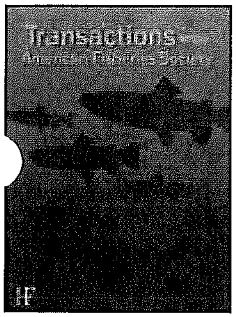

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Transactions of the American Fisheries Society

ISSN: 0002-8487 (Print) 1548-8659 (Online) Journal homepage: http://www.tandfonline.com/loi/utaf20

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Transactions of the American Fisheries Society 141:305–312, 2012
American Fisheries Society 2012
ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2012.662201

NOTE

Occupancy and Detection of Larval Pacific Lampreys and *Lampetra* spp. in a Large River: the Lower Willamette River

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Abstract

Pacific lampreys *Entosphenus tridentatus* (formerly *Lampetra tridentata*) are declining in the Columbia River basin, and the use of large, main-stem river habitats by larvae of this species is unknown. We used a deepwater electrofisher to explore occupancy, detection, and habitat use of larval Pacific lampreys and larval *Lampetra* spp. in the lower Willamette River, Oregon. We used a generalized random tessellation stratified approach to select sampling quadrats (30 × 30 m) in a random, spatially balanced order. Pacific lampreys, *Lampetra* spp., and unidentified lampreys were found in the Willamette River; larvae were detected in all areas except the Multnomah Channel. We calculated reach- and quadrat-specific detection probabilities and the amount of sampling effort required for 80% confidence that larval lampreys were in fact absent when they were not detected. Lampreys were detected in a variety of areas (although relatively low numbers were collected), including shallow, nearshore areas; midchannel areas (depth up to 16 m); and anthropogenically affected areas. Detection probabilities (i.e., in occupied areas) were 0.07 (reach) and 0.23 (quadrat). The sampling effort required for 80% confidence that lampreys were absent when undetected was 20 quadrats (in the lower Willamette River reach) and 6 subquadrats (within a quadrat). Differences in lamprey detection by depth were not observed. A variety of sizes was collected (20–144 mm total length), indicating the likely occurrence of multiple ages of larvae. Our study identifies how the occurrence of larval Pacific lampreys can be quantified with statistical rigor in a large river (i.e., larger than fourth order [1:100,000 scale]). The effect of channel management activities on larval lampreys should be considered in efforts to conserve these important species.

Pacific lampreys *Entosphenus tridentatus* (formerly *Lampetra tridentata*) in the Columbia River basin and other areas have experienced a great decline in abundance, are culturally important to Native American tribes, and are ecologically important within the food web; the decline of this species provides insight into the effect of human actions on ecological function (Close et al. 2002). Information on basic biology, ecology, and population dynamics required for effective conservation and management of Pacific lampreys is lacking.

Pacific lampreys have a complex life history that includes multiple-year larval (ammocoete), migratory juvenile, and adult marine phases (Scott and Crossman 1973). Larvae and juveniles are strongly associated with stream and river sediments. For multiple years after hatching, larvae live burrowed in stream and river sediments, where they consume detritus and organic material by filter feeding (Sutton and Bowen 1994). Larvae metamorphose into juveniles from July to December (McGree et al. 2008), and major migrations are made downstream to the Pacific Ocean in the spring and fall (Beamish and Levings 1991). The sympatric western brook lamprey *Lampetra richardsoni* does not have a major migratory or marine life stage, although adults may locally migrate upstream before spawning (Renaud 1997). For both species, the majority of the information on habitat preferences of larvae comes from Columbia River basin tributary systems (Moser and Close 2003; Torgersen and Close 2004; Stone and Barndt 2005; Stone 2006) and coastal systems (Farlinger and Beamish 1984; Russell et al. 1987; Gunckel et al. 2009).

Larval lampreys are known to occur in sediments of low-gradient streams (smaller than fifth order [1:100,000 scale]; Torgersen and Close 2004), but their use of habitats in relatively deep areas within large rivers is less known. Larval downstream movement, whether passive or active, occurs year-round (Nursall and Buchwald 1972; Gadomski and Barfoot 1998; White and Harvey 2003). Anecdotal observations exist regarding larval lamprey occurrence in large-river habitats, mainly at hydropower facilities (Moursund et al. 2003; CRITFC 2008), where the larvae become impinged on downstream screens, are observed in juvenile bypass facilities, or are observed

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Received March 9, 2011; accepted August 19, 2011
Published online March 12, 2012

EX5059-000002-TRB

during dewatering events. These occurrences are thought to be associated with downstream migration, and specific collections of supposedly migrating ammocoetes have been made in large-river habitats (Beamish and Youson 1987; Beamish and Levings 1991). Ammocoetes of the sea lamprey *Petromyzon marinus* have been documented in deepwater habitats within Great Lakes tributaries, in proximity to river mouths (Hansen and Hayne 1962; Wagner and Stauffer 1962; Lee and Weise 1989; Bergstedt and Genovese 1994; Fodale et al. 2003b), and in the large St. Marys River, which connects Lakes Superior and Huron (Young et al. 1996). References to other species occurring in deepwater or lacustrine habitats are scarce (American brook lamprey *L. appendix*; Hansen and Hayne 1962).

The Willamette River, Oregon, is a large (seventh-order) tributary of the Columbia River that encompasses a drainage area of 29,728 km^2 (Stanford et al. 2005). Pacific lampreys and western brook lampreys inhabit the lower Willamette River, and a large portion of the main stem is unimpounded. Nevertheless, it is unknown whether the lamprey species use the main-stem Willamette and Columbia rivers as rearing areas for larvae in addition to using these systems as migration corridors (i.e., returning adults and outbound juveniles).

Sampling of larvae in deepwater (i.e., >1.0 m) areas is a challenge because of specialized gear requirements and presumed patchy distributions. Bergstedt and Genovese (1994) successfully sampled deepwater areas for larval sea lampreys in tributaries to the Great Lakes by using a modified electrofisher with suction. However, problems associated with the uncertainty in detection probability (*d*) and capture efficiency when sampling for distribution and abundance of organisms can be encountered. In addition, the problem of false absences in estimating occupancy by species that are rare or patchily distributed has been identified (Bayley and Peterson 2001; Peterson and Dunham 2003; MacKenzie et al. 2005).

A goal of this study was to develop a statistically rigorous design to evaluate whether larval lampreys occupy main-stem habitats of a representative large river. In part, rigor can be improved by determining *d*. Knowledge of *d* can be used to (1) determine the number of site visits that are required to achieve a specific certainty of lamprey absence (when not detected) and (2) assist in making inferences about lamprey distribution from collected data. To this end, we sampled the lower Willamette River, which as a whole is known to be occupied by larval lampreys. In general, we documented the presence or absence of larval lampreys throughout the lower Willamette River (i.e., downstream of Willamette Falls, river kilometer [rkm] 42) and determined *d* with deepwater electrofishing. Our specific objectives were to (1) use a deepwater electrofisher to document whether ammocoetes of Pacific lampreys and *Lampetra* spp. occupy various areas of the lower Willamette River, (2) determine the probability of detecting larval lampreys in the lower Willamette River reach (an area known to be occupied), and (3) determine the probability of detecting larval lampreys in an occupied 30- × 30-m quadrat.

METHODS

We estimated occupancy of larval lampreys at several explicit spatial scales within the lower Willamette River by adapting an approach used by Peterson and Dunham (2003) and refined by the U.S. Fish and Wildlife Service (USFWS; USFWS 2008) to evaluate patch occupancy and *d* for bull trout *Salvelinus confluentus*. The approach has several requirements: (1) a site- and gear-specific *d* (assumed or estimated); (2) the probability of presence at a predetermined acceptably low level (given no detection); and (3) random identification of spatially balanced sample sites that allow estimation of presence and the refinement of *d*.

The reach-specific *d* (d_{reach}) was calculated as the proportion of 30- × 30-m sampling quadrats that were occupied by larval lampreys (i.e., larvae were captured) in the lower Willamette River, an area known to be occupied. The posterior probability of reach occupancy given that a larval lamprey was not detected was estimated as

$$P(F \mid C_0) = \frac{P(C_0 \mid F) \cdot P(F)}{[P(C_0 \mid F) \cdot P(F)] + [P(C_0 \mid \sim F) \cdot P(\sim F)]}, \quad (1)$$

where $P(F) =$ the prior probability of larval lamprey presence and $C_0 =$ no detection. Although we knew the lower Willamette River reach was occupied by larval lampreys, a $P(F)$ value of 0.5 (uninformed) was used to inform future study design (i.e., $P[F|C_0]$) for areas where larval lamprey presence was unknown. The term $P(\sim F)$ ($= 1 - P[F]$) is the prior probability of species absence, and $P(C_0|F)$ ($= 1 - d$) is the probability of not detecting a species when it actually occurs (Peterson and Dunham 2003).

The lower Willamette River was sampled during March–October 2009 from Willamette Falls (rkm 42) to the confluence with the Columbia River at Portland, Oregon (Figure 1); the sampled area included the Multnomah Channel (a braid of the Willamette River) and the Portland Harbor Superfund site (rkm 3.2–19.2). A sampling event consisted of using a deepwater electrofisher (Bergstedt and Genovese 1994) in a 30- × 30-m quadrat. This quadrat size was selected based on the previous experience of sea lamprey researchers in the Great Lakes (M. Fodale, USFWS, personal communication), as their sampling approach evolved from systematic to adaptive (Fodale et al. 2003a). The configuration of the deepwater electrofisher is described by Bergstedt and Genovese (1994); sampling was standardized. The bell of the deepwater electrofisher was lowered from a boat to the river bottom. The electrofisher delivered DC at 3 pulses/s with a 10% duty cycle and a 2:2 pulse train (i.e., two pulses on and 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. Suction was produced by directing the flow from a pump through a hydraulic eductor, which prohibited ammocoetes from passing through the pump. Suction began approximately 5 s before electroshocking to purge air

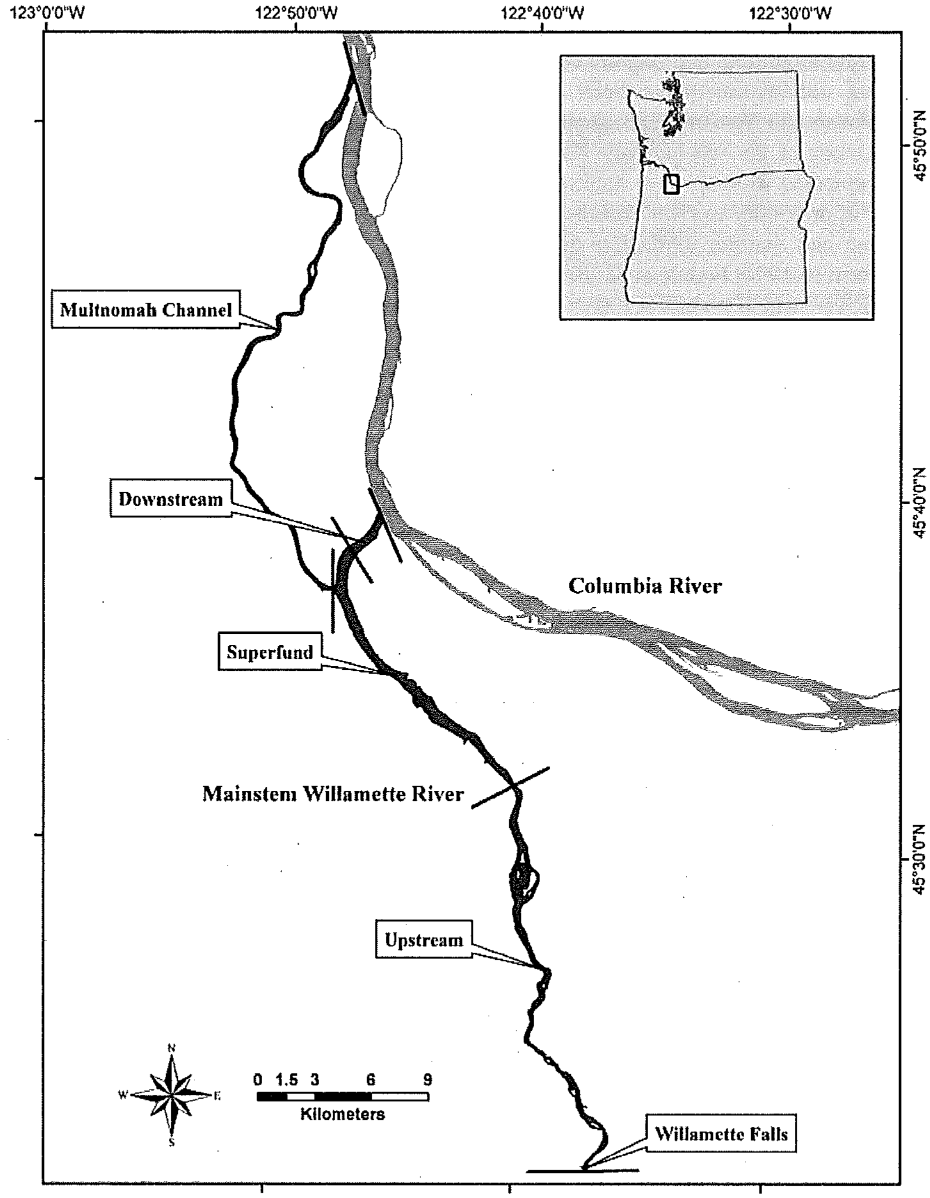

FIGURE 1. The lower Willamette River (Oregon) study area, including strata that were sampled for larval Pacific lampreys and other lamprey species in 2009.

from the suction hose. Shocking was conducted for 60 s, and the suction pump remained on for an additional 60 s after shocking to ensure that the collected ammocoetes passed through the hose and were emptied into a collection basket (27 × 62 × 25 cm; 2-mm wire mesh). The sampling techniques, described in detail by Bergstedt and Genovese (1994), were similar to those used in the Great Lakes region (Fodale et al. 2003b).

We used a generalized random tessellation stratified (GRTS) approach to select sampling quadrats in a random, spatially balanced order (Stevens and Olsen 2004). By using ArcMap version 9.3 (Environmental Systems Research Institute, Redlands, California), we developed a layer of 30- × 30-m quadrats; this layer was overlaid on the entire lower Willamette River (Figure 1). There were 31,306 quadrats from Willamette Falls to the Willamette River mouth. The Universal Transverse Mercator coordinates representing the center point of each quadrat were determined. The GRTS approach was applied to all quadrats to generate a random, spatially balanced sample design for this area of the lower Willamette River. This approach was used to generate an unbiased sample design that would allow *d* to be quantified.

The quadrats were numbered sequentially in order of selection by the GRTS approach, and the lower-numbered quadrats were given the highest priority for sampling. Initially, we set a goal of 56 quadrats (i.e., twice the estimated number required for 80% certainty that lampreys were in fact absent when not detected). To reach this goal, 83 quadrats were ultimately selected and visited; through reconnaissance surveys, sampling was deemed infeasible for 26 (31%) of these quadrats (e.g., they were dewatered, were inaccessible, posed a physical impediment, or had excessive depth for our configuration) and such quadrats were eliminated from the sample. All subsequent quadrats were increased in priority, which resulted in 57 quadrats being sampled. As modifications to the electrofisher configuration allowed sampling at increased depths, additional sites were added to the original design (maintaining the GRTS approach). Ultimately, 247 quadrats were visited and sampling was found to be infeasible for 40 (16%) of these quadrats; thus, a total of 207 quadrats were sampled (depths up to 17 m).

Although we knew that larval lampreys could be found within the lower Willamette River, we did not know whether lampreys occupied certain subreaches of the river. Thus, we longitudinally divided our sampling reach (i.e., the lower Willamette River) into four subreaches to describe subreach-specific occupancy. These subreaches were qualitatively defined as four different habitat areas: (1) an upstream area from Willamette Falls to the Portland Harbor Superfund site (from rkm 42 to rkm 19; hereafter, "upstream subreach"), (2) the Portland Harbor Superfund site (from rkm 19 to rkm 3; "Superfund subreach"), (3) a downstream area (from rkm 3 to the confluence with the Columbia River [rkm 0]; "downstream subreach"), and (4) the Multnomah Channel ("Multnomah Channel subreach"; Figure 1). The overall sampling effort applied to the various subreaches is outlined in Table 1. Depth sampled (among all sites) was further divided into three strata: shallow (depth < 5 m), moderate (depth = 5–10 m), and deep (depth > 10 m). Patterns of detection by depth and by subreach were compared by using the chi-square test for differences in probabilities (Conover 1999).

Given that our occupancy sampling was based on quadrat sampling, we also evaluated how likely we were to detect lampreys in an occupied quadrat (quadrat-specific d [d_{quad}]). A quadrat was considered occupied when at least one larval lamprey was captured in that quadrat. A subsample (n = 10) of occupied quadrats from this study and from concurrent work (Jolley et al. 2010) was selected, and each quadrat was divided into nine 10- × 10-m subquadrats (the minimum cell size that allowed successful boat navigation and holding of position). The Universal Transverse Mercator coordinates were determined at the center of each subquadrat. The subquadrats were sampled, and the d_{quad} was calculated.

Collected lampreys were anesthetized in a solution of tricaine methanesulfonate (MS-222), identified as Pacific lampreys or *Lampetra* spp. according to caudal pigmentation (Goodman et al. 2009), and classified according to developmental stage (i.e., ammocoete, macrophthalmia, or adult). Lampreys were measured (mm total length [TL]), placed in a recovery bucket containing fresh river water, and released after resuming normal swimming behavior. Length frequency histograms were constructed for each lamprey group to describe size structure. All statistical tests were conducted at an α level of 0.05.

RESULTS

Overall, 60 larval lampreys were captured and 54 were measured. They were observed in a variety of habitats, including

TABLE 1. Number of quadrats sampled with deepwater electrofishing, number of quadrats occupied by lampreys, and the lamprey groups (Pacific lamprey [PCL]; *Lampetra* spp. lampreys; and other unidentified lampreys) present at different subreaches in the lower Willamette River, Oregon, during 2009 (d_{reach} = reach-specific detection probability; Superfund = Portland Harbor Superfund site).

Month	Subreach	Quadrats sampled	Quadrats where detected	d_{reach}	PCL	*Lampetra* spp.	Unidentified
Mar	Upstream	20	3	0.15	3	0	1
	Superfund	16	0	0.00	0	0	0
	Downstream	1	0	0.00	0	0	0
	Multnomah Channel[a]	20	0	0.00	0	0	0
Jun	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
Oct	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 excluding Multnomah Channel		145	10	0.07	5	6	1

[a]Six of nine subquadrats in Multnomah Channel were sampled; three subquadrats were dewatered.

shallow depositional areas, main-stem dredged habitats in deep water (>16 m), and the anthropogenically influenced Portland Harbor area. A total of 247 quadrats were visited, of which 207 (84%) were sampled and 40 (16%) were judged infeasible for sampling. Seven quadrats (3%) were deeper than 21 m, our maximum gear capability. Larval lampreys were detected in all subreaches except the Multnomah Channel subreach. Overall, larval lampreys were detected in 10 (7%) of the 145 quadrats sampled in the upstream, Superfund, and downstream subreaches of the Willamette River (i.e., excluding Multnomah Channel; Table 1). Pacific lampreys and *Lampetra* spp. were found in quadrat sampling. In addition, unidentified lampreys were also detected. Unidentified larvae were small individuals that either escaped through the mesh collection basket or could not be visually identified. Given the d_{reach} of 0.07 (i.e., detected in 7% of quadrats sampled) based on deepwater electrofishing in the lower Willamette River, the estimated minimum level of quadrat sampling effort required for 80, 90, and 95% certainty that larval lampreys were absent when undetected was 20, 31, and 41 quadrats, respectively (i.e., $P[F|C_0] = 0.20$, 0.10, and 0.05, respectively; Figure 2). Sixty-two quadrats were sampled in the Multnomah Channel subreach, and no lampreys were detected. If the Multnomah Channel was occupied and the d_{reach} was 0.07 (as observed in the other subreaches), our effort suggests that the probability of larval lampreys occupying the Multnomah Channel subreach would be less than 0.01. In addition, with this amount of effort, if d_{reach} was at least 0.05, then the probability that larval lampreys occupied the Multnomah Channel subreach is less than 0.05. As such, we considered this subreach to be unoccupied and we excluded it from further analyses of *d*.

Eighty-seven subquadrats (10 × 10 m) of 10 occupied quadrats (30 × 30 m) were sampled (three were dewatered; Table 2). Subquadrat *d* ranged from 0.00 to 0.67. Mean d_{quad} was 0.23 (SE = 0.07). Pacific lampreys ($n = 8$), *Lampetra* spp. ($n = 11$), and unidentified lampreys ($n = 7$) were again detected

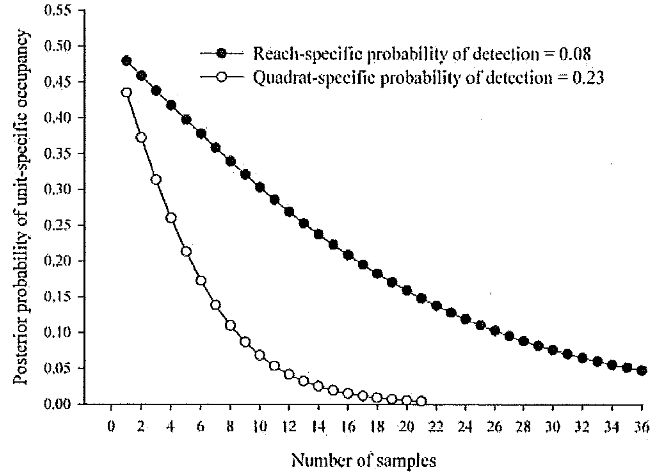

FIGURE 2. Estimated probability that lamprey larvae are actually present when not detected by sampling with deepwater electrofishing; reach- and quadrat-specific detection probabilities (i.e., 0.07 and 0.23, respectively) given varying levels of sampling effort in the lower Willamette River (2009) are presented.

(Table 2). Given the d_{quad} of 0.23 in the lower Willamette River, the estimated minimum level of subquadrat sampling effort (i.e., with deepwater electrofishing) required for 80% and 90% certainty that larval lampreys were actually absent from a quadrat when undetected was 6 and 9 subquadrats, respectively ($P[F|C_0] = 0.20$ and 0.10, respectively; Figure 2). Given the d_{quad} of 0.23, it was not possible to be 95% certain of lamprey absence from a quadrat when undetected because the estimated level of sampling effort required (i.e., 12 subquadrats) was greater than the number of subquadrats within a quadrat (i.e., 9 subquadrats).

Lampreys occupied all depth strata, but *d* did not significantly differ among shallow (i.e., $d = 0.075$), moderate ($d = 0.016$), and deep ($d = 0.078$) strata (chi-square = 3.016, df = 2,

TABLE 2. Number of subquadrats where lamprey larvae were detected by deepwater electrofishing, subquadrat-specific detection probability (d_{quad}), and the lamprey groups (Pacific lamprey [PCL]; *Lampetra* spp. lampreys; and other unidentified lampreys) present at different locations in the lower Willamette River during 2009 (Superfund = Portland Harbor Superfund site). Nine component subquadrats were sampled from each of 10 quadrats that were known to be occupied.

Month	Subreach	Subquadrats (of occupied quadrat) where detected	d_{quad}	PCL	*Lampetra* spp.	Unidentified
Mar	Upstream	4	0.67	4	8	0
		0	0.00	0	0	0
		2	0.22	0	2	0
		2	0.22	1	3	0
Jul	Upstream	3	0.33	2	2	5
	Superfund	0	0.00	0	0	0
		2	0.22	1	1	0
Oct	Superfund	4	0.44	0	4	2
		1	0.11	0	1	0
	Downstream	1	0.11	0	1	0
Pooled		19	0.22	8	22	7

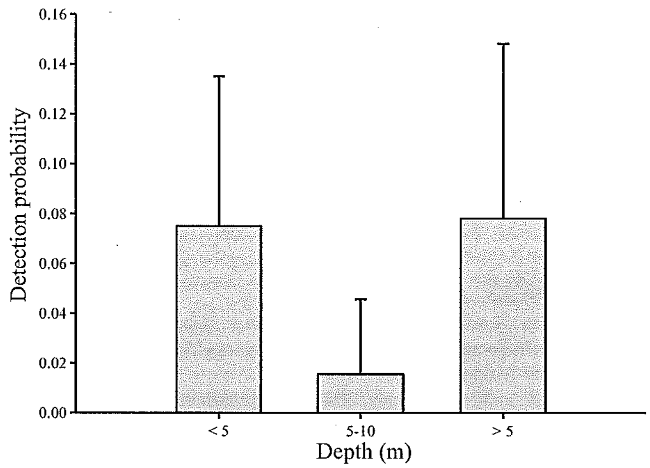

FIGURE 3. Probability of larval lamprey detection (+95% confidence interval) among different depth categories sampled in the lower Willamette River, 2009. Detection probability was similar ($P > 0.05$) among depth categories.

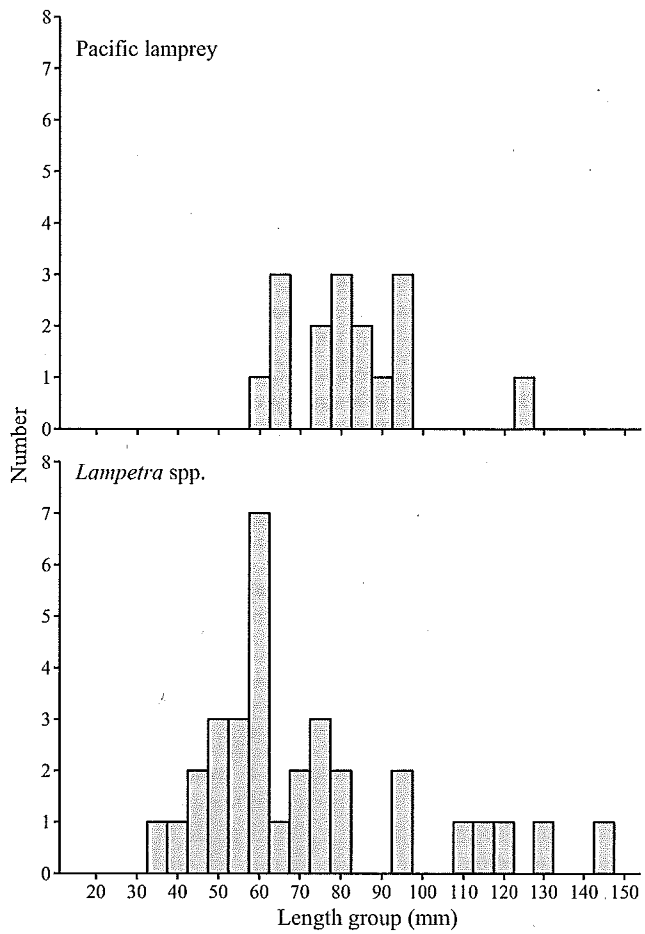

FIGURE 4. Length frequency histogram (total length, mm) describing Pacific lamprey and *Lampetra* spp. ammocoetes collected by deepwater electrofishing in the lower Willamette River, 2009.

$P > 0.05$; Figure 3). Detection probabilities were significantly different among subreaches (chi-square = 6.373, df = 2, $P < 0.05$), although pairwise comparisons were not significant after Bonferroni correction ($P > 0.017$). The upstream subreach had the highest d (0.136), followed by the downstream ($d = 0.047$) and Superfund ($d = 0.027$) subreaches. Of 60 larval lampreys that were captured, 54 were measured. Larvae that escaped were generally small (<20 mm TL). Pacific lampreys ($n = 16$), *Lampetra* spp. ($n = 32$), and unidentified lampreys ($n = 6$) were present. Among the larval lampreys sampled, TL ranged from 20 to 144 mm (Figure 4) and the overall mean TL was 69 mm (SE = 4; Figure 4). Mean TL was 80 (SE = 4) for Pacific lampreys ($n = 16$) and 70 mm (SE = 5) for *Lampetra* spp. ($n = 32$). In addition, a single adult western brook lamprey (144 mm TL) was collected in the downstream subreach during October.

DISCUSSION

Larval Pacific lampreys and *Lampetra* spp. occupied the lower Willamette River. The GRTS approach provided a statistically robust probabilistic technique for estimating the required sampling effort at either the quadrat or subquadrat scale with a reasonable level of confidence in the conclusion. Considering the spatial scale of the study reach (>28 km^2) and the persistent occurrence of larvae (even at presumably low densities), larval use of main-stem habitats, which have been overlooked, may be important to the conservation of the Pacific lamprey. These quantitative techniques form a foundation for comparisons of lamprey occupancy and detection in other main-stem areas; the GRTS approach provides the venue for statistical inference. The increased detection within known-occupied quadrats lends further support to the idea that observed patterns of occupancy correspond to more-densely occupied areas, assuming that density is related to detection. In addition, future research that replicates d_{quad} could incorporate covariates representing a range of biotic and abiotic conditions (e.g., substrate, velocity, and temperature) that would explain variability in detection among quadrats, thus allowing those estimates to be refined. We did not explicitly measure habitat variables in our study, and future iterations of this work will benefit from knowledge of fine-scale habitat information; the GRTS approach can be modified to reflect this information. Finally, Bergstedt and Genovese (1994) estimated 75% capture efficiency (95% confidence interval = 63–93%) of the deepwater electrofisher. Determination of capture efficiency under the conditions examined in the present study will be necessary to provide adjustments to catch data and allow estimation of larval densities.

Although overall detection was low, larval Pacific lampreys and *Lampetra* spp. were present in reaches of the lower Willamette River at depths of up to 16 m. Larval lampreys were found in a variety of areas: shallow depositional areas, main-channel dredged areas, and the industrially influenced Portland Harbor. Larval lampreys were notably absent from the Multnomah Channel. We qualitatively observed hard, clay-type bottom substrates in this area, which may be unsuitable for ammocoetes as was reported by Ojutkangas et al. (1995) for European river lampreys *L. fluviatilis* in Finland. These results highlight the importance of main-stem areas as potential rearing habitat in addition to a migratory corridor.

The origin of the larval lampreys collected in the lower Willamette River is unknown. Frequent redistribution of larval sea lampreys has been suggested (Quintella et al. 2005; Derosier et al. 2007; Jones 2007), but lamprey movements remain poorly studied. In addition, larval sea lampreys and American brook lampreys have been found in lentic areas of the Great Lakes (Hansen and Hayne 1962) and in deepwater tributaries (i.e., depth > 1 m; Bergstedt and Genovese 1994; Fodale et al. 2003b). The presence of a variety of larval sizes in different areas of the Willamette River main stem suggests (1) the potential ability of larvae to disperse considerable distances, (2) spawning near river mouths, or (3) main-stem spawning. Further understanding of larval movement and survival in these habitats is needed.

We documented important biological attributes of the lamprey larvae in our study namely, the occurrence of multiple species of varied sizes. The unidentifiable lampreys (i.e., TL < 20 mm) were probably age 0 (Meeuwig and Bayer 2005). The frequent occurrence of *Lampetra* spp. was an unexpected finding. These lampreys were assumed to be western brook lampreys, and limited information is available regarding the habitat preferences of this species, although it is known to occur in smaller tributaries (e.g., third order or smaller) of the lower Columbia River (Stone 2006). We applied the identification methods outlined by Goodman et al. (2009), but these methods separate lampreys to genus only (Docker et al. 1999); it is possible that river lampreys *L. ayresii* also inhabit the lower Willamette River. The high prevalence of *Lampetra* spp. warrants the use of other available identification techniques for larval river lampreys (Richards et al. 1982; Beamish and Youson 1987).

Main-stem habitats of the Willamette River and adjacent Columbia River should be considered when managing lamprey species. Habitat perturbations, such as dredging, exposure to toxins, and hydrosystem operation, may affect larval lampreys (Beamish and Youson 1987; Ojutkangas et al. 1995; Andersen et al. 2010). We used a technique (deepwater electrofisher and GRTS sampling approach) and platform (*d*) for quantifying larval lamprey occupancy that provide a baseline for comparison with future studies. Further understanding of the distribution and ecology of these understudied species is warranted.

ACKNOWLEDGMENTS

This project was funded by USFWS Region 1. B. Davis, M. Satter, staff of the Columbia River Fisheries Program Office, and staff of the City of Portland provided field assistance. M. Fodale and D. Kochanski (USFWS) provided the deepwater electrofisher and technical guidance, and J. Buck (USFWS) facilitated this exchange. K. Steinke (USFWS) fabricated the electrofisher controls and provided valuable technical expertise. H. Schaller, P. Wilson, and M. Hudson (USFWS) provided technical guidance. The findings and conclusions in this manuscript are those of the authors and do not necessarily represent those of the USFWS. Reference to trade names does not imply endorsement by the U.S. Government.

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