation of Larval Pacific Lamprey Occupancy at Habitat Restoration Sites in the Portland Harbor Superfund Area 1
Table of Contents 3
List of Tables 4
List of Figures 5
Introduction 6
Study Sites 8
Restoration Sites 8
Reference Sites 11
Methods 14
Sample Framework 14
Restoration Sites 15
Reference Sites 17
Reach Sampling in Wadable Habitats 19
Quadrat Sampling in Deep Water Habitats 19
Biological Data Collection 20
Habitat Data Collection 20
Results 34
Restoration Sites 34
Reference Sites 36
Discussion 49
Acknowledgements 52
Literature Cited 53
Appendix 1. 51
U.S. Fish and Wildlife Service 52

List of Tables

Table 1. In 2023, total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small (i.e., less than 60 mm TL) larvae cannot be accurately identified and are classified as unidentified (UNID)…….....39

Table 2. In 2023, habitat variables measured at restoration and reference sites. Capture depth range is the minimum and maximum depths at which lamprey were captured. Sediment collection was transferred to ALS Environmental Laboratory (Kelso, WA)…….…... 40

Table 3. Occupancy results from sampling at Alder Point restoration sites and the paired Ross Island reference site across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID). 41

Table 4. Occupancy results from sampling at Harborton restoration sites and the paired Columbia Slough reference site across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID)….....…42

Table 5. Occupancy results from sampling at Linnton restoration sites and the paired McCarthy reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID)…….……43

Table 6. Occupancy results from sampling at Rinearson restoration sites and the paired Cemetery Creek and Oswego Creek reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID)…..45

Table 7. Occupancy results from sampling at unpaired restoration sites and additional reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID)…….……47

Table 8. Summary table of mainstem habitat types across all years and all sites. Sites are listed from downstream to upstream with reference sites highlighted in gray. Total lamprey (*n*) is the sum of all lamprey collected at the site and habitat type across all years. Average Lamprey/yr is the mean number of lamprey sampled per year at each site and habitat type, with observed minimum and maximum in parentheses. Mean *d* is the mean detection rate at the site and habitat type across all years, calculated from *d* value in Table 3-7……..48

List of Figures

Figure 1. Portland Harbor Superfund study area (orange outline) and the broader focus area (red outline) on the lower Willamette River..... 7

Figure 2. Locations of the restoration (white circles) and reference (gray circles) sites. Note PGE 13.1 is not a NRDA restoration site..... 13

Figure 3. Alder Point restoration site sample sites. Habitats within the sites are confluence quadrats (blue points; each point represent a quadrat center point), shoreline quadrats (yellow points), and slough quadrats (red points). 21

Figure 4. Harborton restoration sample sites with North Tributary quadrats (yellow points), South Tributary quadrats (blue points), and confluence quadrats (red points). 22

Figure 5. Linnton restoration site with shoreline quadrats (yellow points) and confluence quadrats (blue points)..... 23

Figure 6. Triangle Park restoration site with shoreline quadrats (yellow points). 24

Figure 7. Rinearson Natural Area restoration site with confluence quadrats (blue points) and tributary sample reaches (red points). 25

Figure 8. PGE 13.1 restoration site with shoreline quadrats (yellow points). 26

Figure 9. Multnomah Channel reference site with shoreline quadrats (yellow points). 27

Figure 10. McCarthy Creek reference site with confluence quadrats (blue points) and shoreline quadrats (yellow points)..... 28

Figure 11. Columbia Slough reference site confluence quadrats (blue points). 29

Figure 12. Ross Island reference site shoreline quadrats (yellow points)..... 30

Figure 13. Cemetery Creek reference site with confluence (blue points) and shoreline (yellow points). 31

Figure 14. Oswego Creek reference site shoreline quadrats (blue points) and tributary quadrats (red). 32

Figure 15. Miller Creek tributary reference quadrats (red points). 33

Figure 16. *Anodonta* spp. collected in the sediment sample at the Delta Confluence habitat at the Harborton site.……35

Figure 17. Transforming *Lampetra* spp. sampled at the Columbia Slough reference site……..37

Introduction

Pacific Lamprey *Entosphenus tridentatus* in many areas, such as the Columbia River Basin (CRB), appear to have had a decline in abundance (Close et al. 2002) and have been given protected status within the State of Oregon (Kostow 2002). Lampreys are culturally important to Native American tribes, are ecologically important within stream systems and food webs, and are an indicator species whose decline provides further insight into the impact of human actions on ecological function (Close et al. 2002). Information is lacking on the basic biology, ecology, and population dynamics that is required for effective conservation and management of lamprey species (Clemens et al. 2017).

Pacific Lampreys have a complex, anadromous life history that includes a multi-year larval life stage, migratory, parasitic marine juvenile stage, and adult freshwater stage (Scott and Crossman 1973). Larvae live burrowed in stream and river sediments for multiple years after hatching, where they filter feed detritus and organic material (Sutton and Bowen 1994). Larval metamorphosis into juveniles occurs from July to December (McGree et al. 2008) and major migrations of juveniles are made downstream to the Pacific Ocean in the fall and spring (Beamish and Levings 1991). The sympatric Western River/Western Brook Lamprey *Lampetra ayresii*, which display multiple life history strategies (Carim et al. 2023), and Pacific Brook Lamprey *Lampetra pacifica* have been documented in the Willamette River watershed and use the same larval rearing habitats. For both species, much of the information on distribution and habitat preference of larvae comes from CRB tributary systems (Moser and Close 2003; Torgersen and Close 2004; Stone and Barndt 2005; Stone 2006; Renaud 1997; Reid et al. 2011) and coastal basins (Farlinger and Beamish 1984; Russell et al. 1987; Gunckel et al. 2009).

Larval lampreys are known to occur in sediments of low gradient (Torgersen and Close 2004), including large rivers (Jolley et al. 2012; Harris and Jolley 2017), but their use of relatively large river habitats and deep-water areas is not well understood. Downstream movement of larvae, whether passive or active, appears to occur year-round (Nursall and Buchwald 1972; Gadomski and Barfoot 1998; White and Harvey 2003). Larval Sea Lamprey *Petromyzon marinus* have been documented in deep-water habitats in tributaries of the Great Lakes, within the lakes in proximity to river mouths (Hansen and Hayne 1962; Wagner and Stauffer 1962; Lee and Weise 1989; Bergstedt and Genovese 1994; Fodale et al. 2003), and in large water bodies associated with the St. Marys River (Young et al. 1996). However, references to other species occurring in deep-water or lacustrine habitats are scarce (American Brook Lamprey *L. appendix*; Hansen and Hayne 1962). In the Pacific Northwest, observations of larval lamprey occurrence in large rivers have been made. These occurrences have been observed during smolt monitoring operations at Columbia River hydropower facilities, impinged on screens associated with juvenile bypass systems (Moursund et al. 2003; CRITFC 2008), or through observation during dewatering events. Specific collections of larvae have been made in large river habitats in British Columbia, which are thought to be representative of larvae dispersing downstream (Beamish and Youson 1987; Beamish and Levings 1991). More recently, evaluations of larval Pacific Lamprey occupancy and distribution in mainstem river habitats have suggested widespread occurrence in certain areas of the Columbia River and Willamette River mainstem (Jolley et al. 2012; Harris and Jolley 2017; Arntzen and Mueller 2017), with higher probability of presence at locations near tributary mouths (Blanchard et al. 2023).

A portion of the mainstem lower Willamette River, an area that is known to be occupied by

larval Pacific and *Lampetra* spp. (Jolley et al. 2012), was declared a Superfund site in 2000 by the U.S. Environmental Protection Agency. The Superfund study area extends from river kilometer 3.2 to river kilometer 18.9 and has a broader focus area extending from the Columbia River to Willamette Falls, river kilometer 0-42 (Figure 1). To compensate for past environmental damage being identified through the Natural Resource Damage Assessment (NRDA) process, this area is subject to various habitat restoration projects as well as assessments of the effectiveness of these projects. Restoration is being used as a broad and general term intended to capture efforts to revitalize, rehabilitate, replace, or acquire the equivalent (see Rosenzweig 2003; Roni et al. 2008) of those natural resources injured as the result of hazardous substance and oil releases from the Portland Harbor Superfund site (NOAA 2017). Projects in this aquatic environment are primarily focused on recovering juvenile Chinook Salmon *Oncorhynchus tshawytscha*. It is unclear whether these restoration projects will provide benefits to other co-occurring species including Pacific Lamprey. However, these projects provide an opportunity to understand the potential effects of habitat restoration on, specifically, larval and juvenile lampreys.

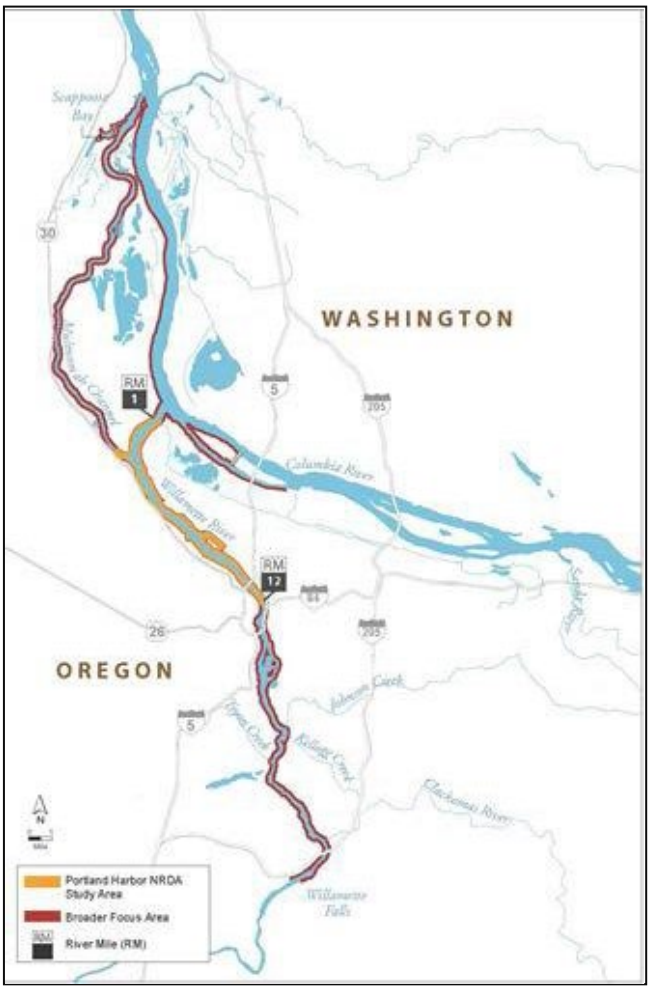

Figure 1. Portland Harbor Superfund study area (orange outline) and the broader focus area (red outline) on the lower Willamette River.

A lamprey monitoring plan (LMP) for habitat restoration projects in the Portland Harbor Superfund area was developed based on a set of monitoring goals and objectives that were identified by the Trustee Council and lamprey biologists in Fall 2011. The LMP priorities included (i) monitoring the impact of projects on larval and juvenile lamprey populations and health in Portland Harbor, and (ii) gathering information about larval and juvenile lamprey life history, biology, and habitat requirements that could be used by the Trustee Council to inform future design and evaluation of habitat restoration projects targeting lamprey. Since lamprey biology and life history are different from many other aquatic biota, the overlap between the LMP and the general monitoring and stewardship plan is not extensive. The LMP differs from the general monitoring and stewardship plan, in part, because the lamprey monitoring is proposed to continue for a period of 20 years. To maximize efficiencies, the Trustee Council will, to the extent possible, use data collected as part of the LMP for general monitoring and stewardship. Biologists

recommended monitoring lamprey for 20 years after the completion of a habitat restoration project, in order to capture data for one to two complete generations. Pre-implementation monitoring will be conducted to the extent practical at each project site. Because lampreys can colonize habitats rapidly, monitoring will be conducted on a yearly basis for the first five years after project implementation, and every five years thereafter. In general, the proposed work is guided by the LMP. However, due to site-specific conditions and constraints, the metrics and timing of monitoring proposed for any given site may differ from those outlined in the LMP.

Understanding larval lamprey usage of areas in and adjacent to rehabilitated habitat is critical to gauging the effectiveness of these projects. Outside of dam removal efforts, minimal information is available on whether lampreys will colonize rehabilitated habitats. Investigation on which life stages may use these restored habitats and for how long is needed for future monitoring efforts and restoration design. A before-after control- impact (BACI, Green 1979) approach is being used to evaluate the effectiveness of habitat restoration projects. The BACI approach allows us to make inferences about whether observed changes in lamprey occupancy are the result of the habitat restoration projects. Our sampling in 2023 was part of an ongoing effort to determine whether larval Pacific Lamprey occupy restoration sites and reference sites both prior to and after project implementation. Our specific objectives for this phase of NRDA monitoring were as follows:

1. Determine whether lamprey occupy restoration sites in the lower Willamette River and Multnomah Channel.
2. Determine whether lamprey occupy reference sites in the lower Willamette River and Multnomah Channel.
3. Determine the types of habitat available at each site and in which habitat types lamprey are detected.
4. Characterize lamprey species and life history stage that occupy each site.
5. Evaluate the health of lamprey detected at each site.

Study Sites

Restoration Sites

Alder Point

The Alder Point site is located at the southern tip of Sauvie Island (Multnomah County, OR), and thus is bordered on the east side by the Willamette River (at approximately river km 6), and on the west side by the Multnomah Channel (Figure 2). As part of a habitat restoration project, slough habitat (henceforth Alder Slough) was constructed through the site, connecting the Willamette River and Multnomah Channel. Unlike the typical confluence habitat in the Superfund area (a tributary or slough having a single confluence with the mainstem), Alder Slough has three distinct confluence habitats, two in the main Willamette River and one in the

Multnomah Channel. Restoration of shoreline habitat also occurred along the Willamette River and Multnomah Channel (Figure 3). Pre-implementation sampling was conducted in 2014. Post-implementation sampling was conducted from 2016 to 2020. Sampling did not occur in 2023 at this restoration site.

Harborton

The Harborton site is located on the southwest side of the Willamette River at river km 5.1, near the confluence of the Multnomah Channel (Figure 2). The site contains the Harborton Wetlands (tributary habitat), a remnant black cottonwood and ash floodplain that provides off-channel habitat, floodplain function, and habitat connectivity between the river and Forest Park. In addition to the tributary habitat, the site also contains confluence habitat (Figure 4). In the case of the Harborton site, monitoring efforts include post-implementation sampling of the tributary habitat, where it is believed to have been inaccessible to fish prior to restoration, and pre- and post-implementation sampling of the confluence habitat. Pre-implementation sampling was conducted in 2017. Post sampling began in 2021 for the tributary habitat and in 2022 for confluence sites. Due to pandemic restrictions and supply chain issues, Harborton tributary was the only habitat sampled in 2021 (post-implementation). Post-implementation sampling continued at all four locations in 2023.

Linnton

The Linnton site is located on the southwest side of the Willamette River at river km 7.5 just upstream of Sauvie Island (Figure 2). It was an industrial property that contained an inactive plywood company and consists of confluence and shoreline habitat (Figure 5). Restoration work was completed at this site in 2019 and included removal of multiple buildings, pilings, and docks. The lower 200 m of Linnton Creek were daylighted to allow fish access, though there is no habitat connectivity past HWY 30, just upstream of the project site. Shallow water habitat was created at the confluence of Linnton Creek and the Willamette River as well as along the banks of the Willamette River. Wood structures, rock piles, and snags were constructed to improve in-water and terrestrial habitat for native species. Monitoring includes the newly restored tributary habitat (post-implementation), the confluence habitat (pre- and post-implementation), and shoreline habitat (pre- and post-implementation). Pre- monitoring was conducted at confluence and shoreline habitats in the Willamette River in 2017 (Skalicky et al. 2018). Post-implementation monitoring began in 2020 and consisted of sampling shoreline and confluence habitats in the Willamette River. Due to pandemic restrictions and supply chain issues, Linnton tributary was the only habitat sampled in 2021 (post-implementation). Sampling was conducted at the Linnton confluence, shoreline, and tributary in 2022 and 2023 for post-implementation monitoring.

Triangle Park

The Triangle Park site is located on the east side of the Willamette River, near the University of Portland, Oregon (Figure 2). There is a proposed action to improve shoreline and riparian habitat and remove pilings from the site (Figure 6). In the Triangle Park site, monitoring includes shoreline habitat and habitat areas around the existing pilings (pre- and post- implementation). Pre-implementation monitoring was conducted at shoreline and piling habitats in the Willamette

River in 2017 (Skalicky et al. 2018). Post-implementation monitoring will consist of sampling shoreline habitats in and areas where pilings had been removed from the Willamette River, however this sampling has not begun as of 2023.

Rinearson Natural Area

The Rinearson Natural Area (RNA) site is located at river km 39. Rinearson Creek flows through the RNA (Clackamas County, OR) and enters the Willamette River from the east, just downstream of the mouth of the Clackamas River (Figure 2). The site has tributary habitat that drains into the Willamette River, as well as associated confluence habitat in the mainstem Willamette River (Figure 7). A project has been implemented to improve and redirect tributary habitat at this site, but a major fish passage barrier exists 600 m upstream of the confluence. Pre-implementation monitoring was conducted at confluence habitat in the Willamette River mainstem as well as wadable depth tributary habitat in Rinearson Creek in 2015 (Silver et al. 2016). Post-implementation monitoring was conducted in 2019 and 2020 and consisted of sampling for larval lamprey in tributary reaches in Rinearson Creek as well as confluence habitats in the mainstem Willamette River. Due to pandemic restrictions and supply chain issues, RNA tributary was the only habitat sampled in 2021 (post-implementation). Sampling was conducted at the Rinearson confluence and tributary in 2022 and 2023 for post-implementation monitoring.

PGE 13.1

In addition to the NRDA restoration sites described above, Portland General Electric (PGE) requested that US Fish and Wildlife Service (FWS) evaluate a similar, non-NRDA restoration action. The PGE 13.1 restoration site is near kilometer 21.1 of the Willamette River (Figure 2). Although restoration at PGE 13.1 is not specifically related to the NRDA process and outside of the official Superfund area, it is within the reach of the Willamette River that bisects the city of Portland. The PGE 13.1 restoration site and the NRDA restoration sites have many commonalities regarding the types of habitats being restored and the biological questions being addressed. FWS is applying a similar lamprey monitoring approach at the PGE 13.1 site and results will be included in this report.

The PGE 13.1 site is located on the east side of the Willamette River. Portland General Electric has proposed a project to rehabilitate the habitat on the east bank of the Willamette River. This site has shoreline habitat with associated city effluents (Figure 8). It was unknown whether lamprey occupied the site. At the PGE 13.1 site, monitoring includes shoreline habitat (pre- and post-implementation). Pre-implementation monitoring was conducted at shoreline habitats in the Willamette River in 2017 (Skalicky et al. 2018). Unlike the design for the NRDA projects, post-implementation sampling for this PGE project was designed to occur in years 2, 4, 6, and 8. As such, post-implementation monitoring was conducted in 2019 but did not occur in 2021 due to pandemic restrictions and supply chain issues. Sampling was shifted to 2022 (5 years post-implementation) and is next scheduled to be conducted in 2025 (8 years post-implementation).

Reference Sites

Seven reference sites have been identified throughout the lower Willamette River and Multnomah Channel (Figure 2). Initially, we attempted to monitor habitats from six of these reference sites. However, some of these habitats were determined to be unsuitable reference sites after several sampling attempts (e.g., barriers near tributary mouths, unwadeable conditions, private ownership with lack of access). Thus, in 2019 we began sampling tributary habitat at the Oswego Creek reference site and one additional reference site (Miller Creek). Currently, various habitat combinations from seven different sites are being used for reference. Reference sites were selected in locations that contained confluence, shoreline, or tributary habitats and in sites not proposed for habitat restoration in the immediate future (relative to when this project began). The reference sites were chosen to provide confluence, shoreline, or tributary habitats that are similar to those which may exist at restoration sites following project implementation.

Multnomah Channel (Shoreline)

The Multnomah Channel site is located just downstream of the McCarthy Creek (near river km 24; Figure 2). The Multnomah Channel reference site contains shoreline habitat (Figure 9). The Multnomah Channel site is not paired with a specific restoration site, but currently serves as a reference site for shoreline habitat and was sampled in 2023.

McCarthy Creek (Confluence and Shoreline)

The McCarthy Creek site is located where McCarthy Creek enters the Multnomah Channel from the southwest, downstream of the Sauvie Island Bridge (near river km 29; Figure 2). The McCarthy Creek reference site provides confluence and shoreline habitats in Multnomah Channel (Figure 10). Although the McCarthy Creek site has a tributary, this habitat is not conducive to sampling (e.g., poor flows, not wadable) and was discontinued as a tributary reference. The McCarthy Creek confluence and shoreline serve as a reference for the Linnton site and were sampled in 2023.

Columbia Slough (Confluence)

The Columbia Slough site is located where the Columbia Slough enters the Willamette River from the east, near the confluence of the Willamette and Columbia Rivers (near river km 2; Figure 2). Confluence habitat occurs in the mainstem Willamette River associated with the mouth of Columbia Slough (Figure 11). The Columbia Slough site serves as a reference for the Harborton site. Sampling was conducted at the Columbia Slough confluence in 2023.

Ross Island (Shoreline)

The Ross Island site, located just upstream of the Ross Island Bridge near downtown Portland (near river km 24; Figure 2), contains shoreline habitat (Figure 12). The Ross Island site serves as a reference for the Alder Point site. Sampling was not conducted at Ross Island in 2023.

Cemetery Creek (Confluence and Shoreline)

The Cemetery Creek site enters the Willamette River from the west, upstream of Ross Island (near river km 27; Figure 2). The Cemetery Creek reference site has confluence and shoreline habitats in the mainstem Willamette River (Figure 13). The Cemetery Creek site, specifically the tributary and confluence habitat, were selected to serve as a specific reference for the RNA site. Although the Cemetery Creek site has a tributary, this habitat is not conducive to monitoring (e.g., lack of Type I habitat and a putative passage barrier) and was discontinued as a tributary reference. In 2019 we began sampling the tributary habitat at the Oswego Creek site, in place of the Cemetery Creek tributary. The confluence and shoreline habitat at the Cemetery Creek site continues to serve as a reference for the RNA site and was sampled in 2023.

Oswego Creek (Confluence and Tributary)

The Oswego Creek site is located where Oswego Creek enters the Willamette River from the west, near the town of Lake Oswego (near river km 34; Figure 2). Confluence habitat occurs where the tributary habitat enters the mainstem Willamette River (Figure 14). Prior to 2019, sampling focused on the confluence habitat at the Oswego Creek site. In 2019, we began sampling the tributary habitat to supplement existing tributary reference sites. The tributary habitat associated with the Oswego Creek site (Cemetery Creek site replacement) was added to serve as a specific reference for the tributary habitat at the RNA restoration site. The confluence habitat associated with the Oswego Creek site is not currently paired with a specific restoration site but serves as a general reference site for confluence habitat. Sampling was conducted at the Oswego Creek tributary and confluence in 2023.

Miller Creek (Tributary)

Miller Creek enters the Multnomah Channel (Figure 2) from the south, just opposite of the upstream end of Sauvie Island (Figure 15). In 2019, we began sampling this habitat to supplement tributary reference sites. The Miller Creek site was added to serve as a specific reference for the Linnton site (McCarthy Creek replacement). Sampling was not conducted at the Miller Creek tributary in 2023.

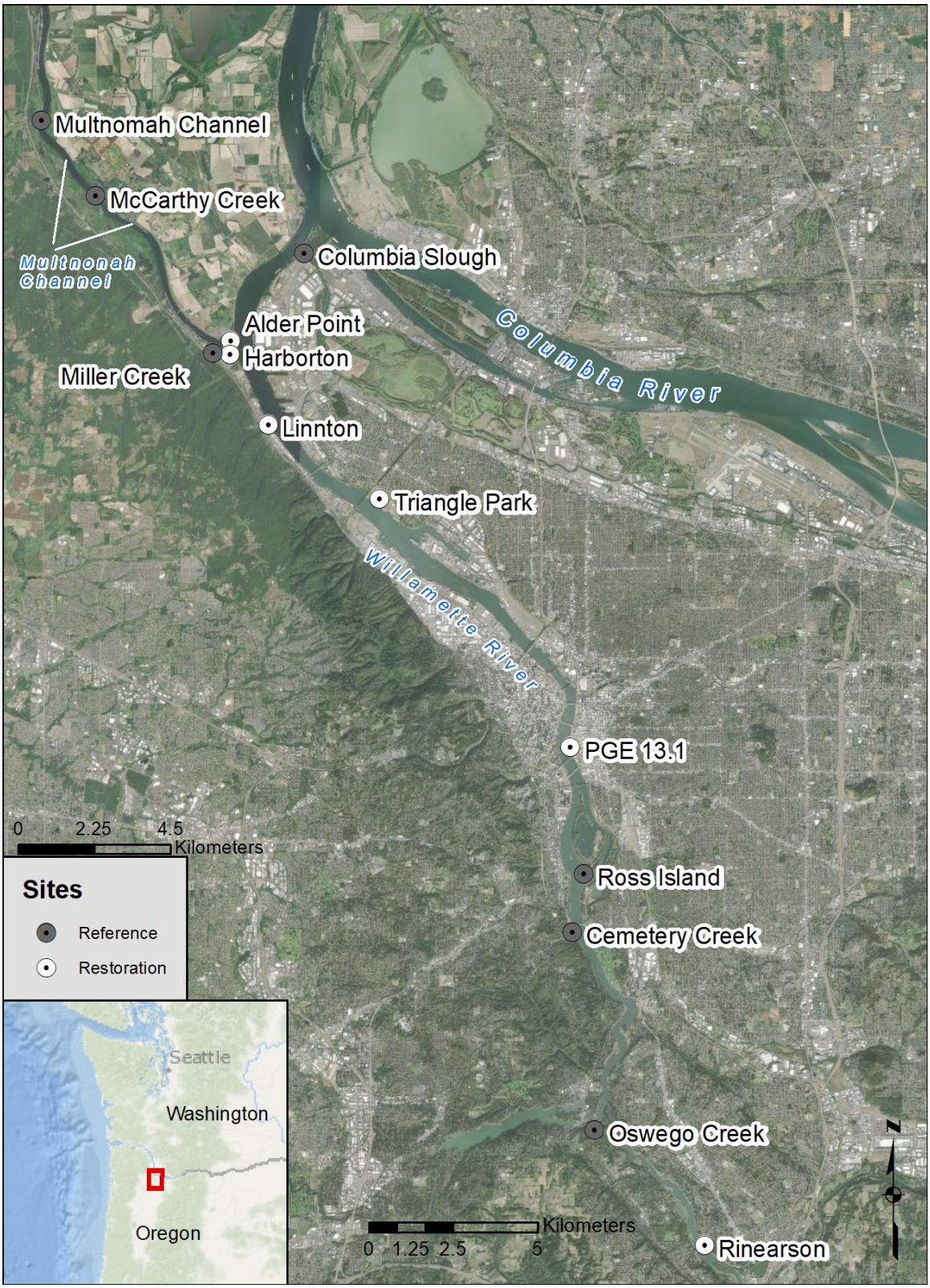

Figure 2. Locations of the restoration (white circles) and reference (gray circles) sites. *Note: PGE 13.1 is not a NRDA restoration site.*

Methods

Sample Framework

We evaluated occupancy of larval lamprey in the restoration and reference sites by using methods that have been previously applied to studies of larval lamprey occupancy in the Columbia River basin in both mainstem and tributary habitats (Silver et al. 2010; Jolley et al. 2012; Jolley et al. 2013; Jolley et al. 2014). The approach has several requirements: 1) a unit- and gear-specific detection probability (assumed or estimated); 2) the probability of presence (given no detection) at a predetermined acceptably low level; and 3) random identification of spatially balanced sample units that allow estimation of presence and refinement of detection probabilities. A unit-specific probability of detection, d_{unit}, was calculated as the proportion of sample quadrats or reaches in which larvae were captured. The posterior probability of area occupancy, given a larval lamprey was not detected, was estimated as:

$$(1)\ P(F|C_o) = \frac{P(C_o|F) \cdot P(F)}{P(C_o|F) \cdot P(F) + P(C_o|\sim F) \cdot P(\sim F)},$$

where P(F) is the prior probability of larval lamprey presence. Although in this case we knew the lower Willamette River was occupied with larval lamprey, a P(F) of 0.5 (uninformed) was used for future study design (i.e., P[$F|C_o$]) in areas where larval lamprey presence is unknown. P(~F), or 1 – P(F), is the prior probability of species absence, and P($C_o|F$), or 1 – d, is the probability of not detecting a species when it occurs (C_0 = no detection; Peterson and Dunham 2003). Random identification of spatially-balanced sample units was achieved by using a generalized random-tessellation stratified (GRTS) approach to delineate sample units in an ordered, unbiased manner (Stevens and Olsen 2004).

Tributary (Wadable Water) Sample Framework

Evaluation of larval lamprey occupancy at wadable depth tributary habitats was conducted at restoration sites pre- and post-implementation. For each tributary habitat longer than 400 m, we developed a layer of 50 m long sample reaches for subsampling. The GRTS approach was again used to delineate sample reaches in a random, spatially-balanced order (Stevens and Olsen 2004). The GRTS method assigns a hierarchical order to the reaches within the creek which is used as an unbiased method of ranking the priority of reaches for sampling. Delineation of sample reaches that are unbiased, randomly selected, and spatially-balanced within a sample universe allows for calculation of unit-specific detection probabilities. In turn, unit-specific estimates of detection probability can be applied to determine sample efforts necessary for achieving a desired level of certainty that a tributary is not occupied by lamprey when they are not detected. As they are selected in the GRTS approach, the lower numbered reaches are given highest priority for sampling. Here we used a subsampling effort (number of sample reaches) that we estimated would allow for at least 80% certainty that larval lampreys do not occupy at least 20% of a tributary habitat when they were not detected (see Bayley and Peterson 2001; Peterson and Dunham 2003). Sample effort is based on estimates of reach-specific detection probabilities generated from previous work and total area at the sampling location (Silver et al. 2010; FWS unpublished data). For wadable depth tributaries, if the area of interest was less than 400 m in length, we sampled all reaches (contiguous 50 m reaches). If the area of interest was 400 m or longer, seven reaches were sampled.

Confluence and Shoreline (Deep Water) Sample Framework

Sample quadrats at confluence and shoreline habitats were derived from the work of Jolley et al. (2012). Quadrats were delineated using the generalized random-tessellation stratified (GRTS) approach scripted in Program R (Stevens and Olsen 2004; Jolley et al. 2012; R Core Team, 2013). The GRTS method assigns a hierarchical order to quadrats, which can be used as an unbiased method of ranking the priority of quadrats for sampling. Delineation of quadrats that are unbiased, randomly selected, and spatially-balanced within a sample universe allows for calculation of unit-specific detection probabilities. In turn, unit-specific estimates of detection probability can be applied to determine sample efforts necessary for achieving a desired level of certainty that an area is not occupied by lamprey when they are not detected. Here we used a sampling effort (number of sample quadrats) that we estimated would allow for at least 80% certainty that larval lampreys do not occupy at least 20% of a confluence or shoreline habitat when they were not detected (see Bayley and Peterson 2001; Peterson and Dunham 2003). Sample effort was based on estimates of quadrat-specific detection probabilities generated from previous work and on total area at the sampling location (Jolley et al. 2012). This sample effort corresponded to sampling of 10 quadrats at each confluence and/or shoreline habitat at both restoration and reference sites. In the case where slough habitat was deep and not wadable, the sample framework described above was also applied to the slough (as a sample unit).

Restoration Sites

Alder Point (Slough, Confluence and Shoreline)

Confluence quadrats at the Alder Point site comprised a subset of quadrats filtered from the lower Willamette River and Multnomah Channel layers. Quadrats were filtered from the larger layers according to the placement of a semicircular buffer of 100 m radius centered on each confluence of the Alder Slough and the Willamette River or Multnomah Channel (Figure 3). The three branches of Alder Slough each form a distinct confluence habitat at Alder Point, two occur on the Willamette River and one occurs on Multnomah Channel. In this case, the confluence quadrat selection process was duplicated at each of the three confluence habitats, resulting in 60 total sample quadrats at the three Alder Slough confluence habitats. At each of the three confluence locations, the 10 lowest numbered of each of the confluence quadrats as ordered by the GRTS method were assigned the highest priority for sampling.

Shoreline quadrats at the Alder Point site also comprised a subset of quadrats filtered from the lower Willamette River and Multnomah Channel layers. Quadrats were filtered from the larger layers according to the placement of a 100 m-wide polygon, from the waterline perpendicular 100 m into the Willamette River or Multnomah Channel. The length of the shoreline polygon was determined by the project area boundaries (Figure 3). The shoreline quadrat selection process resulted in 117 total sample quadrats adjacent to restored shorelines at Alder Point. The 10 lowest numbered shoreline quadrats as ordered by the GRTS method were assigned the highest priority for sampling.

To evaluate larval lamprey occupancy of Alder Slough, a layer of 30 m x 30 m quadrats was developed and overlaid on the newly constructed channel at Alder Point (Figure 3). Using the GRTS approach, quadrats in Alder Slough were delineated in a random spatially-balanced

manner. The lowest 10 numbered quadrats were assigned the highest priority for sampling. Neither the slough, confluence, nor shoreline habitat were sampled in 2023.

Harborton (Tributary and Confluence)

At the Harborton restoration site (Figure 4), the sample effort corresponded to 10 confluence quadrats in each of three confluence habitats (30 total quadrats, to be done pre- and post-implementation). The sample effort will also correspond to seven 50 m reaches in the northern tributary (post-implementation only). Post-implementation sampling in the tributary habitat began in 2021. The three Harborton confluence habitats (north, south, and delta) and the Harborton tributary were sampled in 2023.

Linnton (Tributary, Confluence and Shoreline)

At the Linnton restoration site (Figure 5), pre- and post-implementation sample effort will correspond to 10 confluence quadrats and 10 shoreline quadrates. Post-implementation also includes four tributary reaches, each 50 m in length. Pre-implementation sampling was conducted in 2017 for the confluence and shoreline habitats, and post-implementation sampling began in 2020. Post-implementation monitoring in the four tributary reaches began in 2021 and include the entire newly created tributary habitat at the Linnton project site. Linnton tributary, confluence, and shoreline were sampled in 2023 for post-implementation monitoring.

Triangle Park (Shoreline)

We proposed to determine whether larval Pacific Lamprey occupy the restoration area both prior to and after piling removal. In this unique case, shoreline sample framework was defined as the area 30 m around the line connecting the piling structures (Figure 6). Pre- and post-restoration sample effort would correspond to 21 shoreline quadrats (25% of the total number of quadrats). Restoration efforts at Triangle park have been discontinued. Sampling at the Triangle Park shoreline habitat did not take place in 2023.

Rinearson Natural Area (Tributary and Confluence)

At the Rinearson Natural Area, Rinearson Creek forks into two distributary channels near the Willamette River creating two distinct confluence habitats in the restoration site. In this case, the confluence quadrat selection process was carried out as described above at Alder Point and duplicated at each of the two distinct confluence habitats (Figure 7). The selection process resulted in 34 total sample quadrats at the two confluence habitats. At each of the two confluence locations, the lowest numbered quadrats as ordered by the GRTS method were assigned the highest priority for sampling. Evaluation of larval lamprey occupancy in Rinearson Creek post-restoration began in 2019 and was proposed to occur over an approximately 1,200 m long segment of creek, spanning from the confluence with the Willamette River upstream to the crossing of River Road (Milwaukie, OR; Figure 7). Sample reaches were delineated at a rate of one 50 m reach for every 50 meters of stream. Thus, within the approximately 1,200 m long study area in Rinearson Creek, 24 sample reaches were delineated, of which the lowest numbered reaches, as ordered by the GRTS method, were assigned the highest priority for sampling. Because the area of interest in Rinearson Creek was longer than 400 m, sampling effort will correspond to seven, 50 m long reaches in the creek. Post-implementation sampling

did not occur at the confluence in 2021 due to pandemic restrictions and supply chain issues, but sampling was conducted at the tributary site. Post-implementation sampling was conducted at the Rinearson confluence and tributary in 2023.

PGE 13.1 (Shoreline)

To evaluate larval lamprey occupancy of PGE 13.1, a layer of 30 m x 30 m quadrats was developed and overlaid on the restoration area (shoreline polygon) at PGE 13.1 (Figure 8). Using a GRTS approach, quadrats at PGE 13.1 were delineated in a random spatially-balanced manner. The lowest 10 numbered quadrats were assigned the highest priority for sampling. Post-implementation monitoring at PGE 13.1 began in 2019 and sampling will be conducted every other year. However, sampling did not occur in 2021 due to pandemic restrictions and supply chain issues, therefore sampling was conducted at PGE 13.1 in 2022. Sampling did not occur in 2023.

Reference Sites

Multnomah Channel (Shoreline)

The Multnomah Channel reference site contains shoreline habitat (Figure 9). The quadrat selection process was carried out as described above for shorelines at Alder Point. The length of the shoreline was modeled after that of restoration sites. The 10 lowest numbered shoreline quadrats as ordered by the GRTS method were again assigned the highest priority for sampling. Sampling was conducted at Multnomah Channel, a general reference site for shoreline habitat, in 2023.

McCarthy Creek (Confluence and Shoreline)

The McCarthy Creek reference site consists of tributary, confluence, and shoreline habitat (Figure 10). Upon visiting the site, we discovered that the tributary did not contain a reasonable amount of habitat that could be sampled, and the tributary was removed from the sampling design. In the McCarthy Creek confluence habitat within the Multnomah Channel, quadrat selection was carried out as described above for confluences at Alder Point. The 10 lowest numbered confluence quadrats as ordered by the GRTS method were again assigned the highest priority for sampling. In shoreline habitat within the mainstem Multnomah Channel, quadrat selection was carried out as described above for the shoreline habitat at Alder Point. The 10 lowest numbered shoreline quadrats as ordered by the GRTS method were again assigned the highest priority for sampling. The McCarthy Creek confluence and shoreline serve as a reference, are specifically paired with the Linnton site, and were sampled in 2023.

Columbia Slough (Confluence)

The Columbia Slough reference site contains confluence habitat within the mainstem Willamette River. The confluence quadrat selection was carried out as described above for confluences at Alder Point. The 10 lowest numbered confluence quadrats as ordered by the GRTS method were again assigned the highest priority for sampling (Figure 11). The Columbia Slough site serves as

a reference, is paired with the Harborton site, and was sampled in 2023.

Ross Island (Shoreline)

The Ross Island reference site contains shoreline habitat. The quadrat selection process was carried out as described above for the shoreline at Alder Point. The length of the shoreline was modeled after that of restoration sites. The 10 lowest numbered shoreline quadrats as ordered by the GRTS method were again assigned the highest priority for sampling (Figure 12). The Ross Island site serves as reference, is paired with the Alder Point site, and was not sampled in 2023.

Cemetery Creek (Confluence and Shoreline)

The Cemetery Creek reference site has confluence and shoreline habitats. Though there is a tributary at the site, a barrier precludes the availability of sufficient habitat and the tributary was removed from the reference sample design. For confluence habitat within the mainstem Willamette River, quadrat selection was carried out as described above for confluences at Alder Point. The 10 lowest numbered confluence quadrats as ordered by the GRTS method were again assigned the highest priority for sampling. For shoreline habitat within the mainstem Willamette River, quadrat selection was carried out as described above for shoreline habitat at Alder Point. The 10 lowest numbered shoreline quadrats as ordered by the GRTS method were again assigned the highest priority for sampling (Figure 13). The Cemetery Creek confluence and shoreline habitat serve as a reference, are specifically paired with the Rinearson site, and were sampled in 2023.

Oswego Creek (Confluence and Tributary)

The Oswego Creek reference site contains confluence habitat within the mainstem Willamette River. The confluence quadrat selection was carried out as described above for confluences at Alder Point. The 10 lowest numbered confluence quadrats as ordered by the GRTS method were again assigned the highest priority for sampling (Figure 14). The confluence habitat, a general reference site for shoreline habitat, was sampled in 2023. The Oswego Creek reference site also has tributary habitat. In Oswego Creek, the tributary area of interest was less than 400 m in length. The area spanned from the confluence with the Willamette River upstream approximately 370 m to a reach with significant private ownership. Because the area of interest was not more than 400 m in length, we sampled contiguous 50 m reaches in Oswego Creek up to a total of 350 m (the most downstream 350 m in Oswego Creek). The Oswego Creek tributary habitat serves as a reference, is specifically paired with the Rinearson tributary (replacing the Cemetery Creek tributary habitat) and was sampled in 2023.

Miller Creek (Tributary)

Miller Creek contains reference tributary habitat (Figure 15). In Miller Creek, the tributary area of interest was less than 400 m in length, spanning from the confluence with the Multnomah Channel upstream approximately 350 m to a high gradient reach with significant private ownership. Because the area of interest was not more than 400 m in length, we sampled contiguous 50 m reaches in Miller Creek up to a total of 350 m (the most downstream 350 m in

Miller Creek). The Miller Creek tributary began as a general reference for tributary habitat and in 2019 was designated as a reference site for the Linnton restoration site. Miller Creek was not sampled in 2023.

Reach Sampling in Wadable Habitats

For wadable depth tributary (or slough) habitats, each sampling event consisted of electrofishing 50 m reaches to determine if larval lamprey were present (Silver et al. 2010). Sample reaches were accessed on foot using GPS units with sample reach UTMs for navigation. When a reach could not be sampled (e.g., dewatered conditions, excessive depth, lack of access due to private property) they were eliminated, and subsequent reaches were sampled. Once a sample reach was accessed, a 50 m segment was measured. Water temperature and conductivity were recorded in each reach. The reach was electrofished using an AbP-2 backpack electrofisher. Power output settings for the AbP-2 were adapted from Weisser and Klar (1990). Initially, the electrofisher delivered three DC pulses per second at 25% duty cycle, 125 V, with a 3:1 burst pulse train (i.e., three pulses on, one pulse off). This power output method is designed to stimulate burrowed larvae to enter the water column. Once larvae were observed in the water column, 30 pulses/second were occasionally applied to temporarily immobilize the larvae for capture using a dip net. The time spent electrofishing areas of preferred larval lamprey rearing habitat where depositional silt and sand substrates were dominant (henceforth Type I habitat, Slade et al. 2003) was approximately 30 seconds/m^2. The time spent electrofishing areas with relatively large gravel, hard bedrock, and boulder substrates (henceforth Type II and type III habitats, Slade et al. 2003) was approximately 5 seconds/m^2. All larval lamprey observed were captured and placed in buckets containing stream water until identified and measured.

Quadrat Sampling in Deep Water Habitats

For deep water habitats, each sampling event utilized deep-water electrofishing equipment within the 30 m x 30 m quadrat (Bergstedt and Genovese 1994; Jolley et al. 2012). Quadrats were accessed and sampled by boat, using quadrat center point in Universal Transverse Mercator (UTM) coordinates for navigation. The deep-water electrofishing equipment, equipped with a winch and fiberglass bell, was deployed once within the quadrat. When quadrats could not be sampled (e.g., dewatered conditions, depth less than 0.3 meters, excessive velocity, or depths exceeding 21 meters) they were eliminated, and subsequent quadrats were increased in priority (Table 1). The deep-water electrofisher was comprised of a modified AbP-2 electrofisher (ETS Engineering, Madison, WI) which delivered electrical stimulus to river bottom substrates at electrodes mounted to a fiberglass bell (or hood; 0.61 m^2 in area). The electrofisher delivered three pulses DC per second at 10% duty cycle, with a 2:2 pulse train (i.e., two pulses on, two pulses off). Output voltage was adjusted at each quadrat to maintain a peak voltage gradient between 0.6 and 0.8 V/cm across the electrodes. The electrofisher bell was coupled by a 76 mm vinyl suction hose to a gasoline-fueled hydraulic pump. The hydraulic pump was started approximately 5 seconds prior to shocking to purge air from the suction hose. Suction was produced by directing flow from the pump through a hydraulic eductor, which allows larvae to be collected in a mesh basket (27 x 62 x 25 cm; 2 mm wire mesh) while preventing them from passing through the pump. A 60 second pulse was administered followed by an additional 60 seconds of pumping to further allow displaced larvae to cycle through the hose and into the

collection basket. The sampling techniques are described in detail by Bergstedt and Genovese (1994) and were similar to those used in the Great Lakes region (Fodale et al. 2003) and the Willamette River (Jolley et al. 2012).

Biological Data Collection

Collected lamprey were measured for length (mm) and were classified according to developmental stage (i.e., larvae, juvenile, or adult). When possible (i.e., typically when larvae > 70 mm TL; Goodman et al. 2009, Docker et al 2016), lamprey were identified to genus (i.e., Pacific Lamprey *Entosphenus* or Western River/Western Brook Lamprey or Pacific Brook Lamprey *Lampetra*) according to visual evaluations of caudal fin pigmentation patterns. In some individuals, pigmentation was clear under 70 mm and they were identified to species, otherwise they are denoted as unidentified (UNID). Larvae were released near the area of capture. Physical anomalies (lesions, suspected bird strikes, tumors, etc.) were recorded for all larvae. If abnormalities were observed on larvae, the individual was euthanized and preserved for potential evaluation. In addition, observations of juveniles, adults, or suspected Pacific Lamprey nests were recorded.

Habitat Data Collection

Tributary

Within each sample reach, water temperature (°C) and conductivity (µS/cm) were measured at the beginning of the survey. Depth is measured several times across the 50 m reach where larval sampling occurred and is reported as a mean.

Confluence and Shoreline

Water depth was measured at each quadrat and is presented as mean (± SE) unless otherwise noted. Beginning in 2018, water temperature (°C) and conductivity (μS/cm) were recorded at each habitat visit, where in previous years, these data were recorded at each quadrat. Water temperature and conductivity are sampled at the beginning of each habitat visit and collected at the surface. Concurrent to each sampling event a sediment sample was taken (when possible) from each quadrat with a Ponar bottom sampler (16.5 cm x 16.5 cm). Each sample was mixed thoroughly, and one 250-500 ml subsample was transferred to containers provided by a contracted laboratory. Samples were labeled with the site number and date, placed on ice, returned to the FWS office, and subsequently handled per the instructions provided from the contracted laboratory. Sediment samples collected at each confluence quadrat were transferred to ALS Environmental Laboratory (Kelso, WA) for quantification of parameters such as grain size, grain type, and organic content. See Appendix 1 for information on sediment analyses.

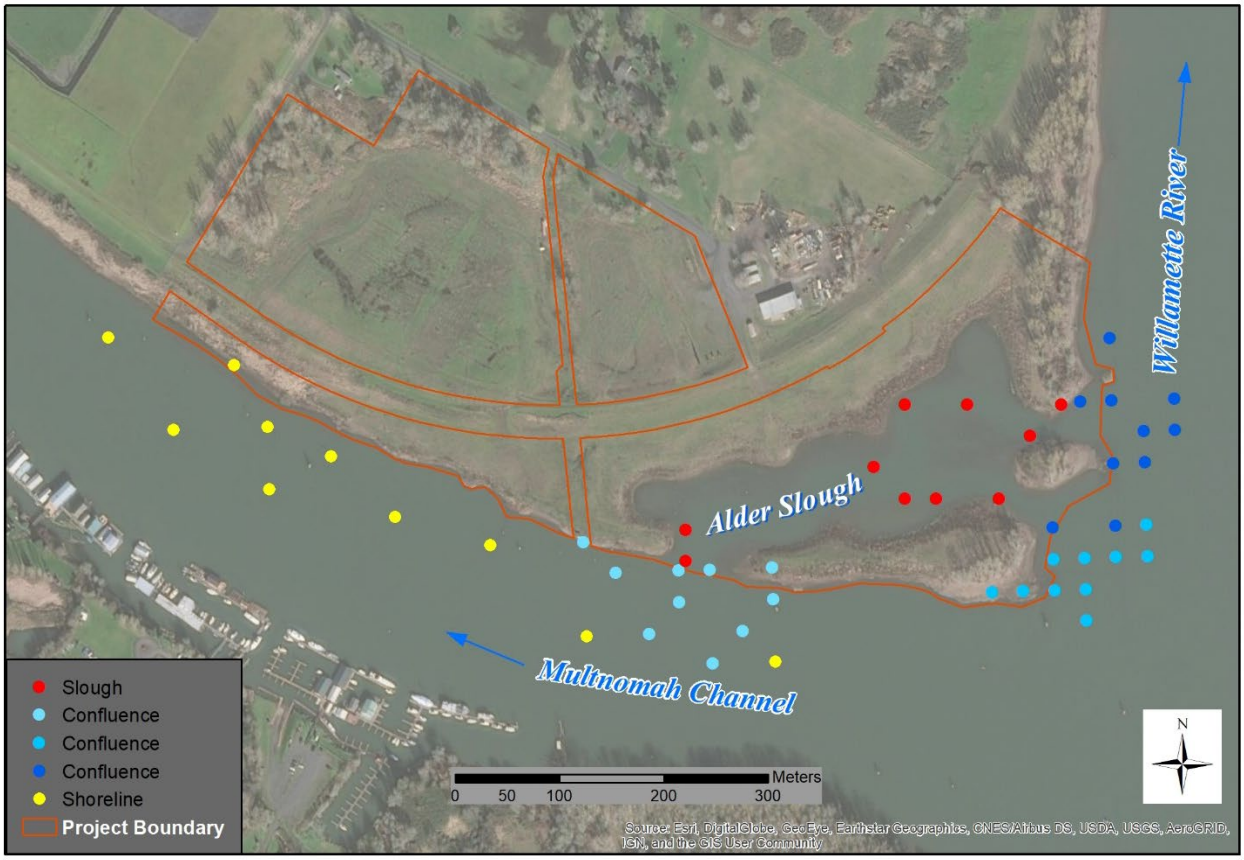

Figure 3. Alder Point restoration site sample sites. Habitats within the sites are confluence quadrats (blue points; each point represent a quadrat center point), shoreline quadrats (yellow points), and slough quadrats (red points). This site was not sampled in 2023.

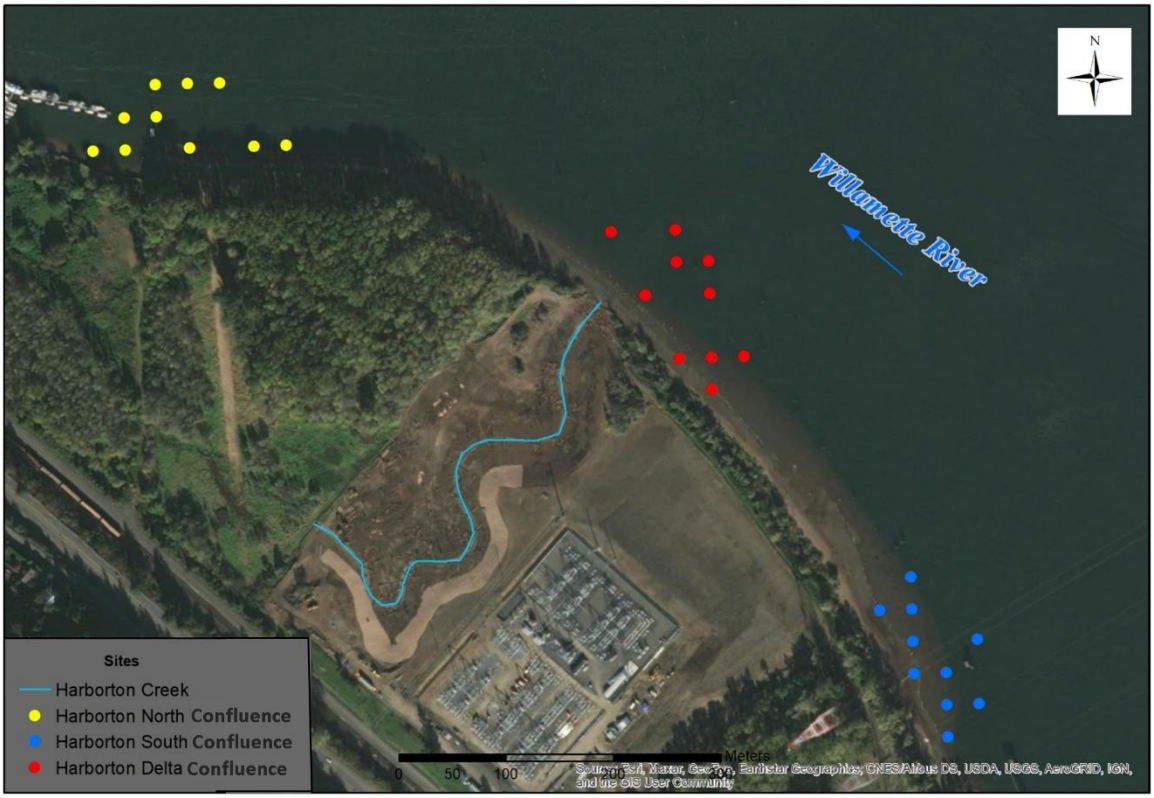

Figure 4. Harborton restoration sample sites with North confluence quadrats (yellow points), South confluence quadrats (blue points), and Delta confluence quadrats (red points).

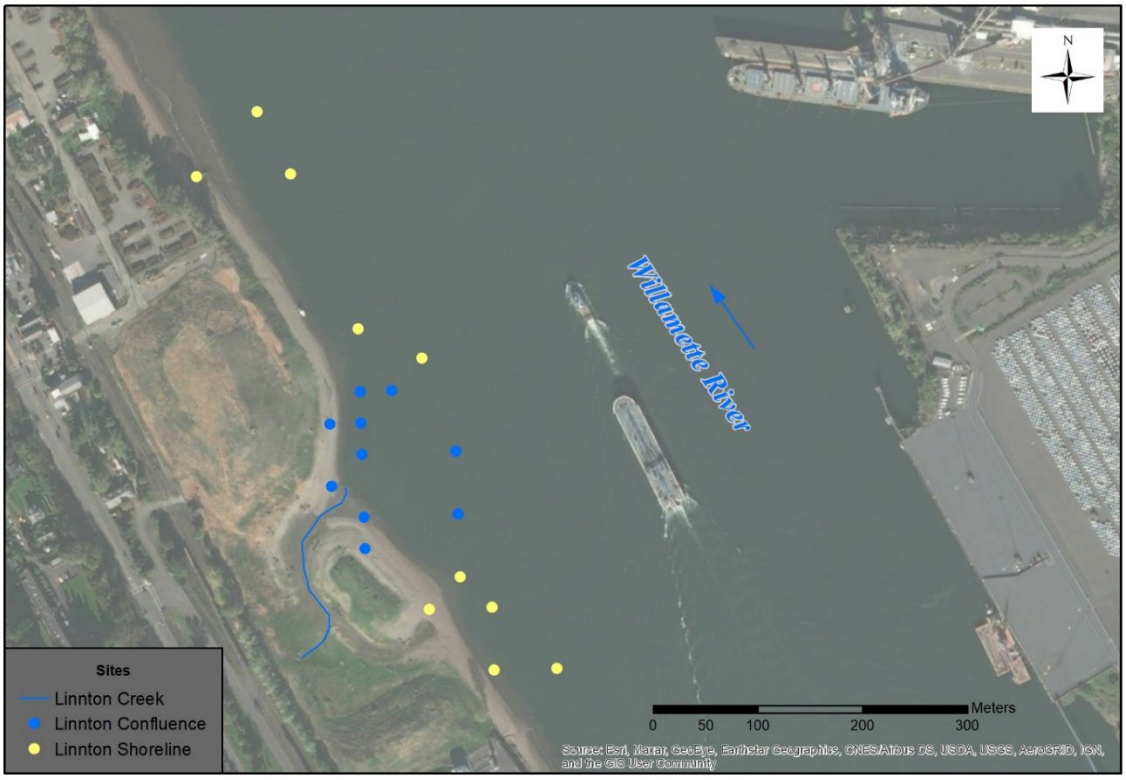

Figure 5. Linnton restoration site with shoreline quadrats (yellow points) and confluence quadrats (blue points).

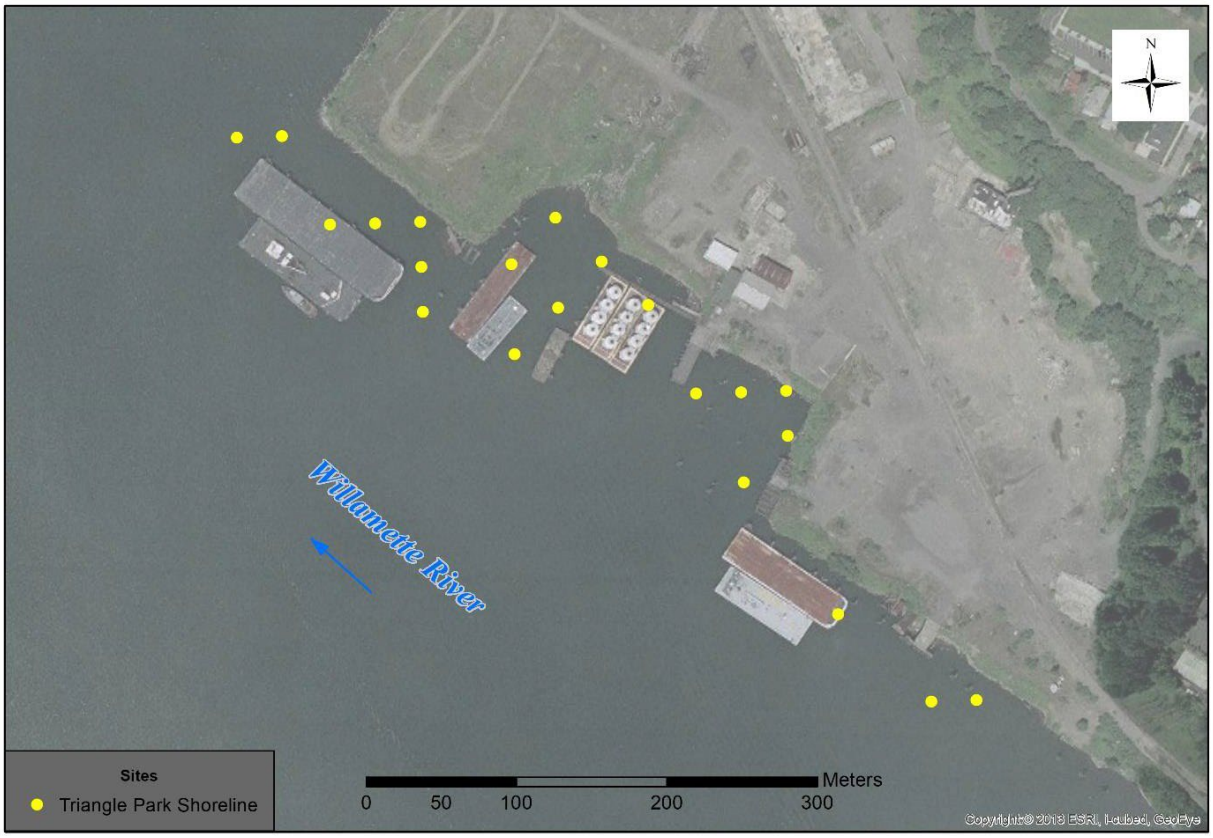

Figure 6. Triangle Park restoration site with shoreline quadrats (yellow points). This site was not sampled in 2023.

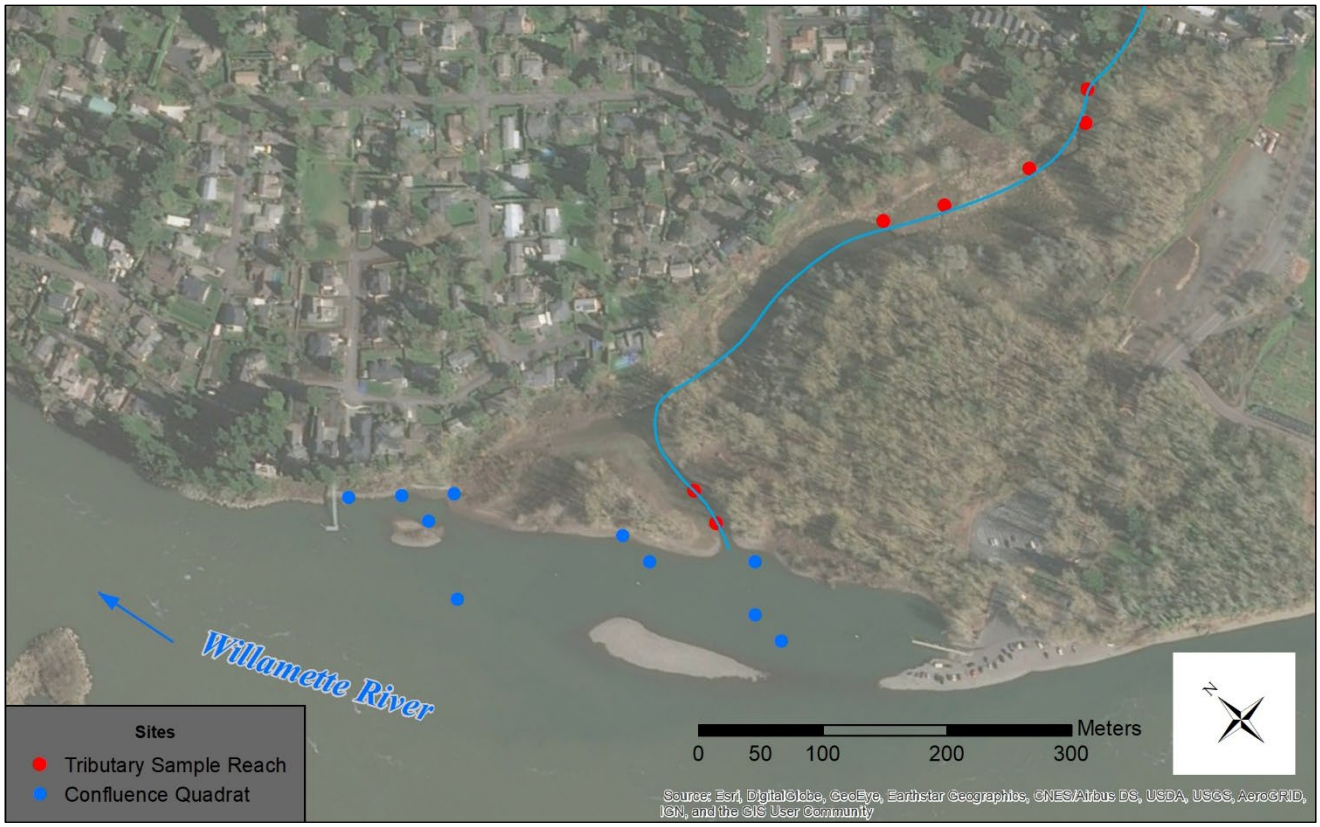

Figure 7. Rinearson Natural Area restoration site with confluence quadrats (blue points) and tributary sample reaches (red points).

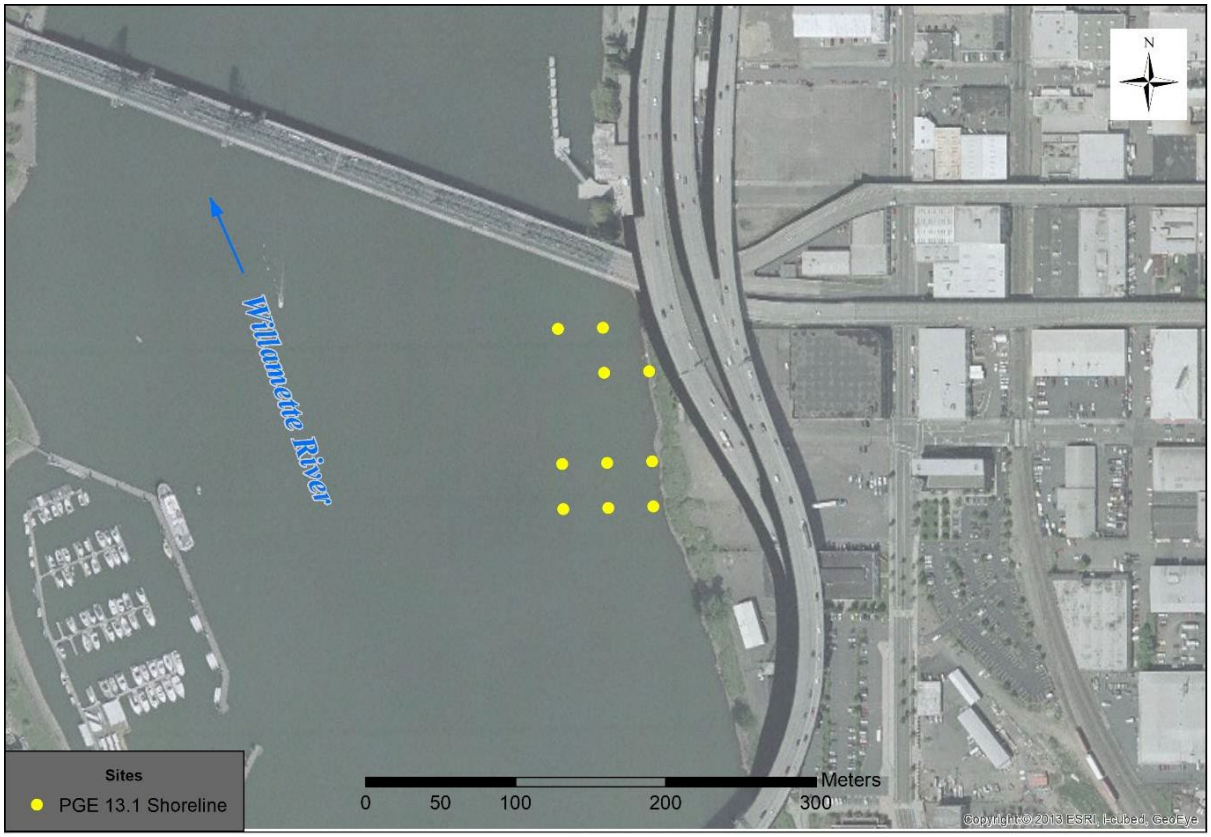

Figure 8. PGE 13.1 restoration site with shoreline quadrats (yellow points). This site was not sampled in 2023.

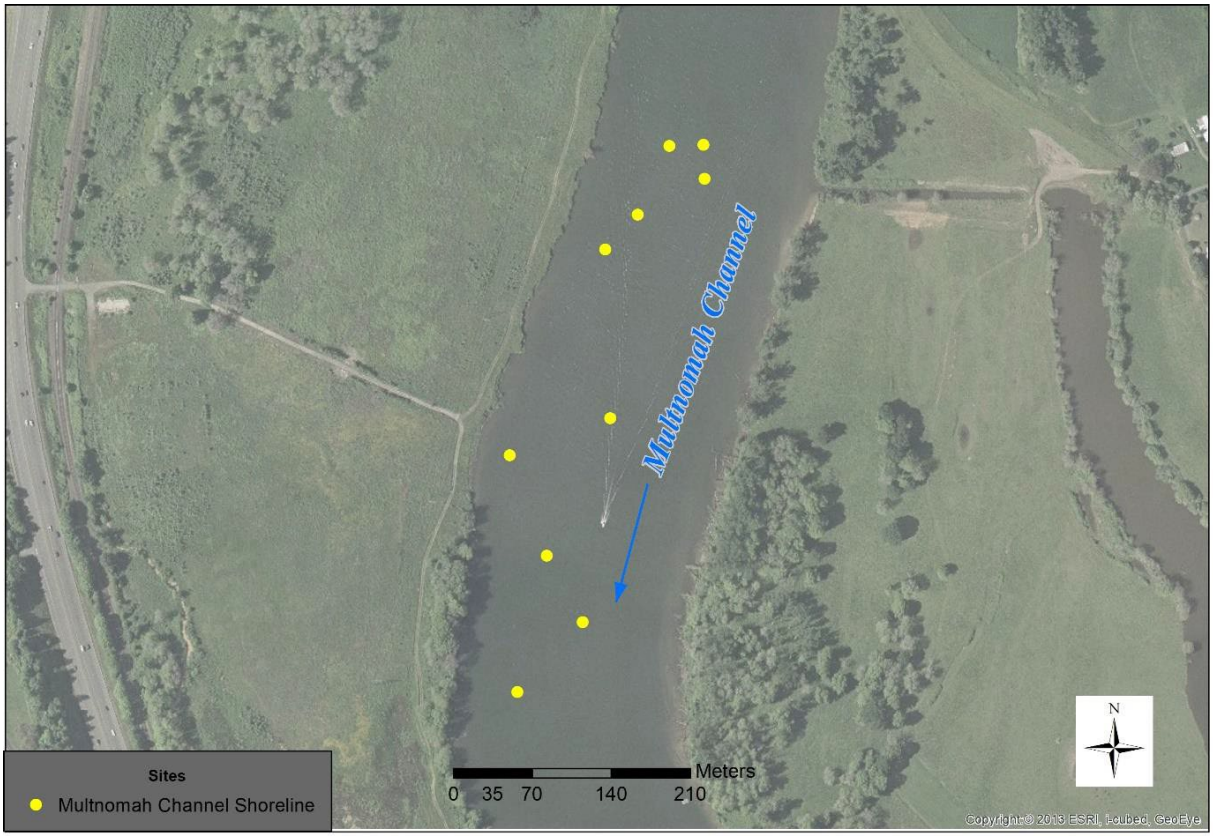

Figure 9. Multnomah Channel reference site with shoreline quadrats (yellow points).

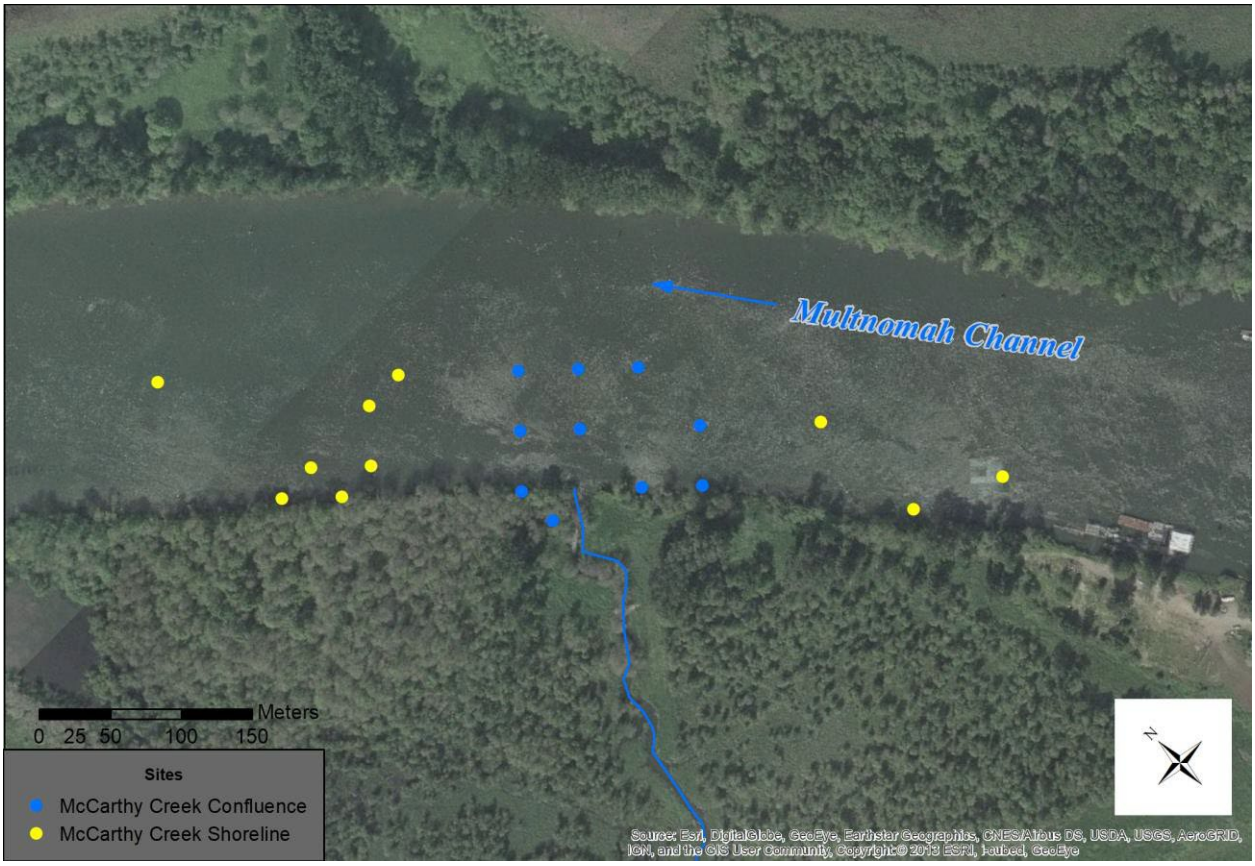

Figure 10. McCarthy Creek reference site with confluence quadrats (blue points) and shoreline quadrats (yellow points).

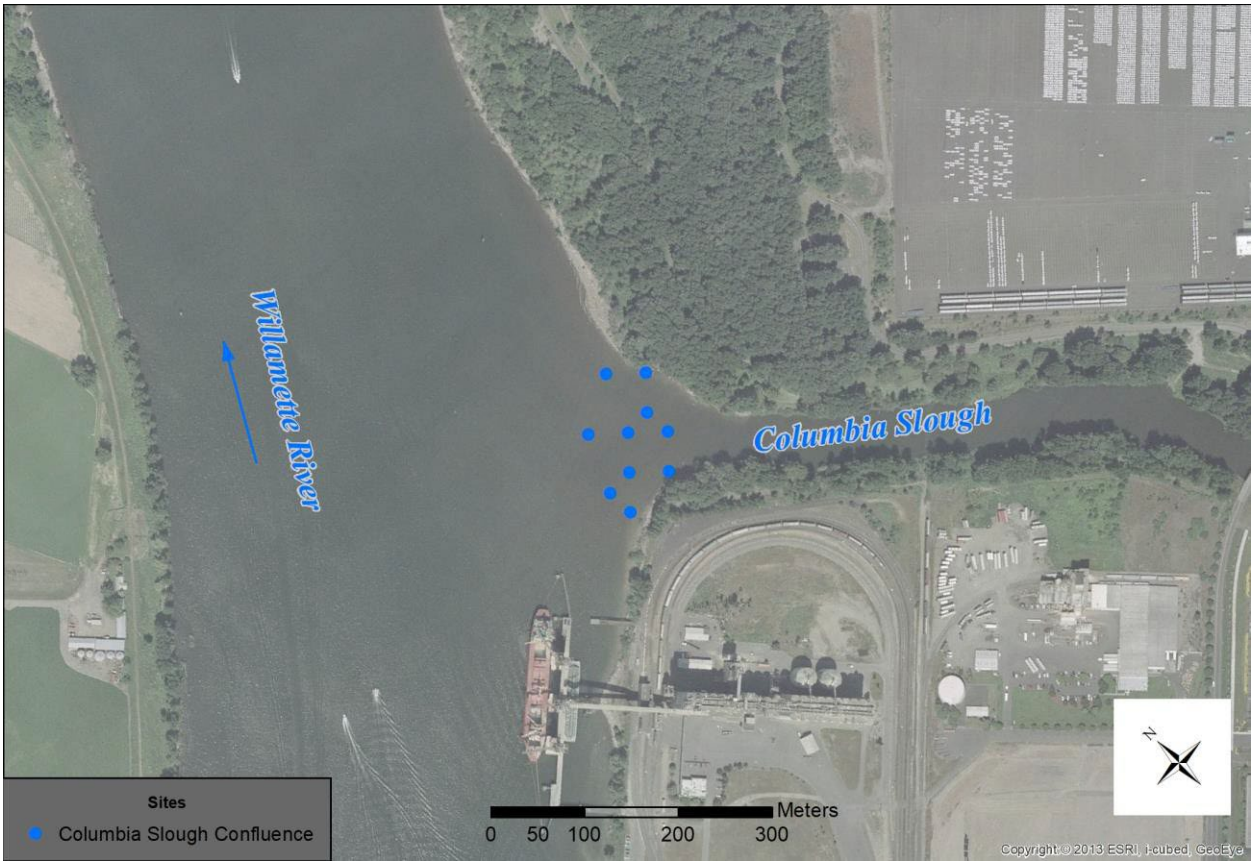

Figure 11. Columbia Slough reference site confluence quadrats (blue points).

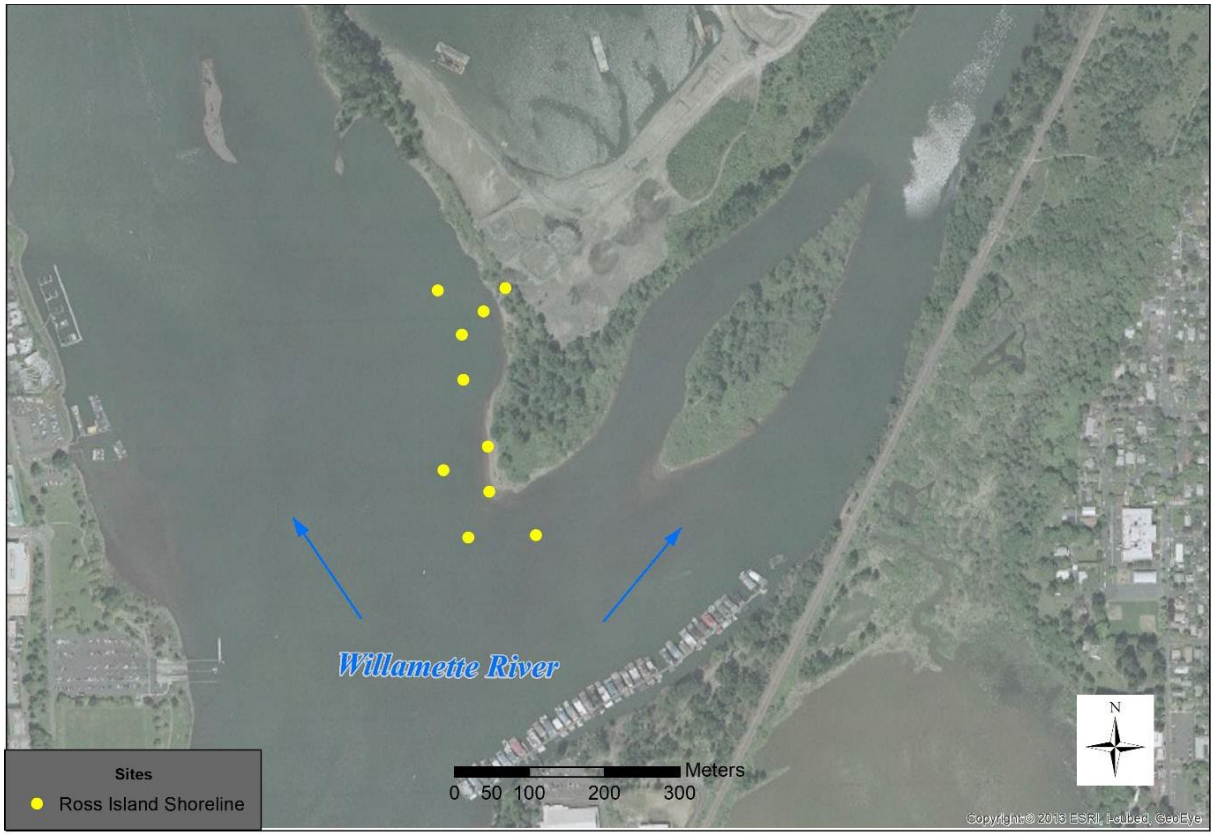

Figure 12. Ross Island reference site shoreline quadrats (yellow points). This site was not sampled in 2023.

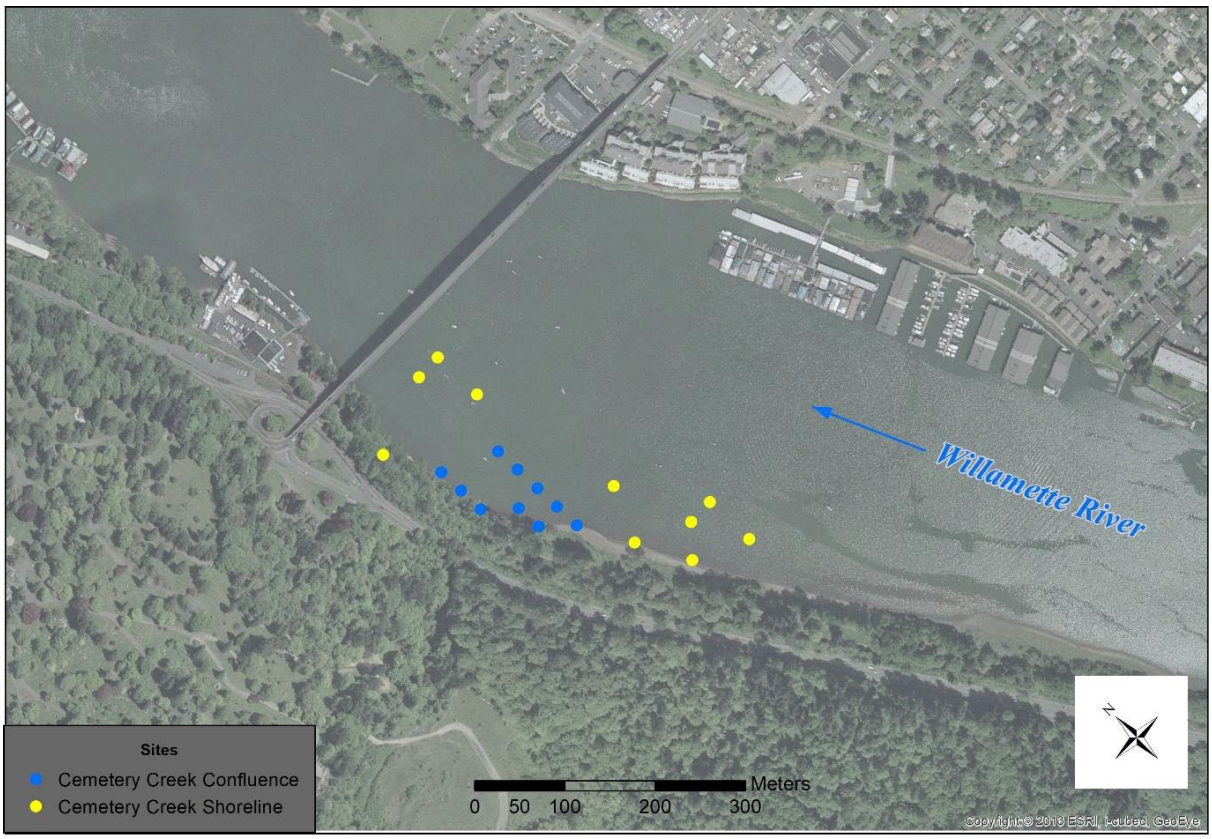

Figure 13. Cemetery Creek reference site with confluence (blue points) and shoreline (yellow points).

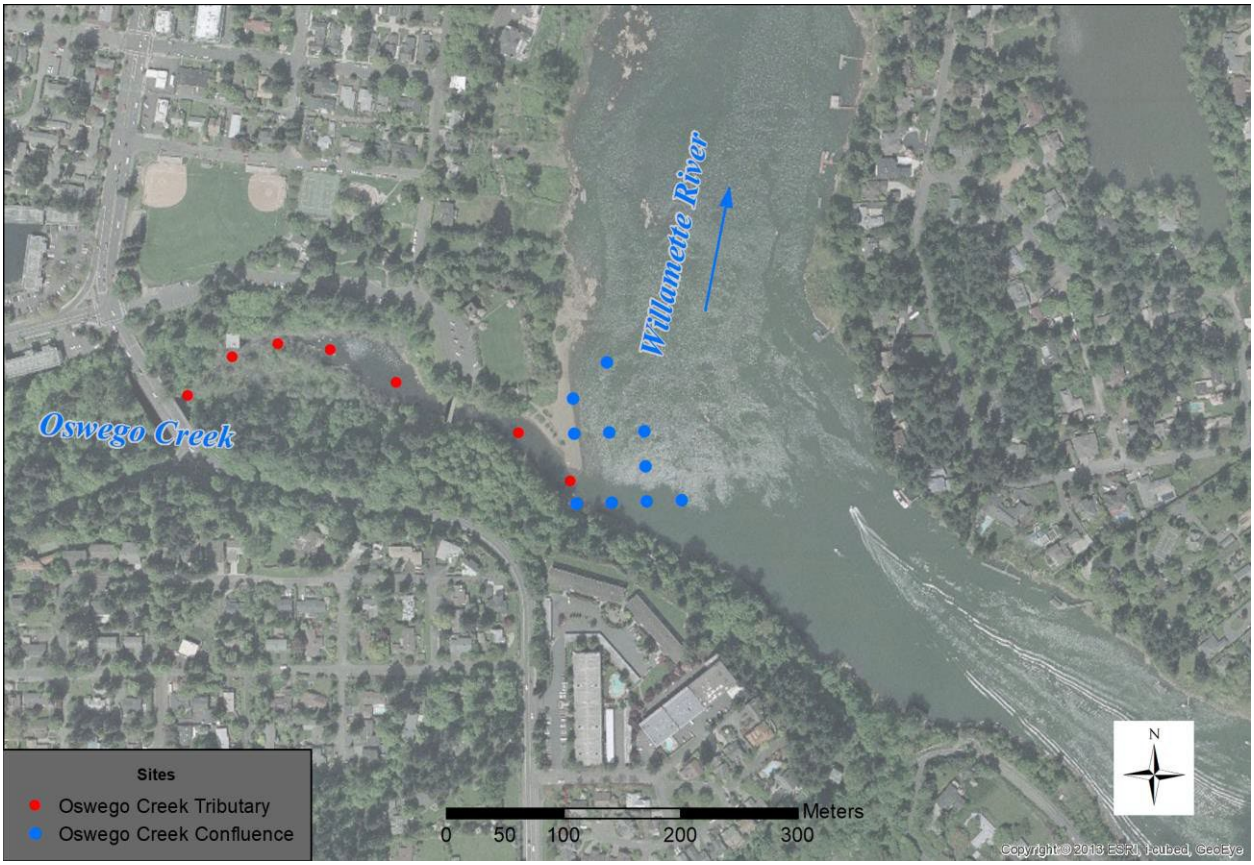

Figure 14. Oswego Creek reference site confluence quadrats (blue points) and tributary sample reaches (red).

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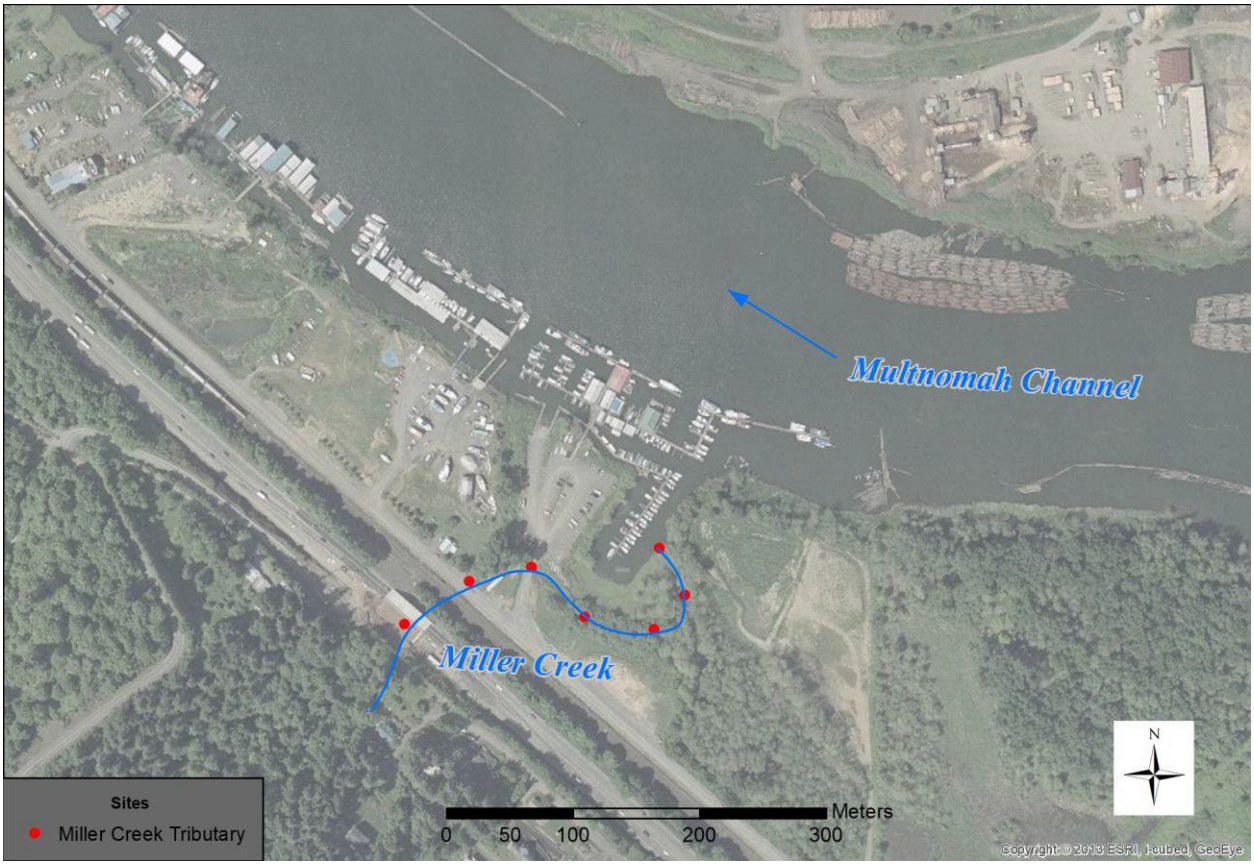

Figure 15. Miller Creek tributary sample reaches (red points). This site was not sampled in 2023.

Results

Restoration Sites

A total of 10 lamprey were observed during 73 sampling events at restoration sites in 2023. All lamprey collected at restoration sites were of the larval life stage. No detections of juveniles or evidence of adults (e.g., live adults, spawning nests) occurred. All larvae collected appeared healthy based on visual observation of external features, and no abnormalities or indications of disease or poor health were observed. Lamprey identification and length measurements are summarized in Table 1 and site-specific depth, temperature, and conductivity are summarized in Table 2**.**

Alder Point Restoration Site

This site was not sampled in 2023.

Harborton Restoration Site

A larval lamprey (n = 1) was detected at one of 10 North Tributary confluence quadrats at the Harborton Restoration Site (d = 0.10, Table 1), sampled on 4 October 2023. The maximum number of lampreys at an individual quadrat was one. Species composition included one Pacific Lamprey (n = 1, TL = 91 mm) captured at a depth of 2.44 m. Sample depths for the 10 quadrats ranged from 1.22 m to 9.45 m (Table 2). Water temperature was 16.4°C and conductivity was 113 μS/cm. Confluence habitat also includes the Delta and South sites, each consisting of 10 quadrats. No lamprey were detected in the Delta or South confluence habitats or tributary habitat at the Harborton restoration site (d = 0.00, Table 1). Delta confluence quadrats were sampled on 4 October 2023, with depths ranging from 0.61 m to 3.66 m (Table 2). Water temperature was 16.3°C and conductivity was 112 μS/cm. South confluence quadrats were sampled on 4 October 2023, with depths ranging from 0.61 m to 8.23 m (Table 2). Water temperature was 15.9°C and conductivity was 106 μS/cm. In the tributary habitat, two 50 m reaches were sampled on 30 June 2023, with depths ranging from 0.15 m to 0.25 m (Table 2). Water temperature was 18.0°C and conductivity was 232 μS/cm. Only two reaches, those closest to the Willamette River, were sampled due to the dense vegetation and very little water in the tributary channel.

Though not a target species, a native floater freshwater mussel *Anodonta* spp. was collected in the sediment sample at the Delta site (Figure 16).

Figure 16. *Anodonta* spp. Collected in the sediment sample at the Delta Confluence habitat at the Harborton site.

Linnton Restoration Site

No lamprey were detected at the confluence, shoreline, or tributary habitats at the Linnton restoration site (d = 0.00, Table 1). Ten confluence quadrats were sampled on 5 October 2023, with depths ranging from 0.61 m to 8.53 m (Table 2). Water temperature was 15.8°C and conductivity was 83 μS/cm in the confluence habitat. Ten shoreline quadrats were sampled on 5 October 2023, with depths ranging from 0.61 m to 10.06 m (Table 2). Water temperature was 15.8°C and conductivity was 82 μS/cm. In the tributary habitat, four 50 m reaches were sampled on 29 June 2023, with depths ranging from 0.15 m to 0.25 m (Table 2). Water temperature was 18.0°C and conductivity was 128 μS/cm.

Triangle Park Restoration Site

This site was not sampled in 2023.

Rinearson Natural Area (Confluences and Tributary)

Lampreys were detected in five of the ten confluence habitat quadrats at the Rinearson Natural Area (d = 0.50, Table 1), sampled on 13 October 2023. Eight larvae were sampled, and species composition included Pacific Lamprey (n = 1, TL = 64 mm) and seven unidentifiable larvae (n = 7, TL = 32-62). The maximum number of larvae sampled at an individual quadrat was two. Capture depths ranged from 0.61 m to 5.49 m and sample depths ranged from 0.30 m to 5.49 m (Table 2). Water temperature was 14.6°C and conductivity was 63 μS/cm. Larval lamprey (n = 1) were detected in one of the seven tributary reaches (d = 0.14, Table 1), sampled on 8 August 2023. Species composition was Pacific Lamprey (n = 1, TL = 110 mm) and the capture depth was 0.08 m. Sample depths ranged from 0.08 m to 0.50 m (Table 2). Water temperature was 15.0°C and conductivity was 187 μS/cm.

PGE 13.1 Restoration Site

This site was not sampled in 2023.

Reference Sites

A total of 14 lamprey were observed during 77 sampling events at reference sites in 2023. All lamprey collected at reference sites were of the larval life stage except for one transforming *Lampetra* spp. individual that was sampled at the Columbia Slough site. No detections or evidence of adults (e.g., live adults, spawning nests) occurred. All individuals collected appeared healthy based on visual observation of external features.

Multnomah Channel (Shoreline)

No lamprey were detected in the 10 shoreline quadrats at the Multnomah Channel reference site (d = 0.00, Table 1), sampled on 3 October 2023. Sample depths ranged from 4.88 m to 10.36 m (Table 2). Water temperature was 16.1°C and conductivity was 93 μS/cm.

McCarthy Creek (Confluence and Shoreline)

No lamprey were detected in the 10 shoreline or the 10 confluence quadrats at the McCarthy Creek reference site (d = 0.00, Table 1), sampled on 3 October 2023. Sample depth for the confluence quadrats ranged from 3.05 m to 9.14 m. Water temperature was 18.8°C and conductivity was 104 μS/cm. Sample depths for the shoreline quadrats ranged from 0.76 m to 11.28 m (Table 2). Water temperature was 16.7°C and conductivity was 106 μS/cm.

Columbia Slough (Confluence)

One transforming, eyed *Lampetra* spp. lamprey (n = 1) was detected at one of 10 confluence quadrats sampled on 4 October 2023 (d = 0.10, Table 1). The individual was 131 mm in length and had developed paired eyes (Figure 16). The transformer was captured at 1.52 m depth. Sample depths from the 10 quadrats ranged from 0.30 m to 3.05 m (Table 2). Water temperature was 17.7°C and conductivity was 121 μS/cm.

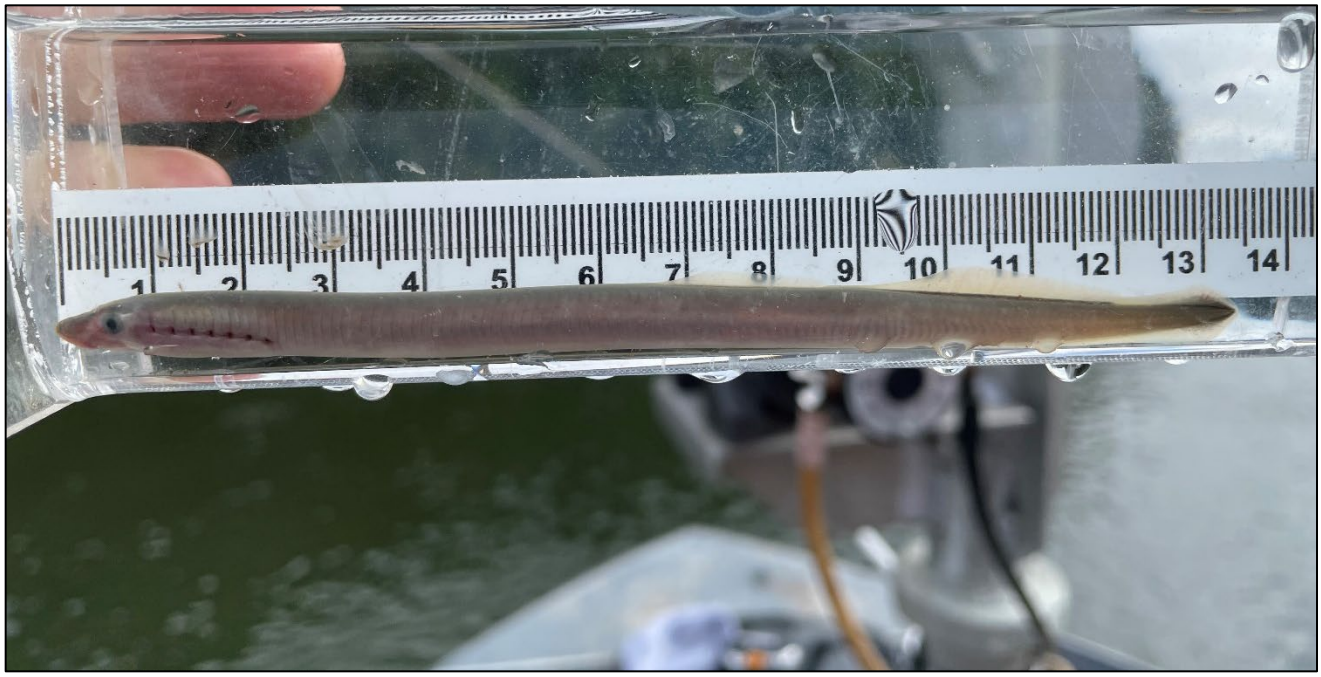

Figure 17. Transforming, eyed *Lampetra* spp. sampled at the Columbia Slough reference site.

Ross Island (Shoreline)

This site was not sampled in 2023.

Cemetery Creek (Confluence and Shoreline)

Larval lamprey (n = 5) were detected in three of 10 confluence quadrats at the Cemetery Creek Reference Site (d = 0.30, Table 1), sampled on 6 October 2023. The maximum number of lampreys at an individual confluence quadrat was two. Species composition included Pacific Lamprey (n = 2, TL = 65, 87 mm), *Lampetra* spp. (n = 1, TL = 102 mm), and unidentifiable lamprey (n = 2, TL = 25, 34 mm) with capture depth ranging from 4.27 m to 8.23 m. Sample depth from the 10 confluence quadrats ranged from 0.61 m to 8.23 m (Table 2). Water temperature was 15.7°C and conductivity was 73 μS/cm. Larval lamprey (n = 4) were detected at three of 10 shoreline quadrats (d = 0.30, Table 1), sampled on 6 October 2023. The maximum number of lampreys at an individual quadrat was two. Species composition included Pacific Lamprey (n = 3, TL = 62-92 mm) and unidentifiable lamprey (n = 1, TL = 42 mm) with capture depth ranging from 1.22 m to 6.10 m. Sample depths from the 10 shoreline quadrats ranged 1.22 m to 12.19 m (Table 2). Water temperature was 15.7°C and conductivity was 73 μS/cm.

Oswego Creek (Tributary and Confluence)

Larval lamprey (n = 4) were detected at two of 10 confluence quadrats at the Oswego Creek reference site (d = 0.20; Table 1), sampled on 6 October 2023. Species composition included unidentifiable lamprey (n = 4, TL = 25-59 mm) captured at 5.19 m to 5.49 m. Sample depths for the confluence site ranged from 2.13 m to 16.76 m, though for three quadrats the depth reader was not working and accurate depths were not recorded (Table 2). Water temperature was 15.6°C and conductivity was 71 μS/cm. No larval lampreys were detected in the seven reaches sampled in the tributary habitat at the Oswego Creek reference site on 18 July 2023. The sample depths for the

tributary site ranged from 0.25 m to 0.65 m (Table 2). Water temperature was 20.0°C and conductivity was 90 μS/cm.

Miller Creek (Tributary)

This site was not sampled in 2023.

Table 1. In 2023, number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID). A single transforming, eyed *Lampetra* spp. was sampled at the Columbia Slough site and is denoted with an *.

							Pacific Lamprey		*Lampetra spp.*		Unidentified		
Site Type	Site	Habitat	Visited	Sampled	Occupied	*d*	*n*	TL(mm) Range	*n*	TL(mm) Range	*n*	TL(mm) Range	Total *n*
Restoration	Harborton	Delta	10	10	0	0.00	0	-	0	-	0	-	0
		North Tributary	10	10	0	0.10	1	91	0	-	0	-	1
		South Tributary	10	10	0	0.00	0	-	0	-	0	-	0
		Tributary	7	2	0	0.00	0	-	0	-	0	-	0
	Linnton	Confluence	10	10	0	0.00	0	-	0	-	0	-	0
		Shoreline	10	10	0	0.00	0	-	0	-	0	-	0
		Tributary	4	4	0	0.00	0	-	0	-	0	-	0
	Rinearson	Confluence	10	10	5	0.50	1	64	0	-	7	32-62	8
		Tributary	7	7	1	0.14	1	110	0	-	0	-	1
Reference	Cemetery Creek	Confluence	10	10	3	0.30	2	65-87	1	102	2	25-34	5
		Shoreline	10	10	3	0.30	3	62-92	0	-	1	42	4
	Columbia Slough	Confluence	10	10	1	0.10	0	-	1	131*	0	-	1
	McCarthy Creek	Confluence	10	10	0	0.00	0	-	0	-	0	-	0
		Shoreline	10	10	0	0.00	0	-	0	-	0	-	0
	Multnomah Channel	Shoreline	10	10	0	0.00	0	-	0	-	0	-	0
	Oswego Creek	Confluence	10	10	2	0.20	0	-	0	-	4	25, 59	4
		Tributary	7	7	0	0.00	0	-	0	-	0	-	0

Table 2. In 2023, habitat variables measured at restoration and reference sites. Capture depth range is the minimum and maximum depths at which lamprey were captured. Sediment collection was transferred to ALS Environmental Laboratory (Kelso, WA). *Depth reader malfunctioning at Oswego Creek Shoreline site so depth so accurate depth was not recorded at four quadrats.

Site Type	Site	Habitat	2023 Date Sampled	Capture Depth (m), range	Sample Depth (m), range	Temperature (°C)	Conductivity (µS/cm)	Sediment Collected?
Restoration	Harborton	Delta Confluence	4-Oct	-	0.61-3.66	16.3	112	Yes
		North Confluence	4-Oct	2.44	1.22-9.45	16.4	113	Yes
		South Confluence	4-Oct	-	0.61-8.23	15.9	106	Yes
		Tributary	30-Jun	-	0.15-0.20	18.0	232	Yes
	Linnton	Confluence	5-Oct	-	0.61-8.53	15.8	83	Yes
		Shoreline	5-Oct	-	0.61-10.06	15.8	82	Yes
		Tributary	29-Jun	-	0.15-0.25	18.0	128	Yes
	Rinearson	Confluence	13-Oct	0.61-5.49	0.30-5.49	14.6	63	Yes
		Tributary	8-Aug	0.08	0.08-0.50	15.0	187	Yes
Reference	Cemetery Creek	Confluence	6-Oct	4.27-8.23	0.61-8.23	15.7	73	Yes
		Shoreline	6-Oct	1.22-6.10	1.22-12.19	15.7	73	Yes
	Columbia Slough	Confluence	4-Oct	1.52	0.30-3.05	17.7	121	Yes
	McCarthy Creek	Confluence	3-Oct	-	3.05-9.14	18.8	104	Yes
		Shoreline	3-Oct	-	0.76-11.28	16.7	106	Yes
	Multnomah Channel	Shoreline	3-Oct	-	4.88-10.36	16.1	93	No
	Oswego Creek	Confluence	6-Oct	5.18-5.49	2.13-16.76*	15.6	71	Yes
		Tributary	18-July	-	0.25-0.65	20.0	90	Yes

Table 3. Occupancy results from sampling at Alder Point restoration sites and the paired Ross Island reference site across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID).

					Quadrats				Lamprey			
Site Type	Site	Habitat	Year	Status	Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Restoration	Alder Point	Confluence 1-3	2016	Post Yr 1	30	30	1	0.03	0	0	1	1
			2017	Post Yr 2	30	30	1	0.03	0	0	1	1
			2018	Post Yr 3	30	30	1	0.03	0	0	1	1
			2019	Post Yr 4	30	30	2	0.07	0	1	1	2
			2020	Post Yr 5	30	30	0	0	0	0	0	0
		Shoreline	2014	Pre Yr 1	30	29	2	0.07	0	3	0	3
			2016	Post Yr 1	10	10	1	0.1	0	1	0	1
			2017	Post Yr 2	10	10	1	0.1	1	0	1	2
			2018	Post Yr 3	10	10	0	0	0	0	0	0
			2019	Post Yr 4	10	10	0	0	0	0	0	0
			2020	Post Yr 5	10	10	0	0	0	0	0	0
		Slough	2016	Post Yr 1	10	10	0	0	0	0	0	0
			2017	Post Yr 2	10	10	0	0	0	0	0	0
			2018	Post Yr 3	10	10	0	0	0	0	0	0
			2019	Post Yr 4	10	10	0	0	0	0	0	0
			2020	Post Yr 5	10	10	0	0	0	0	0	0
Reference	Ross Island	Shoreline	2014	-	28	26	5	0.19	0	6	0	6
			2016	-	10	10	2	0.20	2	0	0	2
			2017	-	13	10	3	0.30	5	0	1	6
			2018	-	10	10	3	0.30	3	2	1	6
			2019	-	10	10	0	0.00	0	0	0	0
			2020	-	10	10	0	0.00	0	0	0	0
			2022	-	10	10	3	0.30	0	0	4	4

Table 4. Occupancy results from sampling at Harborton restoration sites and the paired Columbia Slough reference site across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID).

					Quadrats				Lamprey			
Site Type	Site	Habitat	Year	Status	Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Restoration	Harborton	Delta Confluence	2017	Pre Yr 1	10	10	1	0.1	0	0	2	2
			2022	Post Yr 2	10	10	1	0.1	0	0	1	1
			2023	Post Yr 3	10	10	0	0	0	0	0	0
		North Confluence	2017	Pre Yr 1	10	10	1	0.1	0	1	0	1
			2022	Post Yr 2	10	10	0	0	0	0	0	0
			2023	Post Yr 3	10	10	1	0.1	1	0	0	1
		South Confluence	2017	Pre Yr 1	10	10	0	0	0	0	0	0
			2022	Post Yr 2	10	10	0	0	0	0	0	0
			2023	Post Yr 3	10	10	0	0	0	0	0	0
		Tributary	2021	Post Yr 1	7	7	0	0	0	0	0	0
			2022	Post Yr 2	7	7	0	0	0	0	0	0
			2023	Post Yr 3	7	2	0	0	0	0	0	0
Reference	Columbia Slough	Confluence	2016	-	10	10	0	0	0	0	0	0
			2017	-	11	10	1	0.1	1	0	0	1
			2018	-	10	10	0	0	0	0	0	0
			2019	-	10	10	0	0	0	0	0	0
			2020	-	10	10	2	0.2	0	0	2	2
			2022	-	10	10	3	0.3	0	0	3	3
			2023	-	10	10	1	0.1	0	1	0	1

Table 5. Occupancy results from sampling at Linnton restoration sites and the paired McCarthy Creek and Miller Creek reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID).

					Quadrats				Lamprey			
Site Type	Site	Habitat	Year	Status	Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Restoration	Linnton	Confluence	2017	Pre Yr 1	10	10	0	0	0	0	0	0
			2020	Post Yr 1	10	10	0	0	0	0	0	0
			2022	Post Yr 3	10	10	0	0	0	0	0	0
			2023	Post Yr 4	10	10	0	0	0	0	0	0
		Shoreline	2017	Pre Yr 1	10	10	0	0	0	0	0	0
			2020	Post Yr 1	10	10	0	0	0	0	0	0
			2022	Post Yr 3	10	10	0	0	0	0	0	0
			2023	Post Yr 4	10	10	0	0	0	0	0	0
		Tributary	2021	Post Yr 1	4	4	0	0	0	0	0	0
			2022	Post Yr 2	4	4	0	0	0	0	0	0
			2023	Post Yr 3	4	4	0	0	0	0	0	0
Reference	McCarthy Creek	Tributary (dropped)	2016	-	7	2	0	0	0	0	0	0
			2017	-	7	2	0	0	0	0	0	0
		Confluence	2016	-	10	10	0	0	0	0	0	0
			2017	-	10	10	1	0.1	1	1	0	2
			2018	-	10	10	2	0.2	1	0	1	2
			2019	-	10	10	0	0	0	0	0	0
			2020	-	10	10	0	0	0	0	0	0
			2022	-	10	10	1	0.1	0	0	1	1
			2023	-	10	10	0	0	0	0	0	0
		Shoreline	2016		10	10	1	0.1	0	0	1	1
			2017	-	10	10	0	0	0	0	0	0
			2018	-	10	10	0	0	0	0	0	0
			2019	-	10	10	1	0.1	0	0	1	1
			2020	-	10	10	0	0	0	0	0	0
			2022	-	10	10	0	0	0	0	0	0
			2023	-	10	10	0	0	0	0	0	0

Table 5 continued.

Site Type	Site	Habitat	Year	Status	Quadrats				Lamprey			
					Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Reference	Miller Creek	Tributary	2019	-	7	7	5	0.71	0	4	4	8
			2020	-	7	6	5	0.83	0	24	10	34
			2022	-	7	7	2	0.29	2	0	0	2

Table 6. Occupancy results from sampling at Rinearson restoration sites and the paired Cemetery Creek and Oswego Creek reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID).

					Quadrats				Lamprey			
Site Type	Site	Habitat	Year	Status	Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Restoration	Rinearson	Confluence	2015	Pre Yr 1	13	10	3	0.3	3	0	3	6
			2019	Post Yr 1	10	10	3	0.3	1	0	3	4
			2020	Post Yr 2	10	10	1	0.1	1	0	2	3
			2022	Post Yr 4	10	10	0	0	0	0	0	0
			2023	Post Yr 5	10	10	5	0.5	1	0	7	8
		Tributary	2015	Pre Yr 1	-	7	1	0.14	3	0	0	3
			2019	Post Yr 1	6	4	0	0	0	0	0	0
			2020	Post Yr 2	7	7	1	0.14	1	0	0	1
			2021	Post Yr 3	7	7	2	0.26	2	0	0	2
			2022	Post Tr 4	7	6	2	0.33	2	0	1	3
			2023	Post Yr 5	7	7	1	0.14	1	0	0	1
Reference	Cemetery Creek	Tributary	2015	-	2	2	0	0	0	0	0	0
		Confluence	2015	-	10	10	5	0.5	2	0	6	8
			2016	-	13	10	2	0.2	0	0	2	2
			2017	-	10	10	6	0.6	3	0	7	10
			2018	-	10	10	5	0.5	4	0	4	8
			2019	-	10	10	2	0.2	0	0	4	4
			2020	-	10	10	1	0.1	0	3	1	4
			2022	-	10	10	3	0.3	0	0	3	3
			2023	-	10	10	3	0.3	2	1	2	5
		Shoreline (additional quadrats, not paired)	2016	-	10	10	3	0.3	1	0	2	3
			2017	-	10	10	4	0.4	3	0	3	6
			2018	-	10	10	3	0.3	2	0	4	6
			2019	-	10	10	3	0.3	0	0	5	5
			2020	-	10	10	4	0.4	1	1	6	8
			2022	-	10	10	2	0.2	0	0	2	2
			2023	-	10	10	3	0.3	3	0	1	4

Table 6 continued.

Site Type	Site	Habitat	Year	Status	Quadrats				Lamprey			
					Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total (n)
Reference	Oswego Creek	Confluence (additional quadrats, not paired)	2016	-	10	10	4	0.4	2	1	3	6
			2017	-	10	10	5	0.5	3	0	6	9
			2018	-	10	10	2	0.2	2	0	2	4
			2019	-	10	10	5	0.5	0	0	13	13
			2020	-	10	10	2	0.2	1	1	0	2
			2023	-	10	10	2	0.2	0	0	4	4
		Tributary	2019	-	7	7	1	0.14	0	0	1	1
			2020	-	7	7	2	0.29	1	1	0	2
			2021	-	7	7	2	0.29	0	2	0	2
			2022	-	7	7	1	0.14	1	0	0	1
			2022	-	7	7	0	0	0	0	0	0

Table 7. Occupancy results from sampling at unpaired or non-NRDA restoration sites and additional reference sites across all sampling years (pre and post restoration actions). Total number of quadrats visited, sampled, occupied by larval lamprey, corresponding larval lamprey detection probability (*d*), and lamprey observations by species (*n*). Small larvae (i.e., typically less than 70 mm TL) that cannot be accurately identified and are classified as unidentified (UNID).

					Quadrats				Lamprey			
Site Type	Site	Habitat	Year	Status	Visited	Sampled	Occupied	*d*	Pacific Lamprey	*Lampetra* spp.	UNID	Total n
Restoration	PGE 13.1	Shoreline	2017	Pre Yr 1	10	10	0	0	0	0	0	0
			2019	Post Yr 2	10	10	0	0	0	0	0	0
			2022	Post Yr 5	10	10	2	0.2	0	0	2	2
	Triangle Park	Shoreline	2017	Pre Yr 1	21	21	0	0	0	0	0	0
Reference	Multnomah Channel	Shoreline	2016	-	10	10	1	0.1	0	0	1	1
			2017	-	10	10	0	0	0	0	0	0
			2018	-	10	10	0	0	0	0	0	0
			2019	-	10	10	0	0	0	0	0	0
			2022	-	10	10	0	0	0	0	0	0
			2023	-	10	10	0	0	0	0	0	0

Table 8. Summary table of mainstem habitat types across all years and all sites. Sites are listed from downstream to upstream with reference sites highlighted in gray. Total lamprey (*n*) is the sum of all lamprey collected at the site and habitat type across all years. Average Lamprey/yr is the mean number of lamprey sampled per year at each site and habitat type, with observed minimum and maximum in parentheses. Mean *d* is the mean detection rate at the site and habitat type across all years, calculated from *d* value in Table 3-7.

Site Type	Site	Habitat	Years Surveyed	Total Lamprey (*n*)	Average Lamprey/yr (min-max)	Mean *d*
Reference	Multnomah Channel	Shoreline	6	1	0.2 (0-1)	0.02
Reference	McCarthy Creek	Confluence	7	5	0.7 (0-2)	0.06
Reference	McCarthy Creek	Shoreline	7	2	0.3 (0-1)	0.03
Reference	Columbia Slough	Confluence	7	7	1 (0-3)	0.10
Restoration	Alder Point	Confluence	5	5	1 (0-2)	0.03
Restoration	Alder Point	Shoreline	6	6	1 (0-3)	0.05
Restoration	Alder Point	Slough	5	0	0	0.00
Restoration	Harborton	Confluence	3	5	0.4 (0-2)	0.03
Restoration	Linnton	Confluence	4	0	0	0.00
Restoration	Linnton	Shoreline	4	0	0	0.00
Restoration	Triangle Park	Shoreline	1	0	0	0.00
Restoration	PGE 13.1	Shoreline	3	2	0.67 (0-2)	0.07
Reference	Ross Island	Shoreline	7	24	3.4 (0-6)	0.18
Reference	Cemetery Creek	Confluence	8	44	5.5 (2-10)	0.34
Reference	Cemetery Creek	Shoreline	7	34	4.9 (2-8)	0.31
Reference	Oswego Creek	Confluence	6	38	6.3 (1-13)	0.33
Restoration	Rinearson	Confluence	5	21	4.2 (0-8)	0.24

Discussion

Degradation of native fish habitat in the Willamette River basin is due to a variety of causes including ongoing industrialization, chemical pollution, and river channelization. These anthropogenic impacts have caused a decline in suitable habitat by altering low velocity floodplains necessary for native fish survival (Lundin et al. 2019). Native fish assemblages have been impacted throughout this river system and restoration actions have been implemented to increase habitat for juvenile Chinook salmon *Oncorhynchus tshawytscha.* Other native fishes, such as the Pacific Lamprey *Entosphenus tridentatus* and *Lampetra spp.*, utilize similar habitat in their larval life stage and may benefit from these restoration actions. Monitoring of larval lamprey presence pre- and post-implementation can increase the benefit of restoration actions for multiple species in the future. Restoration actions are complete at five sites, Alder Point, Harborton, Linnton, Rinearson, and PGE 13.1.

Alder Point and its paired reference shoreline site, Ross Island, were not sampled in 2023, but at both sites larval lamprey were observed in 2014, pre-implementation at Alder Point, and subsequently in years since implementation (Table 3). Since monitoring began, Ross Island has a higher average detection rate than the Alder Point Shoreline habitat, but both sites have had years of no detection as well as years with detection. The newly created slough habitat is the only habitat type to have no lamprey detected during the 5-year post-implementation monitoring. The post restoration, ten-year monitoring will be conducted in 2025.

Monitoring at the three Harborton confluence habitats can be directly compared to the reference site at Columbia Slough, which is sampled concurrently (Table 4). Surveys in 2023 are the third year of post-implementation monitoring at the Harborton site. Lamprey were found to occupy the confluence habitats at both the restoration and reference sites in 2023 as well as in previous years sampled. Lamprey have been observed at the Delta and North confluences habitats in pre- and post-implementation years. Lamprey have never been detected at the South confluence habitat, or the newly created tributary habitat at the Harborton restoration site. The tributary is increasingly challenging to sample for lamprey as the wetland vegetation has responded to restoration efforts and is growing thickly at the site. The channel is often obscured and only the reaches closest to the confluence with the Willamette River are visible and have enough water to sample in June. This tributary also becomes disconnected from the Willamette River and there is no presence of spawning habitat in this tributary. If lamprey are using this habitat they would likely drift in seasonally with high flows, however year round persistence may be limited by the lack of low flow wetted lamprey habitat. Continued monitoring of larval lamprey occupancy at the Harborton restoration site and its paired reference site is warranted and will provide a better understanding of larval lamprey colonization rates at newly available habitats.

Surveys in 2023 are the fourth-year post-implementation monitoring for the Linnton restoration site mainstem habitats and the third year for the tributary habitat. McCarthy Creek acts as a reference site for the shoreline and confluence habitat and Miller Creek acts as a reference for the tributary habitat (Table 5). No larval lamprey were detected in the shoreline, confluence, or tributary habitats at the Linnton restoration site ($d = 0$). This occupancy data follows patterns documented over the sampling period, as non-detections has been consistent for pre- and post-restoration years. In 2023 no lamprey were detected at the McCarthy Creek confluence habitat ($d = 0$) nor at the shoreline habitat ($d = 0$). Detections at these reference sites have been varied during the sampling period. Miller Creek was not sampled in 2023, but lamprey have been

detected during all prior years of sampling.

Monitoring at the Rinearson site can be directly compared to the reference site habitats at Cemetery Creek and Oswego Creek, which are sampled concurrently (Table 6). Surveys in 2023 are the fifth-year post-implementation surveys. Larval lamprey have generally occupied confluence habitat at both sites pre- and post-restoration. Larval lamprey were not detected in the Rinearson confluence habitat in 2022, as they were in previous years, but the highest detection rate to date ($d = 0.5$) was observed in 2023. Larval lamprey occupied tributary habitat within the Rinearson site pre-restoration. The first larval lamprey was detected post-restoration in 2020 and lamprey have consistently been detected in the Rinearson tributary habitat since that time. All larval lamprey detections in the tributary post restoration have occurred in the two reaches nearest the confluence, where backwater from the Willamette River commonly inundates the tributary. These reaches are downstream from the section of the roughen channel, multiple beaver pond reaches, and an old dam. Conclusions regarding lamprey use of the Rinearson restoration site cannot be made without further monitoring, however, lamprey are being detected in its tributary habitat post-restoration. Similarly, lamprey have only been detected in the lower two reaches of Oswego Creek, which are also backwatered by the Willamette River. Lamprey have been found to occupy all other reference site habitats (Cemetery Creek and Oswego Creek) during the study period and continue to do so based on our 2023 sampling.

Although restoration at PGE 13.1 is not an NRDA site, in an attempt to further understand the utility of the restoration in the broader NRDA area of the Willamette River, FWS samples this location for lamprey presence. There is no specific reference site for PGE 13.1 (Table 7). PGE 13.1 post-restoration sampling is scheduled to occur every other year. The site was previously sampled in 2019. Due to pandemic restrictions and supply chain issues, PGE 13.1 was not sampled in 2021 (as scheduled) but was sampled in 2022. Lamprey were found to occupy PGE 13.1 for the first time since sampling began in 2017 (pre-restoration). PGE 13.1 was not sampled in 2023 and will be sampled again for the final time in 2025. Although conclusions regarding the use of the PGE 13.1 restoration site require further monitoring, lamprey were found to occupy the shoreline restoration habitat at this location. Additional shoreline habitat sampling also occurs in the Multnomah Channel (Table 7), and over the course of the six years of sampling only one larval lamprey has been detected in that habitat.

Reference site monitoring is an important component of the lamprey monitoring program associated with the Portland Harbor Superfund project. Patterns of larval lamprey occupancy at reference sites will provide a baseline for evaluating changes in larval lamprey occupancy at restoration sites over time and assessing the utility of restoration actions for larval lamprey. After 2017 sampling, we eliminated the wadable portions of Cemetery Creek and McCarthy Creek as reference sites due to sampling and habitat conditions that prevent these sites from being useful and appropriate. In 2019, Miller Creek tributary was added as a replacement for the McCarthy Creek tributary and Oswego Creek tributary was added as a replacement for Cemetery Creek tributary.

In addition to reference site monitoring, it may be useful to evaluate the entire project geographically. Over the course of this project to date, 194 lamprey have been collected in mainstem habitats, of which 161 (83%) have been observed upstream of the core downtown Portland area (upstream river kilometer 22.5) (Table 8). The upstream sites include the Rinearson restoration shoreline habitat as well as the reference habitats Owego Creek

confluence (unpaired), Cemetery Creek confluence and shoreline, and Ross Island shoreline. These five habitats have notably higher mean detection rates as well as higher mean lampreys sampled per year than all habitats downstream. The upstream habitats average 4.9 lamprey (n = 3.4-6.3) sampled per year over the course of the study, while downstream habitats average less than one lamprey per year (n = 0-1). The mean detection rates in upstream habitats was 0.28 (d = 0.18-0.34), while in downstream habitats the mean detection rate was 0.03 (d = 0.0-0.1). This stark division in lamprey detection rate could be due to many factors, but geographic location within the system is likely influencing lamprey densities, recolonization rates, and habitat use at restoration sites. Continued monitoring within the study area will help elucidate these broad scale lamprey distribution patterns and contextualize expected lamprey project use.

All documented Pacific Lamprey spawning near the Portland Harbor Superfund sites are in tributaries that enter the mainstem Willamette River upstream of the NRDA study area and the downtown Portland core (Oregon Department of Fish and Wildlife 2020). These tributaries, including the Clackamas River, Abernethy Creek, and Johnson Creek, are likely contributing a proportion of the larval Pacific Lamprey observed in this study. Though less formally documented, there is likely *Lampetra* spp. spawning occurring in these tributary systems as well as in smaller tributaries entering the study area, including Miller Creek. Evidence suggesting dispersal of larval lamprey out of tributaries and into mainstem habitats has been observed previously in the mainstem Columbia River and Willamette River basins (Jolley et al. 2012; Jolley et al. 2013; Jolley et al. 2014) and may occur over extensive distances (Scribner and Jones 2002; Derosier et al. 2007). Larval lamprey from nearby tributaries could disperse into the Portland Harbor Superfund study area and spend multiple years rearing in the Willamette River. The higher detection rates and higher numbers of observed larvae in the upstream sites could be related to the closer proximity to known spawning locations. Pacific Lamprey have been documented spawning directly adjacent to the Portland Harbor Superfund study area at the confluence of the Clackamas and Willamette rivers (B. Walczak, Oregon Department of Fish and Wildlife, personal communication), as well as in the lower mainstem of the Lewis River (J. Doyle, PacifiCorp, personal communication), suggesting that Pacific Lamprey spawning in relatively large rivers (5^{th} order, and possibly larger) is plausible where suitable substrate and flow regimes occur. Pacific Lamprey spawning has not been documented in the mainstem Willamette River of the Portland Harbor Superfund area. However, it is possible that some of the larval Pacific Lamprey detected at restoration and reference sites originated from spawning within the Portland Harbor Superfund area.

Similar to the results of previous years, (Jolley et al. 2015; Silver et al. 2016; Skalicky et al. 2018, Skalicky et al. 2019, Skalicky and Whitesel 2020, Blanchard et al. 2021; Kelley et al. 2022) we observed a combination of larval Pacific Lamprey and *Lampetra* spp. in the Portland Harbor Superfund area. Mainstem habitats associated with the Harborton and Rinearson restoration sites, as well as habitats at many reference sites, continue to appear suitable and available for colonization by larvae in the mainstem Willamette River and Multnomah Channel. This was evidenced by the presence of larvae in confluence and shoreline habitats.

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Appendix 1.

Results from sediment sampling have been provided to and can be obtained from:

Industrial Economics, Inc.
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