Occurrence, Detection, and Habitat Use of Larval Lamprey in Mainstem Environments: the Lower Willamette River.

2009 Annual Report

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Introduction

Pacific lamprey *Entosphenus tridentatus* in the Columbia River Basin (CRB) have declined to a remnant of their pre-1940s populations concerning tribal and regional agencies (Close et al. 2002). Lamprey are culturally important to Native American tribes, are ecologically important within the food web, and are an indicator species whose decline provides further insight into the impact of human actions on ecological function (Close et al. 2002). Pacific lamprey populations are declining and have been given protected status within Oregon due to declines along the coast and in the Columbia River Basin (Close et al. 2002; Kostow 2002). Increased knowledge of the biology, population dynamics, ecology, and identification of Pacific lamprey and sympatric species such as the western brook lamprey *Lampetra richardsoni* will help managers understand and conserve these important species.

Pacific lampreys have a complex life history that includes a three to seven year larval (i.e., ammocoete), migratory juvenile (i.e., macrophthalmia) and adult phases (Scott and Crossman 1073). Ammocoetes and macrophthalmia are strongly associated with stream and river sediments. Ammocoetes live burrowed in stream and river sediments for periods up to seven years after hatching, where they filter feed detritus and organic material (Scott and Crossman 1973; Sutton and Bowen 1994). Ammocoetes metamorphose into macrophthalmia from July to December (McGree et al. 2008) and migrate downstream to the Pacific Ocean. The timing, duration, and habitat use at the ammocoete and macrophthalmia life stage are poorly understood. For Pacific lamprey, the majority of the information on habitat preference of larvae comes from CRB tributary systems (Moser and Close 2003; Torgersen and Close 2004; Stone and Barndt 2005; Stone 2006). Lamprey ammocoetes are known to occur in shallow stream sediments but their use of comparatively large river habitats in relatively deeper areas is unknown. Anecdotal observations exist regarding larval lamprey occurrence in mainstem habitats, mainly at hydropower facilities or in downstream bypass reaches (CRITFC 2008), impinged on downstream screens, or through observation during dewatering events. Occurrences at hydropower facilities are generally thought to be associated with downstream migration. However, larval lamprey apparently rearing in nearshore areas have also been observed (USFWS, unpublished data). Sea lamprey *Petromyzon marinus* ammocoetes have been documented in deepwater habitats in tributaries of the Great Lakes and in proximity to river mouths (Bergstedt and Genovese 1994; Fodale et al. 2003).

The Willamette River has been the recipient of decades of toxic materials through industrial discharge and agricultural runoff including harmful levels of heavy metals, PCBs, dioxins, and pesticides. Elevated levels of these substances have been found in resident fish and near-shore sediment samples from the highly urbanized reaches of the Lower Willamette River and Portland Harbor (Oregon Department of Environmental Quality 2004). Larval lamprey using river sediment habitats are likely at risk from toxic sediment exposure. In addition, channel management activities (i.e., dredging) may affect this vulnerable life stage of lamprey. A deep-draft trade vessel passage from the Pacific Ocean to the Port of Portland is maintained on the Lower Willamette and Columbia rivers and the channel requires frequent maintenance. Currently, however, whether lampreys use the mainstem Willamette and Columbia rivers as a rearing area for larvae in addition to a migration corridor for returning adults and outbound juveniles is unknown.

A pilot study of Pacific lamprey and western brook lamprey use of mainstem habitat of the Willamette and Columbia rivers was initiated in 2007 (Silver et al. 2008). Pacific lamprey and western brook lamprey of a broad range in size were found utilizing nearshore areas of the

Willamette and Columbia Rivers. Specific locations included areas near Ross Island, Oswego Creek, and the Clackamas River on the Willamette River, the Cowlitz River delta, the Government Island area, and the Cottonwood Island area of the Columbia River. Those surveys were made using an AbP-2 backpack electrofisher in wadeable areas less than 1 m deep. Thus, although larval lamprey generally occupied these mainstem areas, knowledge of lamprey presence in deeper areas was unknown.

Sampling of ammocoetes in deepwater areas is a challenge because of specialized gear requirements as well as presumed patchy distributions. Successful sampling of deepwater areas for sea lamprey ammocoetes has occurred in tributaries to the Great Lakes using a modified electrofisher with suction (Bergstedt and Genovese 1994). This technique has shown promise in pilot studies in the Lower Willamette River (Windward Environmental 2005). However, one problem encountered when sampling for distribution and abundance of infaunal organisms is associated with the uncertainty in detection probabilities and capture efficiencies. One goal of this study was to develop a statistically robust design to evaluate the distribution (i.e., occupancy) of larval lamprey in the Willamette River. In part, statistical robustness can be improved by determining detection probability (DP). Knowledge of detection probabilities can inform sample design (e.g., required site visits giving 80% certainty of lamprey absence when not detected) and data analysis.

To this end we sampled deepwater areas of the Lower Willamette River (an area known to be occupied) to further document the presence of larval lampreys. In general, we attempted to document presence or absence of larval Pacific and western brook lampreys throughout the Lower Willamette River (i.e., downstream of Willamette Falls, RKM 42) and determine detection probabilities using a deepwater electrofisher. Our specific objectives were as follows:

1) Document presence or absence of lamprey ammocoetes through the Lower Willamette River.
2) Determine the probability of detecting larval lamprey in the Lower Willamette with a deepwater electrofisher.
3) Determine the probability of detecting larval lamprey in a 30 x 30 m quadrat.
4) Evaluate larval lamprey spatial clustering.
5) Describe the age (i.e., size) distribution of larval lamprey in the Lower Willamette River.
6) Describe the species composition of larval lamprey in the Lower Willamette River.

Methods

The Willamette River (Columbia River Basin) was sampled from Willamette Falls (RKm 42) to the confluence of the Columbia River at Portland, Oregon (Figure 1). The sampling area included the Multnomah Channel (a braid of the Willamette River) and the Portland Harbor Superfund site (RKm 3.2-19.2), an area known to be occupied with larval lamprey. Sampling occurred in three seasonal phases (i.e., March, June, and October).

We developed a layer of 30 m x 30 m quadrats using ArcMap 9.3 (ESRI [Environmental Systems Research Institute], Redlands, California) which was overlaid on the entire Lower Willamette River (Figure 2). There were 31,306 quadrats from Willamette Falls to the Willamette River mouth. The Universal Trans Mercator (UTM) coordinates representing the center point of each quadrat were determined. A Generalized Random Tessellation Stratified (GRTS) approach was applied to select quadrats in a random, spatially-balanced order (Stevens and Olsen 2004). The GRTS approach was applied to all quadrats to generate a spatially explicit sample design for this area of the Lower Willamette River. This approach was used to generate an unbiased, sample design that would allow the quantification of detection probabilities.

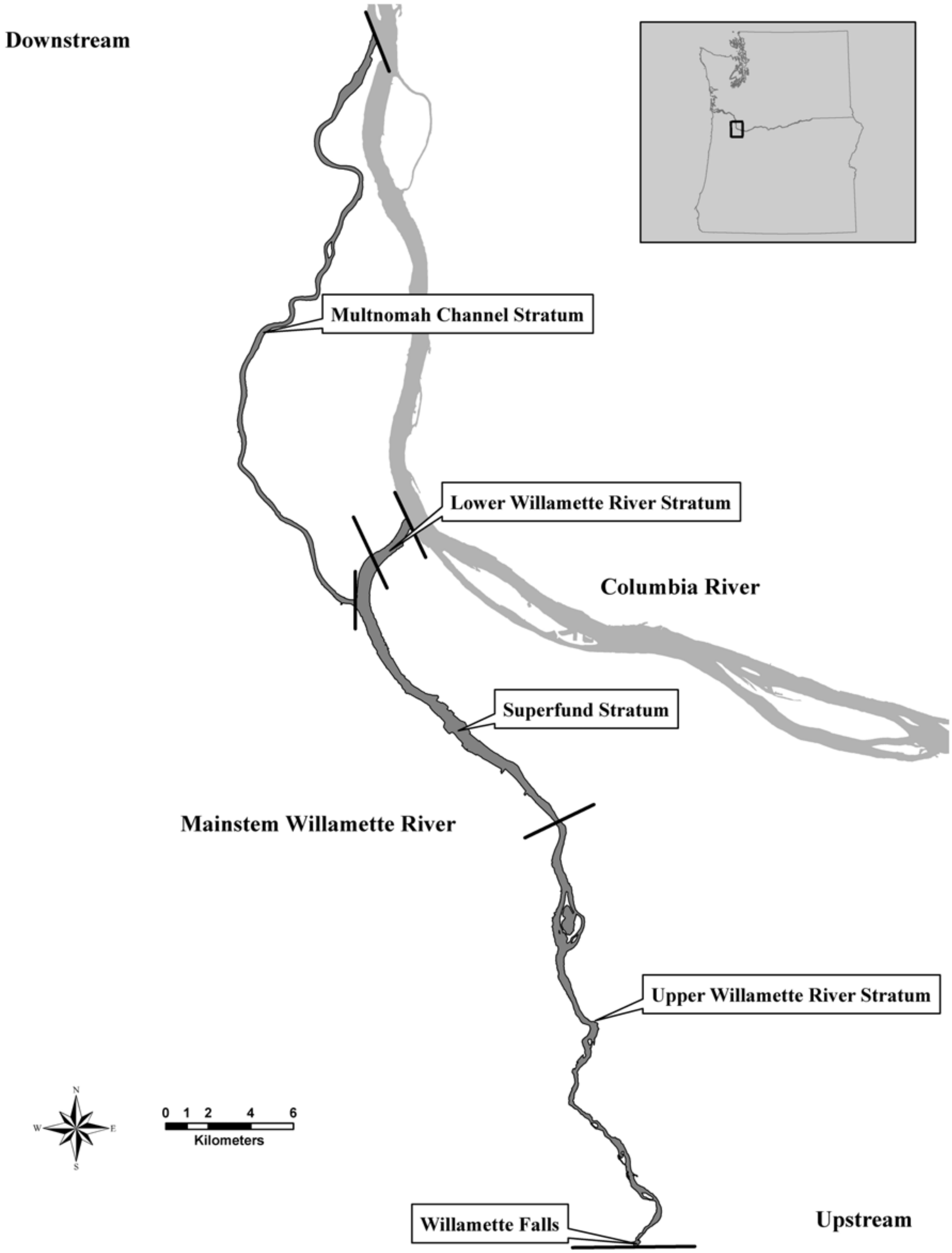

Figure 1. Map of the study area including sampling strata for lampreys on the Lower Willamette River, Oregon, 2009.

It is ideal but impractical and unnecessary to sample all 31,306 quadrats. The actual number of quadrats that were ultimately necessary was determined based partly on the power associated with a given sample design and the desired level of confidence. Using a deepwater electroshocker, the detection probability and capture efficiency for larval lamprey in the Lower Willamette River was unknown but, during selective fishing with a backpack electroshocker, ranged from 14-31%. At an assumed detection probability of no less than 0.10, we estimated that 28 sample quadrats would provide the statistical power for 80% certainty of larval lamprey absence when not detected. Based on previous sampling efforts which found larval lamprey in the area, we estimated sampling effort of 28 quadrats to allow calculation of a specific detection probability and provide additional distributional information. We identified quadrats using the GRTS approach to sample. The quadrats were ordered sequentially as they were selected in the GRTS approach and the lower numbered quadrats were given highest priority for sampling. Unfeasible quadrats (e.g., dewatered, inaccessible, physical impediment, excessive depth for our configuration) were eliminated from the sample through reconnaissance surveys and all subsequent quadrats were increased in priority. To maintain a random design and statistical robustness, quadrat modifications were determined prior to initiation of sampling.

Our sampling effort was expanded several times to be thorough both in overall effort and temporal scale. In addition, modifications to the electrofisher configuration allowed sampling at increased depth and 21 sites within the lowest stratum were added (depth > 13.7 m). We divided our sampling reach (i.e., Lower Willamette River) into four strata to investigate reach-specific occupancy. These were defined as an upstream reach from Willamette Falls to the Superfund site (RKm 42-19), the Superfind site (RKm 19-3), downstream area (RKm 3 to the mouth), and the Multnomah Channel (Figure 1). Strata are hereby referred to as upstream, Superfund, downstream, and Multnomah Channel. Table 1 outlines the overall sampling effort applied at the various strata of the Lower Willamette River.

An increased sampling effort is necessary to allow calculation of detection probability within an occupied quadrat in which only one sampling event occurs. A quadrat is considered occupied when one larval lamprey was captured in that quadrat. A subsample of occupied quadrats were selected and divided into nine, 10 m x 10 m subquadrats and the UTM coordinates were determined at the center of each (Figure 2). The subquadrats were sampled and quadrat-specific probability of detection was calculated. We sampled four sets of subquadrats in the upstream stratum in March. In June, subquadrats were sampled in the upstream (n=1 set) and Superfund strata (n=2 sets) and two sets were sampled each in the Superfund and downstream area in October. To evaluate potential clustered distributional patterns and attempt to increase the number of larvae captured we sampled each of the eight adjacent quadrats to occupied quadrats (n=3 sets).

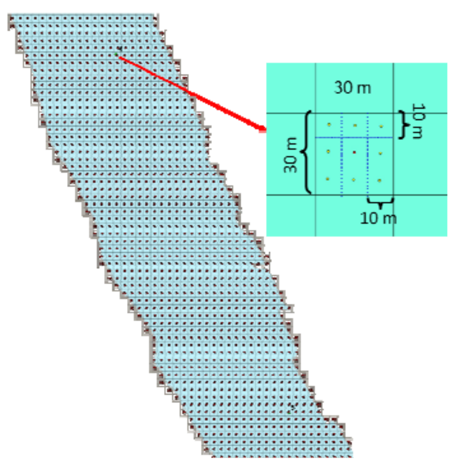

Figure 2. A schematic showing a section of the Lower Willamette River divide into 30 x 30 m quadrats and associated UTM center points. Inlay shows a quadrat divided into its component 10 m x 10 m subquadrats.

Depth may influence distribution of larvae. In August, we added an exploratory component to study. We selected transects in the upstream (n=3), Superfund (n=2), and downstream (n=1) strata. Transects were selected based on previous or presumed larval presence. Samples were taken at the center of every adjacent quadrat (i.e., every 30 m along a transect, perpendicular to the river channel, from bank to bank). The proportion of quadrats occupied along transects was examined by depth and patterns of occupancy were examined among quadrats. Depth sampled (among all sites) was further divided into three strata defined as shallow (depth < 5 m), moderate (depth 5-10 m), and deep (depth >10 m). In addition, patterns of occupancy by depth were compared using the Chi-square test for differences in probabilities (Conover 1999).

A sampling event consisted of lowering the bell of the deepwater electrofisher/dredge to the river bottom within the 30 m x 30 m quadrat. The sampling techniques were described in detail by Bergstedt and Genovese (1994) and were similar to those used in the Great Lakes region (Fodale et al. 2003). 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 suction is produced by directing the flow from a pump through a hydraulic eductor prohibiting ammocoetes from passing through the pump. Suction begins approximately 5 seconds prior to shocking to purge air from the suction hose. Shocking was conducted for 60 seconds, and the suction pump remained on for an additional 60 seconds after shocking to ensure collected ammocoetes passed through the hose and emptied into the collection basket.

Collected lampreys were anesthetized in a solution of tricaine methanesulfonate (MS-222), identified to species (Pacific lamprey, western brook lamprey, or lamprey spp.) according to caudal pigmentation (Goodman et al. 2009), and classified according to developmental stage (i.e., ammocoete, macrophthalmia, or adult). Lampreys were measured (TL in mm) and weighed (wet weight in g) and caudal fin tissue was collected for genetic analysis to confirm genus identification. Lampreys were placed in a recovery bucket of fresh river water and released after resuming normal swimming behavior.

Average TL was compared between species and among seasons and strata for each species using two-way ANOVA. Post-hoc comparisons were conducted using the Tukey multiple range test. Correlation analysis was used to explore the relationship of individual larval lamprey TL and depth. All statistical tests were conducted at an alpha level of 0.05.

Results

A total of 262 quadrats were visited of which, 208 (79%) were sampled and 54 were not sampled because they were not feasible (e.g., excessive depth, dewatered conditions, located within private marinas). Larval lampreys were detected in all strata except the Multnomah Channel. Two lampreys were collected in March at the northern end of Sauvie Island at the confluence of the Columbia River and Multnomah Channel among abandoned wood pilings. In addition, the lampreys were collected from shallow water (<0.5 m) and were not considered to be in the Multnomah Channel proper. Excluding this site, 63 quadrats were sampled in the Multnomah Channel and no lampreys were detected. Assuming a minimum DP of 0.10, this effort suggests the probability that larval lamprey occupy the strata is < 0.05. As such, we did not consider this stratum to be occupied and omitted it from further analyses of detection probability.

Table 1. Number of quadrats sampled and occupied and species present at different locations in the Lower Willamette River 2009. Species were Pacific lamprey (PCL) and western brook lamprey (WBL).

Month	Stratum	Quadrats sampled	Quadrats where detected	Detection probability	PCL	WBL	Unidentified
March	Willamette River	37	3	0.08	3	0	1
	Multnomah Channel[a]	20	1	0.05	1	1	0
June	Upstream	24	3	0.13	2	2	0
	Superfund	21	1	0.05	0	1	0
	Downstream	21	0	0.00	0	0	0
	Multnomah Channel[a]	22	0	0.00	0	0	0
October	Upstream	0	-	-	-	-	-
	Superfund	0	-	-	-	-	-
	Downstream	21	1	0.05	0	1	0
	Downstream (>13.7 m)	21	2	0.10	0	2	0
	Multnomah Channel[a]	21	0	0.00	0	0	0
Total		208	11	0.08	6	7	1

[a]Quadrats from the Multnomah Channel omitted from analyses of overall detection probability given it appears to be an area that is not occupied.

Overall, larval lampreys were detected in 11 (8%) of the quadrats sampled in the three strata of the Willamette River (Table 1). Pacific lamprey (n=8) and western brook lamprey (n=7) were found in quadrat sampling. In addition, unidentified lampreys were also detected. These excessively small individuals escaped through the mesh collection basket or precluded visual identification. Tissue was removed from collected individuals for genetic confirmation of genus; genetic analyses are ongoing. Given the quadrat-specific DP of 0.08, the estimated level of quadrat sampling effort to be 80% certain that larval lamprey are absent when undetected, was 17 quadrats (Figure 3).

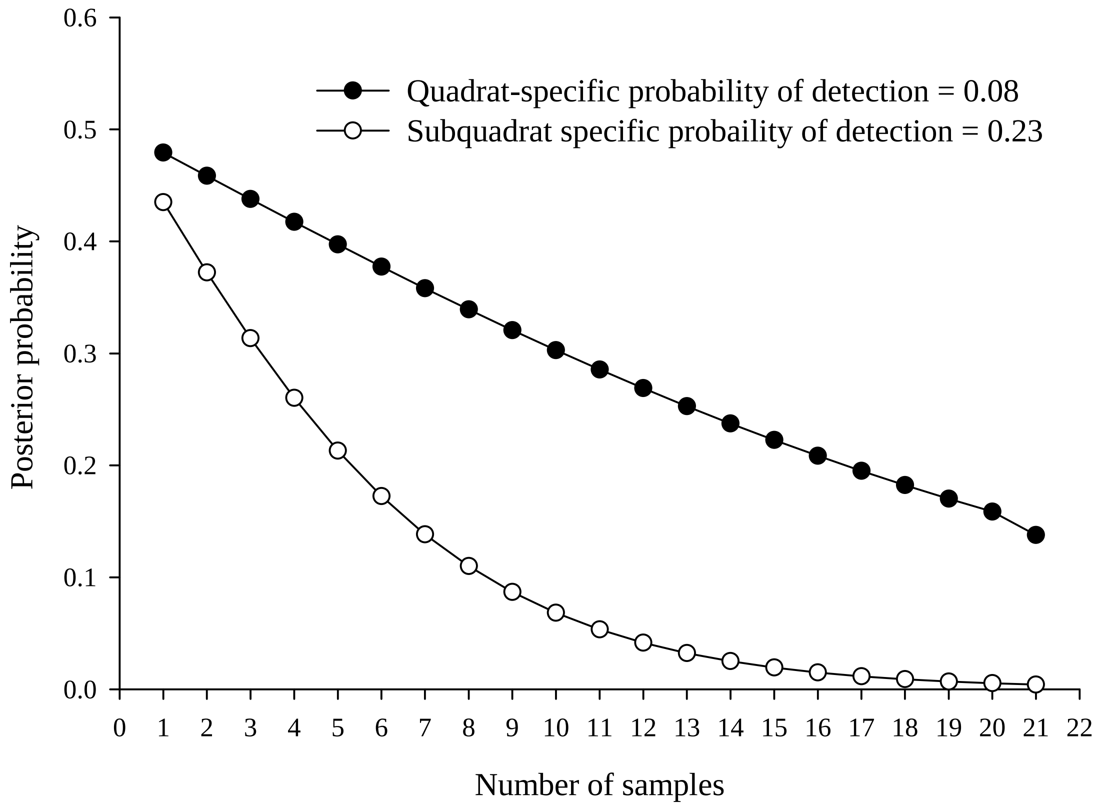

Figure 3. Quadrat- and subquadrat-specific detection probability given varying levels of sampling effort in the Lower Willamette River in 2009.

Subquadrats of 10 occupied quadrats were sampled (Table 2). Subquadrat detection probability ranged from 0 to 0.67. Average detection probability was 0.23 (SE ± 0.7). Pacific lamprey (n=8), western brook lamprey (n=11), and unidentified lamprey (n=7) were again detected (Table 2). The predicted level of subquadrat-specific required sampling effort, with 80% certainty of lamprey absence when undetected, was 6 subquadrats, assuming a 0.23 prior probability of detection (Figure 3).

Table 2. Number of subquadrats (of occupied quadrats) occupied of a possible 9, consequent detection probability, and species present at different locations in the Lower Willamette River 2009. Species were Pacific lamprey (PCL) and western brook lamprey (WBL).

Month	Stratum	Quadrat	Subquadrats where detected	Detection probability	PCL	WBL	Unidentified
March	Willamette River	17[a]	4	0.67	4	8	0
		54	0	0.00	0	0	0
		X1	2	0.22	0	2	0
		X2	2	0.22	1	3	0
July	Upstream	101	3	0.33	2	2	5
	Superfund	110	0	0.00	0	0	0
		105	2	0.22	1	1	0
October	Superfund	X3	4	0.44	0	4	2
		X4	1	0.11	0	1	0
	Downstream	867	1	0.11	0	1	0
Pooled			19	0.22	8	22	7

[a]6 of possible 9 subquadrats were sampled. 3 subquadrats were dewatered.

Quadrats adjacent to three occupied quadrats were also evaluated (Table 3). We were able to sample 17 of 24 possible (71%) adjacent quadrats. Seven adjacent quadrats were not viable. A western brook lamprey was detected in one (6%) adjacent quadrat and mean detection probability in adjacent quadrats was 0.04 (SE ± 0.04). There was no difference in the detection probabilities between randomly selected quadrats and quadrats adjacent to occupied quadrats (chi-square=2.615, df=1).

Table 3. Number of quadrats adjacent to occupied quadrats that were also occupied, detection probability, and species present at 3 sites of the upstream strata of the Lower Willamette River in March 2009. There were 8 possible adjacent quadrats. Species were Pacific lamprey (PCL) and western brook lamprey (WBL).

Quadrat	Adjacent quadrats occupied	Detection probability	PCL	WBL	Unidentified
33	0	0.00	0	0	0
17	0	0.00	0	0	0
54	1	0.13	0	1	0

Transects were sampled across the entire river channel in summer and fall of 2009. Two transects were sampled in the upstream and Superfund strata and one transect was sampled in the downstream strata (Table 4). The mean proportion of transect-specific quadrats occupied was 0.14 (SE ± 0.04) and lampreys were found at depths ranging from 0.6 to 8 m with no apparent trend related to depth. Overall, larval lampreys were detected at all depth strata and the proportion occupied of quadrats ranged from 0.02 to 0.08. No differences were detected in proportion of quadrats occupied among depth strata (chi-square=3.015, df=2; Figure 4).

Overall, 60 larval lampreys were captured and 54 were measured. Larvae that escaped were generally small (likely < 20 mm TL). Species present were Pacific lamprey (n=16) and western brook lamprey (n=32) as well as unidentified individuals (n=6). Of those sampled, larval lamprey TL ranged from 20 to 144 mm (Figure 5) and had a mean overall TL of 69 mm (SE ± 4, Figure 5). Mean TL of Pacific lamprey (n=16) and western brook lamprey (n=32) was 80 (SE ± 4) and 70 mm (SE ± 5), respectively. In addition, an adult western brook lamprey (TL = 144) was collected in the fall in the downstream stratum. No differences were detected in mean TL between species (F=1.99, P= 0.16) or among seasons or strata (F=1.13, P=0.36).

Lamprey were found in water up to 16 m deep and correlation analysis indicated a positive relation between larval lamprey TL and sample depth (*r*=0.30, *P*=0.03; Figure 6).

Table 4. Number of transects of occupied quadrats, detection probability, and species present at 3 sites of the upstream strata of the Lower Willamette River in March 2009. Species were Pacific lamprey (PCL) and western brook lamprey (WBL).

Month	Stratum	Quadrats sampled	Quadrats occupied	Detection probability	Mean depth occupied	PCL	WBL	Unidentified
August	Upstream	8	1	0.13	8 (-)	2	0	0
	Upstream	8	2	0.25	4 (3)	0	0	2
October	Superfund	8	1	0.13	6 (-)	0	0	1
		5	1	0.20	3 (1)	0	1	1
	Downstream	13	0	0.00	-	0	0	0

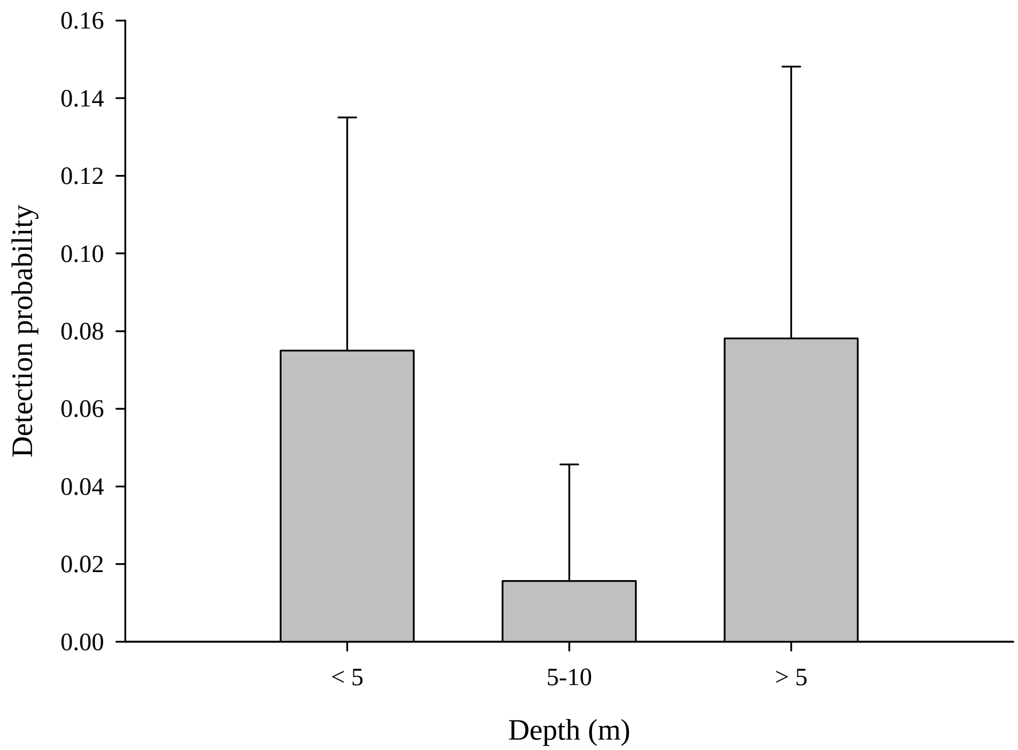

Figure 4. Detection probability (± 95% CI) among different depth categories in the Lower Willamette River in 2009.

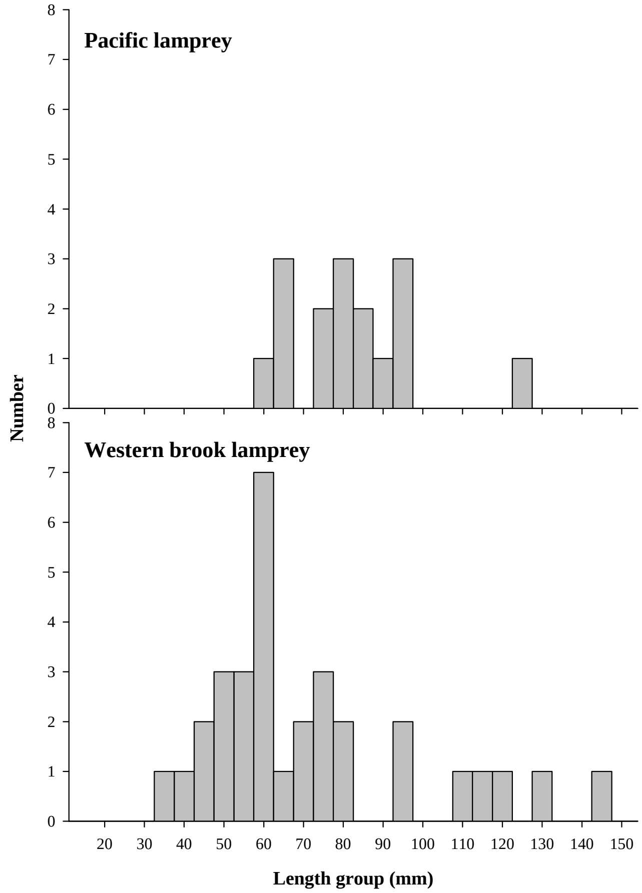

Figure 5. Length-frequency histogram for Pacific lamprey and western brook lamprey ammocoetes collected from the Lower Willamette River in 2009.

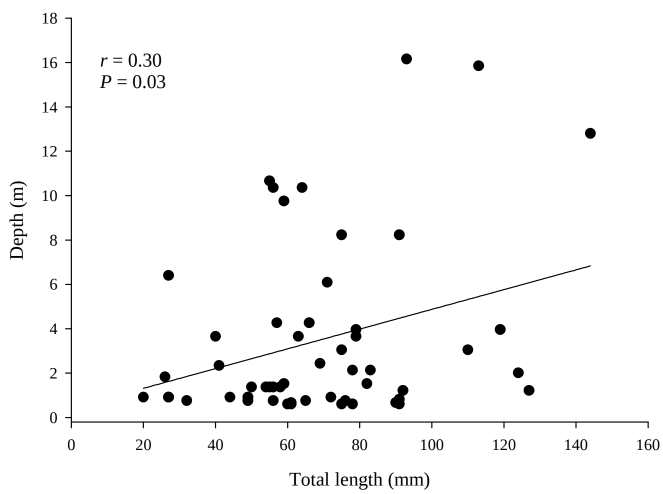

Figure 6. Relation of total length (TL) and depth captured of Pacific lamprey and western brook lamprey ammocoetes collected from the Lower Willamette River in 2009.

Findings

Larval Pacific and western brook lamprey occupy the lower Willamette River. Pacific lampreys and western brook lampreys were present throughout most of the lower Willamette River at a range of depths up to 16 m. Distribution of larval lampreys was widespread and not associated with particular depth. Larval lampreys were notably absent from the Multnomah Channel despite the presence of presumably suitable habitat. We did not find any evidence that lamprey were distributed in a clustered or patchy manner. The GRTS approach provided a statistically robust probabilistic technique for estimating the required sampling effort at either the quadrat or subquadrat scale.

It is unknown if the larval lampreys collected in the Willamette River mainstem migrated (actively or passively), were washed out of tributary habitats, or hatched there. Deepwater spawning of lamprey has not been documented although lentic spawning has been observed (Russell et al. 1987). In addition, larval sea lamprey and American brook lamprey *Lethenteron appendix* have been found in lentic areas of the Great Lakes (Hansen and Hayne 1962) and in deepwater tributaries (Bergstedt and Genovese 1994; Fodale et al. 2002). Larvae found in the mainstem were of a range of sizes, indicating mainstem use by different ages of fish. Although, length-frequency modal separation of age-groups was ambiguous, it is likely that Pacific lamprey larvae ranged from age 2 to at least age 5 and that western brook lamprey larvae ranged from age 1 to age 5 (Meewig and Bayer 2005). The unidentifiable lamprey (i.e., TL<20 mm) were likely age 0. The presence of a broad range in size and presumably age of larvae in the mainstem indicates the potential ability of ammocoetes to disperse considerable distances, spawning near river mouths, or mainstem spawning. It also highlights the importance of Willamette River mainstem areas, including the impaired Superfund reach, as rearing habitat and not just as a migration corridor. Further research directed at ammocoete movement is warranted. Larval lamprey distribution and habitat usage of mainstem areas in the Columbia River, including above and below hydropower projects, is largely unknown. In addition, studies of ammocoete movement would benefit from investigations on migration distance, as well as passage routes and survival rates at Columbia River dams.

Shipping channel and harbor modifications (i.e., dredging) may negatively affect larval lampreys. Although river lamprey *L. ayresii* macrophthalmia have been found in dredge spoils from the Fraser River, British Columbia (Beamish and Youson 1987), it is unknown if lamprey are removed in dredging activities of the Columbia River. Sampling of Columbia River dredge spoils has been recommended (Kostow 2002) and investigation of in situ occurrence of lamprey in pre- and post-dredge areas may be useful. Examination of larval lamprey mainstem river habitat use is ongoing.

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