Migration Behaviors of Adult Pacific Lamprey (*Entosphenus tridentatus*) at Large Hydropower Dams on the Columbia River

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AUTHORIZATION TO SUBMIT THESIS

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Abstract

While a number of anthropogenic threats have been attributed to the population declines of Pacific lamprey (*Entosphenus tridentatus*), the poor passage of this species at hydropower dams on the Columbia River (~50% success rate) has been identified as a leading causative agent. This thesis sought to examine the potential mechanisms at hydropower dams responsible for limiting passage by exploring Pacific lamprey behavior at three different scales. The first scale was dam-wide, which sought to understand differences in behavior between Pacific lamprey and Chinook salmon (*Oncorhynchus tshawytscha*), a species which has high passage success at these dams (>95%). The second scale was a fishway-scale, where an acoustic camera was placed within fishways to identify the swimming behavior and potential mechanisms inducing passage failures. The final scale was a site-specific study, which attempted to identify the hydraulic and structural elements at a known passage bottleneck responsible for liming Pacific lamprey passage.

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Chapter 1

Thesis Introduction

Pacific lamprey (*Enthosphenus tridentatus*) are a primitive, anadromous fish that are native to the Pacific Coast of North America. The species exhibits an unusual life history with an extended freshwater larval stage (3-7 years) where it burrows into stream sediments as a filter/deposit feeder (Torgersen and Close 2004). The species then metamorphoses and emigrates into the marine environment as a parasitic adult for 2-3 years. Like species of Pacific salmon (*Oncorhynchus spp.*), they return to freshwater streams where they spawn and die. Pacific lamprey have significant cultural value to many of the Native American tribes in the Columbia River basin who harvested them historically as a food source and for ceremonial purposes. Ecologically valuable, adults serve as 1) a source of marine derived nutrients to nutrient poor streams, 2) an important prey species and predation buffer for economically valuable salmonid species, and 3) the larval stages are likely important components in the nutrient retention capabilities of stream ecosystems (Moser and Close 2003; Clemens et al. 2010). As awareness of the species' importance has increased in both the scientific and non-scientific communities, Pacific lamprey have slowly earned recognition in western culture as being an integral part of the Pacific Northwest.

The number of Pacific lamprey returning to the Columbia River basin has declined considerably, with some of the lowest counts of returning adults occurring within the past decade (Columbia Basin Research 2014). While several factors have been proposed to explain these declines such as habitat degradation and ocean conditions (Moser and Close 2003, Murauskas et al. 2013), anthropogenic barriers appear to be one of the leading causative agents. The Columbia River is one of the most heavily regulated river systems in the world, where fish returning to the Upper-Columbia River must overcome up to nine hydroelectric dams and fish returning to the Upper Snake River must overcome up to eight of these barriers (Keefer et al. 2004). Telemetry studies have revealed that the four dams on the lower mainstem of the Columbia River have low passage efficiency (~50%) for upstream migrating adult Pacific lamprey (Moser et al. 2002; Keefer et al. 2013a). As a result, only a miniscule number of fish (~5% of the total run) pass these dams to distribute into the spawning reaches of the Upper-Columbia and Snake Rivers (Keefer et al. 2013b).

The general consensus for explaining the poor passage of Pacific lamprey has been that the species is ill-adapted for negotiating the fishways present at these dams. Historically, to prevent these hydroelectric projects from obstructing the migrations of Pacific salmon to their natal spawning grounds, volitional fish passage systems were installed at many projects based on the optimal passage criteria designated for Pacific salmon. However, the design and criteria implemented for passage of Pacific salmon is likely to be very different than the optimal passage criteria of Pacific lamprey given differences in morphology, behavior, and swimming capabilities of these two taxa (Mesa et al. 2003; Keefer et al. 2010, 2011).

Many of these technical fishways were comprised of similar features, beginning with entrances in the tailrace that had relatively high flows in order to attract fish into the fishway. Migrants would then be funneled into collection channels and eventually into a transition area. This section of the fishway transitioned fish from the previously low-elevation gradient to the steeper gradient experienced in the fish ladder, which was also composed primarily of pool-and-weir type fishways. Many of the larger projects also included a vertical-slot weir section at the top of the ladder that migrants had to overcome before reaching the fishway exit (Clay 1995). The objective of the thesis described herein was to advance our knowledge of the passage behaviors of Pacific lamprey when negotiating these fishways and identify the potential mechanisms responsible for explaining why this species experiences poor passage.

This thesis is composed of three major chapters, which evaluated Pacific lamprey passage behaviors at three different spatial scales. Chapter two involved the use of network theory to describe the movements of Pacific lamprey and Chinook salmon (*Oncorhynchus tshawytscha*) at the 'dam-wide' scale for one hydroelectric project on the Columbia River. The chapter sought to test hypotheses regarding potential motivation differences in passage between Pacific lamprey and Chinook salmon based upon patterns of spatial distribution. Chapter three involved the use of an acoustic camera to directly observe Pacific lamprey swimming behavior and estimate rates of swimming activity at several locations within fishway environments. We used these observations of swimming behavior in order test hypotheses about the potential mechanisms eliciting poor passage of Pacific lamprey at these locations. Similarly, chapter four focused on evaluating Pacific lamprey swimming performance under experimental conditions and how swimming performance may explain the poor passage observed at one particular fishway location. Finally, chapter five concludes with a brief summary and holistic interpretation on the contribution of this work to the science and management of Pacific lamprey within the Columbia River.

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Chapter 2

Network analyses reveal intra- and interspecific differences in migration behavior during passage of a complex barrier

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Abstract

While traditional movement models have focused on understanding how ecological processes drive patterns of individual movement, very few studies have explored how individual variation in movement translates into behavioral differences at the intra- or interspecific levels. Network theory provides a novel perspective on interpreting movement data for individual animals by evaluating the relationships between the detection locations (nodes) and animal movements (links) in spatially complex environments. The purpose of this study was to use network analyses to test hypotheses regarding differences in the migration behavior and success rates of two anadromous fish species, Pacific lamprey (*Entosphenus tridentatus*) and Chinook salmon (*Oncorhynchus tshawytscha*), when ascending a large multi-fishway hydroelectric project (Bonneville Dam, WA, USA). Network analyses revealed greater variation in network metrics for Pacific lamprey compared to Chinook salmon, which was largely due to differences in passage success between the two species (46% vs. 98%, respectively). Salmon that passed the dam had networks consisting of more direct passage routes with fewer overall movements compared with lamprey that passed Bonneville. Lamprey that did not pass the dam exhibited a wide range of movement patterns, from approaching only one fishway entrance to visiting every fishway site. These differences in movement are consistent with expectations based on motivation in a philopatric species (Chinook salmon) passing facilities designed to salmon

passage criteria versus a nonphilopatric species (Pacific lamprey) passing fishways largely designed without consideration for lamprey swimming capacities. Our results provide new evidence that the poor passage observed for Pacific lamprey may result from individual differences associated with motivation-based processes. Our study further highlights the diversity of animal movement questions that network analyses can address in basic (i.e., inferring population level processes from individual variation) and applied ecology (i.e., assessing animal movement and behavior in altered migration corridors or landscapes).

Introduction

Individual variation in behavior has important biological consequences, contributing to processes such as life history diversification and speciation (Sih et al. 2004). Migratory species, as an example, often exhibit wide variability in migration behaviors with regards to when to migrate, how far to migrate, and even whether to migrate (Dingle and Drake 2007; Dingle 2014). Such variability can be influenced by any set of intrinsic (e.g., conditional state, genetic, sex) or extrinsic (i.e., environmental) factors (Brodersen et al. 2008; Morales et al. 2010; Papastamatiou et al. 2013), acting through the expression of behavioral plasticity, phenotypic plasticity, or genotypic differences (Sih et al. 2004; Moser et al. 2013). Hence, understanding the causes and consequences of individual variability can often be challenging, particularly for migratory species that move at large spatiotemporal scales or locally through spatially complex habitats (Alerstam et al. 2003; Chapman et al. 2011).

Recent advancements in telemetry have provided improved capabilities for tracking and quantifying animal movements at both large and fine scales, which has been critical for elucidating patterns of movement (Cooke et al. 2004). The advancements have also spurred development of new conceptual approaches for analyzing movement (e.g., Nathan et al.

2008). Telemetry datasets can often be enormous in size and difficult to analyze with traditional statistical techniques, which leaves potentially valuable biological information inaccessible (Cagnacci et al. 2010, Jacoby et al. 2012). The advancement of new mathematical approaches and statistical models has provided new ways for ecologists to interpret movement data more comprehensively (Schick et al. 2008; Morales et al. 2010; Finn et al. 2014). Importantly, the inferences from many approaches are linked to the spatial and temporal structure of the monitoring network, often resulting in context-dependent outcomes. As a result, ecologists are simultaneously gaining a greater ability to understand the evolutionary and ecological consequences of movement, while also continuing to refine analysis approaches toward greater generality.

Within the past decade, ecologists have begun using network theory for understanding the connectivity of ecological systems. Network theory is a component of graph theory, which is a body of mathematics and computer science that characterizes the connectivity of systems based on interactions between different components (Wey et al. 2008; Eros et al. 2012). Network theory has been applied to various ecological questions such as understanding metapopulation structure (Fortuna et al. 2006) and how landscape connectivity influences habitat conservation (Galpern et al. 2011). Network methods have recently been used to describe and summarize movements at the individual level (Jacoby et al. 2012, Finn et al. 2014). To our knowledge, the methods have not been used to summarize movements at the population level or for direct hypothesis testing, both of which are crucial for identifying the mechanisms that explain larger patterns of movement and migration (Cagnacci et al. 2012). Here, we used network theory to 1) test for differences in behavior of two species of migratory fishes and 2) test for associations among individual traits within each species at a large and complex hydroelectric dam.

Pacific lamprey (*Entosphenus tridentatus*) and Chinook salmon (*Oncorhynchus tshawytscha*) share parallel semelparous and obligate anadromous life histories (Quinn 2005; Moser and Close 2003). The two taxa also exhibit important differences in migration behavior that make them good models for a comparative approach with network methods. Whereas Pacific salmonids generally exhibit a philopatric (i.e., homing) migration strategy (Dittman and Quinn 1996; Keefer and Caudill 2014), evidence suggests Pacific lamprey do not home to natal streams (Spice et al. 2012) and that migration distance is influenced by body size and genetics (Keefer et al. 2009; Hess et al. 2014). When passing large hydropower dams on the Columbia River, Chinook salmon have much faster passage times compared with Pacific lamprey (<2 days for salmon, 4-6 days for lamprey; Moser et al. 2002a, Keefer et al. 2004) and passage success is nearly twice as high for Chinook compared with Pacific lamprey (95% vs. 50%, respectively; Moser et al. 2002a, 2002b; Keefer et al. 2013). Understanding this difference in passage has been important not only for understanding the biological differences between these two species, but also to help improve Pacific lamprey passage in the Columbia River basin (Moser and Close 2003).

We used network analyses to test two competing biological hypotheses about the potential processes causing limited passage in Pacific lamprey. Both hypotheses make explicit, yet contrasting predictions regarding how network size and connectivity pertains to movement and behavior (Table 2.1). The first hypothesis, the 'performance filter' hypothesis, assumes that all individuals are equally motivated to pass the dam (e.g., individuals homing to upstream locations). We predict that metrics of network size and connectivity will be higher for fish that do not pass the dam than those that do because poor performing fish are frequently forced back out of the dam due to passage challenges and are forced to test alternative routes of passage (Moser et al. 2002a, Keefer et al. 2013a). Conversely, network size and connectivity will be lower for fish passing the dam because higher performing fish will be able to pass challenging locations within the fishways (Keefer et al. 2013, 2014), resulting in less exploration of alternative routes and less 'milling' behavior than those that do not pass.

The second hypothesis we tested was a 'motivation filter' hypothesis, which assumes similar swimming capacity (e.g., burst swim speed) among all individuals such that the probability of passing a location on the first attempt is similar, but that individuals vary in some motivational threshold and 'giving up time'. The motivation filter hypothesis predicts that network size and connectivity should be higher for fish that passed the dam compared with fish that did not pass the dam, since individuals with higher motivation to pass would test more routes, explore more locations, and be more likely therefore to find a suitable route, whereas those with low motivation would quickly give up and move downstream out of the network (Table 2.1).

Methods

Study site – We monitored the movements of Pacific lamprey and Chinook salmon at Bonneville Dam (45.6°N, 121.9°W), which borders the states of Oregon and Washington on the lower Columbia River in the Pacific Northwest (Figure 2.1A). The dam is the first hydropower dam on the Columbia River (river kilometer [rkm] 235 from the Pacific Ocean) encountered by anadromous fishes during upstream spawning migrations. The dam has two powerhouses and one spillway, which results in three channels separated by two islands (inset Figure 2.1A). Fishways at Bonneville Dam are unusually complex because there are multiple and branched routes by which fish may ascend the dam. The dam has a total of eight entrances and four primary routes, which lead fish to two different exits at the top of the dam. Notably, all three main fishway routes (PH1, Spillway, and PH2) have similar zones defined by gradient and structural features: a lower fishway section with minimal gradient (entrance-collection channel-transition area), a steeper (1:10 slope) gradient fish ladder with pool-and-weir fishway designs, and an upper fishway section with counting stations, serpentine weirs, and fishway exits.

As a brief overview, there is a south (A) and north entrance (C) at Powerhouse 1 (PH1), which is located adjacent to the Oregon shore (Figure 2.1C). Collection channels inside each entrance (B, D) lead fish along the “A branch” of the Bradford Island fish ladder and eventually to the Oregon shore fishway exit (I). There are two entrances next to the spillway (SP), which is the center channel at the dam (inset Figure 2.1A). One entrance is at Bradford Island to the south (J) that leads to the “B branch” of the fish ladder and up into the Bradford Island ladder junction (F). The second entrance is to the north at the Cascade Islands fishway (M) (Figure 2.1D), which leads fish through Powerhouse 2 (PH2) via the upstream migrant channel (P) and intersects with the PH2 fishway (BB), which leads to the Washington shore fishway exit (EE). The Washington-shore fishway has two sets of paired entrances at PH2 with two entrances to the north (V, X) and two to the south (R, T) (Figure 2.1B). Collection channels from each of the four entrances funnel all fish into a transition area (Z) that leads fish up through a pool-and-weir fish ladder (AA) to the Washington shore fishway exit (EE). Table 2.2 provides a descriptive list of the monitored locations in Figure 2.1.

Fish collection and tagging –Both Pacific lamprey (Johnson et al. 2012; Moser et al. 2002a) and Chinook salmon (Keefer et al. 2004a, 2004b) tagging protocols have been described previously. Briefly, Pacific lamprey were collected at night using traps situated adjacent to fishway weirs in the Adult Fish Facility (AFF) on the Washington Shore ladder (Figure 2.1B). Lamprey were anesthetized using clove oil or eugenol (concentration mix of 4-5 mL solution/50 L water) and were measured for body length, weight, and girth. A small incision was made on the ventral side of the body nearly parallel to the first dorsal fin. A radio transmitter (8 mm by 18 mm and 2.1 g, Lotek Wireless, Newmarket, Ontario) was inserted into the body cavity and incisions were closed with sutures. Lamprey were allowed to recover in holding tanks for 8-12 hours before being released at dusk approximately 3 km downstream of Bonneville Dam at two sites, one on the Washington and one on the Oregon shore.

Chinook salmon were diverted from the Washington-shore ladder into the AFF where individual fish could be selected for tagging. Sampled Chinook were diverted into a large holding tank where they were anesthetized using clove oil or eugenol (concentration mix of 22 mL solution/L water). Salmon were measured for total length, weight, sex, and identified as either natural or hatchery origin based on the presence of fin clips. Salmon were gastrically implanted with a radio transmitter (8.3 cm by 1.6 cm and 29 g, Lotek Wireless, Newmarket, Ontario) and placed within a large, 2,275-L oxygenated transport tank for recovery. All salmon were released approximately 7 km downstream of Bonneville Dam at two sites, one on the Washington and one on the Oregon shore. All collection, handling, and tagging procedures were approved by the Animal Care and Use Committee at the University of Idaho and under the authority of appropriate scientific collection permits.

Monitoring and telemetry data - Movements at Bonneville Dam were monitored using an extensive array of radio receivers composed of both aerial and underwater antennas. Radio transmitters emitted digital signals every ~5 seconds that were unique to individual fish, which were identified by receivers at each site. All sites in Figure 2.1 were monitored and multiple receivers along fishway segments produced detection efficiencies near 95-100% for each site (Keefer et al. 2006, 2013b). However, any missed detections that could be reasonably inferred were subsequently entered (e.g., movements along linear pathways). Detection histories were analyzed for individual fish and time-stamped activity codes were assigned to specific events at each site (e.g., first entrance approach, first entrance, successful passage, etc.). The movements between sites were then defined as either upstream (entrance towards exit), downstream (exit towards entrance), or between entrances (entrance to entrance). Final outcomes of all passage events were classified as either ‘Pass’ or ‘Did not pass.’

We calculated the passage times, hereafter referred to as ‘time in network,’ of individual fish to compare the spatial component of movement at Bonneville Dam with a temporal element of behavior. We also wanted to statistically control for variation among time spent in the network since we expected network connectivity to increase with increasing time in the network. ‘Time in network’ for fish that passed the dam was calculated from the time of first approach at an entrance until the time of last detection at an exit. ‘Time in network’ for fish that did not pass the dam was conservatively calculated from the time of first approach until the time of last known detection at a fishway site. Any fish that passed and were subsequently redetected downstream of Bonneville Dam were classified as “fallbacks” (Naughton et al. 2006) and only movements from their first upstream passage event were included.

Network analyses – We randomly selected 255 Pacific lamprey and 240 Chinook salmon from radio-telemetry studies that were conducted at Bonneville Dam in 2010 for these analyses. The system of fishways at Bonneville Dam was treated as the network of interest (Figure 2.1). Networks are composed of nodes and edges, whereby nodes may represent a location, population, or individual, while an edge represents an interaction between nodes (Wey et al. 2008; Jacoby et al. 2012). In this study, monitored fishway locations were defined as nodes and fish movements between nodes were defined as edges, which were classified as either upstream, downstream, or between entrances. All networks were composed of either 30 nodes for Chinook salmon or 33 nodes for Pacific lamprey; one node for the release location downstream of the dam, 28 (salmon) or 31 (lamprey) nodes for all the fishway locations at Bonneville Dam in Figure 2.1, and one node at an upstream or downstream location indicating whether fish passed or did not pass the dam. The network was increasingly more constrained moving upstream through the fishway, whereby any edge was possible among tailrace sites and only a single upstream and single downstream edge was possible within the relatively linear middle and upper fishways.

The detection histories of fish were imported into and analyzed using Cytoscape (version 2.8.3). Cytoscape is an open source network platform that was originally designed for molecular and bioinformatics use, but has a broad application for all network studies (Shannon et al. 2003, Smoot et al. 2011). Networks were constructed and reviewed for all salmon and lamprey individually. Once Cytoscape constructed a visual map from the imported telemetry data, the ‘network analysis’ plugin (Shannon et al. 2003) was used to calculate a number of quantitative metrics that described patterns of connectivity throughout the network. We selected several maps of representative, individual lamprey to: 1) demonstrate how network visualization captured the spatial patterns of movements that fish exhibited at Bonneville Dam and 2) compare how network metrics differed between individuals.

We selected six metrics to characterize the movements of individual fish across fishway locations. Each metric described a particular element of network size or connectivity that has bearing on the hypotheses of interest. The first network metric, **diameter**, was the greatest distance (i.e., path length with the greatest number of edges) between any two nodes in the network (Wey et al 2008; Jacoby et al. 2012). Diameter was an estimate of the network size and described the route length of fish when ascending the dam. Under the motivation filter hypothesis, we predicted network diameter to be higher for fish that passed the dam. The second metric was the **clustering coefficient**, which was defined as the number of edges shared by one node and all of its neighbors divided by the maximum number of edges possible between that node and its neighbors (Wey et al. 2008). More simply put, it was calculated as the number of triangles that occur among nodes divided by the total number of triangles possible within the network. Given the relatively linear nature of fishways in the middle and upper sections, where few triangles occur, the clustering coefficient provided a good indicator for the degree of movements that individuals exhibit across fishway entrances. However, 70% of lamprey and 83% of salmon had a clustering coefficient of zero (i.e., paths did not form triangles among nodes). Thus, we limited consideration of the clustering coefficient to descriptive summaries.

The third and fourth metrics were highly correlated and described similar elements of network connectivity. The **average number of neighbors** was defined as the number of edges for each node in the network divided by the total number of nodes in the Bonneville network (Smoot et al. 2011). Average number of neighbors described the average connectivity of one node with all other surrounding nodes and served as an indicator of the amount of movements that an individual exhibited across sites. Similarly, **network density** was the number of edges in the network divided by the total number of edges possible in the network (Wey et al. 2008), which also served as an indicator for the extent of travel across the network. High connectivity associated with these metrics would mostly be attributed to the connectivity across fishway entrances given the linear nature inside fishways, similar to the clustering coefficient. We predicted both of these metrics to be higher for fish that passed the dam and lower for fish that did not pass the dam under the motivation filter hypothesis.

The fifth metric, the number of **multi-edge node pairs**, was the number of node pairs in the network that shared more than one edge (Smoot et al. 2011). The number of multi-edge nodes described how linear fish movements were (i.e., A → B→ C versus A ↔ B ↔ C) and how frequently fish moved between the same sites (i.e., moving upstream to a site, then downstream to the previous site). This metric was not only associated with higher rates of connectivity and movement, but also indicated possible milling behaviors between the same sites. Finally, we calculated an **edge to diameter ratio** (number of edges divided by the network diameter) for each individual fish which compared the amount of movements standardized by a measure of path length. Higher ratios were indicative of passage routes populated with more movements, and thus indicated potential exploration and milling behaviors. Hence, we predicted that both these metrics will be higher for fish that passed the dam under the motivation filter hypothesis. Important to note is that although individual fish may have made the same movements between the same nodes multiple times, current network metrics do not account for multiple movements made in the same direction. Hence, our results represent underestimates regarding the total number of movements fish exhibited.

Statistical analyses – Multiple regression analysis was used to test whether network metrics were significantly ($P < 0.05$) associated with Pacific lamprey outcome (passed versus did not pass the dam). The model we used was:

$$\text{Metric}_i = \text{outcome} + \text{body length} + \text{tag date} + \text{'time in network'} + \text{outcome} \times \text{'time in network'}$$

We evaluated the effect of lamprey body length in our model as a secondary objective, whereby we predicted that lamprey body size would be negatively associated with network size and connectivity because larger lamprey are more likely to reach upstream sites (Keefer et al. 2009, 2013c; Hess et al. 2014). Hence, we expected larger lamprey to generate more power for overcoming potential velocity barriers than smaller lamprey (assuming a similar level of motivation). The model also included tag date (e.g., indicator of runtime and seasonal environmental conditions at Bonneville Dam) and log-transformed ‘time in network’, to control for variation in these additional factors. The outcome × ‘time in network’ interaction tested whether metrics increased at the same rate with ‘time in network’ for lamprey that did or did not pass. Variance inflation factors (VIF) were calculated to test for multicollinearity among the four predictor variables (VIF < 3 are indicative of no collinearity; Zuur et al. 2009). A similar model was tested for Chinook salmon, though the salmon model excluded outcome because only four salmon did not pass the dam (see Results).

Following the multiple regression analyses, we used univariate regression to describe inter- and intraspecific differences in network metrics between Chinook salmon that passed the dam, Pacific lamprey that passed the dam, and Pacific lamprey that did not pass the dam. These results are presented to provide a graphical comparison of the differences in network metrics for these three groups. Post-hoc Tukey's "honestly significant difference" (HSD) tests were used to test for further mean differences between these three groups. All network metrics were log-transformed for all analyses to meet the assumption of normality in the residuals. All analyses were completed using generalized linear models in R, version 3.0.3 (R Development Core Team 2014).

Results

Visualization and metric comparisons

Examination of networks for three Pacific lamprey with similar body sizes and tag dates revealed very different spatial patterns (Table 2.3; Figure 2.2). Lamprey 39689 and 39619 had higher clustering coefficients given the number of entrances these fish moved between, while Lamprey 39664 had a clustering coefficient of zero since it only approached one entrance. Density, average number of neighbors, and number of multi-edge nodes was also higher for Lamprey 39689 and 39619 due to the greater number of movements exhibited, particularly between entrances. Lamprey 39689 had a higher diameter due to the long path length from the wider range of movements across all sites. While Lamprey 39664 and 39619 both had the same diameter, the higher edge: diameter ratio for 39619 indicated a passage route highly populated with movements and milling behaviors (Figure 2.2C). While we expected a general positive association between ‘time in network’ and network metrics, comparison of Lamprey 39689 and 39664 illustrate variability in behavior among individuals. Although both fish took 17 days to pass the dam, the long passage time for one fish consisted of actively exploring all passage routes (39689; Figure 2.2B) and the long passage time for another fish consisted of slowly traversing just one route (39664; Figure 2.2A).

Population-level differences

Variation in network metrics was higher for Pacific lamprey compared with Chinook salmon, which was largely due to the differences in passage success between both species. Of the 240 salmon that were detected approaching a Bonneville Dam entrance, 236 salmon passed the dam (passage rate = 98.33%). The four salmon that did not pass had similar network characteristics composed of short path lengths and very few movements, which are expressed as the number of edges (Figure 2.3A, 2.3C). In contrast, only 118 of the 255 lamprey that approached an entrance passed the dam (passage rate = 46.27%) and the variation in path length and number of movements was high for the 137 lamprey that did not pass (Figure 2.3B, 2.3D).

All predictor variables for the multiple regression analyses had low VIFs, suggesting acceptably low collinearity among the variables. Overall, multiple regression models incorporating outcome, body length, tag date, and ‘time in network’ explained anywhere from 28-62% of the variation observed in network metrics for Pacific lamprey (Table 2.4). The multiple regression model was significant for network diameter ($R^2 = 0.63$, $P < 0.001$), average number of neighbors ($R^2 = 0.53$, $P < 0.001$), network density ($R^2 = 0.51$, $P < 0.001$), multi-edge node pairs ($R^2 = 0.35$, $P < 0.001$), and the edge: diameter ratio ($R^2 = 0.28$, $P < 0.001$). Outcome was a significant predictor for all five lamprey metrics ($P \leq 0.007$). ‘Time in network’ was also a significant predictor for all network metrics, suggesting that longer times at the dam corresponded to higher metrics of network size and connectivity (Figure 2.5E) and that accounting for this variation improved the ability to detect other effects. Contrary to our prediction, lamprey body size was not a significant predictor for any of the network metrics.

Network diameter, density, and average number of neighbors were all higher for Pacific lamprey that passed compared with Pacific lamprey that did not, which was consistent with the predictions of the motivation filter hypothesis (Table 2.1; Table 2.4). In contrast, multi-edge node pairs and the edge: diameter ratio was higher for Pacific lamprey that did not pass, indicating more milling behavior in this group among individuals spending the same amount of time in the network and providing support for the performance filter hypothesis (Table 2.4). This pattern actually arises from controlling for ‘time in network’ in the model and can be explained from the outcome × ‘time in network’ interaction observed for multi-edge node pairs. Specifically, several individuals who did not pass had very short ‘times in network’ (<3 hours) and also moved frequently between the same fishway sites in the lower fishway, indicating that these individuals likely had high motivation to pass during that short timeframe (Figure 2.4). The same pattern is observed for the edge: diameter ratio since these fish had small passage routes that were highly populated with movements during only a short passage experience. These results demonstrate how intertwined our two biological hypotheses can be given the observation that high motivation may also be associated with rapid decisions to abandon further passage attempts.

Multiple regression models for Chinook salmon were significant for all network metrics as well, although the explanatory power of the models was lower compared with Pacific lamprey (Table 2.4). The model was significant for network diameter ($R^2 = 0.13$, $P < 0.001$), average number of neighbors ($R^2 = 0.31$, $P < 0.001$), network density ($R^2 = 0.32$, $P < 0.001$), multi-edge node pairs ($R^2 = 0.23$, $P < 0.001$), and the edge: diameter ratio ($R^2 = 0.33$, $P < 0.001$). Salmon body size did not have any significant effect on network metrics. However, 'time in network' and tag date did display significant, positive relationships with network metrics. The positive relationship with tag date revealed that later run salmon appeared to exhibit more movements across sites compared with earlier run salmon (i.e., spring versus summer Chinook salmon; Figure 2.5B). Figure 2.5 shows a linear regression for the relationships observed between one network metric (average number of neighbors) and the three continuous predictor variables for both Chinook salmon and Pacific lamprey that passed the dam.

Although network metrics of connectivity frequently differed among the three groups examined (Chinook salmon that passed, Pacific lamprey that passed, Pacific lamprey that did not pass), metrics of connectivity were higher and passage routes were generally more circuitous for lamprey that passed the dam compared with salmon. Network diameter differed between the three groups ($F_{2, 488} = 261.40$, $P < 0.001$) with salmon and lamprey that passed having longer path lengths than lamprey that did not pass (Figure 2.3, Figure 2.6A). Average number of neighbors ($F_{2, 488} = 66.23$, $P < 0.001$) and network density ($F_{2, 488} = 77.17$, $P < 0.001$) were higher for lamprey that passed and salmon compared with lamprey that did not pass (Figure 2.6B). The number of multi-edge node pairs differed significantly between all three groups ($F_{2, 488} = 13.34$, $P < 0.001$) with mean pairs higher for lamprey that did pass the dam compared with lamprey that did not pass the dam, contrary to the multiple regression analysis. Salmon exhibited the most directed and "linear" paths through the dam (Figure 2.6A). Finally, the edge: diameter ratio differed between the three groups ($F_{2, 488} = 17.28$, $P < 0.001$) with ratios lower for salmon compared with lamprey (Figure 2.6C). The coefficient of variation in the edge: diameter ratio was also lower for salmon (CV=0.31) compared with lamprey that did (CV=0.40) and did not pass (CV=0.53). This indicated more consistency in Chinook salmon movement patterns, which generally consisted of medium path lengths and relatively few lateral movements.

Discussion

Movement patterns and behaviors are becoming increasingly integrated in ecological studies from individual scales to ecosystem scales as the result of improved technology and theoretical frameworks (Cooke et al. 2004; Nathan et al. 2008; Jacoby et al. 2012). Notably, ecologists studying movement typically have a qualitative understanding of movement patterns. Few of the patterns observed in this study were surprising to us given our long experience with the telemetry dataset and prior experiences from coding individual movements at this site. However, the network analyses presented here provided a spatially explicit framework for quantifying the patterns of movement both within and among populations, which provided a bridge between telemetry techniques, qualitative assessments of movement, testable mechanistic hypotheses, and established statistical approaches.

Pacific lamprey and Chinook salmon behavioral differences

A large amount of attention has been placed on studying how mismatches between fishway conditions (e.g., design, hydraulics) and Pacific lamprey swimming capacity explain the poor passage observed for this species (Moser et al. 2002a; Johnson et al. 2012; Keefer et al 2013a). The majority of these studies have concluded that Pacific lamprey simply have poor performance in overcoming these obstacles. While evidence does exist that performance-related mechanisms associated with limited swimming capacity (e.g., low burst swimming speed) and structural impediments explain poor passage at some locations (Keefer et al. 2010, 2011), network analyses herein showed that network metrics were typically higher for lamprey that passed the dam compared with lamprey that did not. While some lamprey were detected at nearly every fishway location (e.g. Figure 2.2B), a large number of lamprey were only detected once before quickly opting to leave the dam (Figure 2.3). This wide range of behavioral variability is not likely a sole result of performance-based variation in passage ability.

The level of individual variability observed for Pacific lamprey in this study fits the concept of a behavioral axis, whereby some individuals may be more reactive to certain environmental conditions and exhibit more plasticity regarding their decision-making processes (Sih et al. 2004). Large individual variation has been previously documented in Pacific lamprey with regard to their migration rates and passage times (Moser et al. 2002a; 2013), and network metrics herein provided further evidence. This behavioral plasticity could be in response to previous passage experiences (e.g., tagging effects), maturation status, or experiencing high levels of predation risk (Sih et al. 2004; Kirk et al. Chapter 3). In contrast, Chinook salmon exhibited less variability in their network metrics. Even the four salmon that did not pass the dam all exhibited relatively few movements before opting to leave the network (e.g., Figure 2.3A, 2.3C), possibly because these individuals were native to downstream tributaries and exhibited 'tributary overshoot' behavior (Keefer et al. 2008) or may have been predation events in the tailrace by marine mammals (Keefer et al. 2012). Hence, nonphilopatric Pacific lamprey may have a more flexible decision structure compared with Chinook salmon, and may be more responsive to local environmental conditions during upstream migrations (Dingle and Drake 2007; Waldman et al. 2008).

Additional evidence has shown that Pacific lamprey passage and migration distance is size-dependent in the Columbia River basin (Keefer et al. 2009, 2013b), which has been used as further evidence that poor passage may be associated with limited swimming capacity for smaller individuals. The primary mechanisms put forth to explain this size-selectivity have been energetic-related, in that larger lamprey have 1) greater endurance capabilities, 2) higher energetic reserves to make repeat attempts at passage (i.e., larger energetic threshold), and 3) higher burst swimming capabilities to overcome velocity barriers (i.e., greater swimming power). However, body size did not show a significant relationship with any network metrics (e.g., Figures 2.3B, 2.5C). Hence, the extent of movements that lamprey exhibit across the dam may not be associated with any of the potential size-selective processes determining Pacific lamprey passage. In fact, the observation that many large lamprey had small path lengths (e.g., Figure 2.3B) actually contradict these hypotheses, since these fish would have been expected to make more passage attempts and overcome passage barriers easier.

Although network metrics showed no association with lamprey body size, a logistic regression analysis with the Pacific lamprey population in this study revealed that probability of passage over Bonneville Dam was indeed higher for larger lamprey (*n* = 255, $\chi^2 = 14.13$, $P < 0.001$). Additional multiple logistic regression analyses using both network metrics and body size revealed that network metrics explained more variation in passage probability than body size (*Kirk, unpublished data*). These results present a very interesting paradox in that both network connectivity and body size explain probability of passage, but they do not exhibit a relationship with one another. An alternative hypothesis is that size-selective filters exist at upper fishway locations (Keefer et al. 2103b), which would have been masked in this study due to similar connectivity for individuals that did and did not pass. However, recent experiments simulating an upper fishway bottleneck showed that no size-selective processes were associated with the probability of passage at this location (Kirk et al. Chapter 4). Previous analysis of telemetry results have also shown that while Pacific lamprey passage is size-selective at the dam-wide scale, there are no size-selective relationships at the route-specific scale (Keefer et al. 2013a). Overall, it appears that the size-selective processes operating at Columbia River dams was spatially diffuse and was undetectable using network metrics examined here.

If energetic and performance-related mechanisms do not explain the size-selective processes at Columbia River dams, genetic differences are certainly plausible. Recent evidence suggests that there may be a genetic component to migration distance, which appears to be linked with individuals of larger body size (Hess et al. 2014). Hence, phenotypic differences associated with Pacific lamprey size may be derived from genetic differences. Similar genetic mechanisms may also influence decisions of whether to pass barriers. Network results presented here provide new evidence that individual differences associated with intrinsic traits (e.g., motivation, physiology, genotype; Sih et al. 2004) may be important for explaining Pacific lamprey passage. Gaining a better understanding of those behavioral mechanisms will be critical for future efforts to improve the passage of this species in the Columbia River basin.

In addition to our hypotheses regarding Pacific lamprey, network analyses quantified an interesting new element of Chinook salmon behavior, which further highlights the potential for these methods to test intraspecific hypotheses about movement and behavior. The positive relationship between network metrics with salmon tag date (spring versus summer Chinook salmon) was unexpected since faster passage times have been previously observed for summer Chinook salmon, while some spring Chinook have been observed to take weeks to pass Bonneville Dam (Keefer et al. 2004a). While the underlying mechanism remains unknown, these spatial patterns may be due to early arrival of late-season stocks. Stock-specific migration timing differences reveal Upper Columbia River stocks were most frequent during summer runs (Keefer et al. 2004b). Up-river migrants may engage in more upstream-downstream and zig-zag movements as they attempt to locate necessary olfactory cues from more distant and laterally mixed tributary plumes (Keefer et al. 2006). These potential differences in stock-specific movement are further supported by the demarcation that exists in network metrics after the June 1 cutoff date for summer run fish (Figure 2.5B). Temperature gradients between the bottoms and tops of ladders are larger at Bonneville Dam during summer months (though temperature differences are smaller in magnitude than at upstream dams) and these gradients may also contribute to milling behavior in summer Chinook salmon (Caudill et al. 2013).

Applied implications

The network methods applied in this study have wide application to both aquatic and terrestrial systems, particularly for environmental monitoring. For example, one of the most frequently cited threats to migratory fish species are anthropogenic barriers, and the installation of fish passage systems at both large and small dams world-wide has been a primary mediation tool for this problem (Clay 1995; Caudill et al. 2007). Although many fish passage facilities have long term monitoring programs to observe and document migratory fish runs (Katopodis and Williams 2012), fishways can be complex spatial systems that affect fish behaviorally in more complex ways than just population size (Naughton et al. 2006). Additionally, movement patterns and decisions made prior to entering fishways often have large effects on passage rate during both up- and downstream movement. As a result, traditional metrics developed by the fish passage community to monitor passage, such as 'efficiency', 'passage time', or 'success' (Moser et al. 2002a, 2002b; Keefer et al. 2013a), may overlook important elements of behavior. Consider the two Pacific lamprey visualizations provided in this study (Figure 2.2A, 2.2B). Without considering the spatial distributions of each fish, the tremendous variation in behavior from movement patterns would not have been captured had only passage times been compared.

Similarly, roads, highways, and culverts present another type of anthropogenic barrier that can alter the behaviors of wildlife. Even though roads are a relatively transparent barrier for animals to overcome, studies have shown many organisms exhibit strong avoidance behaviors to these barriers (Shepard et al. 2008; Beyer et al. 2014). Mitigation techniques for reducing mortality at road crossings have received much attention and prioritization of where to improve habitat connectivity is critical (Dodd et al. 2004).

Network analyses have the potential to identify road crossings with high connectivity to fragmented habitat patches via node-removal manipulations (Eros et al. 2012; Jacoby et al. 2012). Hence, network analyses provide a promising addition to the monitoring toolbox for assessing changes in animal movement in the face of environmental change. Future applications of network methods would be most valuable within the context of a before-and-after, control-impact (BACI) experimental design. Such evaluations could include evaluating the impacts local-scale mitigation efforts (Naughton et al. 2007, Johnson et al. 2012), large-scale environmental manipulations (Caudill et al. *in review*) or ecosystem development (Kim et al. 2012) on patterns of movement and space use.

Although 'migratory delay' at anthropogenic barriers has been frequently cited as a critical threat to migratory species (Budy et al. 2002; Caudill et al. 2007), most of the fitness related consequences have been placed on the temporal component. This is primarily because movement times are easier to analyze compared with the larger elements of data associated with spatial distributions (Zabel 2002; Jacoby et al. 2012). Bridging the temporal and spatial elements of movement are needed to provide a more complete view of the fitness consequences of an individual's migration experience (Morales et al. 2010). Reconsider the two Pacific lamprey visualizations previously discussed. Although both species experienced depleted energy reserves due to longer 'times in network', Lamprey 39689 likely expended more energetic reserves given the distance this individual traversed across the dam.

A similar argument could be made regarding the fitness consequences for the increase in movements observed for summer versus spring Chinook salmon (Figure 2.5B). The extra energetic costs associated with these milling behaviors may be significant if summer Chinook are indeed from up-river stocks and are required to travel farther distances upstream. Our results suggest that the 'delay' behaviors previously documented for migratory fish (i.e., longer passage times, higher milling behavior; Moser et al. 2002a; Caudill et al. 2007, 2013; Keefer et al. 2013a) may incur greater energetic costs than previously considered with the inclusion of spatial movements. Consideration of these spatial dynamics in animal movements will thus be important for accurately assessing the fitness consequences of potential 'delay' behaviors.

Implications for future movement studies

Making inferences from the application of these methods involves addressing some important limitations, both for our and future studies. First, the outcome of network analyses for telemetry data depends on the structure of monitoring locations. Our monitoring array only captured movements within the Bonneville Dam fishway environment and did not capture movements within the tailrace downstream of the dam. Some fish, especially Pacific lamprey, went undetected for long periods of time and it is impossible to know whether these fish were actively moving through the tailrace, holding in the tailrace, or temporally went downstream of the dam. Accounting for those movements could improve the strength of these methods for explaining individual variation in network metrics, especially when Pacific lamprey have been shown to spend considerable time in tailrace environments (Noyes et al. 2014). However, our network closely matched the available migration pathways through this landscape and thus should have largely captured important elements of movement.

Second, although some fish had missed detections at fishway locations, the majority of missed detections could be reasonably inferred due to the linear nature of fishways and the large spatial coverage of receivers at Bonneville Dam. However, missed detections or limited spatial coverage in some systems can lead to the formation of incorrect inferences (Cagnacci et al. 2010; Papastamatiou et al. 2012). Careful consideration needs to be given to developing a monitoring array *a priori* to ensure limited measurement error and that the coverage area will generate results compatible with the researcher's question of interest. For instance, applying similar techniques in other habitats such as the pelagic zone of lakes or marine systems will require careful consideration of the scale of movements relative to the detection distances and spacing of nodes in the network of interest. Most critical will be to ensure that detections are equally likely for all classes of interest (e.g., species, body sizes, etc.) to avoid systematic biases that would compromise hypothesis tests. Third, there were slight differences in the network size between Pacific lamprey and Chinook salmon that may have affected inter-specific comparisons. This is because lamprey are capable of entering the auxiliary water supply in the upper fish ladder (Sites H, CC; Table 2.2) where fish can either exit back to the ladder or pass via a lamprey specific passage system at these locations. However, preliminary analyses indicated inclusion or exclusion of these sites did not affect the outcome of the study and the difference had no significant effect on network metrics when network size was controlled between the two species.

Finally, and perhaps most importantly, these methods did not account for any processes associated with individual movements at a fine temporal resolution. Traditional application of movement models, such as random walks or state-space models, have analyzed whether movement paths result from random or ecological processes (e.g., foraging, reproduction, competition) at finer temporal resolutions (Schick et al. 2008; Avgar et al. 2013; Papastamtiou et al. 2013). Environmental features (e.g., flow rate, temperature, predator density) may have explained movement probabilities at some fishway locations, given the environmental complexity observed within tailrace and fishway environments (Caudill et al. 2007; Keefer et al. 2013a). However, such fine-scale analyses would have been beyond the scope of our project given the relatively limited movements that most fish exhibited across locations and the potentially long lag times between detections outside the fishways. Nonetheless, being able to statistically control for those ‘time in network’ effects is an important first step for helping to elucidate the relationships of interest in any movement study (Papastamtiou et al. 2013; Finn et al. 2014). Notably, the time spent moving in a network will affect network metrics, as these will grow larger with longer time spent moving in the network (e.g., under a random walk). Future analyses should strive to develop network metrics accounting for time and multiple movements among nodes.

A major challenge for movement models is the assumption that under similar environmental conditions, all individuals within a group or population will have similar movement distributions (Morales et al. 2010; Langrock et al. 2014), though clearly these results reveal large variation in movement among individuals. Studies that have considered individual differences in movement have observed as much variability explained by individual effects as environmental effects (Avgar et al. 2013). Hence, movement patterns may be driven by some intrinsic condition (e.g., phenotype, genotype, motivation) as much as external, environmental conditions. Network analyses provide empirically-derived metrics for describing connectivity that can be statistically compared among individuals or populations. Future studies that directly test for association between metrics and factors such as genotype (Hess et al. 2014), morphology, or physiological condition (Pon et al.

2009) could test the relative contribution of environmental versus intrinsic factors in determining individual movement patterns.

While network analyses have been most frequently applied for ecological studies of population and habitat connectivity (Fortuna et al. 2006; Galpern et al. 2011, Eros et al. 2012), recent studies have used these methods to summarize patterns of individual movement behavior and habitat use (Jacoby et al. 2012; Finn et al. 2014). However, these studies focused on only a few individuals, which limited the ability to make strong population-level inferences (Cagnacci et al. 2012). The ability to develop population-level inferences is an important step for the development of mechanistic models attempting to characterize individual movements (Schick et al. 2008), which is a major strength of the methods documented here. Another important strength of our application of these methods is demonstrating their application for testing *a priori* hypotheses about movement. In particular, network analyses uncovered new and interesting patterns of movement that helped elucidate new hypotheses regarding the migration behaviors of the two species in this study.

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Table 2.1: The predicted relationships of all six network metrics under the two hypotheses being tested for each passage group.

Hypothesis	Fish outcome	Predicted relationship
‘Performance-filter’	Pass	-
	Did not pass	+
‘Motivation-filter’	Pass	+
	Did not pass	-

Table 2.2: Monitored sites at Bonneville Dam sorted by fishway location and fishway section. Site codes refer to the labels in Figure 2.1.

Fishway name	Fishway section	Site code	Site name
Bradford Island “A” branch	Lower fishway	A	PH-1 South entrance (outside)
		B	PH-1 South entrance (inside)
		C	PH-1 North entrance (outside)
		D	PH-1 North entrance (inside)
	Ladder	E	“A” branch transition area
		F	“A” and ”B” branch ladder junction
	Upper ladder	G	Ladder/Count window
		H*	Auxiliary water supply/Lamprey passage system
		I	Fishway exit
Bradford Island “B” branch	Lower fishway	J	Bradford Island entrance (outside)
		K	Bradford Island entrance (inside)
	Ladder	L	“B” branch transition area
Cascade Island	Lower fishway	M	Cascade Island entrance (outside)
		N	Cascade Island entrance (inside)
	Ladder	O	Cascade Island transition area
		P	Upstream migrant channel (UMC)
		Q*	Auxiliary water supply
Washington-shore	Lower fishway	R	South downstream entrance (outside)
		S	South downstream entrance (inside)
		T	South upstream entrance (outside)
		U	South upstream entrance (inside)
		V	North downstream entrance (outside)
		W	North downstream entrance (inside)
		X	North upstream entrance (outside)
		Y	North upstream entrance (inside)
	Ladder	Z	Washington-shore transition area
		AA	Main ladder
		BB	UMC junction area
	Upper fishway	CC*	Auxiliary water supply/Lamprey passage system
		DD	Count window/serpentine weirs
		EE	Fishway exit

*Indicates the three receivers monitored only for lamprey.

Table 2.3: Body length, tag date, ‘time in network’, and network metrics for the three Pacific lamprey selected for visual representation in Figure 2.2.

Traits/Network metric	Lamprey 39664	Lamprey 39689	Lamprey 39619
Figure number	2.2A	2.2B	2.2C
Body length (cm)	64	69	66
Tag date	June 8	June 9	June 6
Passed dam (Y/N)	Y	Y	N
‘Time in network' (days)	17.03	17.73	47
Clustering coefficient	0	0.102	0.047
Diameter (nodes)	7	13	7
Average number neighbors (edges/node)	0.424	1.515	1.515
Network density	0.013	0.047	0.047
Multi-edge node pairs	1	16	15
Edge: Diameter	2.00	4.92	7.14

Table 2.4: Multiple regression results (ß = slope, *P* = whether ß was significantly different from 0 at α = 0.05) for five network metrics and the predictor variables (outcome, body length, tag date, ‘time in network’) for Pacific lamprey and Chinook salmon. Outcome comparison is passed: did not passed (i.e., positive coefficient indicates higher values for lamprey that passed). Bolded values are significant (*P* <0.05).

	Network metrics									
	Diameter		Network density		Average number of neighbors		Multi-edge node pairs		Edge: diameter ratio	
	ß	*P*	ß	*P*	ß	*P*	ß	*P*	ß	*P*
Pacific lamprey (n = 255)										
Outcome	**0.283**	**<0.001**	**0.144**	**<0.001**	**0.162**	**<0.001**	**-0.413**	**0.007**	**-0.648**	**0.005**
Length	-0.001	0.746	<0.001	0.931	0.001	0.808	-0.002	0.884	0.021	0.260
Date	0.001	0.173	<-0.001	0.377	<-0.001	0.327	-0.001	0.946	**-0.007**	**0.036**
Time	**0.095**	**<0.001**	**0.134**	**<0.001**	**0.141**	**<0.001**	**0.388**	**<0.001**	**0.525**	**<0.001**
Outcome*Time	-0.027	0.301	0.032	0.390	0.019	0.606	**0.528**	**<0.001**	0.218	0.303
Chinook salmon(n = 236)										
Length	-0.001	0.200	<0.001	0.470	<0.001	0.490	0.009	0.304	0.013	0.0375
Date	**0.001**	**<0.001**	**0.002**	**<0.001**	**0.002**	**<0.001**	**0.015**	**<0.001**	**0.059**	**<0.001**
Time	**0.005**	**<0.001**	**0.015**	**<0.001**	**0.014**	**<0.001**	**0.065**	**<0.001**	**0.013**	**<0.001**

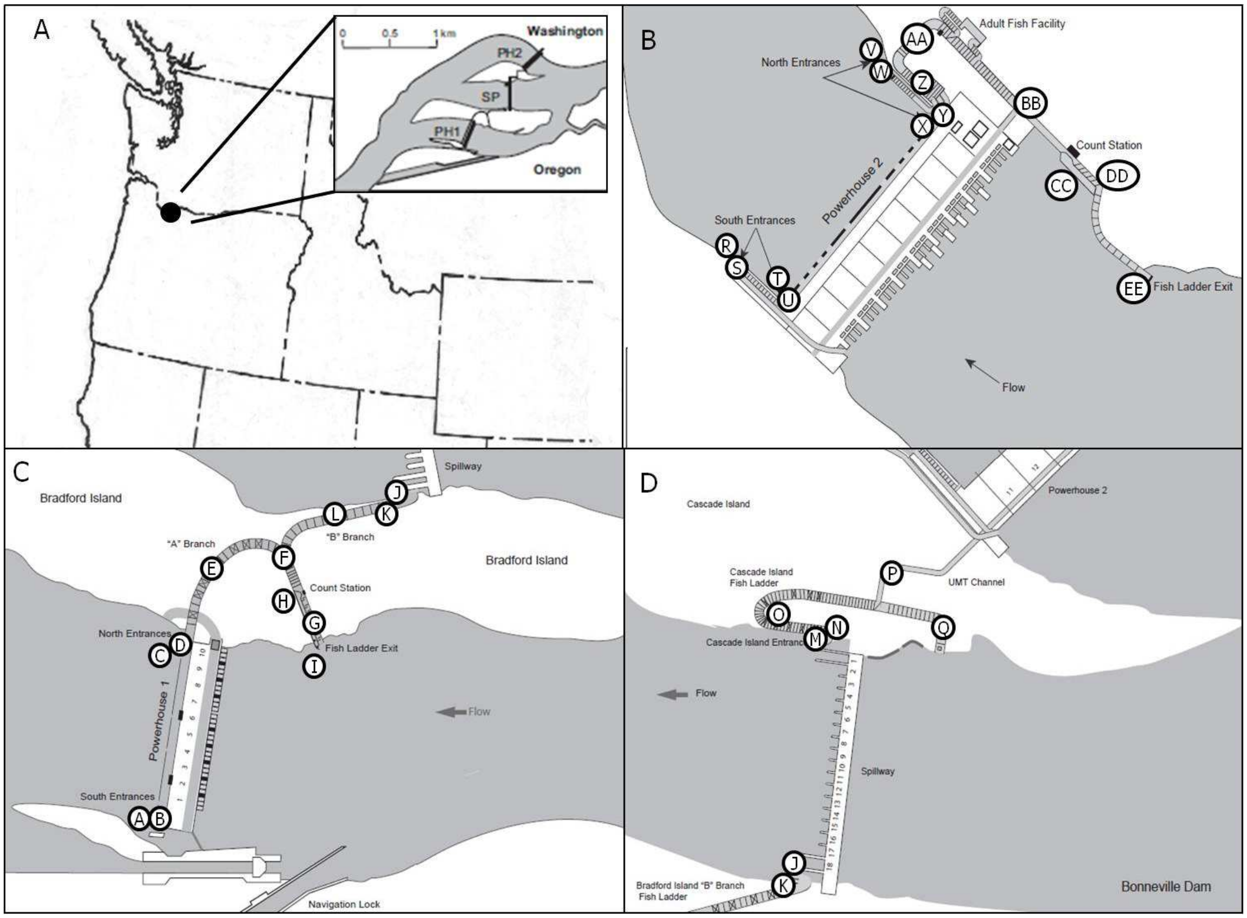
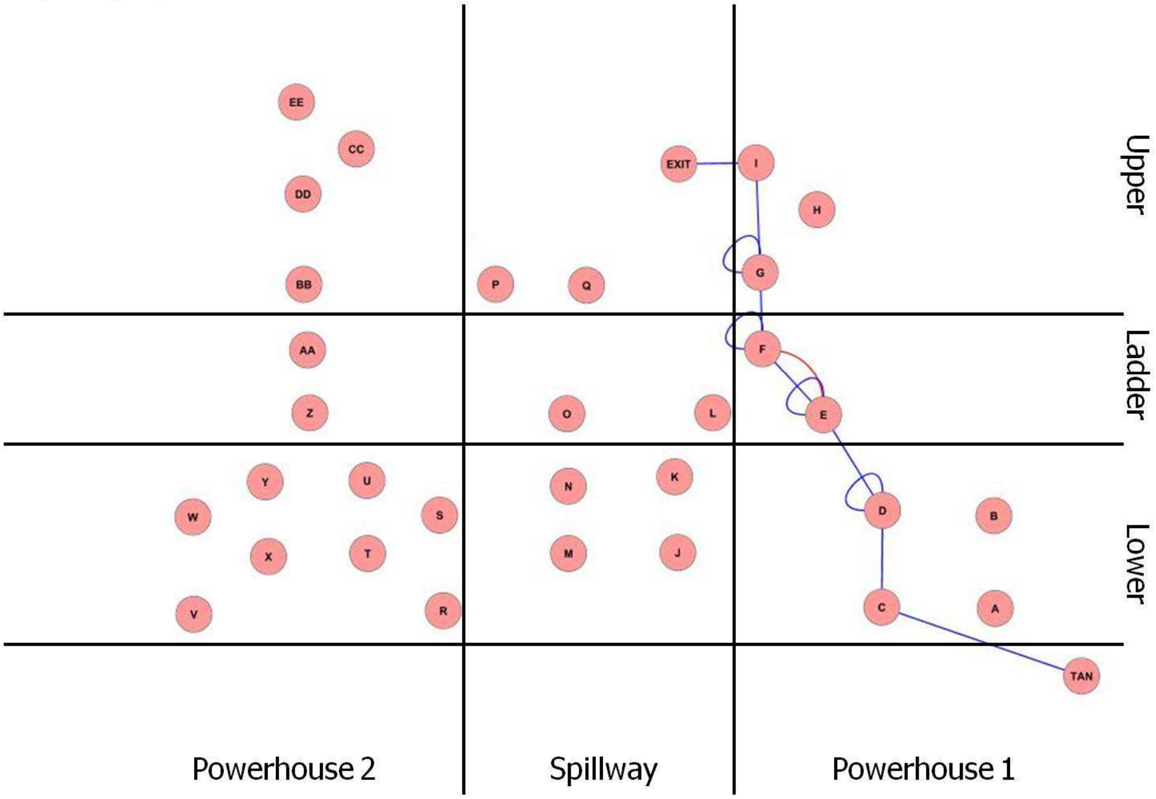
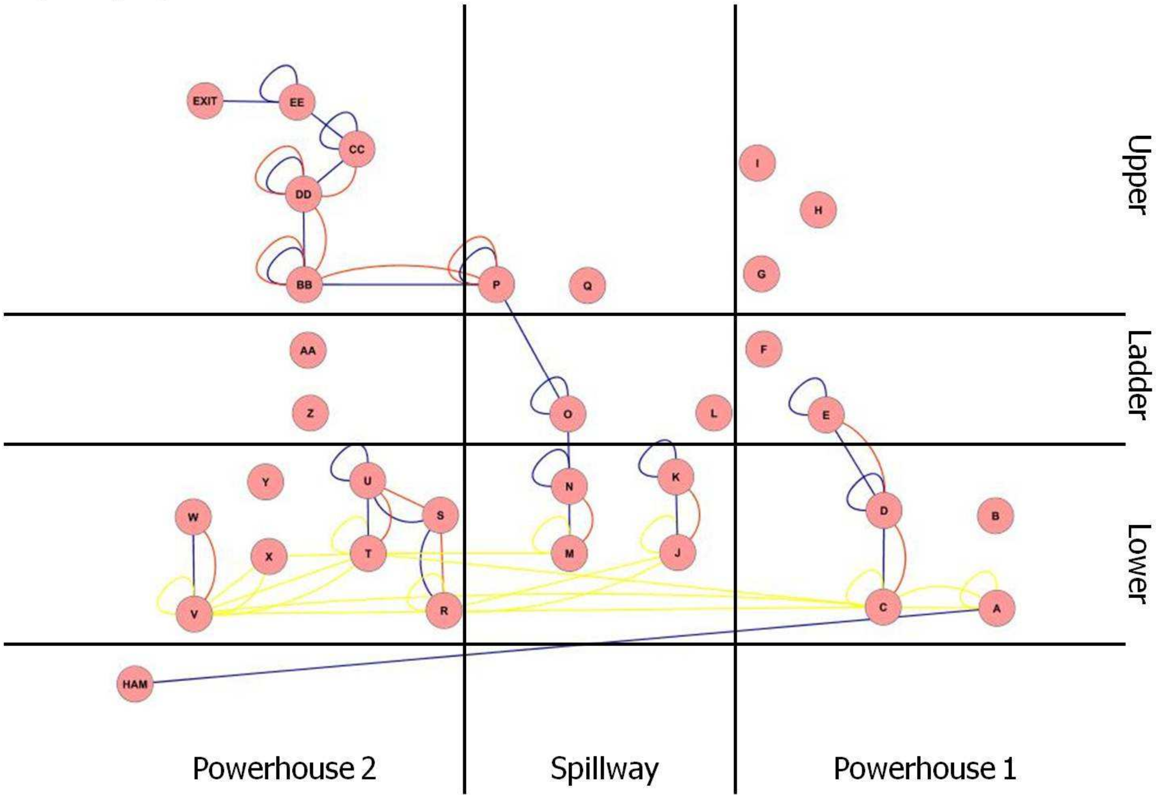


Figure 2.1: Map showing all of the monitored locations in the (B) Washington-shore Powerhouse-2, (C) Bradford Island Power-house 1, and (D) Cascade Island fishways at Bonneville Dam located in the Pacific Northwest, USA (A).

A) Lamprey 39664



B) Lamprey 39689



C) Lamprey 39619

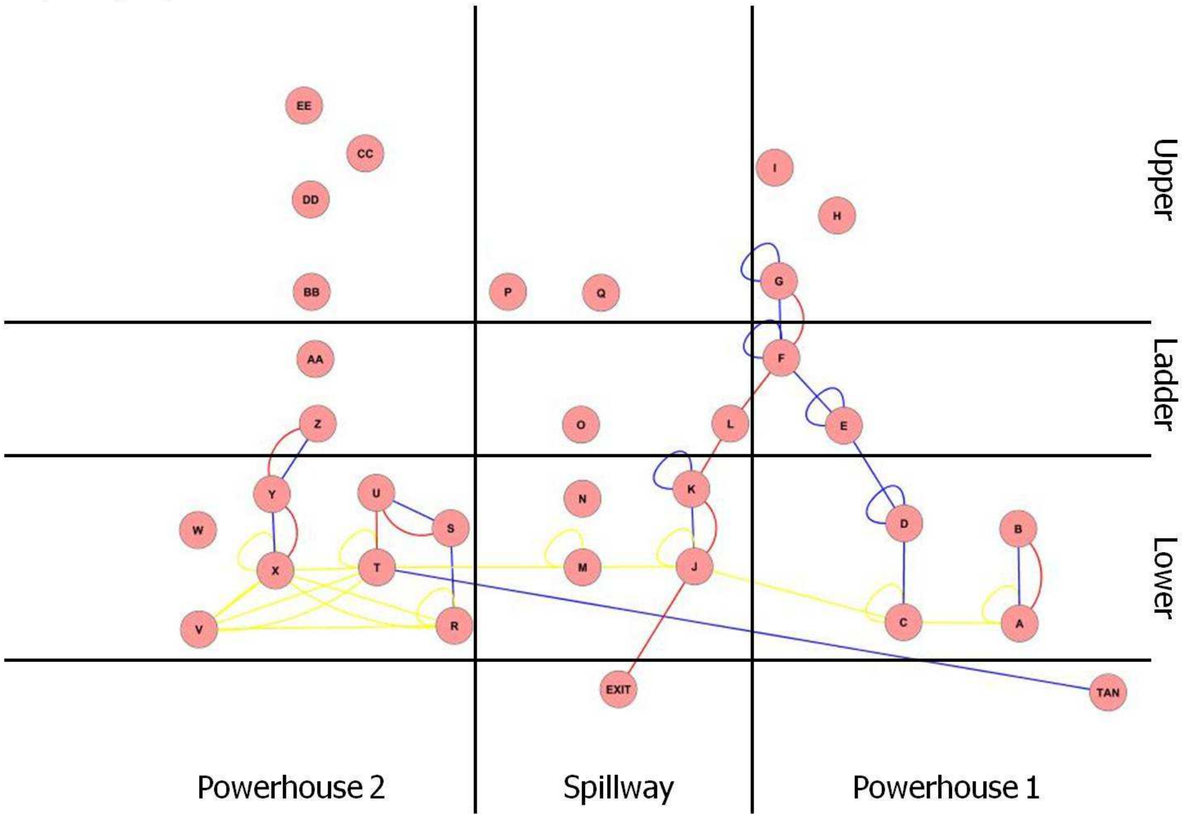


Figure 2.2: Top-down visualizations of Bonneville Dam and movement paths for three Pacific lamprey. Black lines separate the three different channels at Bonneville Dam (Powerhouse 1, Spillway, Powerhouse 2) and the separate fishway sections (lower, ladder, upper). HAM and TAN represent the release locations downstream of the dam and EXIT indicates the last known detection at the dam. Blue edges indicate upstream movement, red indicate downstream, and yellow indicates between-entrance movements.

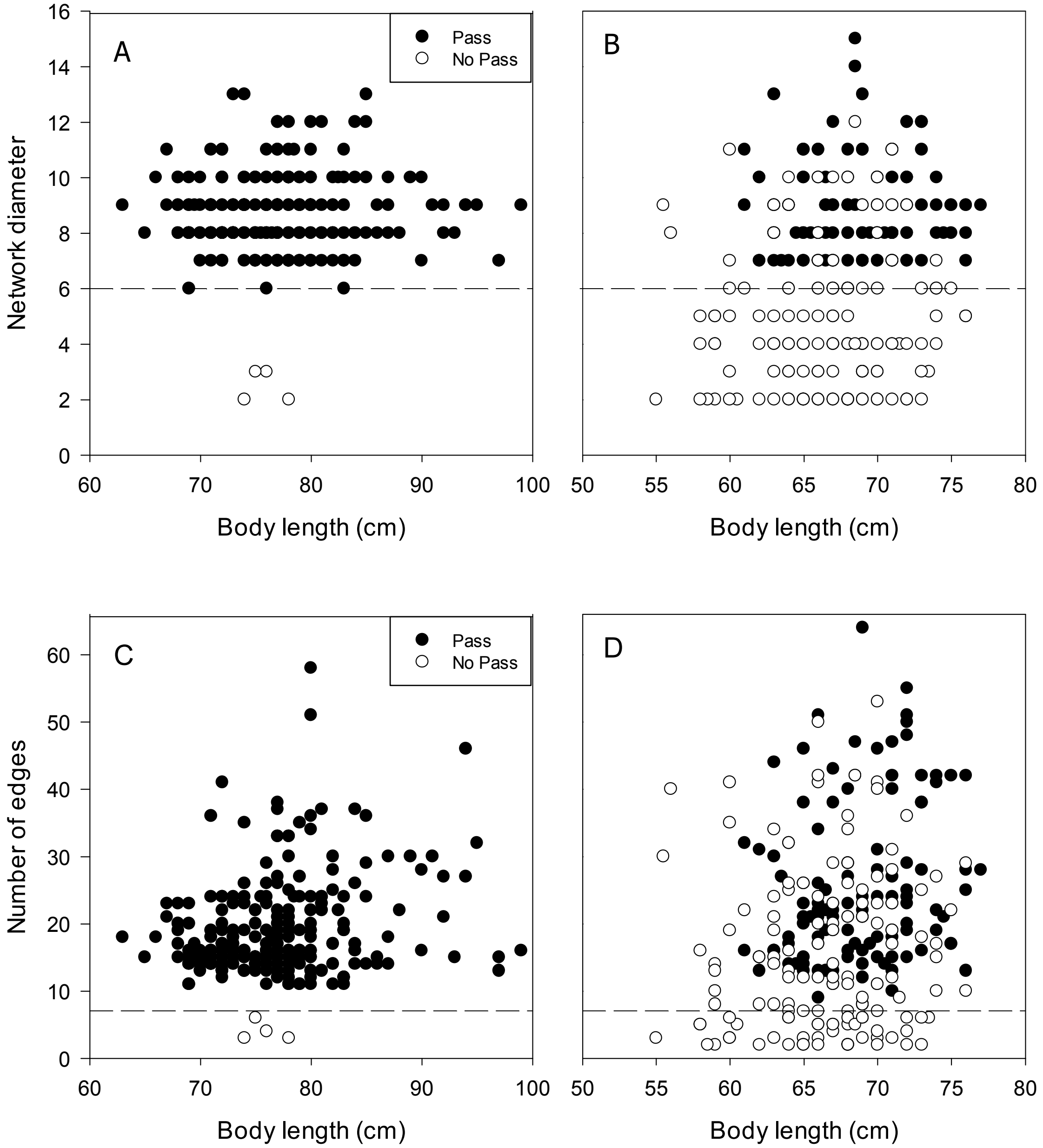


Figure 2.3: Relationships between network diameter, number of edges, and body length for Chinook salmon (A and C) and Pacific lamprey (B and D) sorted by fish that passed and fish that did not pass Bonneville Dam. Dashed horizontal lines indicate the minimum path length and minimum number of movements required to pass the dam.

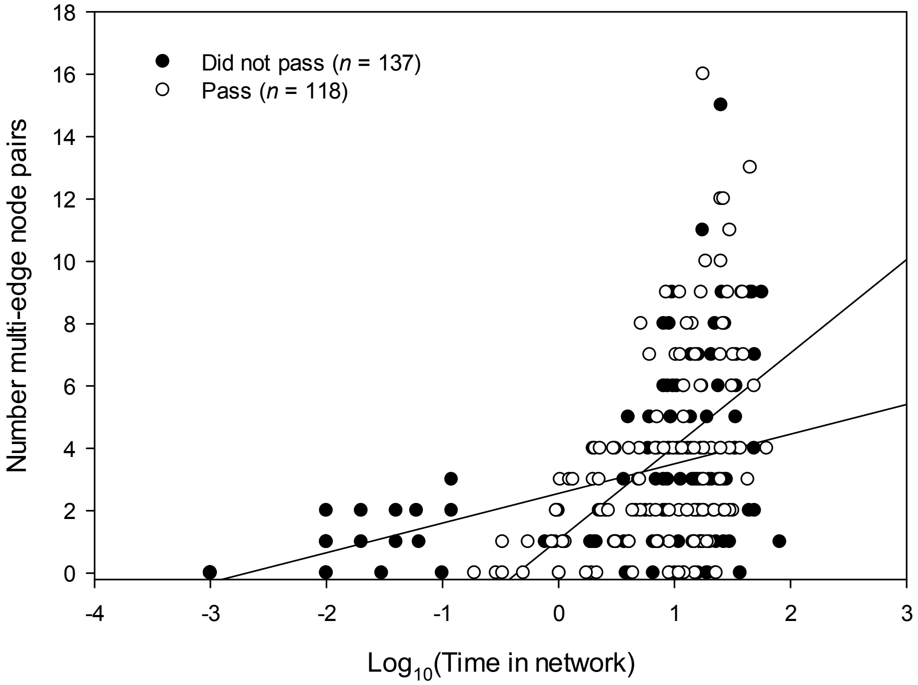


Figure 2.4: Relationship between number of multi-edge node pairs and log-transformed 'time in network' for Pacific lamprey that passed and did not pass the dam. Mean multi-edge node pairs was higher for lamprey that did pass (mean = 3.68) compared with lamprey that did not (mean = 2.82). See Figure 6A as well.

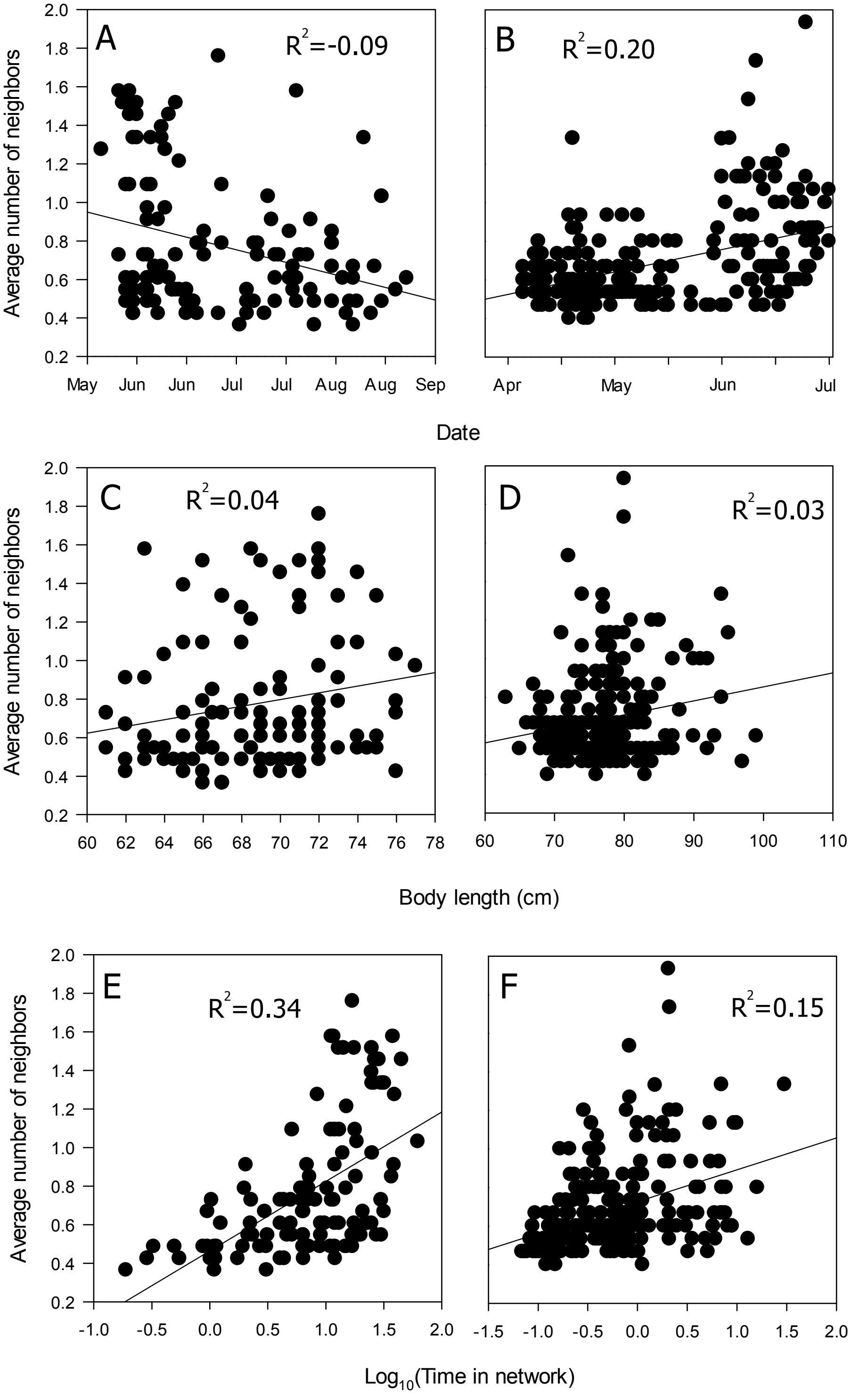


Figure 2.5: Linear regressions between a selected network metric (average number of neighbors) and fish tag date (A and B), body length (C and D), and 'time in network' (E and F). Plots for Pacific lamprey that passed Bonneville Dam (*n* = 118) are on the left (A, C, E) and plots for Chinook salmon that passed Bonneville Dam (*n* = 236) are on the right (B, D, F).

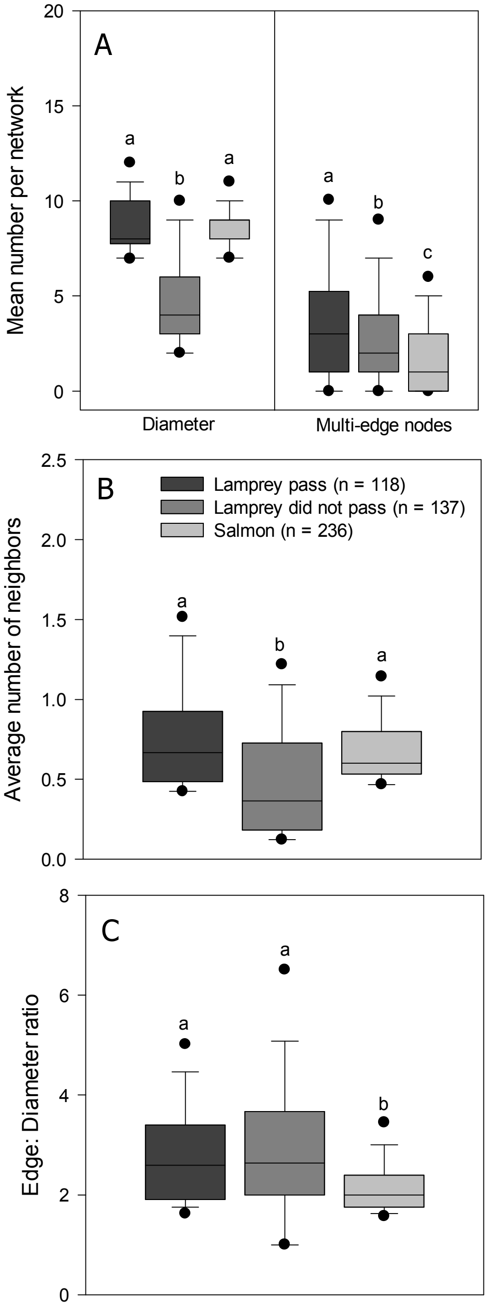


Figure 2.6: Comparison of (A) network diameter and multi-edge node pairs, (B) average number of neighbors, and (C) edge to diameter ratio for Chinook salmon, Pacific lamprey that passed Bonneville Dam, and Pacific lamprey that did not pass Bonneville Dam. Letters indicate significantly different populations based on Tukey's HSD ($P < 0.05$). Box plots show 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, and 95^{th} percentiles.

Chapter 3

Characterization of adult Pacific Lamprey swimming behavior in relation to environmental conditions within large dam fishways

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Abstract

Understanding the migration behaviors of adult Pacific Lamprey (*Entosphenus tridentatus*) inside fishways is critical to resolving why the species has low passage rates at large hydropower dams on the Columbia River. We used a Dual-Frequency Identification Sonar (DIDSON) camera to make *in situ* observations on the swimming behaviors of Pacific Lamprey at two different dams (Bonneville and John Day), in order to develop inferences regarding potential mechanisms influencing passage within lower fishway sections (entrances, collection channels, transition areas). The spatial distribution of lamprey appeared to support a model of increasingly demersal behaviors with increasing water velocity. Lamprey were primarily free-swimming in the mid- and upper water column at Bonneville Dam with relatively low mean water velocities at monitored locations (0.78 m/s at fishway entrance, 0.39 m/s in transition area). In contrast, lamprey encountered higher velocities at similar locations at John Day Dam (1.55 m/s at fishway entrance, 0.72 m/s in transition area) where lamprey were more likely to orient to the fishway floor. Behavior in the Bonneville Dam transition area appeared to be affected by poor guidance and the presence of predatory White Sturgeon (*Acipenser transmontanus*). Higher tail-beat frequencies (TBF) and lower swim efficiencies (the ratio of ground speed to swimming speed) were observed at fishway entrances and in the John Day Dam collection channel

compared with low-velocity transition areas. High TBF and low swim efficiencies supports the hypothesis that fishway entrances may act as short, high-velocity barriers for Pacific lamprey, while passage through the lower John Day Dam fishway may be limited by endurance requirements of passing a long collection channel.

Introduction

Providing adequate passage conditions at dams can be challenging, particularly when accommodating the morphological and behavioral differences of multiple taxa (Haro et al. 2004; Bunt et al. 2012). Historically, the design process of upstream fish passage systems has given primary consideration to economically and socially valuable fishes such as salmonid and clupeid species and information on the swimming performance of non-target species was typically unavailable to help guide design (Clay 1995; Katopodis and Williams 2012). Under some circumstances, failure to consider passage requirements for multiple fish morphologies has contributed to significant population declines of non-focal species and the potential loss of ecosystem functions (Agostinho et al. 2007; Mallen-Cooper and Brand 2007; Moser et al. 2011).

Pacific Lamprey (*Entosphenus tridentatus*) is an anadromous species native to western North America, where management for Pacific salmonids (*Oncorhynchus* spp.) has dominated for more than a century. When most fishways at hydropower dams in the Pacific Northwest were constructed in the twentieth century, they were specifically designed and operated to accommodate the passage of Pacific salmonids (Clay 1995). Pacific Lamprey have experienced significant population declines in past decades and were petitioned for listing under the U.S. Endangered Species Act in the early 2000s (Moser and Close 2002). Large hydropower dams have been implicated as a leading cause for these declines because

Pacific Lamprey passage success (~50%) is approximately one half that observed for Pacific salmonids at Columbia and Snake River dams (~95%; Moser et al. 2002a, Keefer et al. 2013b).

A variety of factors acting at multiple scales may explain the difference in passage success between species, including differences in morphology, migration strategy, swimming behavior and physiology. For example, the sub-carangiform swimming style of Pacific salmon is capable of generating greater burst velocities than the anguilliform style exhibited by Pacific Lamprey (Borazjani and Sotiropoulos 2009). Hence, lamprey may be less capable than salmon to overcome the high-velocity and turbulent conditions within fishways. Pacific Lamprey also exhibit a different migration strategy, with limited homing to natal streams (Spice et al. 2012) in comparison to the highly philopatric anadromous salmonids (Keefer and Caudill 2014). Migration distance in the Columbia River has been associated with size (Keefer et al. 2009) and genotype (Hess et al. 2014), though it remains unknown whether these associations result from selection during long-distance migration (e.g., energetic depletion) or selection for traits allowing passage at dams (e.g., burst swimming ability). While both taxa migrate long distances to interior spawning sites, the apparent lack of philopatry in Pacific Lamprey suggests there may be differences in the level of motivation to overcome encountered obstructions. The above mechanisms are not mutually exclusive and developing a better understanding of the passage behaviors of this species can simultaneously elucidate mechanisms of poor passage at local scales and identify future passage improvements.

Very little is known about the swimming behaviors of Pacific Lamprey inside fishways. Previous radiotelemetry studies have quantified movements in fishways at resolutions of ~ 10 m and have identified several areas of poor passage including fishway entrances, transition areas, counting stations, and vertical slot weirs (Moser et al. 2002a, 2002b; Keefer et al. 2013b). We used these studies, in conjunction with a series of tests in an experimental fishway (Keefer et al. 2010, 2011), to generate hypotheses regarding the specific mechanisms that cause Pacific Lamprey passage failures. To test these hypotheses, we used a dual-frequency identification sonar (DIDSON) camera as a non-invasive method for observing Pacific Lamprey behaviors inside fishways. These direct observations at scales of ~1-10 m tested hypotheses regarding lamprey passage at three fishway locations: 1) fishway entrances, 2) lower fishway channels, and 3) the transition between the lower fishway and fish ladders (hereafter "transition area").

Our first objective was to document the swimming mode of Pacific Lamprey at these three locations. Pacific Lamprey are considered to be demersal, substrate-oriented organisms that employ their suctorial disc during saltatory swimming movements (i.e., attach-and-burst) to propel themselves upstream in turbulent and high-velocity areas (Moser et al. 2002b, 2011; Keefer et al. 2011). This behavioral paradigm has been reinforced from experiments demonstrating that Pacific Lamprey use their oral disc to help them climb vertical surfaces (Kemp et al. 2009). However, other observations suggest the use of saltatory swimming or free-swimming modes is dependent upon passage conditions (Quintella et al. 2004). We hypothesized that swimming mode would depend on site-specific conditions within fishways. To describe the swimming mode of lamprey at each location, we characterized the: 1) frequency of attachment events, 2) vertical distribution, 3) lateral distribution, and 4) movement direction (upstream versus downstream).

The second objective was to observationally test hypotheses about potential mechanisms (i.e., hydraulic, structural, or biological) generating the distribution and behavioral patterns observed in objective one. We calculated water velocity using DIDSON particle tracking and estimated the activity of White Sturgeon (*Acipenser transmontanus*) as two different environmental descriptors (hydraulic and predatory) for each location. We also tested for associations between Pacific Lamprey behavior in relation to: 1) diffuser grating along the fishway floor and 2) at two different fish ladder structures (submerged weir orifice versus overflow weir) to determine whether these structural components affected behavior.

The third objective was to quantify elements of Pacific Lamprey swimming activity to understand whether short-term, swimming costs could be associated with poor passage at fishway sites. We were particularly interested in the role of potential high-velocity barriers at fishway entrances (Johnson et al. 2012a). Individual lamprey swim speeds were estimated to calculate a swimming efficiency index (ratio of ground speed to swim speed; Hinch and Rand 2000) that characterized the energetic efficiency of upstream movements. Tail-beat frequency (TBF; Mueller et al. 2010) was also estimated for individual lamprey as a second measure of swimming activity and surrogate for potential metabolic and energetic costs. We hypothesized that higher TBF and lower swim efficiency would be observed at high-velocity fishway sites (e.g., entrances) compared to low-velocity sites (e.g., transition areas).

Methods

Study sites and monitoring locations.- We deployed a DIDSON (Sound Metrics Corp., Bothell, WA) camera at a fishway entrance, a lower fishway channel, and a transition area in the Washington-shore fishway at Bonneville Dam (river kilometer [rkm] 235 from the Pacific Ocean), which is the first dam returning adults encounter when migrating upstream on the lower Columbia River (inset Figure 3.1A). Bonneville Dam spans three channels separated by islands; our observations were in the Washington-shore Fishway at Powerhouse 2 to the north. The Washington-shore fishway has a total of four entranceways (paired up- and downstream entrances on the north and south shore) that merge into a junction pool and transition area at the base of the pool-and-weir section of the fish ladder (Figure 3.1A).

We also deployed the DIDSON at similar sites (entrance, collection channel, and transition area) inside the north fishway at John Day Dam, the third dam that upstream migrants encounter (rkm 347; inset Figure 3.1A). The John Day Dam north fish ladder has a single entrance leading to a collection channel that proceeds upstream to the transition area at the base of the fish ladder (Figure 3.1B). Entrances at Bonneville Dam are vertical slot entrances with adjustable weirs at the bottom used to regulate attraction flow, whereas the John Day Dam north entrance has a variable-width ("keyhole") weir with a fixed floor. During the winter of 2011-2012, a bollard field (floor-mounted steel pegs; 20.3 cm. wide by 30.5 cm. high) was installed inside the John Day Dam entrance area (inset Figure 3.1B). The bollard field was designed to improve Pacific Lamprey passage by providing a reduced velocity field along the fishway floor.

The three Bonneville Dam locations were monitored from May-August of 2011-2013 (Table 3.1). The DIDSON was deployed at the north downstream entrance (NDE) in all three years to evaluate lamprey behavior under high-velocity conditions in a relatively large, open water environment common to fishway entrances. We deployed the DIDSON in a lower fishway channel at the junction pool (JP) in 2012 to evaluate lamprey interactions with: 1) diffuser grating near the fishway floor where pumped attraction water upwells, and 2) interactions with adult White Sturgeon that occur in high densities in both the junction pool and transition area. The DIDSON was deployed in the transition area upstream of the junction pool (JPU) in 2013 at the second weir of the fish ladder to determine how lamprey responded to different openings on the weir (weir orifice versus overflow weir). The fishway weir was fully inundated during the study period as is typical during passage at Bonneville Dam.

At the John Day Dam north fishway, we deployed a single DIDSON camera at four locations in July and August of 2012 and 2013 (JD 1-4; Figure 3.1B). Deployments occurred later in the summer at John Day Dam because fish arrive later in the migration season. Observations of the entrance area were made from two sample volumes. The first oriented the DIDSON to an area of high-velocity conditions several meters upstream of the entrance (JD1), and a second was closer to the fishway entrance near the bollard field (JD1_ELONG) to observe lamprey behavior in the reduced velocity zone created from this structural modification. Two locations were within an 80-m long collection channel (JD2, JD3) upstream of the entrance. The transition area location (JD4) was directly below the first weir of the fish ladder to observe behavior at the weir openings, similar to Bonneville Dam. White Sturgeon activity was monitored at all sites. Table 3.1 provides a general summary of all deployments.

Data collection.**-** We deployed a standard-range DIDSON mounted with an X2 dual-axis rotator that allowed the camera to be tilted and panned in the directions of interest. The camera and rotator were attached to an aluminum trolley that was lowered onto steel I-beams fixed to the fishway walls and retrieved using a portable davit crane. The camera was deployed for a 24 h period at each site and sample volumes were normally alternated on a daily basis. The DIDSON was typically deployed with the transducer facing across the fishway channel, which resulted in the highest probability of insonifying fish and the highest level of identification success (Johnson et al. 2012b). However, the camera orientation depended upon the objectives for each location (Table 3.1).

The DIDSON was operated in high-frequency mode (1.8 MHz), which produced 96 acoustic beams with 0.3° spacing to generate a 28.8°sample volume. High-frequency mode produces the best high resolution video for the short ranges (<10 m) common in fishway environments and preliminary observations suggested lower resolutions were insufficient to reliably differentiate Pacific Lamprey from other species (Johnson et al. 2012b). The sample window length was normally set to 5 m and the most frequent sample volumes encapsulated a range of 2-7 m away from the camera, which typically captured images of the opposing fishway wall. The frame rate was set to 10 frames/s and video files were saved in 10 min increments to facilitate data review.

Camera orientation.- The DIDSON only produces a two dimensional image (x = image width, y = distance from camera) from a three-dimensional sample volume (x, y, z; sample volume defined by a 28° field of view in the x dimension and 14° in the z dimension), so we deployed the camera in two different orientations to provide a more robust sample of all three fishway dimensions. In ‘landscape mode’, the x and y axes were oriented horizontally, which characterized the lateral distribution of targets. In ‘portrait mode’, the y and z axes were oriented horizontally and the x axis was oriented vertically to the water column, which sampled a vertical cross-section and provided information on the vertical distribution (depth) of targets. The only portrait orientations occurred at the north downstream entrance (NDE_P) at Bonneville Dam and the entrance at John Day Dam (JD1_P) (Table 3.1).

Two methods were used to capture variation in the vertical distribution of lamprey when the camera was deployed in landscape mode: an automatic tilting program and manual alteration of camera depth. We used a manual depth sampling protocol at Bonneville Dam in 2012 where the camera was deployed in a fixed orientation either near the surface or near the bottom of the water column for a full overnight period at NDE ($z = -7°$ to $+7°$). The camera was deployed in a fixed orientation near the surface, middle, and bottom of the water column in the junction pool. An automatic tilting feature was used at John Day Dam and for the transition area locations (JPU) at Bonneville Dam in 2013. The tilting program altered the angle of the DIDSON upward or downward every ten minutes to image upper ($z = 0°$ to $+14°$) and lower sample volumes ($0°$ to $-14°$).

Data processing and analysis.- All video files were reviewed using the DIDSON v5.25.25 Control and Display (Sound Metric Corp.) software. Six to eight reviewers watched data files each year. Given the high volume of data collected, one reviewer randomly subsampled files to distribute to other reviewers. Reviewers recorded the number of lamprey observations (defined as a lamprey event) that occurred during each ten minute file viewed. The random subsampling design was weighted toward nighttime files because Pacific Lamprey are predominately nocturnal during their upstream migrations at dams (Keefer et al. 2013a). For the analyses presented here, we restricted all analyses to nighttime data only (2100-0530).

Reviewers were trained and tested with a training set of videos prior to scoring files to ensure that lamprey identification was reasonably consistent among reviewers. Scoring criteria were established to help distinguish Pacific Lamprey from other species that were also present (Pacific salmonids, American Shad *Alosa sapidissima,* and Peamouth *Mylocheilus caurinus*). The most reliable criterion for identifying lamprey was their anguilliform swimming motion, which typically generated an S-shaped image compared to sub-carangiform swimmers such as salmon that exhibited a C-shaped image. Additional criteria that helped distinguish Pacific Lamprey from other species included target size (50-80 cm), body shape (low width: length ratio), and whether fish were observed attaching to fishway surfaces. A confidence level (low, medium, high) was also scored by reviewers for each event based on how strongly the event matched these criteria. Confidence levels were compared across reviewers to determine the level of agreement among reviewers for lamprey identification. Use of quality control measures and the four identification criteria helped standardize lamprey identification and reduced potential differences among viewers (Johnson et al. 2012b)

For every lamprey event, the reviewer recorded: 1) the time the fish was first and last observed, 2) the range of the fish (defined as the distance from camera along the y-axis), 3) movement direction (upstream or downstream), 4) where and when any attachment events occurred, and 5) a White Sturgeon index of activity, which was defined as the number of White Sturgeon observed per video file. Individual sturgeon could not be identified and thus the index represented a combined metric of density and activity. The index was defined as the number of times White Sturgeon entered and exited the frame of view during a ten minute file, and was translated into an hourly index for number of events per hour.

Because the DIDSON field of view increased with distance from the camera, the probability of detecting lamprey events increased with distance as well. We weighted our estimates of density along the lateral (y) transects by the volume of the observed area in 1 m increments along the y axis (hereafter referred to as bins) to account for the smaller sample volumes in bins closer to the camera. The geometric formula we used to calculate volume was for a 'truncated rectangular pyramid':

$$\text{Volume} = (1/3*H)*(A*B + \text{sqrt}[A*B*C*D] + C*D)$$

where H = the bin width (i.e., 1 m increments in distance from camera), A = width at the near end of the bin, B = height at the near end of the bin, C = width at the far end of bin, and D = height at the far end of the bin. We calculated lamprey depth with respect to the DIDSON for portrait data using:

$$\text{Depth} = X*\tan(ø)$$

where X = range of the event and ø = angle of the event with respect to the y axis.

Water velocity and swim speed estimates.- We estimated water velocity at several sites from DIDSON footage to serve as a first order descriptor of the hydraulic conditions Pacific Lamprey experienced. We used the fish measuring tool in the DIDSON software to measure velocity as the distance an individual suspended particle traveled across the image field divided by the time required to travel that distance. A previous study (Mueller et al. 2008) explored the feasibility of calculating x-direction velocity and found that such calculations were successful for distinguishing between passive and actively moving objects in the flow field. Ten estimates of particle velocity were made for each video file and the average of those ten estimates was the estimated mean water velocity for that file.

Ground speeds of Pacific Lamprey were estimated similarly using the fish measuring tool by measuring the distance fish swam divided by time. Fish swim speeds through water (hereafter 'swim speed') were calculated as the sum of the mean water velocity for each file and the ground speed of each fish. A swim efficiency index described by Hinch and Rand (2000) was calculated as ground speed divided by swim speed to describe fish swimming activity. The index serves as a surrogate measure for the energetic costs of movement by describing how energetically efficient upstream movements were for individual fish (i.e., lower scores indicate more work per unit upstream progress). Two important assumptions were made regarding these estimates of water velocity and swim speed. First, both methods assume co-planarity and do not account for any velocity component along the z axis, and thus the estimates represent minimum values. However, the magnitude of this bias was likely small because the x-y plane of the camera was oriented parallel to the bulk flow field in most locations. We also assumed passive transport of all particles in the flow field (i.e., entrained air, debris, sediment).

Tail-beat frequency estimates.- We followed a method developed by Mueller et al. (2010) to estimate tail-beat frequencies (TBF) from DIDSON footage as a second measure of Pacific Lamprey swimming activity. TBF has been frequently used as a swim performance metric that often correlates with fish energetic expenditure and metabolic rates (e.g., Standen et al. 2002; Steinhausen et al. 2005). We used the echogram procedure available in the DIDSON software to estimate TBF, which identifies the frequency of body undulations based upon the timing of the maximum acoustic intensity of the tail or body undulation represented as peaks on a time-stamped echogram. Following Mueller et al. (2010), the TBF of an individual lamprey was calculated with the equation:

$$\text{TBF} = \frac{(n-1)}{\sum_{i=1}^{n-1}(t_i + 1 - t_i)}$$

where n is the number of peaks and t_i is the time of each peaks. Time t represents the interval between peaks (i.e., shorter time generates higher TBF estimates). We only estimated TBF for lamprey oriented upstream and that had at least four consecutive, discernible peaks in the echogram.

Statistical analyses.- Analyses were conducted within year, except for analysis of TBF and swim efficiency at John Day Dam (2012 and 2013 combined). We conducted one-way factorial analysis of variance or Student's t-tests to test for differences in event rates across locations and at different depths using ten minute video files as independent replicates. Welch's t-tests were used to test for differences in lamprey and sturgeon activity rates when data violated the assumption of equal variances (Welch 1947). We used Student's t-tests to test for differences in the swim efficiencies and TBFs of individual lamprey across sites at Bonneville Dam, as well as for differences between years at John Day Dam. For these analyses, individual lamprey events were treated as independent replicates. Linear regression was used to test for correlations between TBF, swim efficiency, and water velocity. All analyses were completed using R, version 3.0.3 (R Development Core Team 2014).

Results

Behavior and Distribution

The majority of Pacific Lamprey were observed free swimming at all locations except at the bollard field at the John Day Dam entrance. Attachment events were relatively rare at Bonneville Dam, where the behavior was observed only at NDE (NDE_L, 21 of 516 events, 4%; Table 3.2). Attachment events were also infrequent (<2.5%) at John Day Dam, except in the bollard field sample volume (JD1_ELONG) where 74% (70 of 95 events) of all events observed in the bollard field involved an attachment to either a bollard, along the fishway floor, or along the fishway wall. Also notable was that the event rate of the bollard field sample volume was the highest of all locations at John Day Dam (Table 3.2).

Pacific Lamprey were not substrate-oriented at NDE at Bonneville Dam, but rather were free-swimming and were distributed in the upper water column in a portrait sample volume (DIDSON depth = 1.8m; 42% vs. 58% of 48 events in the upper and lower half of the portrait sample volume, respectively; Figure 3.2A). Similarly, lamprey were observed more frequently in the upper water column in landscape observations at most locations at Bonneville Dam, including the high-velocity NDE site. Event rates were higher in upper sample volumes at NDE (NDE_L, $t = -3.80$, $P < 0.001$), junction pool west (JP_W, $F_{2,106} = 11.05$, $P < 0.001$), and the transition area (JPU_LONG, $t = -7.00$, $P < 0.001$) compared with lower and middle sample volumes (Figure 3.3A). No differences were observed between depths at junction pool north (JP_N, $F_{2,121} = 0.74$, $P = 0.479$), and lamprey were rarely observed in the lower water column near diffuser grating along the fishway floor. Event rates also differed among the four Bonneville Dam locations ($F_{3,390} = 12.42$, $P < 0.001$) with the highest rates observed in the transition area (Table 3.2).

In contrast to Bonneville Dam, Pacific Lamprey event rates exhibited a clear longitudinal decline in the John Day Dam north fishway ($F_{3,219} = 6.05$, $P < 0.001$; Figure 3.3B). More free-swimming events were also observed in the lower half of the John Day Dam portrait sample volume upstream from the fishway entrance (DIDSON depth = 1.5 m; 77% of 30 total events in lower), which included monitoring near the fishway floor (Figure

3.2B). Event rates were higher in the lower sample volumes at the fishway entrance and through the collection channel at JD1 (JD1_XSECT, $t = 1.96$, $P = 0.055$), JD2 (JD2_XSECT, $t = 2.96$, $P = 0.005$), and JD3 (JD3_XSECT, $t = 1.95$, $P = 0.059$). No differences were observed in the transition area at JD4 (JD4_XSECT, $t = -0.83$, $P = 0.410$).

The distribution of upstream and downstream movements at fishway entrances differed both laterally and vertically between dams. At Bonneville NDE (NDE_L), lamprey were observed more frequently in the middle of the fishway when swimming upstream. Over 60% of all upstream events occurred in the two range bins in the middle of the sample range for both the lower (42% and 23%; Figure 3.4A) and upper sample volumes (29% and 34%; Figure 3.4B). Additionally, the highest percentage of events in both the upper (63%; 232 of 366 events) and lower (80%; 118 of 147 events) sample volumes were upstream movements.

At the John Day Dam entrance (JD1_XSECT), upstream events were observed more frequently along the fishway wall in the upper sample volume. Nearly half of all upstream movements (49%) occurred at the farthest distance from the camera with fewer events in the remaining range bins (range: 0-26%; Figure 3.4D). In contrast, upstream events were more uniformly distributed across all range bins (range: 8-25%) in the lower sample volume (Figure 3.4C). In contrast to Bonneville Dam, upstream movements at John Day Dam were more frequent in the lower water column (79%, 41 of 52 events) while downstream movements were more common in the upper water column (70%, 26 of 37 events).

Associations with White Sturgeon

Pacific Lamprey event rates were lowest when White Sturgeon activity rates were highest at both dams. Sturgeon activity differed across the four Bonneville locations ($F_{3,390} = 52.01$, $P < 0.001$), with higher activity in the transition area (JPU_LONG, mean = 69.42 events/h, SD = 60.44), junction pool north (JP_N, mean = 30.39 events/h, SD = 27.22) and junction pool west (JP_W, mean = 21.09 events/h, SD = 26.34) than at NDE (NDE_L, mean = 8.08 events/h, SD = 13.15). Sturgeon activity was higher in the lower sample volumes at junction pool west ($F_{2,106} = 18.22$, $P < 0.001$), junction pool north ($F_{2,121} = 3.03$, $P = 0.052$), and in the transition area ($t = 9.89$, $P < 0.001$); no differences were observed at NDE ($t = 0.85$, $P = 0.852$). Across locations, a negative, non-linear relationship was observed between lamprey events and sturgeon activity rates (Figure 3.5A).

White Sturgeon activity also differed among John Day Dam sites ($F_{3,219} = 28.65$, $P < 0.001$), with higher activity at JD4 (JD4_XSECT, mean = 29.35 events/h, SD = 35.63) than at JD1 (JD1_XSECT, mean = 4.32 events/h, SD =14.69). The lowest activity rates were observed in the collection channel at JD2 (JD2_XSECT, mean = 0.55 events/h, SD = 2.65) and JD3 (JD3_XSECT, mean = 0.54 events/h, SD = 2.63). More sturgeon events were observed in the lower versus upper sample volumes at JD1 ($t = 2.23$, $P = 0.036$) and JD4 ($t = 4.96$, $P < 0.001$), but not at JD2 ($t = 1.54$, $P = 0.134$) and JD3 ($t = 1.54$, $P = 0.135$). Similar to Bonneville Dam, a negative and non-linear relationship was observed between the number of lamprey events and sturgeon activity rates across the John Day Dam locations (Figure 3.5B).

Overflow and orifice weir sections

In the Bonneville Dam transition area, Pacific Lamprey were observed more frequently in sample volumes oriented at overflow weirs (i.e., upper sample volume) than at sample volumes capturing submerged orifice sections (i.e., lower sample volumes) (JPU_SHORT, $t = -9.15$, $P < 0.001$). In addition, White Sturgeon activity was high in the lower sample volume capturing orifice sections (mean = 49.45 events/h, SD = 34.22), although there was no significant difference in rates of sturgeon activity between upper and lower sample volumes ($t = 0.93$, $P = 0.36$; Figure 3.6A). Lamprey were rarely observed ($n = 1$; 0.11 events per hour) at either the overflow or orifice sections in the transition area at John Day Dam (JD4_UP). Similar to the trend at Bonneville Dam, White Sturgeon activity was higher in the lower sample volumes near the orifice section (mean = 45.45, SD = 34.22) than in the upper sample volumes ($t = 5.11$, $P < 0.001$; Figure 3.6B).

Water velocity and swimming activity

At Bonneville Dam, estimated water velocity was higher at the NDE fishway entrance (NDE_L, mean = 0.78 m/s, SD = 0.23, $n = 88$) than the transition area (JPU_LONG, mean = 0.39 m/s, SD = 0.17, $n = 44$). TBF estimates were higher at NDE (mean = 3.99 beats per second (bps), SD = 0.47) and suggested more strenuous swimming compared with TBF estimates from the transition area (mean = 2.87 bps, SD = 0.63) ($t = -9.52$, $P < 0.001$; Figure 3.7C). Similarly, swim efficiencies indicated lamprey were achieving less upstream progress per unit effort at NDE (mean = 0.42, SD = 0.14) than the transition area (mean = 0.70, SD = 0.09) ($t = 13.63$, $P < 0.001$; Figure 3.7A).

At John Day Dam, mean water velocity estimates declined upstream through the fishway, but were overall higher than the estimates from Bonneville Dam. The highest velocities were inside the entrance (JD1_XSECT, mean = 1.55 m/s, SD = 0.15, n = 113,) with similar velocities at JD2 (JD2_XSECT, mean = 1.34 m/s, SD = 0.14, n = 66) and JD3 (JD3_XSECT, mean = 1.27 m/s, SD = 0.11, n = 40), while the lowest estimated velocities were in the transition area (JD4_XSECT, mean = 0.72 m/s, SD = 0.09, n = 24). While there was no significant difference between TBF and year at John Day Dam (t = -0.53, P =0.198), swim efficiency estimates were significantly higher in 2013 than in 2012 (t = -2.69, P =0.009). TBF patterns were similar to those at Bonneville Dam, with higher estimates at JD1 near the fishway entrance (mean = 3.92 bps, SD = 0.76) than in the transition area at JD4 (mean = 2.82 bps, SD = 0.72). However, only two estimates were calculated from the JD4 location due to low event rates. TBF estimates in the collection channel at JD2 (mean = 3.55 bps, SD = 0.47) and JD3 (mean = 3.82 bps, SD = 0.60; Figure 3.7D) were comparable to estimates from JD1 near the entrance. Low swim efficiencies at JD1 (mean = 0.25, SD = 0.06) and in the collection channel at JD2 (mean = 0.22, SD = 0.07) and JD3 (mean = 0.32, SD = 0.12) also suggested that upstream progress was slow at these locations (Figure 3.7B).

When individual lamprey were pooled together from all sites at both dams, swimming activity measures were correlated with water velocity and with each other. Swimming efficiency was negatively correlated (r = -0.83, $P < 0.001$, n = 210, Figure 3.8B) and TBF was positively correlated (r = 0.51, $P < 0.001$, n = 174, Figure 3.8A) with water velocity. TBF was also negatively correlated with swimming efficiency (r = -0.48, $P < 0.001$, n = 138) and positively correlated with lamprey swim speeds (r = 0.50, $P < 0.001$), but was not correlated with lamprey ground speeds (r = -0.14, P = 0.101).

Discussion

Caveats and assumptions

Several important caveats need to be considered that apply to our analyses. First, our estimates of Pacific Lamprey event rates may have underestimated the true rates in a given ten minute file. Our comparison of scoring results among viewers of the same data indicated differences in the number of events scored for identical files. Hence, it was likely that reviewers did not classify all lamprey events due to short duration or ambiguous image quality (Johnson et al. 2012b). Two other important assumptions for our inter-annual comparisons are that 1) the passage rate and run size of Pacific Lamprey was similar between years, and 2) migrating lamprey experienced similar conditions at each dam across all study years. Although the daytime lamprey counts at Bonneville Dam were ~20% higher in 2012 (29,224) than in 2013 (23,970), the counts at John Day Dam were ~30% higher in 2013 (6,645) than 2012 (4,567; Columbia Basin Research 2014). Hence, event rates in the Bonneville transition area may have been underestimated relative to estimates from NDE and the junction pool.

No major operational changes occurred at Bonneville Dam during the study period that would affect our conclusions, particularly since data for each location was only presented for one year. At John Day Dam, however, higher flow volumes were maintained through the diffusers in the collection channel near JD1, JD2, and JD3 in 2012 due to fishway pump modifications that were not completed until Spring 2013 (M. Zyndol, USACE, *personal communication*). The significantly higher swim efficiency estimates observed for fish in 2013 suggests Pacific Lamprey experienced higher flows at JD1, JD2 and JD3 in 2012. Additionally, lamprey event rates and net upstream movements rates were higher at JD3 in 2013 compared with 2012 (*Kirk, unpublished DIDSON data*), further suggesting that behavior at this location was different between years. Hence, strenuous conditions within the collection channel may be an important factor limiting Pacific Lamprey passage through the John Day Dam North fishway. Overall, this provided a novel opportunity to evaluate the effects of altered flow conditions on lamprey passage rather than just being a limitation of our results.

Finally, our estimates of water velocity and lamprey speed may represent underestimates of true velocity if the assumptions of co-planarity and passive particle movement were not met. Thus, the relationships observed likely include estimation error and also represent conservative estimates of the effects on lamprey movement. We compared our estimates to hydraulic evaluations conducted by the U.S. Army Corps of Engineers (USACE) using numerical simulations from fishway models or field measurements with a Price meter to evaluate the potential magnitude of any bias from DIDSON estimates. DIDSON-derived estimates (0.78 m/s) at Bonneville Dam were lower than USACE operational criteria for reduced nighttime velocities at NDE (1.23 m/s; Johnson et al. 2012b; S. Schlenker, USACE, *personal communication*). The reduced velocity conditions were implemented during all video files that water velocity estimates were made for this study (USACE, *unpublished data*). In contrast, velocity estimates in the Bonneville Dam transition area (JPU) were more similar (USACE = 0.46 m/s, DIDSON = 0.39 m/s; USACE 2005). Velocity estimates in the John Day Dam transition area from DIDSON (0.72 m/s) were higher compared to USACE field measurements (0.41-0.50 m/s; USACE 2012).

Potential differences in these estimates can be attributed to sampling and modeling different points across time (i.e., seasonal changes) and space (i.e., changes in water depth, distance from entrance slot), in which hydraulic changes are known to occur. For example, DIDSON estimates at NDE near the water surface (1.16 m/s) were more similar to the estimate observed by the USACE (1.23 m/s) compared with DIDSON estimates in the lower water column (0.33 m/s) (*Kirk, unpublished DIDSON data*). Overall, the similarity between DIDSON and independent estimates suggest that the magnitude of the bias was relatively small and unlikely to affect qualitative conclusions about Pacific Lamprey swimming performance.

A framework for understanding Pacific Lamprey passage behavior

The direct observations of Pacific Lamprey in this study allowed us to test hypotheses about the preferred swimming modes in three different fishway environments. Previous studies have characterized Pacific Lamprey as being primarily demersal and substrate-orientated with a preference for moving along surfaces or edges to which they can attach (Moser et al. 2002b, 2011; Keefer et al. 2010). In fact, lamprey at John Day Dam were more frequently in the lower water column along the fishway floor, especially when moving upstream. When fish were observed moving upstream in the upper water column at John Day Dam, they were most frequently near the fishway wall. These patterns were consistent with the edge and substrate orientation effects described previously for the species. In contrast to this expectation, event rates at Bonneville Dam were highest in the upper water column and lamprey were most frequently observed free swimming in the open water column, even at the highest velocity location (NDE).

Similarly, ‘portrait mode’ observations at both fishway entrances also revealed substantial proportions of free-swimming lamprey in the open water column (compare Fig.

2A and 2B; we note the spatial inference at NDE was limited), while the majority of events in the John Day portrait orientations were observed in the lower water column. Thus, by altering the camera orientation, our results demonstrate that DIDSON images can be used to quantify either horizontal or vertical distributions. We note, however, that the orientation simultaneously affects key elements of imagery. Pacific Lamprey identification was more difficult for reviewers in ‘portrait’ orientations because events were imaged as lateral cross-sections as they swam through the sample volume (Johnson et al. 2012b). Overall, these distribution patterns suggest that Pacific Lamprey may alter their swimming mode, free-swimming in the open channels versus free-swimming and substrate-oriented versus saltatory swimming, depending upon the environmental conditions fish experience.

At least two factors may explain the difference in Pacific Lamprey swimming behavior between Bonneville and John Day dams. First, the reduced nighttime water velocities at Bonneville Dam that is implemented to improve Pacific Lamprey passage (i.e., Johnson et al. 2012a) may facilitate free swimming and open channel behavior. In contrast, the higher velocities observed at John Day Dam likely contributed to the more demersal behavior of lamprey at those locations. The second potential mechanism pertains to fishway scale and the presence of rheotactic cues, in that the NDE channel is nearly three times deeper than the channel of the John Day Dam entrance (see Figure 3.2). The high velocity plume at NDE is most prevalent near the surface and very diffuse in the lower water column, which leaves little potential for combined rheotactic and substrate guidance. In contrast, the attraction plume at John Day Dam is located directly above the substrate and lamprey are capable of using both guidance cues simultaneously. The perception of hydraulic cues is probably the primary sensory modality used during nocturnal passage and each of these potential mechanisms highlights the important role that hydraulic conditions have in the swimming behavior of Pacific Lamprey.

Saltatory swimming, when lamprey progress upstream via attach-and-burst movements, has been frequently observed under high-velocity conditions (Quintella et al. 2004, Keefer et al. 2010, 2011). However, events that involved lamprey attaching to a fishway surface (i.e., wall, floor, or bollards) were relatively infrequent under the high-velocity conditions experienced at Bonneville and John Day Dams, except within the bollard field near the John Day Dam entrance. The reduced bulk velocity in the bollard field is a result of the turbulence generated by these structures and the lateral and lifting forces may compel lamprey to attach to the substrate. Concurrent experiments in an experimental flume comparing turbulent to non-turbulent conditions also suggest that the frequency and duration of attachments increases under high turbulence and high velocity (Kirk et al., Chapter 4). Turbulence is hypothesized to be an important, yet understudied, element of fish behavior (Silva et al. 2012), and the combination of turbulence and velocity conditions near walls or substrate may serve as important detection cues for whether Pacific Lamprey employ free-swimming or saltatory behaviors.

Linking mechanisms with telemetry studies

The *in-situ* observations of Pacific Lamprey allowed us to test several hypotheses generated from previous studies. First, previous telemetry studies have hypothesized that diffuser grating along fishway floors create inadequate attachment surfaces or misleading attraction cues that impede Pacific Lamprey passage (Moser et al. 2002a; Keefer et al. 2013b). While adult Pacific Lamprey have been stranded below gratings during dewatering events at some fishway locations (Moser et al. 2008), we observed no evidence of a response to diffuser gratings in the junction pool. Instead, lamprey were most frequently observed free-swimming well above the diffuser sections in this area. Although we did not directly quantify the behavioral response to the diffusers, our observations suggest movements through gratings are low in areas where lamprey are free-swimming since we did not directly observe any reaction of Pacific Lamprey to the interface between solid floor and diffuser gratings.

Second, we tested for spatial associations between Pacific Lamprey and White Sturgeon because predation risk from sturgeon has also been previously suggested as a potential mechanism for poor passage based on anecdotal evidence (Keefer et al. 2013a, 2013b). The negative relationship observed between lamprey event rates and sturgeon activity in this study suggests that Pacific Lamprey behavior and distribution inside the fishways may be partially driven by predator avoidance behaviors. Pacific Lamprey were observed more frequently in the upper water column whereas White Sturgeon activity was greater in the lower water column at the Bonneville Dam transition area and junction pool. Circumstantially, we also observed lamprey ceasing upstream movements and quickly exiting the field of view upon encountering groups of sturgeon. Pacific Lamprey may detect and avoid sturgeon using chemosensory cues, as many lamprey species have highly developed olfactory systems (Yun et al. 2011). Several recent studies have shown lamprey aversion to odors associated with predators and with lamprey injury and mortality (e.g., Imre et al. 2010; Wagner et al. 2011; Di Rocco et al. 20114). Further studies are needed to verify that Pacific Lamprey respond to the presence of White Sturgeon, to identify the sensory mechanism employed (i.e. chemosensory, vibrational, visual), and to directly quantify the effect of sturgeon or sturgeon cues on upstream passage.

Past radiotelemetry observations have revealed poor passage through the transition areas at Bonneville Dam and limited guidance cues have been hypothesized as one mechanism limiting passage at these locations (Moser et al. 2002a; Keefer et al. 2013b). Transition areas are known for flow conditions that provide limited guidance for salmon passage (Naughton et al. 2007; Keefer et al. 2008), primarily at the lowest weirs in the ladder which are typically fully inundated and reduce attraction flow for fish at the surface and weir orifices. The Washington-shore transition area monitored in this study is known for high Pacific Lamprey turn-around rates (Keefer et al. 2013b), and it is likely that the high event rates observed at this location resulted from lamprey moving repeatedly upstream and downstream (i.e., “milling behavior”) through the lower transition area. Low attraction flows at the submerged weir orifices also likely influenced the low event rates at these passage structures. Inferring which process explains these patterns from our observational data is difficult, particularly since the impacts of both the ‘predator avoidance’ and ‘guidance limited’ hypotheses overlapped significantly in the transition area. Taken together, these data suggest that Pacific Lamprey behavior in the transition area is dictated by a complex interaction of hydraulic, structural, and biological factors.

The lowest Pacific Lamprey event rates were observed in the John Day Dam transition area. This may have been due to either poor passage through the turn-pool upstream of JD3 (see Figure 3.1B), high White Sturgeon densities in the transition area, or from the attrition that occurs through the high-velocity segment in the collection channel (see Figure 3.3B). Given the high levels of fatigue that have been observed for lamprey during long swimming bouts at high-velocities (Mesa et al. 2003; Quintella et al. 2004), passage through the lower fishway at John Day Dam may be limited by Pacific Lamprey endurance while swimming at or near ‘critical swimming speed’ for long distances (e.g., the ~80 m distance between the entrance and transition area). Recent installation of a lamprey passage system (LPS, Moser et al. 2011) downstream of the collection channel near the fishway entrance and bollard field also supports this hypothesis given the collection rates observed in the LPS. Overall these data indicate that passage through the John Day Dam North fishway was predominately limited by processes acting in the collection channel rather than upstream in the transition area.

Implications of TBF and swimming speed estimates

The novel application of making *in-situ* estimates of TBF and swim speeds has important implications for understanding both how to improve Pacific Lamprey passage and estimating the swimming activity associated with migration conditions. Mueller et al. (2010) only briefly touched on the application of TBF from DIDSON echograms to bioenergetic studies by showing that TBF estimates were higher for salmon during a falling tide compared with a rising tide. While several studies have examined the swim speed capabilities of lamprey species in the laboratory (Mesa et al. 2003; Katopodis and Gervais 2012), few studies have tested specific questions about the energetic implications from *in situ* estimates of swimming metrics such as TBF (Standen et al. 2002). Although swim speed measurements like ‘critical swim speed’ can be considered an indicator of both swimming endurance and aerobic capacity (Katopodis and Gervais 2012), it does not describe the amount of work that fish are exhibiting relative to environmental conditions like TBF or swimming efficiency (Hinch and Rand 2000).

Our estimates of TBF and swimming efficiency have provided new insight into how Pacific Lamprey swimming capacity may limit success at passage bottlenecks. While TBF estimates from this study support the 'velocity barrier' hypothesis previously suggested for why fishway entrances limit passage (Moser et al. 2002b; Johnson et al. 2012; Keefer et al. 2013b), the physiological demands from burst swimming that short distance (~10 m) are likely small. The high TBF estimates through the John Day Dam collection channel also supports the 'velocity barrier' hypothesis, but the sustained high-velocity fields (~80 m) at this location likely exceed lamprey swimming endurance. Hence, the endurance capabilities of swimming at burst or critical swim speed will be important for understanding whether potential energetic barriers constrain lamprey passage. In contrast, the low TBF's observed in transition areas suggests a 'guidance limited' hypothesis rather than passage limitations associated with swimming capacity. These methods would be beneficial in identifying potential locations for velocity reduction experiments to improve lamprey passage, such as in the John Day Dam collection channel.

Quantifying the endurance time associated with the maximum TBF of lamprey would be an important step for understanding whether potential bottlenecks exhibit short-duration (entrances) or long-duration (collection channel) energetic costs. Additionally, TBF could be linked with physiological metrics such as oxygen consumption or metabolic rate (Standen et al. 2002; Steinhausen et al. 2005), which could be used in conjunction with *in situ* observations to directly estimate the physiological costs of particular environmental conditions (e.g., high velocity entrances).The methods described here offer a promising tool both for the basic and applied aspects of understanding and improving fish migrations, particularly at anthropogenic barriers.

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Table 3.1: Locations, deployment abbreviations, orientation of the DIDSON, and description of captured sample volumes.

Dam	Location	Years	Orientation	Sample volume description
Bonneville	North Downstream Entrance (NDE_P)	2011 2012	Portrait	Upper-water column aimed perpendicular to channel
	North Downstream Entrance (NDE_L)	2012	Landscape	Upper and lower-water column aimed perpendicular to channel
	Junction Pool West (JP_W)	2012	Landscape	Upper, middle, and lower-water column aimed to the Southwest
	Junction Pool North (JP_N)	2012	Landscape	Upper, middle, and lower-water column aimed perpendicular to channel to the North
	Transition area (JPU_LONG)	2013	Landscape	Upper and lower tilts aimed perpendicular to channel at a pool and weir section
	Transition area (JPU_SHORT)	2013	Landscape	Upper and lower tilts aimed upstream parallel to channel at a pool and weir section
John Day	North fish ladder entrance (JD1_P)	2012 2013	Portrait	Mid-water column aimed perpendicular to channel
	Bollard field (JD1_ELONG)	2012	Landscape	Upper and lower tilts aimed downstream parallel to channel into the bollard field
	North fish ladder entrance (JD1_XSECT)	2012 2013	Landscape	Upper and lower tilts aimed perpendicular to channel inside of entrance
	Collection channel (JD2_XSECT)	2012	Landscape	Upper and lower tilts aimed perpendicular to channel inside collection channel
	Collection channel (JD3_XSECT)	2012 2013	Landscape	Upper and lower tilts aimed perpendicular to channel inside collection channel
	Transition area (JD4_XSECT)	2012 2013	Landscape	Upper and lower tilts aimed perpendicular to channel just downstream of a pool and weir section
	Transition area (JD4_UP)	2012	Landscape	Upper and lower tilts aimed upstream parallel to channel at a pool and weir section

Table 3.2: Quantity of DIDSON footage analyzed, collected, number of Pacific Lamprey events observed for all files, mean event rates per hour across all files, and percent attachment events observed for all nighttime data at each location.

Dam	Location (year of data)	Number of files watched	Hours watched	Total hours collected	Number of total events	Mean event rate per hour	Percentage of attachment events
Bonneville	NDE_P (2011)	231	38.5	87.1	140	3.6	0
	NDE_L (2012)	98	16.3	34.0	516	29.82	4.1
	JP_W (2012)	109	18.2	56.0	503	27.69	0
	JP_N (2012)	124	20.7	42.0	38	1.84	0
	JPU_LONG (2013)	65	10.8	46.5	496	46.34	0
	JPU_SHORT (2013)	69	11.5	61.3	112	9.74	0
John Day	JD1_P (2012)	68	11.3	26.0	54	4.80	1.9
	JD1_ELONG (2012)	28	4.7	25.0	95	21.11	74.7
	JD1_XSECT (2012, 2013*)	57	9.5	41.0	78	8.21	1.3
	JD2_XSECT (2012)	55	9.2	26.0	48	5.45	0
	JD3_XSECT (2012, 2013*)	56	9.3	25.0	40	4.28	2.5
	JD4_XSECT (2012, 2013*)	55	9.2	40.0	15	1.64	0
	JD4_UP (2012)	57	9.5	19.0	1	0.11	0

*Data from 2012 and 2013 were only combined for TBF and swim efficiency estimates.

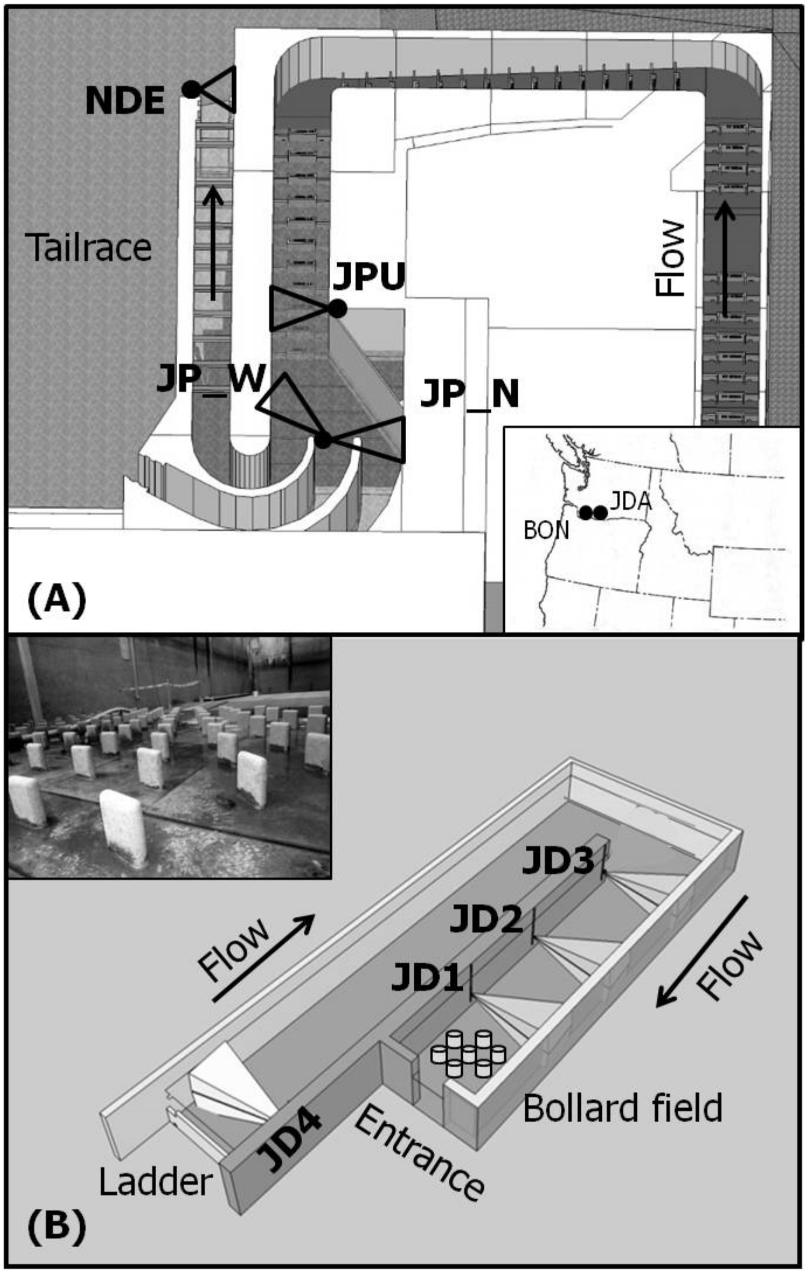

Figure 3.1: Three-dimensional diagrams of lower fishway segments monitored in this study at (A) the Washington-shore fishway at Bonneville Dam and the (B) John Day Dam north fishway. Monitoring sites at Bonneville Dam included (downstream to upstream): the north downstream entrance (NDE), the two junction pool locations (JP_W and JP_N), and the transition area (JPU) at the base of the fish ladder. Monitoring sites at John Day Dam included (downstream to upstream) a location near the fishway entrance (JD1), two locations inside the collection channel (JD2, JD3) downstream of the turn-pool, and in the transition area (JD4) near the first orifice and overflow section of the fish ladder. Triangles represent approximate sample volumes for each location.

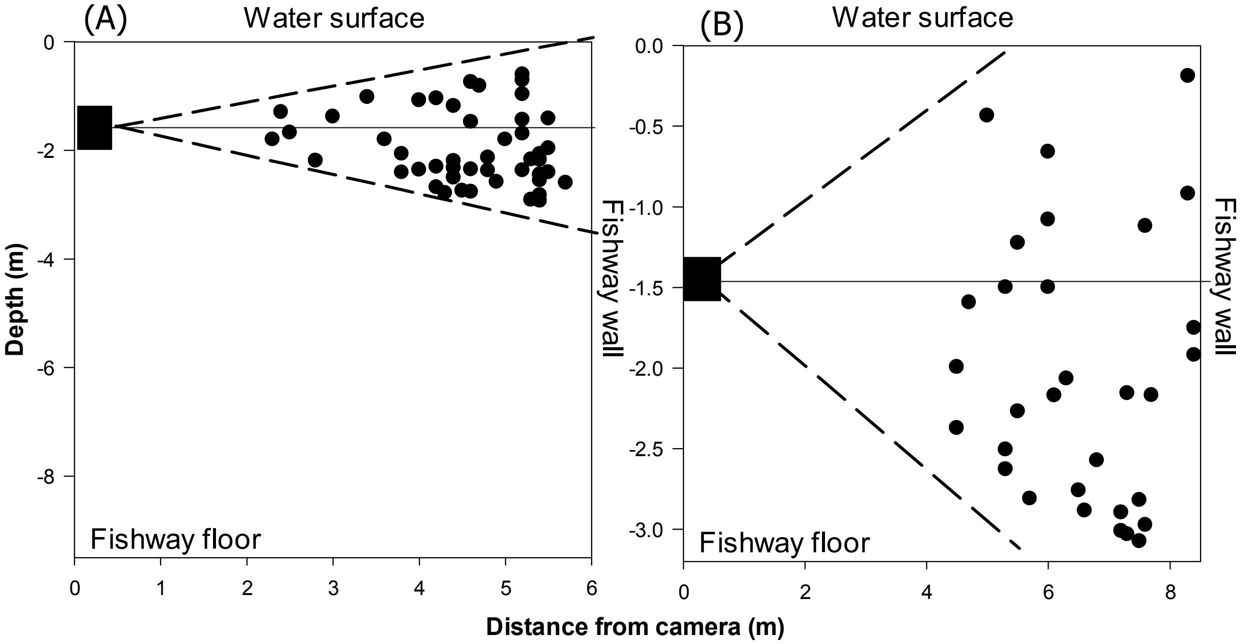

Figure 3.2: Vertical distribution of Pacific Lamprey targets (●) from DIDSON files during a single night at the (A) north downstream entrance at Bonneville Dam (NDE_P) and (B) the John Day Dam north fish ladder entrance (JD1_P). Black squares represent the DIDSON and dotted lines represent sample volumes with the camera in portrait orientation. Note differences in camera depth (y-axis) and distance from camera (x-axis) for the two locations.

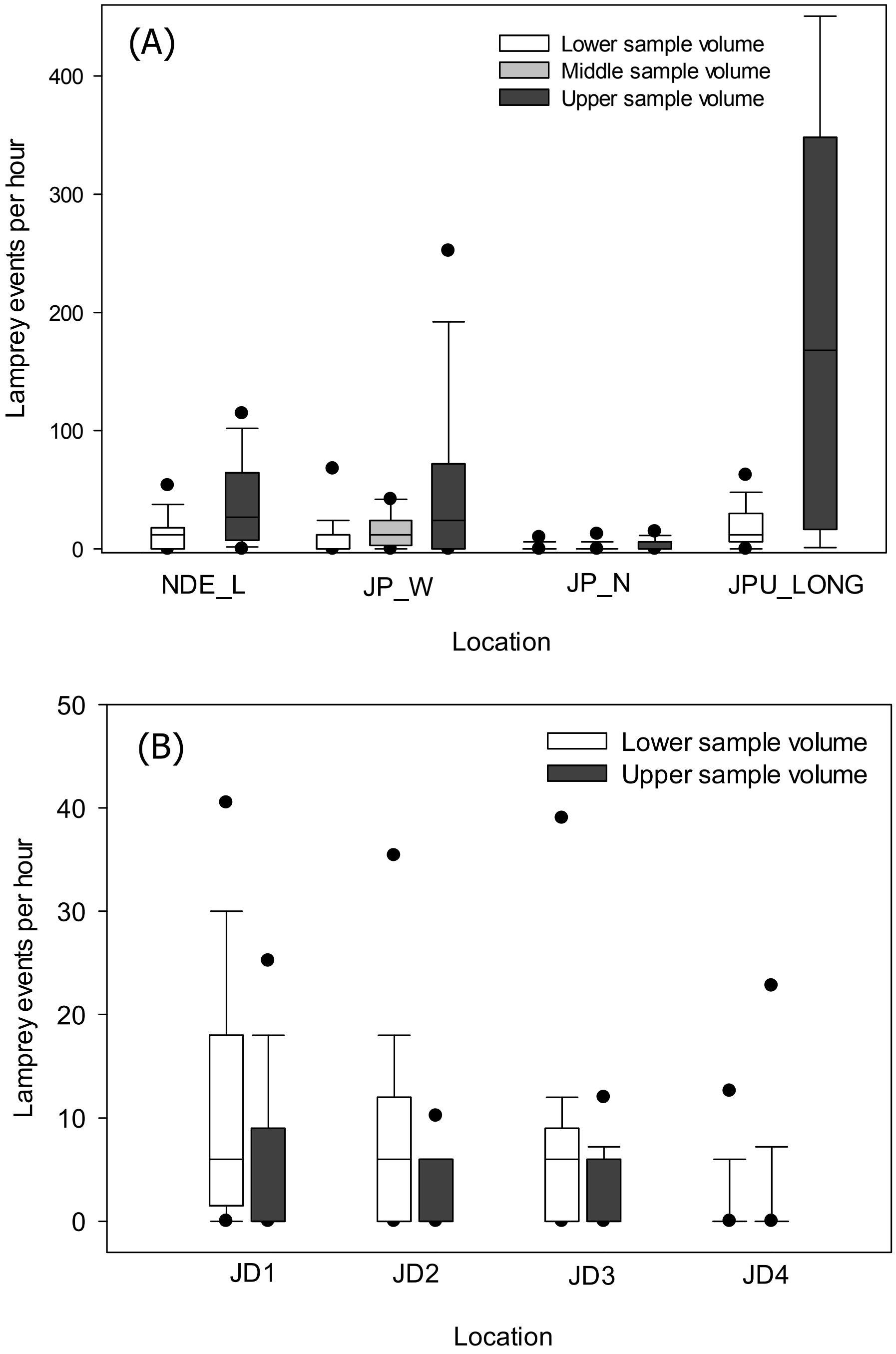

Figure 3.3: Vertical distributions of Pacific Lamprey event rate from ten minute DIDSON files in landscape orientation at (A) Bonneville Dam and (B) John Day Dam. Lower, middle, and upper sample volumes refer to deployments that occurred in the lower, middle, and upper water column. JP_W denotes junction pool west and JP_N denotes junction pool north locations. Box plots show 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles.

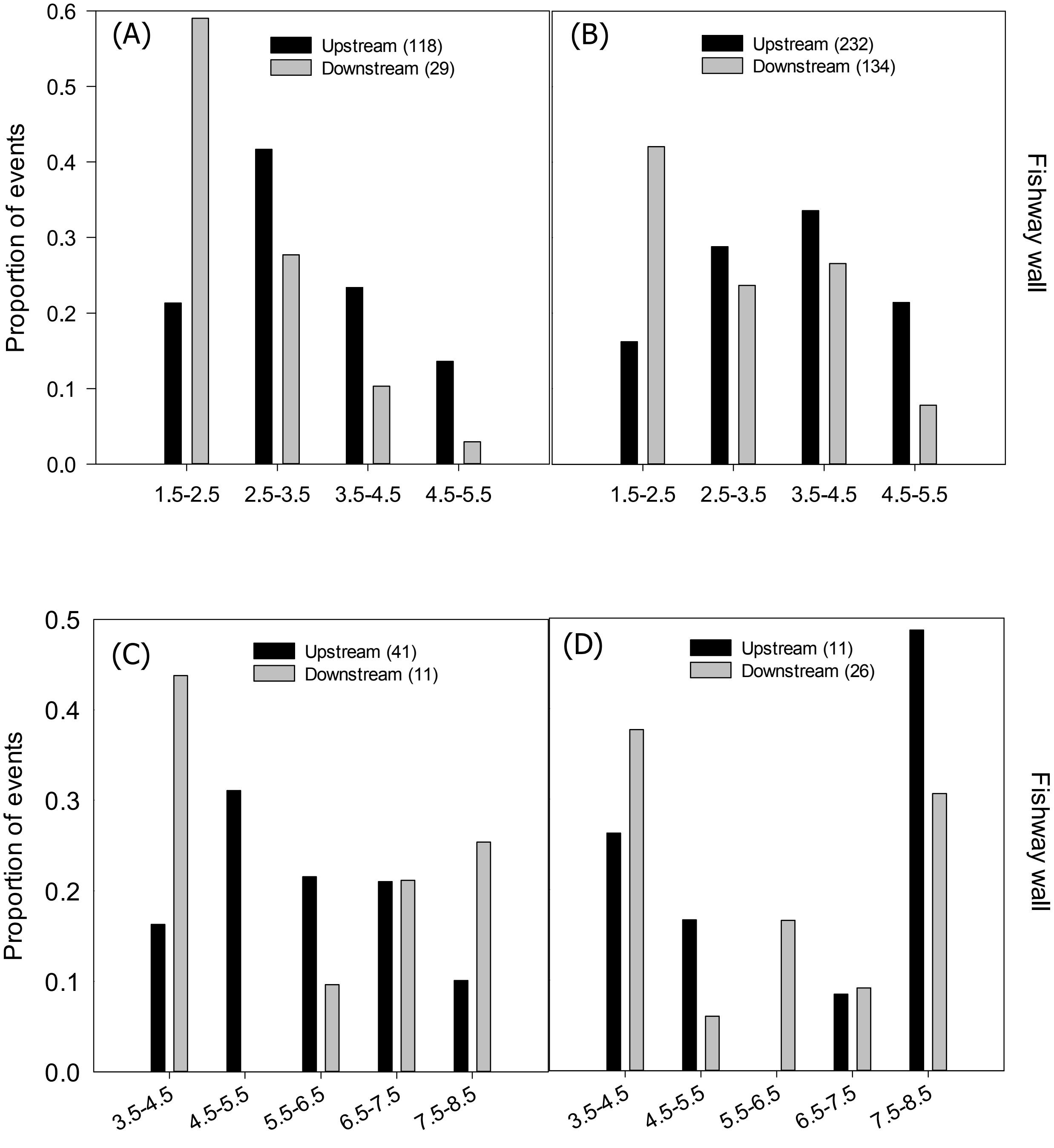

Figure 3.4: Weighted estimates of the lateral distribution of Pacific Lamprey movements (upstream, downstream) in the (A) lower and (B) upper sample volumes at NDE at Bonneville Dam and in the (C) lower and (D) upper sample volumes at JD1 at John Day. Numbers of upstream and downstream events are in parentheses. Weighted estimates account for differences in the DIDSON's sample volume. Maximum distance from camera at 5.5 and 8.5 m for each figure represents the opposing fishway wall.

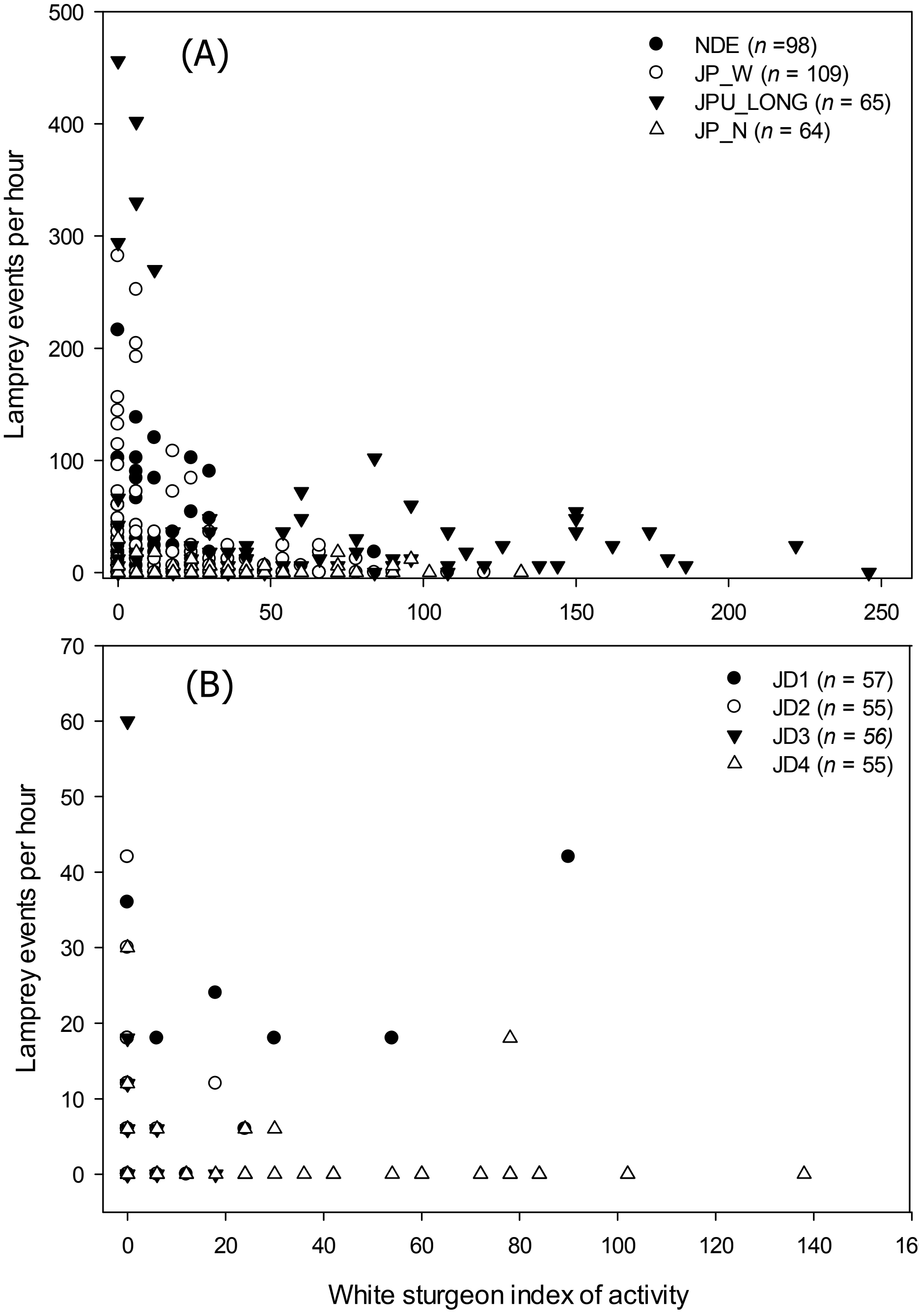

Figure 3.5: Relationship between number of Pacific Lamprey events per hour and a White Sturgeon index of activity per hour at four locations at (A) Bonneville Dam and four locations at (B) John Day Dam. Each point represents a ten minute file and numbers in parentheses are number of files watched for each location.

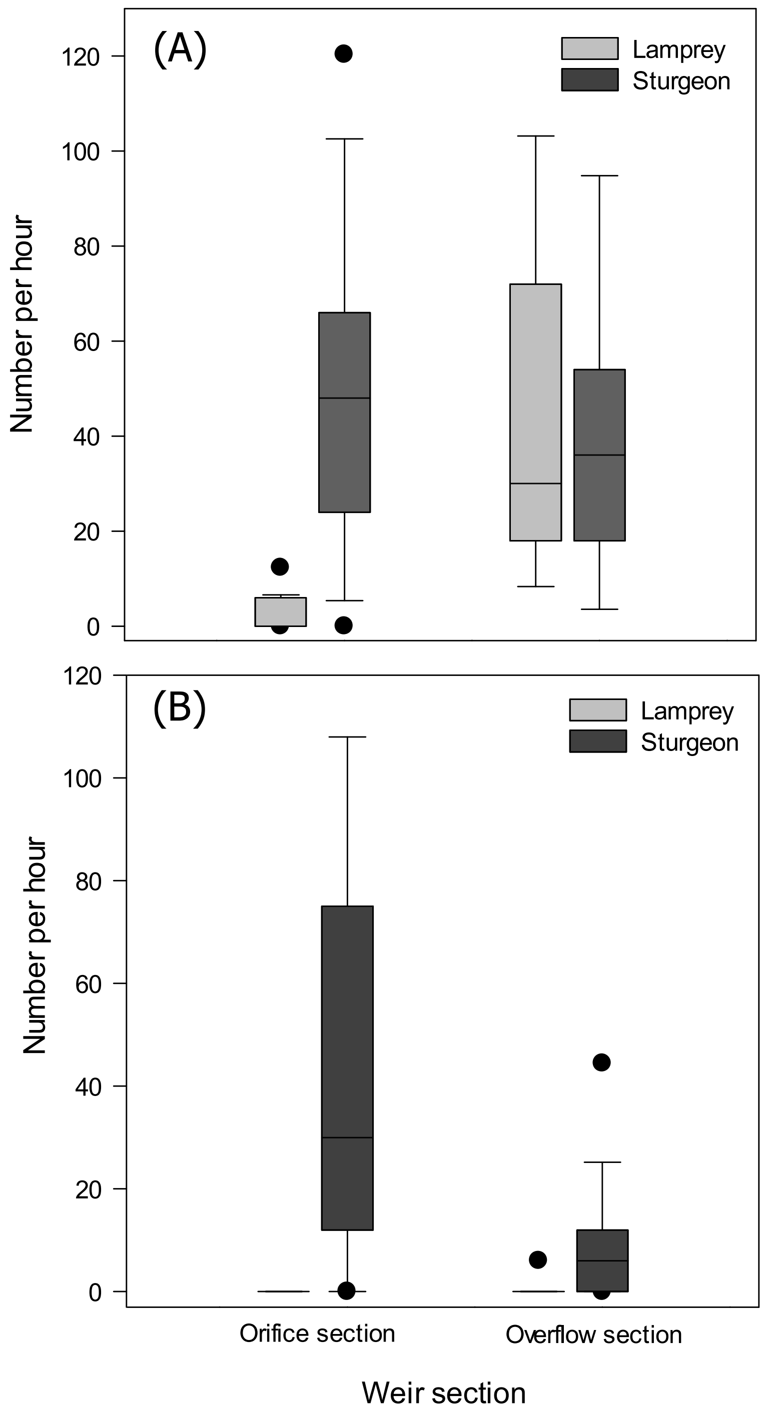

Figure 3.6: Distribution of Pacific Lamprey events per hour and White Sturgeon index of activity per hour at the orifice section (lower sample volumes) and overflow section (upper sample volumes) in the transition area at (A) Bonneville Dam (JPU_SHORT) and (B) John Day Dam (JD4_UP). Box plots show 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles.

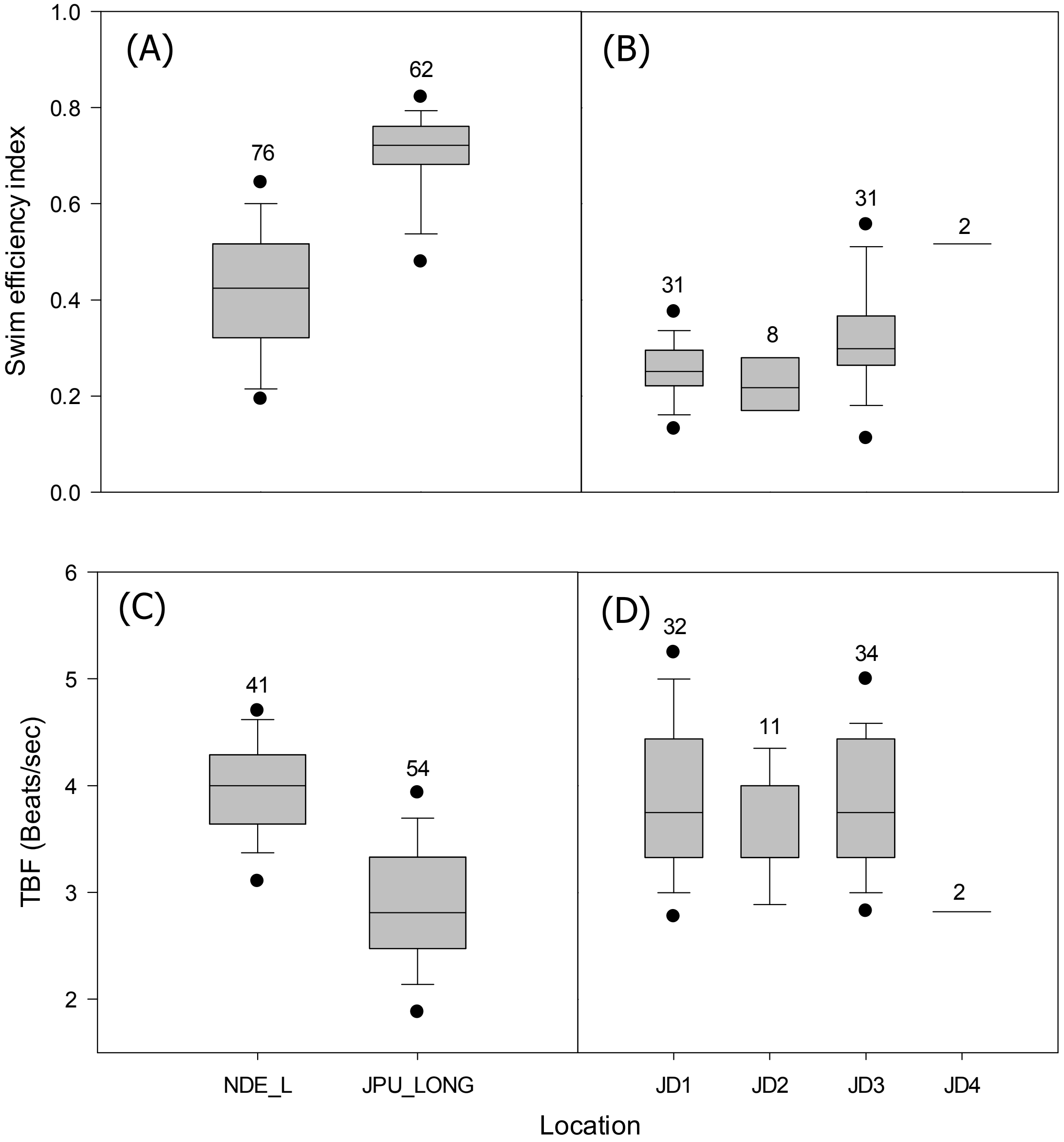

Figure 3.7: Swimming activity measures of swim efficiency index (A and B) and tail beat frequency estimates (C and D) for individual Pacific Lamprey at Bonneville Dam (A and C) and John Day Dam (B and D) locations. Box plots show 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles. Numbers above bars are sample sizes of fish.

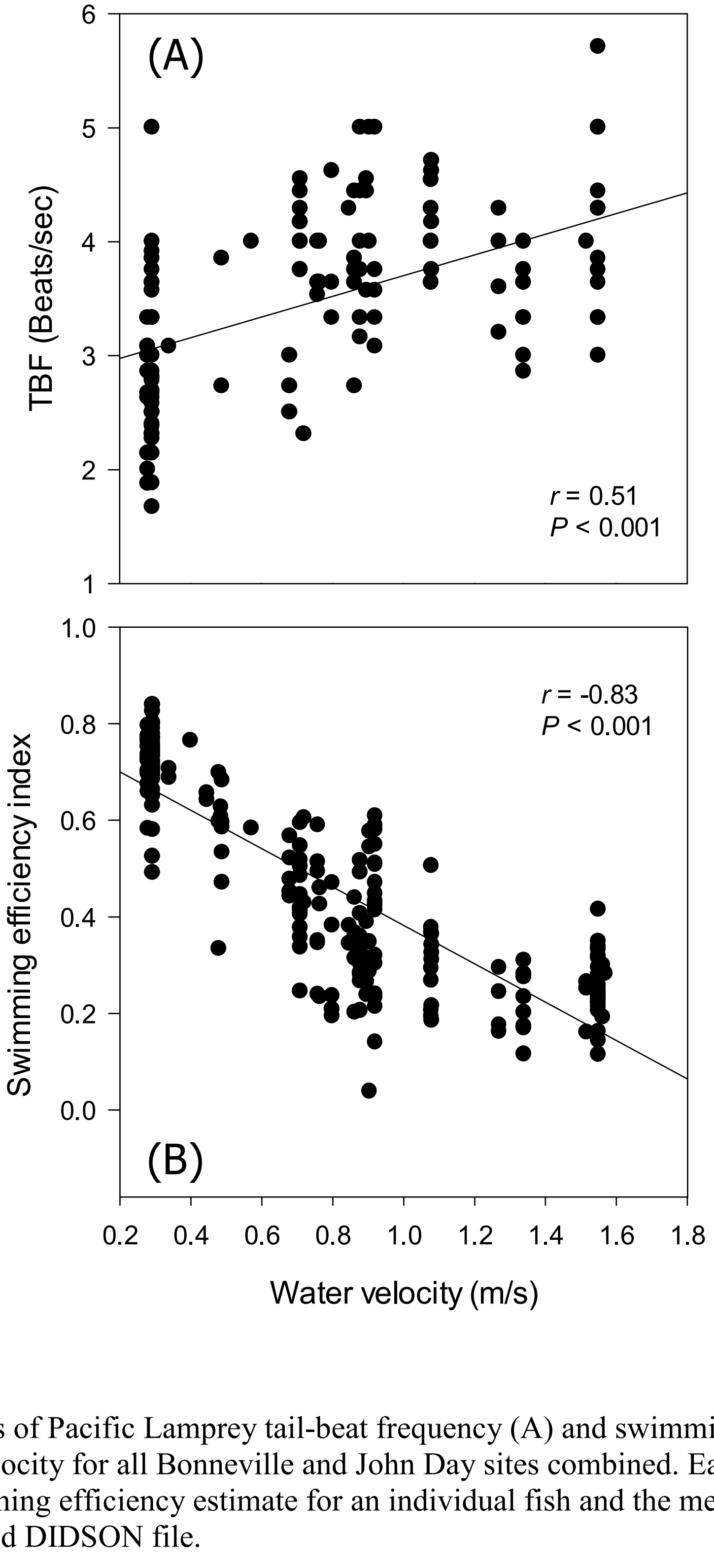

Figure 3.8: Plots of Pacific Lamprey tail-beat frequency (A) and swimming efficiency (B) versus water velocity for all Bonneville and John Day sites combined. Each point represents a TBF or swimming efficiency estimate for an individual fish and the mean water velocity for the associated DIDSON file.

Chapter 4

Pacific lamprey swimming behavior and performance in relation to passage barrier velocity, distance, and turbulence

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Abstract

Many species of freshwater lamprey have difficultly passing migratory barriers with volitional fish passage. The general conclusion has been that current fish passage designs do not adhere to the behavior and swimming characteristics of anguilliform swimmers, because these systems were designed specifically for sub-carangiform swimmers like salmonids (*Oncorhynchus* spp.) The objective of this study was to test the behavior and performance of Pacific lamprey (*Entosphenus tridentatus*) with regards to different passage conditions associated with velocity, turbulence, and barrier distance (e.g., slot length). We evaluated Pacific lamprey behavior and performance in an experimental flume where we simulated the hydraulic and structural conditions associated with a known passage bottleneck (serpentine weirs) for this species. Compared with past observations, success rates were surprisingly high for the majority of treatment conditions (>80%). We observed a turbulence × distance interaction, in which the lowest success rates were observed for the high turbulence, long barrier distance treatments (69%) compared with high turbulence treatments at shorter distances (>90%). A similar interaction was observed for the propensity and duration of which lamprey used their oral disc to attach to the substrate whereby attachment increased non-linearly under high velocity, high turbulence treatments. There was no indication body size affected passage probability. Although challenging conditions at local barriers do not

appear to limit Pacific lamprey passage, the strenuous conditions observed under some treatment conditions (high velocity and turbulence) suggest that poor passage may be manifested through cumulative effects. Specifically, the energetic costs associated with traversing ~15 serpentine weirs in the fishway may exceed the endurance-capabilities of this species. Future studies quantifying elements of Pacific lamprey swimming capacity, such as endurance and fatigue, will assist in further elucidating the potential mechanisms responsible for passage failures.

Introduction

Evaluating how fish respond to their hydraulic environment can be challenging given that fish behavior is as dynamic as the physical environment itself. The biological consequences of hydraulic parameters can often be a ‘double-edged’ sword with both positive and negative consequences. Water velocity, for example, has been frequently cited as an important factor that affects the swimming performance of fishes since high velocities require substantial energy use (Haro et al. 2004; Kemp et al. 2010; Peterson et al. 2013). Turbulence has also been shown to negatively influence swimming performance due to increased energetic costs of traversing chaotic flows (Enders et al. 2003, 2005). However, both water velocity and turbulence can also positively influence fish behavior by creating attraction cues and increasing individual motivation (Castro-Santos 2004; Silva et al. 2012). Additionally, these hydraulic components are not independent from one another, and each is influenced by other elements of the physical environment such as water depth, substrate, and channel structure (Guiny et al. 2005; Rodriguez et al. 2006; Goettel et al. 2014). Hence, discerning how multiple elements of the physical environment interact plays an important role in determining the physiological and behavioral responses elicited by fish.

From an applied perspective, understanding the biological-physical interactions that occur has been critical for predicting fish behavior in the face of anthropogenic change. The natural flow regimes of river systems worldwide have been altered due to the construction of anthropogenic barriers. Barriers such as hydropower dams have elicited numerous environmental changes, including disruptions to ecosystem processes and loss of freshwater biodiversity (Poff and Hart 2002; Poff et al. 2007). One of the most frequently cited concerns is the obstruction of river corridors for migratory fish species, for which the primary solution has been the development of fish passage systems (Clay 1995; Bunt et al. 2012; Noonan et al. 2012). However, one of the greatest challenges to developing efficient fish passage has been ensuring that passage systems are "transparent" or "porous" and allow for the passage of a diversity of fishes (Moser et al. 2011).

Fish size, morphology, behavior, and life-history variation occurs both within and among species, and such variation should be accounted for when implementing passage designs. Past research efforts have primarily focused on economically valuable species (e.g., salmonids, clupeids), whereas less charismatic species have been largely overlooked (Kemp et al. 2012, Silva et al. 2012). Morphological differences between species are particularly important to consider in fish passage design because morphology affects elements of swim speed, endurance, and fatigue (Katopodis and Gervais 2012). Swimming capacities can be positively correlated with body size (Rodriguez et al. 2006) and some evidence suggests that large barriers act as size-selective filters against smaller individuals (Agostinho et al. 2007; Keefer et al. 2009). Hence, previous focus on passage criteria for single taxa (e.g., salmonids) has unintentionally selected against other species. This problem has appeared worldwide, and consequently large proportions of native fish assemblage have difficulty in traversing passage systems (Moser et al. 2002a; Mallen-Cooper and Brand 2007).

The Columbia River, one of the most highly regulated river systems in the world, is one location where a recent focus has been placed on improving the passage of a non-salmonid species. Efforts to improve the passage of native, anadromous Pacific lamprey (*Entosphenus tridentatus*) have grown in the last decade, in part due to the considerable population declines in their native range along the Pacific coast of North America (Moser and Close 2003). Anthropogenic barriers have been hypothesized as one of the leading causative agents for these declines; primarily on the Columbia River where poor passage of this species has been observed in salmonid-specific fishways (Moser et al. 2002a, 2002b; Keefer et al. 2013b). While Pacific lamprey are generally considered to have relatively low swimming capacity compared to salmonids (i.e., low burst swimming speeds), the species possesses a unique adaptation for overcoming migratory challenges. Pacific lamprey employ their oral disc for attaching to substrate during high-velocity and turbulent conditions (Keefer et al. 2010, 2011), which intuitively would provide fish a resting opportunity without being swept downstream.

Prototype lamprey-specific passage systems (LPSs; Moser et al. 2011), which provide inclined ramps for Pacific lamprey to scale using their oral discs, have been placed at several migratory barriers on the Columbia River to improve passage. These systems have been quite successful and are becoming a popular alternative for passing Pacific lamprey in the basin. However, future development of additional passage improvements will benefit from a mechanistic understanding of the limits on passage in technical fishways. While several studies have explored how water velocity and the structural components of fishways limit Pacific lamprey passage (Johnson et al. 2012; Keefer et al. 2010, 2011), a more basic understanding of how hydraulic conditions influence behavior is needed given the high frequency of passage bottlenecks observed at mainstem Columbia River dams (Keefer et al. 2013b).

The objective of our study was to simulate the conditions at a known location of poor passage (serpentine weirs), in order to identify the potential hydraulic and structural mechanisms that limit Pacific lamprey success. Serpentine weir sections within Columbia River fishways are a structurally and hydraulically dynamic environment, which potentially elicit a variety of different behaviors. Hydraulic complexity has been hypothesized as one of the potential mechanisms responsible for the high rates of Pacific lamprey passage failure through this fishway section (Clabough et al. 2012, Keefer et al. 2013b). We evaluated the effects of three different fishway features: velocity, turbulence, and weir distance in a factorial experiment to test for additive and interactive effects of these three treatment variables on elements of Pacific lamprey behavior and success. We hypothesized that success rates would be lower for treatment conditions of high velocity, high turbulence, and long distances.

We then tested three additional hypotheses regarding the potential biological mechanisms responsible for limiting success. First, we predicted that success would be positively related with metrics of body size because previous studies have shown that larger Pacific lamprey have higher rates of passage success at Columbia River dams and migrate farther distances (Keefer et al. 2009a, 2013c, Hess et al. 2014). Secondly, we predicted that longer distances, higher velocities, and higher turbulence would increase both the frequency of lamprey attachments and the time required to pass the experimental challenge. Finally, we hypothesized that lamprey movement paths would differ between treatment conditions. Specifically, we predicted fish would have more constrained swim paths in higher velocity and turbulence treatments.

Methods

Fish collection.- Adult Pacific lamprey (*n* = 300) were collected overnight from May 18, 2014 until July 12, 2014 from trap boxes adjacent to fishway weirs near the adult fish facility (AFF) at Bonneville Dam (45.6°N, 121.9°W) on the lower Columbia River. Fish were removed from traps the following morning and were held in large aluminum tanks (92 × 152 × 122 cm) that had constant, re-circulating river water. Lamprey were anesthetized with AQUI-S 20E using a concentration of 45-50 mL solution/50 L river water. Fish were weighed, measured for total length and body girth, and had a fin clip removed for complementary genetic studies. A measurement was also made between the two dorsal fins, which has been shown to be a potential marker for maturation status and energetic condition in Pacific lamprey (Clemens et al. 2009). A small incision was then made on the fish's ventral side and a half-duplex passive integrated transponder tag (HD-PIT tag; 4 by 32 mm, Texas Instruments, Dallas, Texas) was inserted into the body cavity. Tagged fish were allowed to recover for 8-12 h in the large holding tanks at densities less than 20 individuals per holding chamber prior to experiments. All surgical and handling methods were approved by the University of Idaho Animal Care and Use Committee. Further details on collection and tagging procedures can be found in Moser et al. (2002a) and Johnson et al. (2012).

Experimental Design.- Experiments were conducted in an experimental flume (11.6 m long by 1.2 m wide by 2.4 m high) inside the adult fish facility at Bonneville Dam. The flume consisted of a 9 m long experimental section with a 10% slope, a downstream chamber with a removable gate where fish could be acclimated prior to experiments, and an upstream chamber at the top of the flume with fyke nets that fish could enter, but not exit (Figure 4.1A). The flume was supplied with river water via two supply pipes that were capable of generating flow rates of 835 L/s. Further details of the flume can be found in Keefer et al. (2010, 2011).

We constructed an experimental section within the flume simulating a vertical slot design, which allowed for manipulation of all three treatment variables. We had three levels of velocity (2.4 m/s, 1.8 m/s, 1.2 m/s), three levels of distance (1 m, 0.66 m, 0.33 m), and two levels of turbulence (treatment and control) for a total of 18 treatment combinations with a target of three replicates per treatment combination. However, distance treatments were unbalanced due to several preliminary experiments that occurred during the final week of tests (Table 4.1). Velocity was manipulated by changing the flow rates of the supply pipes and by altering hydraulic head through wooden boards placed on the downstream overflow section. Treatment velocities were determined from measurements at the hydraulic jump of the weirs and along the flume floor using a Marsh-McBirney flow meter (Hach Company, Loveland, Colorado). Measurements indicated that velocities near the surface and velocities at the floor were relatively consistent (Appendix I).

A 3-m long false floor was fixed to the bottom and placed in the middle of the flume, and three pairs of 2.44 m high weirs were placed within this experimental section. Each weir was 33 cm long and 38 cm wide, which created a 46-cm wide slot that fish had to traverse under manipulated distances of 0.33 m, 0.66 m, and 1 m. The first set of weirs was permanently fixed in the false floor and represented the short distance treatment (Figure 4.1D), whereas the other two sets were removable in order to create the medium (Figure

4.1C) and long (Figure 4.1B) distance treatments. Two aluminum HD-PIT antennas were custom designed to slide into the frame of the first and last set of weirs to monitor individual fish movements. A 2.44 m high turbulence wall was placed ~1.5 m upstream of the weirs and projected halfway out into the flume (Figure 4.1). Turbulence was induced by the addition of this wall (treatment), which produced large, circular eddies upstream of the experiment weirs. The control tests involved removal of the turbulence wall.

Experimental Trials.- All experiments were conducted at night (2100-0520), since Pacific lamprey are primarily nocturnal during their migration phase (Keefer et al. 2010, 2013a). Five to six lamprey were first placed in the acclimation section of the flume for 10 minutes at low velocity conditions (1.2 m/s). The velocity was then raised to test conditions and fish were allowed to acclimate for another 10 minutes. 90 minute tests were initiated by raising the gate of the acclimation section, which allowed fish to move upstream through the flume. Lamprey movements and behaviors were video-recorded using infrared lamps and cameras placed on the upstream and downstream sections of the experimental weirs (X's in Figure 4.1). Placement of the cameras allowed for near full coverage of lamprey activity in the experimental weirs and ~ 0.5 m upstream and downstream of the weirs. Behaviors were tied to individual fish from time-stamps of video files and HD-PIT antenna records. Upon completion of each trial, the flume was drained and the position of all fish was noted. Fish were removed by hand from the fykes or netted from the flume, scanned for HD-PIT tags, and allowed at least 30 minutes recovery before being released upstream at Stevenson, WA following all experiments.

Data analysis.- Video and HD-PIT data were used to quantify six elements of Pacific lamprey performance and behavior; three at the trial-level (success, fallbacks, and percent of attachment events) and three at the individual fish level (approach time, weir passage time, and total passage time). Success was defined as the number of unique fish that passed the experimental weirs divided by the number of unique fish that approached the weirs in each trial; individuals that never approached the experimental weirs were censored from further analyses. The number of fallbacks was defined as the number of times in each trial that a fallback occurred from upstream of the weirs to the bottom of the weirs. The percentage of attachment events was the percentage of all observed upstream movements in a trial that involved an attachment by a fish, including all passage events for those individuals falling back and reascending. Approach time was measured as the time from the initiation of a trial (S1) until an individual fish approached the first downstream camera or the first detection at the downstream antenna (A1). Weir passage time (WPT) was calculated as the time when individual fish were first observed on the downstream camera or the first detection on the downstream antenna (A1) until an individual fish passed the last upstream camera or the last detection at the upstream antenna (P1). Finally, total passage time (TPT) was calculated as the time from the initiation of a trial (S1) until an individual fish passed the last upstream camera or the last detection at the upstream antenna (P1).

Swim tracks were estimated for a random subset of individual fish from each trial (6-9 fish per treatment combination) to provide more ‘fine-scale’ evaluations of Pacific lamprey responses to the hydraulic environment, and are presented here for descriptive purposes. A simple grid system was established within the experimental weirs consisting of 6 cells, (left-right; upper, middle, lower; each cell was 6.23 cm. × 31 cm.) to compare the movement of fish on each side of the flume (turbulence wall was adjacent to right side; Figure 4.1). Swim tracks were only selected for fish that had a known grid of entrance and exit and moved upstream. Fish were denoted as right-side or left-side based on their nose position relative to the flume centerline, and the lateral precision of these estimates was within +/- 2-3 cm.

Statistical analysis.- The experimental design was a split-split-plot because all variables were not replicated together. Instead, it was a restricted randomization design where each factor was hierarchically randomized. Distance was the whole plot factor that was randomized first, turbulence was the split-plot factor that was randomized next within distance treatments, and velocity was the split-split-plot factor that was randomized last within turbulence treatments (Kuehl 1999). This design minimized the time and effort associated with the logistical constraints of manipulating the distance and turbulence conditions for each experiment (Figure 4.1). Treatments were randomized within three blocks across time to account for potential seasonal differences in Pacific lamprey behavior. The blocking variable was defined as ‘run date’ and the blocking groups were early (trials from May 18 to June 3), middle (June 4 to June 25), and late (June 26 to July 12). All factors were treated as fixed effects. A linear mixed effects model (lmer, lme4 packages in R v. 3.0.3, R Development Core Team, 2014) was used to evaluate the effects of the different treatment conditions on the six behavioral metrics. The model equation was:

Behavior/Success = run date + distance + turbulence + velocity + distance × turbulence + distance × velocity + turbulence × velocity + distance × turbulence × velocity + Error1 (run date × distance) + Error2 (run date × distance × turbulence) + Error3 (residual sum of squares)

A mixed effects model ($\alpha < 0.05$) was necessary to account for the additional error effects from the different randomization levels at the whole plot and split-plot levels (Kuehl

1999). Data for the number of fallbacks and weir passage times were log-transformed to satisfy the assumption of homogeneity of variances. Further differences in treatment groups were analyzed using Tukey's post hoc test based on least square means comparisons (lsmeans package in R v. 3.0.3). We also used time to event analyses (survival package in R v. 3.0.3; Hosmer and Lemeshow 1999) to model potential relationships between approach times and passage times for all treatment conditions.

To test the hypotheses that Pacific lamprey success was dependent upon body size, we used logistic regression (GLM function with binomial distribution, R v. 3.0.3) with 'passed the weirs' and 'did not pass the weirs' as the two binary response variables. The logistic regression model included all three treatment variables, run date, fish weight, dorsal distance, and residual weight estimated from a linear regression between length and weight, hereafter referred to as 'condition' (Brodersen et al. 2008).

Results

Lamprey passage success

The majority of treatment conditions did not drastically influence Pacific lamprey success rates, which were relatively high for all treatments. The blocking variable of run date had a near significant effect on success ($F_{2,34} = 3.12$, $P = 0.057$) where mean success rates were lower for early run groups (69.8%) compared with the middle (84.9%) and late (88.3%) groups (Figure 4.2). No main effects from the three hydraulic variables had any significant effects on success (Table 4.2). The only significant hydraulic effect was a turbulence × distance interaction ($F_{2,34} = 4.35$, $P = 0.021$), where mean success rates within the high turbulence treatments were lowest for long distances (69.4% across all velocity treatments) compared with the short (91.7% across all velocity treatments) and medium distances (90.8% across all velocity treatments). Control turbulence treatments had increasing passage success with increasing distance (Figure 4.3A).

Multiple logistic regression analyses showed that the morphological metric of dorsal distance was the only significant predictor of passage probability. Odds ratio indicated that passage probability for lamprey increased 2.5 times for every centimeter increase in dorsal distance ($\chi^2 = 7.40$, $P = 0.007$). In contrast, the metrics of body weight and condition displayed no relationship with probability of success (Table 4.4).

Lamprey passage behaviors

Treatment effects on Pacific lamprey behavior were complex and differed among the five passage behaviors. Velocity had a significant effect on both the percent of attachment events for each trial ($F_{2,34} = 14.54$, $P < 0.001$; Figure 4.4) and weir passage times for individual fish ($F_{2,96} = 23.11$, $P < 0.001$; Figure 4.5), with both metrics increasing with higher velocities. The higher weir passage times were associated with fish being attached for longer durations rather than multiple passage attempts. There was also a significant turbulence × velocity interaction on weir passage times ($F_{2,96} = 5.85$, $P = 0.004$), whereby lamprey were observed holding nearly 3 times longer during high-velocity treatments in the turbulence treatment (12.5 minutes) compared with the turbulence control (4.7 minutes).

Relationships were similar between approach times and total passage times for the different treatment conditions (Table 4.3), which was a result of the correlation between these two metrics ($r = 0.74$, $P < 0.001$). There was a significant three-way interaction ($F_{2,174} = 4.75$, $P = 0.001$) and a distance × velocity interaction ($F_{2,171} = 2.66$, $P = 0.034$) on lamprey approach times. Specifically, passage times were twice as fast for short distance, high-velocity conditions under the turbulence controls (mean = 22.7 minutes) compared with turbulence treatments (mean = 46.4 minutes; Figure 4.6). However, this interaction was hard to interpret given the large variability observed in the individual approach time among fish (0.5-89 minutes). Pairwise comparisons revealed no differences between any of the treatment conditions (all $P > 0.115$).

Time to event analyses revealed no further insight into the potential relationships between approach times and treatment conditions. There were no significant differences in approach times for the survival curves of distance ($\chi^2 = 1.40$, $P = 0.507$), velocity ($\chi^2 = 0.60$, $P = 0.732$), or turbulence ($\chi^2 = 0.40$, $P = 0.507$). There was, however, a significant difference between the survival curves for run date with a higher probability of fish approaching the experimental weirs in the late season group ($\chi^2 = 6.70$, $P = 0.035$; Figure 4.7). Cox proportional hazard models including lamprey body size, treatment conditions, and run date revealed no additional relationships for either approach or total passage times.

The average number of swim tracks constructed for each treatment combination was 7.5; with most treatment combinations having 7-9 lamprey that fit the necessary criteria. Pacific lamprey appeared to show a propensity for moving upstream along the right side of the experimental weirs, regardless of treatment. In both turbulence treatments for the long and medium distances, nearly every fish exhibited one of two behaviors. Lamprey either 1) entered and exited along the right-side of the weirs or 2) entered along the left-side of the weirs before switching sides to exit on the right-side (Figure 4.8, 4.9). Hence, some preferential passage conditions may have existed on the right-side of the experimental weirs. There were two notable differences between the turbulence treatment and control pairs as well: at high velocity and short distance, lamprey were uniformly distributed under low turbulence and passed on the right side in the high turbulence treatment. The converse was true at medium velocity (Figure 4.8, 4.9). Although cameras may have been biased towards right side detections (Figure 4.1), the detection efficiency for cameras averaged 85% for all upstream movements based on comparisons with HD-PIT detections.

Discussion

Notes on lamprey behavior

Understanding how the hydraulic environment dictates patterns of behavior and passage has important biological consequences for any fish species. These cues are likely to be especially important for Pacific lamprey, given the nocturnal nature of their upstream migrations and the poor vision of most lamprey species (Binder and McDonald 2007; Keefer et al. 2013a). The majority of Pacific lamprey showed a preference for moving upstream while skimming along the fishway floor regardless of hydraulic condition, which reflects the demersal nature and use of tactile cues that has been previously documented for lamprey species (Keefer et al. 2010, 2011; Kemp et al. 2010). However, whether fish were demersal and free-swimming versus demersal and saltatory was dependent upon hydraulic conditions. Lamprey were observed most frequently free-swimming along the flume floor during low-velocity and low-turbulence conditions. We would also frequently observe lamprey approach the experimental weirs in the upper water column at the hydraulic jump during low velocity treatments, before “falling” straight down to the floor and moving upstream through the weirs along the bottom.

Pacific lamprey altered their behavior as velocity and turbulence increased, with a greater prevalence of saltatory movements under these treatments (i.e., lamprey exhibiting attach-and-burst movements; Figure 4.4, 4.5). These behavioral patterns have been previously observed under physiologically demanding passage conditions with high turbulence and high velocity for several different lamprey species (Kirk et al. Chapter 3; Quintella et al. 2004; Kemp et al. 2010). The most strenuous conditions observed in our study were under these treatments, where lamprey would hold location for long periods of time before incrementally bursting upstream to a new attachment point. This is likely an energy-conserving behavior (Hinch and Rand 2000), which minimizes the risk of lamprey being pushed downstream against the current and provides resting opportunities after periods of fatigue and exhaustion (Kemp et al. 2009, 2010). Lamprey may also employ attachment behaviors as a stabilizing mechanism given their lack of paired pectoral fins (Liao 2007), particularly under turbulent conditions with strong upwelling currents. These observations, along with behaviors from *in situ* fishway observations (Kirk et al. Chapter 3), have provided a behavioral framework for understanding how Pacific lamprey respond to more 'coarse-scale' hydraulic cues within fishway environments.

Swim track data on the other hand provided novel evidence for the ability of Pacific lamprey to detect 'fine-scale' changes within their hydraulic environment. Lamprey showed an affinity for both entering and exiting on the right side of the flume, and individuals that did enter the left side were most frequently observed exiting on the right side (Figure 4.8, 4.9). We propose two potential hypotheses for these movement observations. The first is that lamprey were responding to changes in hydraulic cues, which could include either avoidance or attraction to turbulent forces (Silva et al. 2012; Goettel et al. 2014). Eventually, hydraulic data collected from a complementary study using an Acoustic Doppler Velocimeter (ADV) will provide further insight into how Pacific lamprey detect and respond to flow properties surrounding the experimental weirs (*Syms et al., University of Idaho, unpublished data*). The second hypothesis is that Pacific lamprey follow a similar swim path of previous conspecifics (Goettel et al. 2014). Lamprey were frequently observed attached in groups within only a few centimeters of each other, especially in the high velocity and high turbulence treatments. It is possible that these two mechanisms are complementary, whereby lamprey use social cues to reduce the costs of finding low cost, passable routes identified by earlier lamprey. Regardless of the mechanism, these data provide preliminary evidence that Pacific lamprey movements are driven by hydraulic or biological cues at a scale smaller than previously documented within fishway environments.

The lower success rates in the early season group were likely a result of behavioral or physiological changes associated with temperature differences. During these early season trials, more lamprey were never observed making an approach to the experimental weirs than later in the season. Warmer water temperatures during late season trials may have raised the metabolic activity of lamprey and promoted faster upstream movement (Booth et al. 1997; Lee et al. 2003; Reinhardt et al. 2009). Warmer temperatures may have also promoted quicker recovery from potential tagging effects during late season trials (Wilkie et al. 1997). While success rates may have been higher if experiments lasted longer than 90 minutes, the vast majority of lamprey approached and attempted to pass during middle and late season groups. Early season Pacific lamprey exhibiting slower migration times has been observed previously in field populations and the specific mechanism, whether behavioral or physiological, remains unknown (Keefer et al. 2009a, 2009b).

An important limitation for our results was inferring how patterns of behavior were affected in the lower section of the flume downstream of the experimental weirs. The area below the weirs was not monitored, thus we only included those lamprey that approached the experimental weirs in our analyses. However, unidentified mechanisms in the downstream section of the flume may have explained other patterns of behavior. For example, the significant three-way interaction and distance by velocity interaction on approach times may have been determined by either downstream hydraulics, individual differences in fish recovery (Wilkie et al. 1997), or motivation (Casto-Santos 2004; Reinhardt et al. 2009). However, time to event analyses did not show any potential differences in attraction between treatments or any substantial tagging effect on treatment effects. Sampling error associated with small sample sizes may have been responsible in part for these patterns, especially given the high variability observed in approach times among fish (0.5-89 minutes). Regardless, our results do not indicate a systematic bias in behavior during approach that would likely have affected observations in the experimental weirs, which was the primary area of interest.

Lamprey passage implications

Poor passage of lamprey species is not restricted to the Pacific Northwest, but to river systems worldwide (Lucas et al. 2009; Russon et al. 2011; Foulds and Lucas 2013). The resulting conclusion has been that many fishway designs are inefficient for passing lamprey. The primary mechanism suggested for this inefficiency arises from the lower swimming capacity of lamprey compared with salmonid species, for which many of these passage structures are specifically designed (Clay 1995; Rodriguez et al. 2006). However, the overall success rates observed for Pacific lamprey in the fishway challenge simulated in this study were high (86.6% across all trials when excluding early run groups). In fact, the ability of Pacific lamprey to overcome the highly turbulent, high velocity conditions in these experiments is actually a testament to the species swimming capabilities. The high rate of success in our study contrasts with the poor performance observed for lamprey at other experimental challenges at similar scales (>50% success; Keefer et al. 2010, 2011; Kemp et al. 2010) and at the fishway site replicated in this study (Keefer et al. 2013b).

This apparent contradiction may have resulted from differences in fishway and experimental conditions and/or scale. An important assumption for any laboratory-based study is that the experimental conditions replicated matched the conditions observed in the natural environment. The serpentine weir design simulated in our study had a similar design to vertical slot weirs (Figure 4.1), which have been previously shown to be one of the more efficient passage designs among those tested for lamprey species, but only under operational criteria of low velocity and low turbulence (Laine et al. 1998; Foulds and Lucas 2013). Qualitative observations from video analysis at Bonneville Dam revealed that velocities in the serpentine weir sections actually appeared to be most comparable to the low or medium velocity treatments observed in the flume. Thus, we think that the spatial scale and hydraulic conditions in the single experimental weir were representative of the conditions in the fishway.

Serpentine weirs do not provide a linear passageway for fish to ascend, but rather consist of a ‘snake-pattern’ design that generates large eddies with highly chaotic flow patterns instead of laminar flows. The vortices and eddies induced by the turbulence treatment were representative of the conditions observed within the serpentine weir sections. The range of weir distances simulated in this study was also representative of the ranges observed within the fishway. Despite our effort to replicate similar hydraulic conditions, preliminary observations from video analyses revealed differences in Pacific lampery behavior between the flume and serpentine weirs. Field data suggested that many Pacific lamprey did not show a strong demersal preference, attachment events were relatively infrequent compared with flume studies, and many individuals were observed free-swimming (*Kirk and Dunkle, unpublished data*). Although differences in scale and 'edge effects' certainly may have driven the patterns of demersal behavior observed in the experimental flume given the narrower channel width, the scale of the experimental weirs themselves were representative of the weirs within the fishway.

Since our experiments likely captured the range of conditions present at individual slots, we propose three hypotheses for explaining why poor passage is observed for Pacific lamprey through these fishway sections. First, limited passage may be associated with weirs of long distances (~1 m) and turbulent conditions. Success rates through the short (0.33 m) and medium (0.66 m) weir treatments in this study were ~25% higher compared with the long (1 m) weir treatments under high turbulence conditions (Figure 4.3A). While it may be possible that the distance of the high velocity barrier is the mechanism of poor performance (Castro-Santos 2004, 2006; Haro et al. 2004), the similarly low success rates between the low and high velocities suggest a potentially different mechanism (Figure 4.3A). Our data suggests that some hydraulic interaction between the flow and structural environment (e.g., weir wall) may be responsible for inducing changes in Pacific lamprey performance. The reduced success may have been from greater shear stress and turbulence associated with the longer boundary layer for the long distance treatments (Vogel 1996; Weissburg 2000). The eddies generated from the turbulence wall also may have dissipated at a faster rate for short and medium treatments given the farther distance between the weirs and turbulence wall (Figure 4.1).

The second hypothesis is that the poor passage observed through these sections resulted from a cumulative effect of passing multiple weirs. Hence, we propose an ‘endurance-based’ hypothesis related to physiological exhaustion or motivation for explaining the patterns of poor passage. While Pacific lamprey only had to overcome one obstacle in these experiments, they must overcome ~15 of these weirs in the Washington-shore and Bradford Island fishways at Bonneville Dam (Figure 4.10). Preliminary observations intended to quantify Pacific lamprey response to exhaustive exercise revealed that experiments had produced physiologically demanding conditions for fish to overcome (Figure 4.11). When modeling the cumulative passage success of Pacific lamprey through the actual serpentine weir section based on the average passage success observed from our results for the slot length of each weir (91% for short and medium distances, 69% for long distances), a population of lamprey would suffer 92% attrition through all 15 weirs.

Interestingly, high passage rates through the pool-and-weir sections of the fish ladder at Bonneville Dam (~90%; Moser et al. 2002a) suggest that endurance is not a problem through these sections where velocity can be upwards of 2.4 m/s. However, resting areas present in these passage sections do not contain the turbulent and chaotic flows present within the pools between serpentine weirs. Pacific lamprey require 1-4 hours to recover from exhaustive exercise (Mesa et al. 2003), so the constant exposure to these high turbulence conditions during periods of recovery and attachment (e.g., Figure 4.5) may inflict additional endurance-based costs for lamprey (Enders et al. 2003, 2005). Further research is needed regarding the physiological costs of both attachment behaviors and saltatory movements, as well as how these behaviors may aid in physiological recovery while simultaneously under exhaustive conditions.

The third and final hypothesis is that the high rate of turning associated with the ‘snake-like’ design of serpentine weir sections may disrupt the orientation of Pacific lamprey. Fish must overcome 15 weirs involving sharp turns at either 90º or 180º (Figure 4.10A), which are conditions that are not present within natural riverine systems. Given that vision plays a minimal role in the migrations of lamprey species (Binder and McDonald 2007), Pacific lamprey may be dependent upon hydraulic cues and additional sensory systems for correct orientation (Quinn 2005; Putnam et al. 2013). Hence, these sections may present an orientation barrier to Pacific lamprey if the turbulent conditions dilute the necessary attraction flows for passing from weir to weir. Simple maze experiments could be conducted to test how the effects of turning would influence elements of behavior, aversion, and success.

Body size relationships

There was some marginal evidence to support the hypothesis that body size was a significant predictor of success, with passage probability being higher for Pacific lamprey with longer length-specific dorsal distances. Dorsal distance has been shown to be correlated with energetic status and maturation state in Pacific lamprey, in which shorter dorsal distances are associated with decreases in body weight (Clemens et al. 2009). This measurement has also been speculated as a morphological distinction between first year and second year migrants, since lamprey may spend an additional winter holding in riverine environments before spawning a year after beginning their migrations (Robinson and Bayer 2005). Hence, fish that have suffered a reduction in body weight or are physiologically close to spawning stage may be less motivated or lack the energetic capabilities required for traversing passage barriers. It is also plausible that this shorter distance may have altered some biomechanical feature resulting in lower performance for these fish. However, it is worth noting that there was a sampling bias towards shorter dorsal distances in long distance and high turbulence treatments (Appendix II). This was purely coincidental in that the high turbulence, long distance treatments overlapped with the earliest group of migrants and occurred during the peak migration days, where river conditions sparked large quantities of fish to initiate upstream movements (~200 fish being trapped). Future studies should seek to confirm the relationships between dorsal distance, energetic status, and passage.

The mechanisms proposed to explain the patterns of size-selective passage in the Columbia River basin have included larger Pacific lamprey having higher energetic condition, as well as the homing of larger lamprey to upstream locations (Keefer et al. 2009a, 2013c; Hess et al. 2014). While we provided indirect evidence here for the role of energetic condition in describing passage, we previously proposed two additional hypotheses regarding how size-selective processes could explain these patterns (Kirk and Caudill Chapter 2), which we revisit here. The first hypothesis was that larger lamprey have a higher amount of energetic reserves which allow them to overcome more migratory obstacles, subsequently referred to as the ‘energetic-threshold hypothesis.’ The second hypothesis was that larger lamprey have greater burst swimming capabilities which allow them to overcome migratory barriers more successfully, subsequently referred to as the ‘power hypothesis.’ Although numerous studies have documented a correlation between swimming capacity and body size (Beamish 1974; Haro et al. 2004), our results reject the ‘power hypothesis’ since this hypothesis would have predicted that smaller lamprey would have had lower success under the high velocity, high turbulence, and long distance treatments. Overall, our results suggest that these ‘size-selective’ hypotheses do not appear to be that important for describing Pacific lamprey passage success through difficult passage areas at a fine scale (Keefer et al. 2013b). Instead, body size effects may manifest through motivation and/or giving-up thresholds associated with passage attempts at multiple challenges.

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Table 4.1: Summary data for the number of replicates, number of Pacific lamprey, and the mean length of lamprey for each treatment in this study.

Factor	Level	Number of replicates	Number of lamprey	Mean length (cm)	Standard deviation
Velocity	2.4 m/s	18	102	68.2	4.81
	1.8 m/s	18	100	68.4	4.46
	1.2 m/s	18	98	67.6	4.16
Turbulence	Treatment	27	150	67.8	4.48
	Control	27	150	68.3	4.49
Distance	1.00 m	21	115	68.2	4.48
	0.66 m	16	90	67.7	4.79
	0.33 m	17	95	68.2	4.22

Table 4.2: Mixed model results for the effects of run date, distance (D), turbulence (T), and velocity (V) on the success rates, number of fallbacks, and percentage of all upstream events involving an attachment for all trials. Numbers below each metric indicate number of trials. Bolded values are significant (*P* < 0.05).

	Success (n = 54)				Fallbacks (n = 54)				Percent attachments (n = 54)			
Source	nDF	dDF	F	P	nDF	dDF	F	P	nDF	dDF	F	P
Run date	2	34	3.12	0.06	2	34	0.01	0.93	2	34	2.69	0.08
D	2	34	0.10	0.91	2	34	0.24	0.78	2	34	1.33	0.28
T	1	34	0.53	0.47	1	34	0.70	0.41	1	34	0.45	0.50
V	2	34	1.31	0.28	2	34	2.08	0.14	**2**	**34**	**14.54**	**<0.001**
D*T	**2**	**34**	**4.35**	**0.021**	2	34	1.20	0.31	2	34	0.43	0.66
D*V	4	34	1.08	0.38	4	34	0.63	0.64	4	34	1.71	0.17
T*V	2	34	0.53	0.59	2	34	1.21	0.31	2	34	0.76	0.47
D*T*V	4	34	0.20	0.94	4	34	0.15	0.97	4	34	1.61	0.19

Table 4.3: Mixed model results for the effects of run date, distance (D), turbulence (T), and velocity (V) on the total passage time, weir passage times, and approach times for all Pacific lamprey. Numbers below each metric indicate number of individuals. Bolded values are significant (*P* < 0.05).

	Total passage time (n = 170)				Weir passage time (n = 119)				Approach times (n = 197)			
Source	nDF	dDF	F	P	nDF	dDF	F	P	nDF	dDF	F	P
Run date	2	3	0.33	0.41	2	4	0.14	0.87	2	3	0.14	0.88
D	2	3	0.02	0.98	2	4	0.24	0.80	2	3	0.18	0.84
T	1	5	0.02	0.88	1	5	0.13	0.74	1	5	0.58	0.48
V	2	146	0.60	0.55	**2**	**96**	**23.11**	**<0.001**	2	172	0.01	0.99
D*T	2	5	1.73	0.27	2	5	1.24	0.37	2	5	1.21	0.38
D*V	4	147	2.03	0.09	4	97	1.01	0.41	**4**	**171**	**2.66**	**0.034**
T*V	2	147	0.40	0.67	**2**	**96**	**5.85**	**0.004**	2	172	1.73	0.18
D*T*V	**4**	**148**	**3.24**	**0.014**	4	96	1.78	0.16	**4**	**174**	**4.75**	**0.001**

Table 4.4: Multiple logistic regression results testing for the effects of Pacific lamprey body size on probability of passage. Bolded values are significant ($P < 0.05$).

Factor	χ^2	P	Odds ratio
Weight	0.15	0.694	1.00
Dorsal	**7.29**	**0.007**	**2.60**
Condition	0.14	0.706	6.30
1 m – 0.66 m	0.01	0.914	1.06
1 m – 0.33 m	0.47	0.491	0.71
1.2 m/s – 2.4 m/s	1.13	0.287	0.61
1.8 m/s – 2.4 m/s	0.75	0.386	1.67
Treatment - Control	0.01	0.913	0.96
Late-Early run date	2.07	0.150	2.10
Mid-Early run date	1.30	0.253	1.81

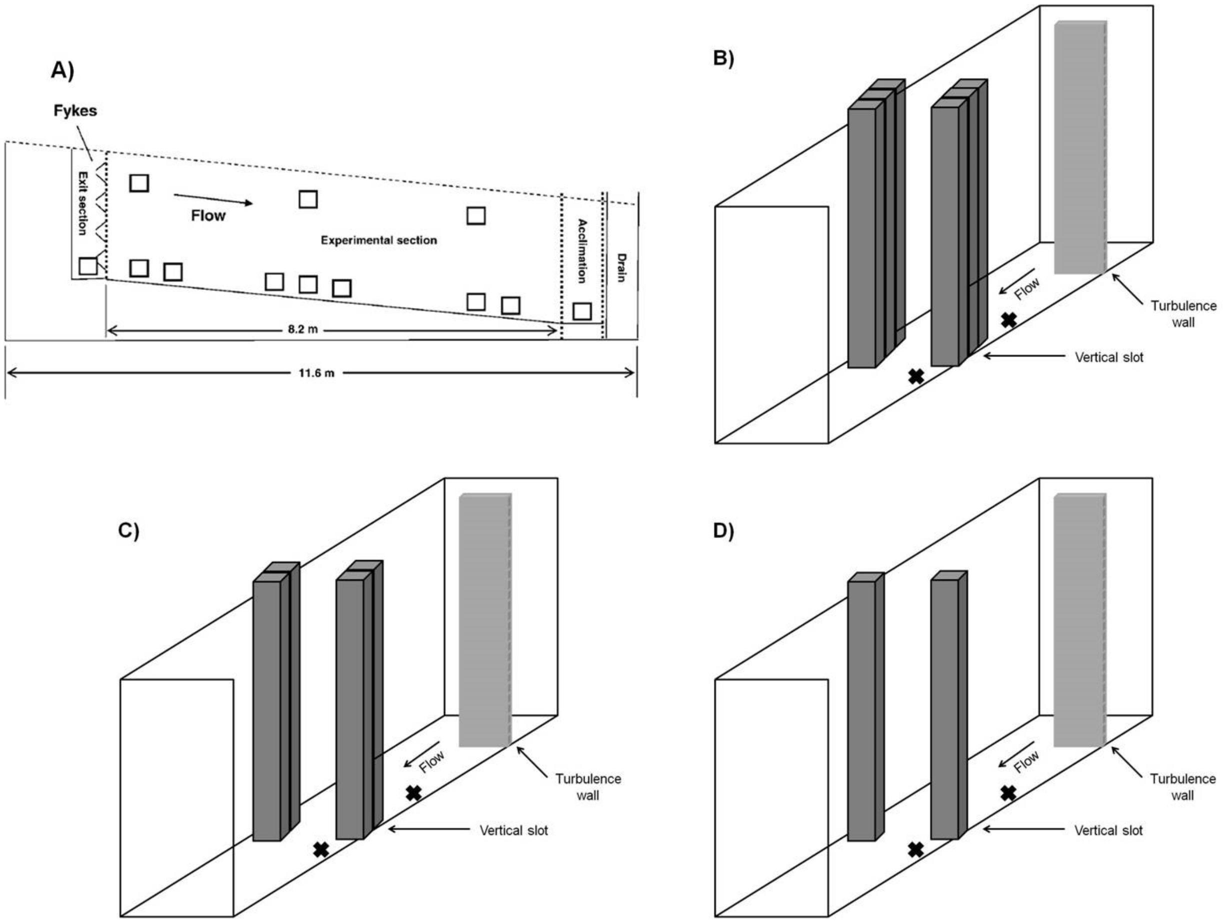


Figure 4.1: Upper left panel is a side view of the experimental flume (A) with the different flume sections. Three-dimensional cross sections of the flume show how the 6 experimental weirs manipulated a vertical slot design with varying distances of (B) 1.00 m (long), (C) 0.66 m (medium), and (D) 0.33 m (short). The turbulence wall was removed from the experimental flume for control treatments. X’s indicate the location of cameras. Note that the figure is not drawn to scale.

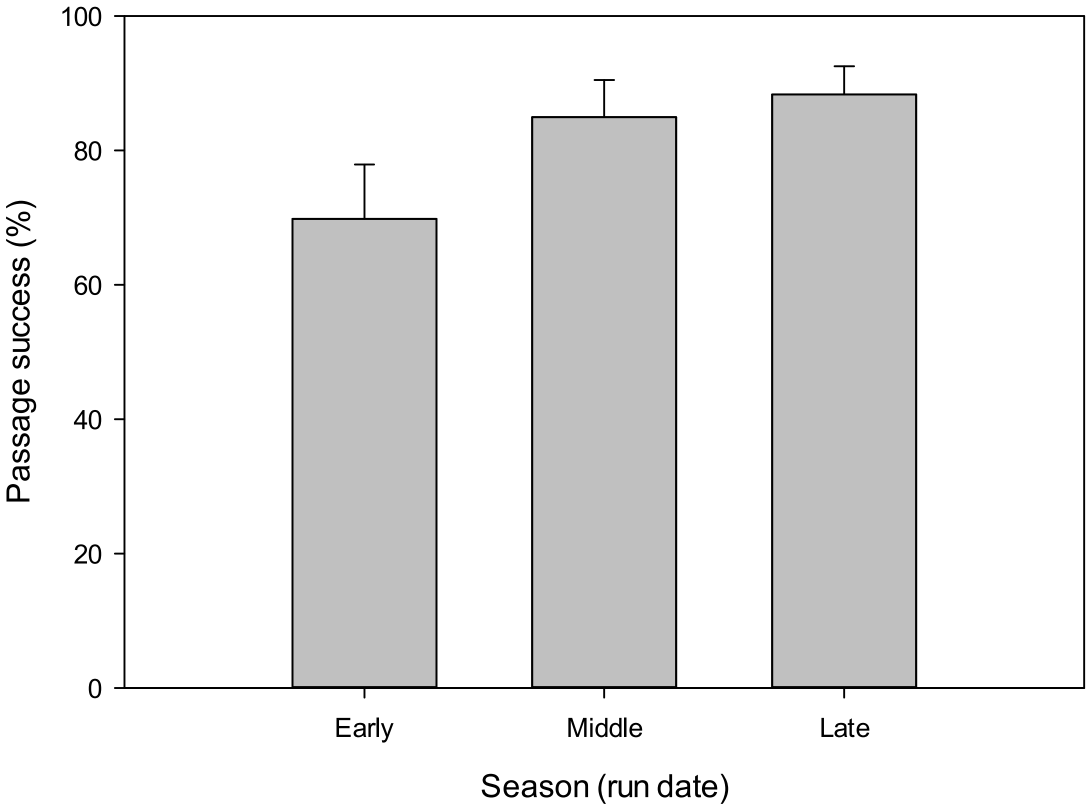


Figure 4.2: Mean success rates (+SE) for Pacific lamprey by the blocking variable run date.

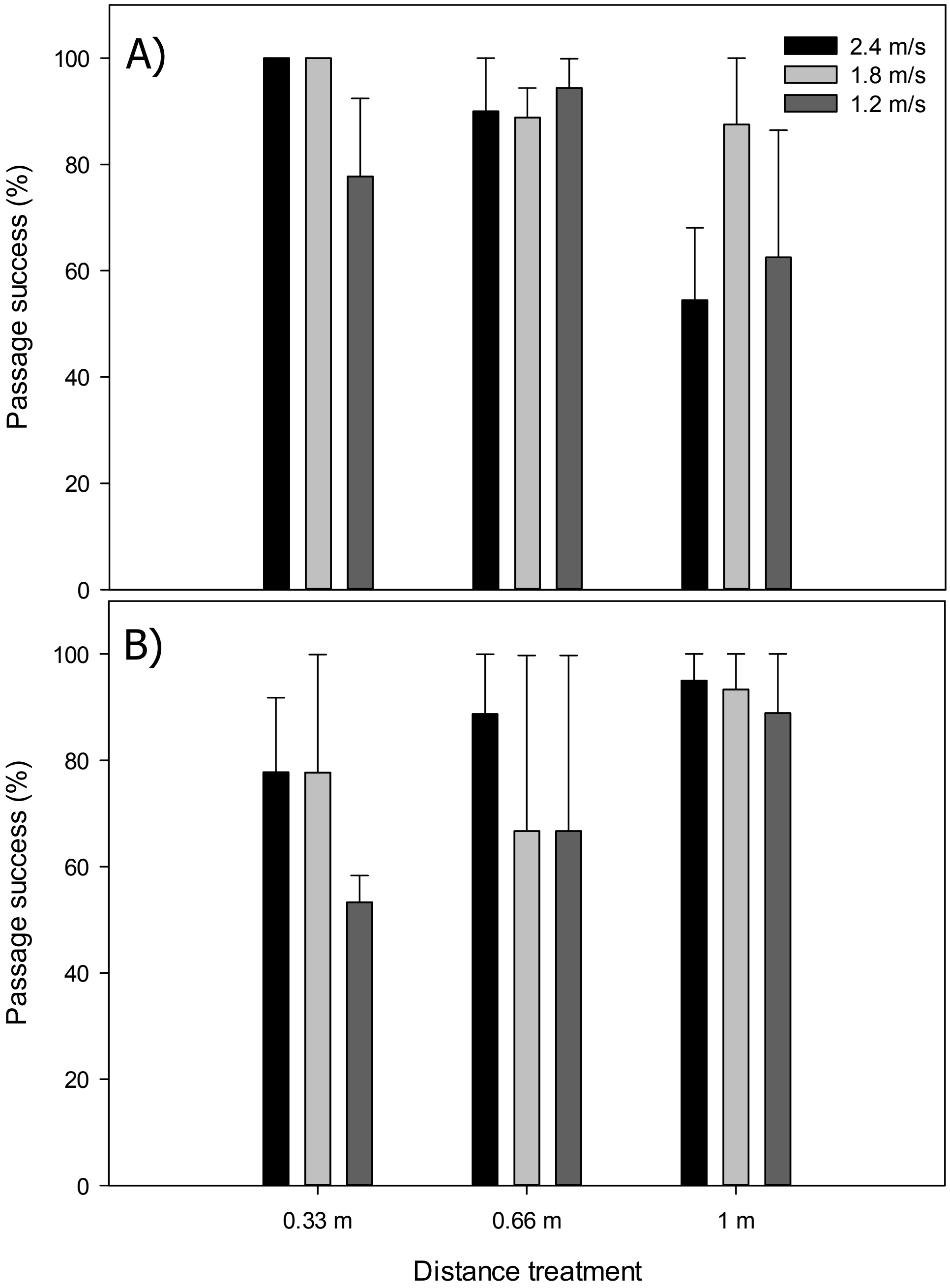


Figure 4.3: Mean success rates (+SE) of Pacific lamprey for all trials (*n* = 54) sorted by distance and velocity treatments for (A) turbulence and (B) control treatments.

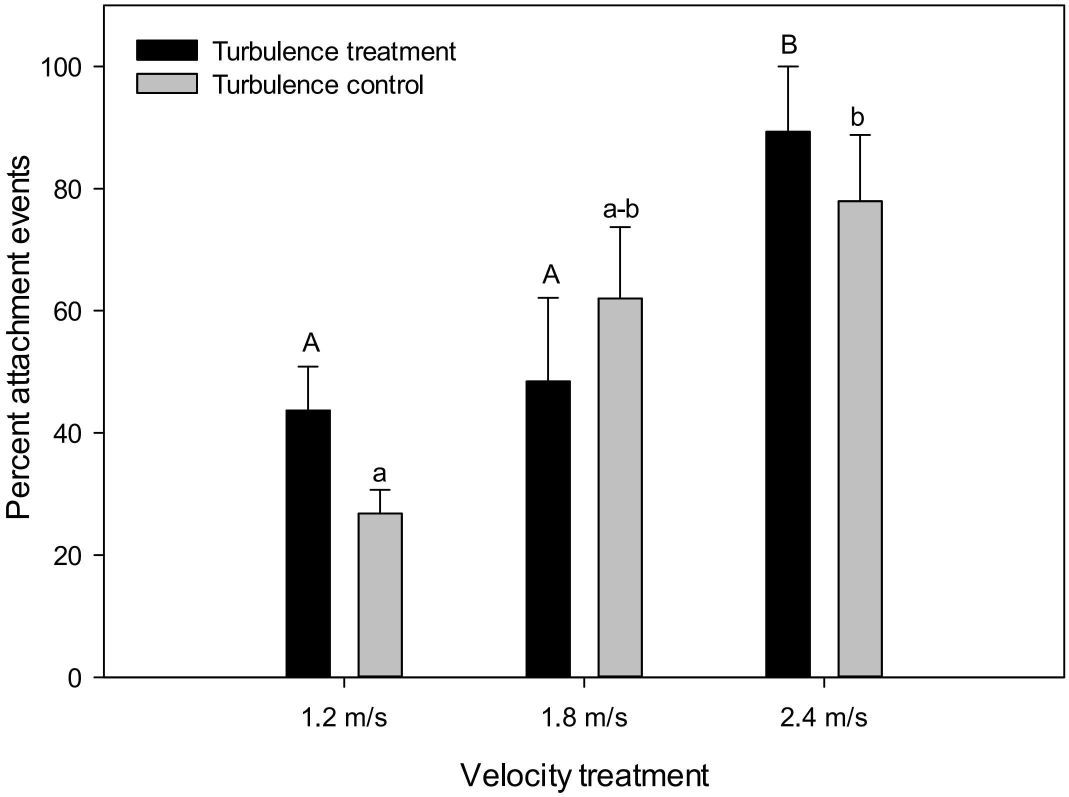


Figure 4.4: Percentage of upstream events (+SE) that involved an attachment for all velocity treatments sorted by turbulent (black) and non-turbulent (gray) conditions. Treatments with different capital letters were significantly different from each other within turbulent conditions and treatments with lower-case letters were significantly different from each other within non-turbulent conditions ($P < 0.05$)

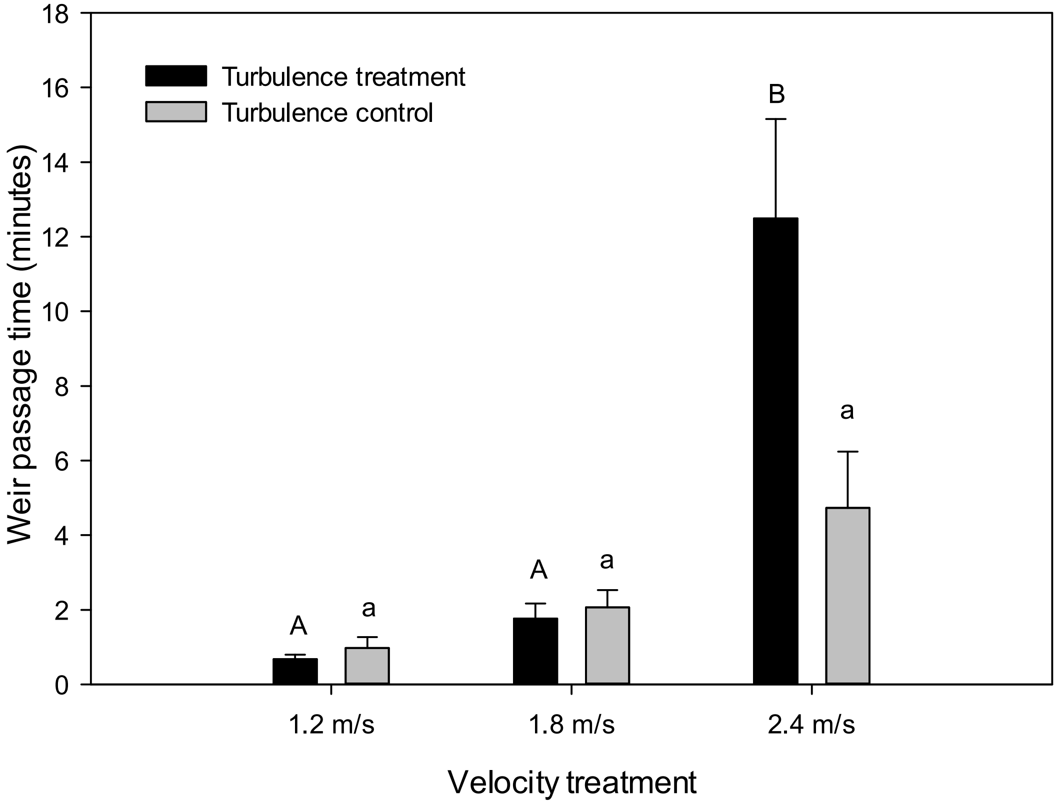


Figure 4.5: Weir passage times (+SE) for all velocity treatments sorted by turbulent (black) and non-turbulent (gray) conditions. Treatments with different capital letters were significantly different from each other within turbulent conditions and treatments with lower-case letters were significantly different from each other within non-turbulent conditions ($P < 0.05$).

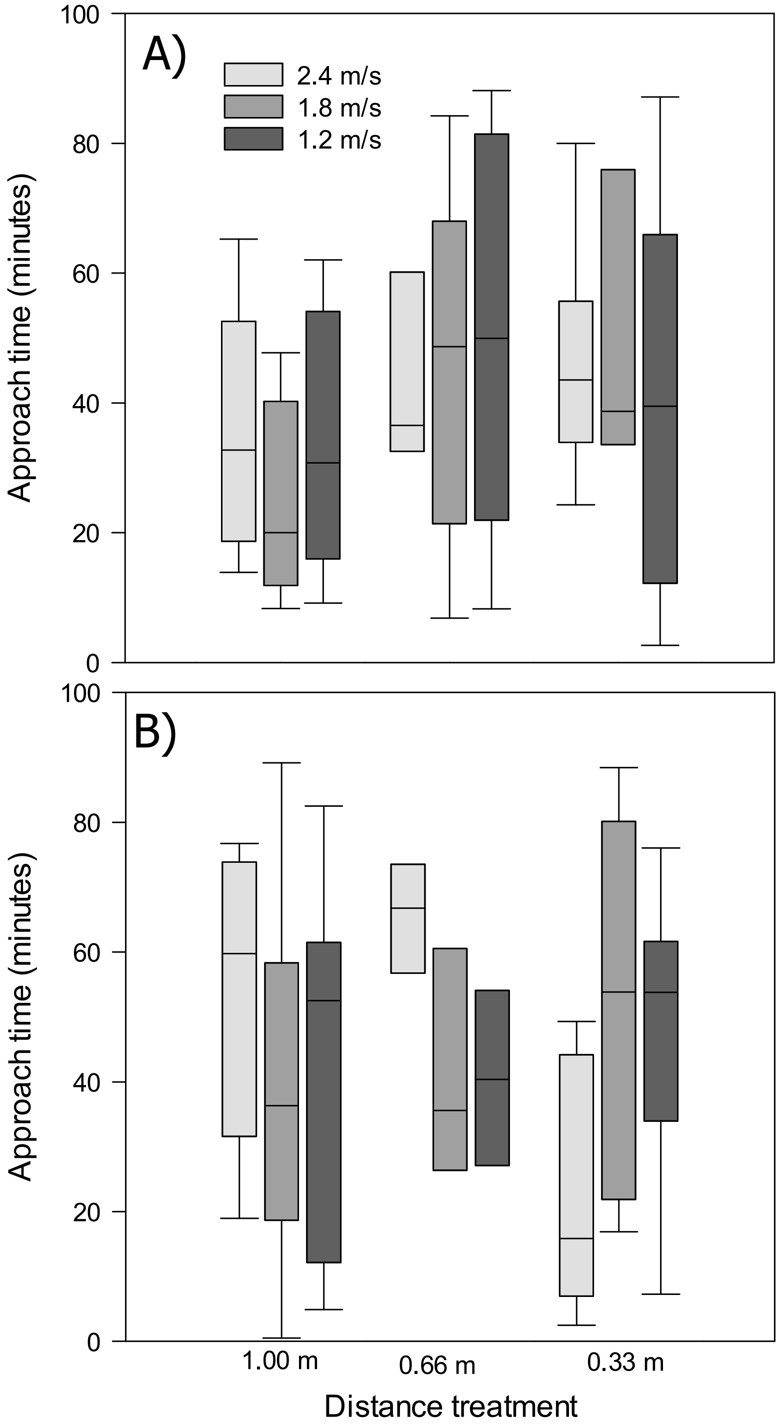


Figure 4.6: Approach times of Pacific lamprey sorted by distance and velocity conditions for (A) turbulence and (B) control treatments. Box plots show 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles.

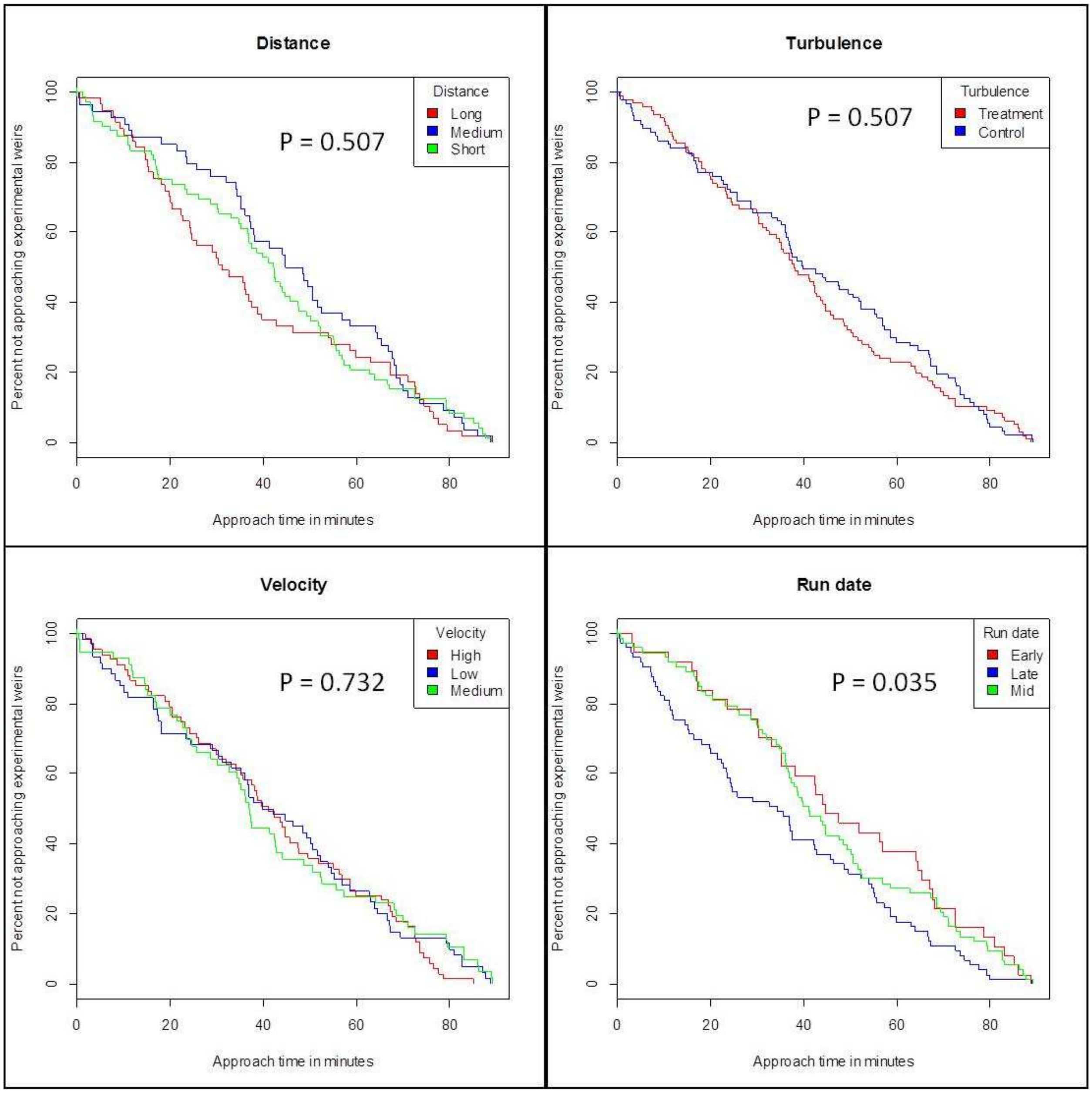


Figure 4.7: Time to event curves of Pacific lamprey approach times for the treatment variables of distance (upper left), turbulence (upper right), and velocity (lower left), as well as for the blocking variable of run date (lower right).

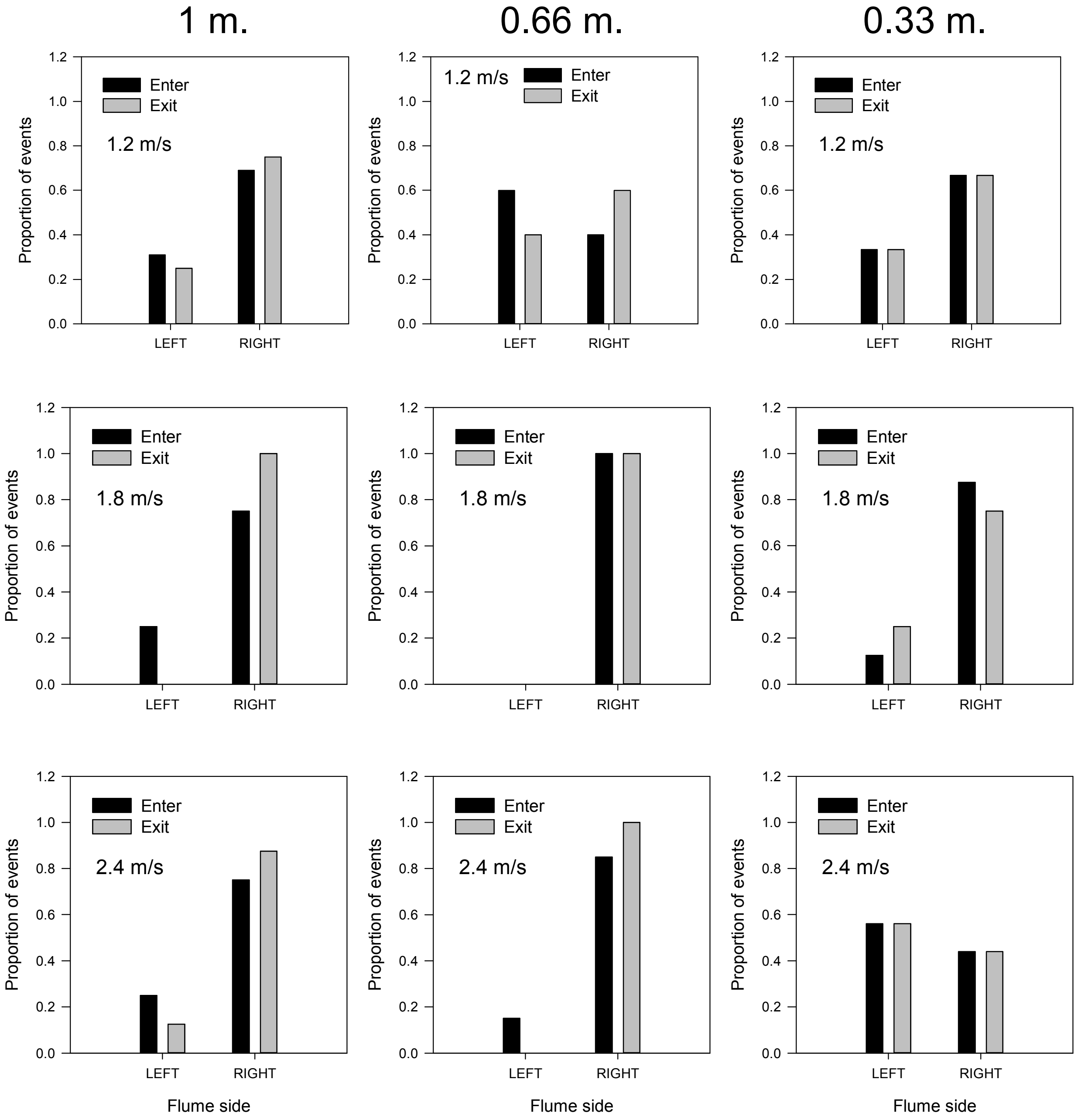


Figure 4.8: Proportion of upstream movements in low turbulence treatments for Pacific lamprey entering (black bars) and exiting (gray bars) the experimental weirs on the left and right side of the flume. The direction of flow would be from top to bottom. Columns are sorted by distance (1 m = left, 0.66 m = middle, 0.33 m = right) and rows are sorted by velocity (top = 1.2 m/, middle = 1.8 m/s, bottom = 2.4 m/s).

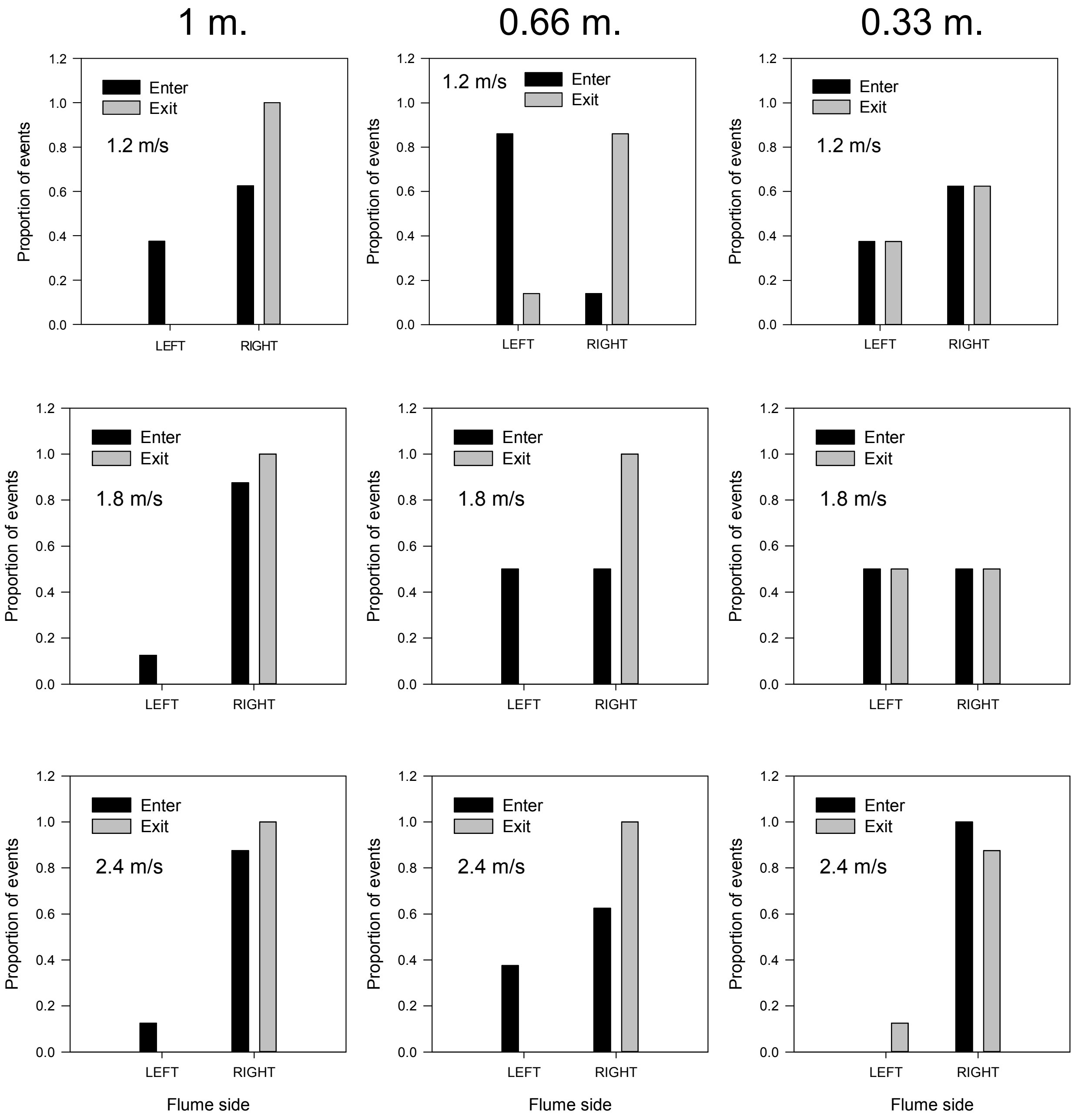


Figure 4.9: Proportion of upstream movements in high turbulence treatments for Pacific lamprey entering (black bars) and exiting (gray bars) the experimental weirs on the left and right side of the flume. The direction of flow would be from top to bottom. Columns are sorted by distance (1 m = left, 0.66 m = middle, 0.33 m = right) and rows are sorted by velocity (top = 1.2 m/, middle = 1.8 m/s, bottom = 2.4 m/s).

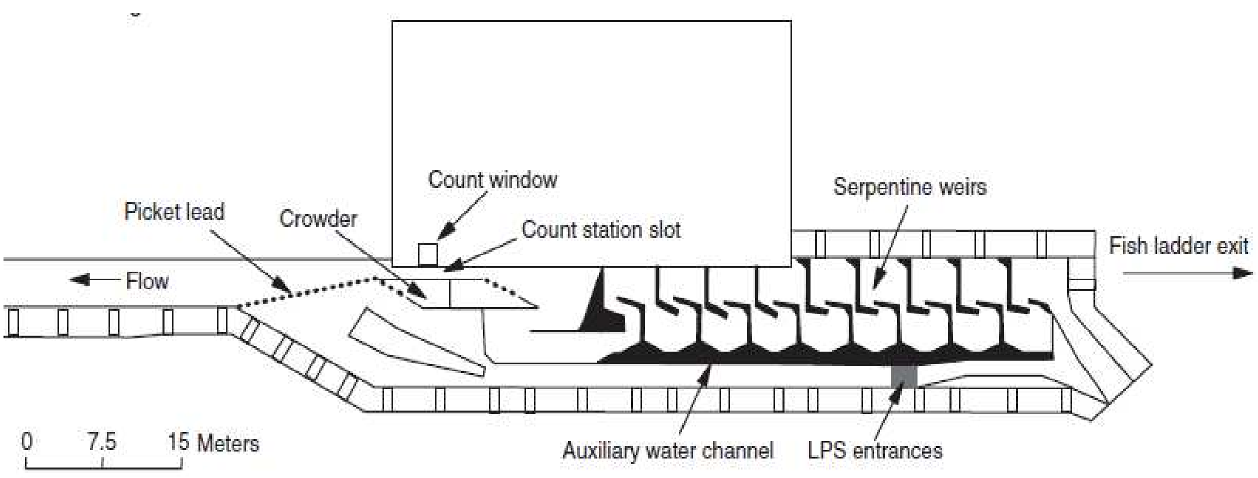


Figure 4.10: Overhead view of the Washington-shore fishway serpentine weir section at Bonneville Dam. There are approximately 15 weirs which fish must overcome to reach the fishway exit.

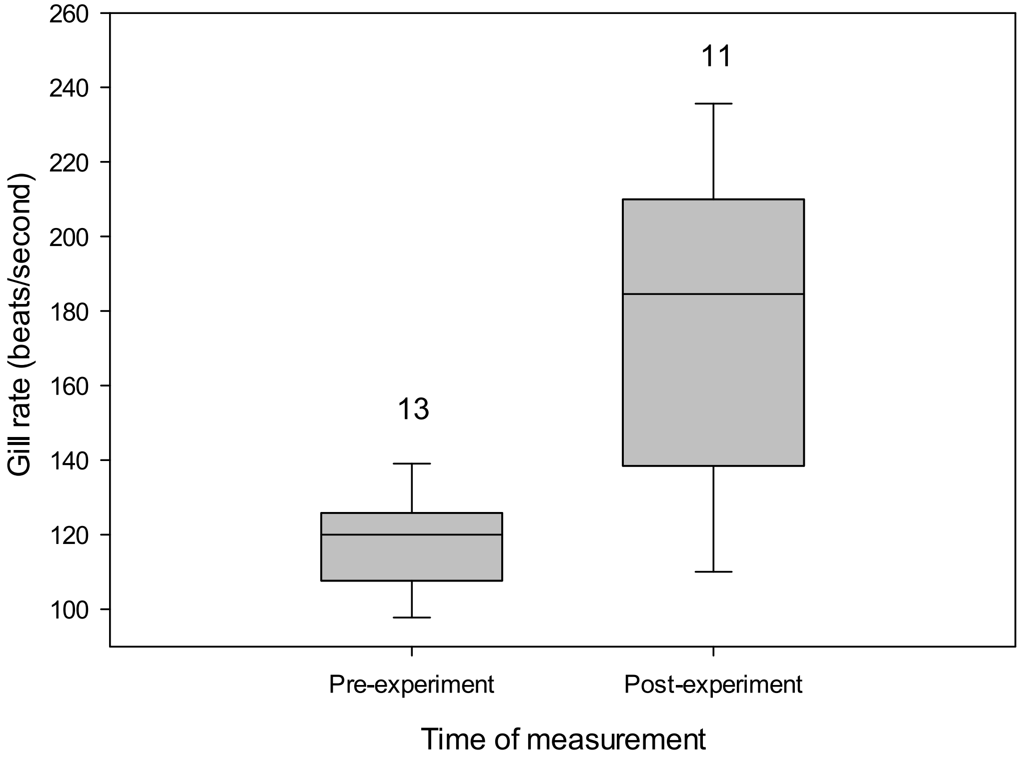


Figure 4.11: Estimates of Pacific lamprey gill beat rates before and after four experimental trials. Box plots show 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles. Numbers above bars are samples size of fish.

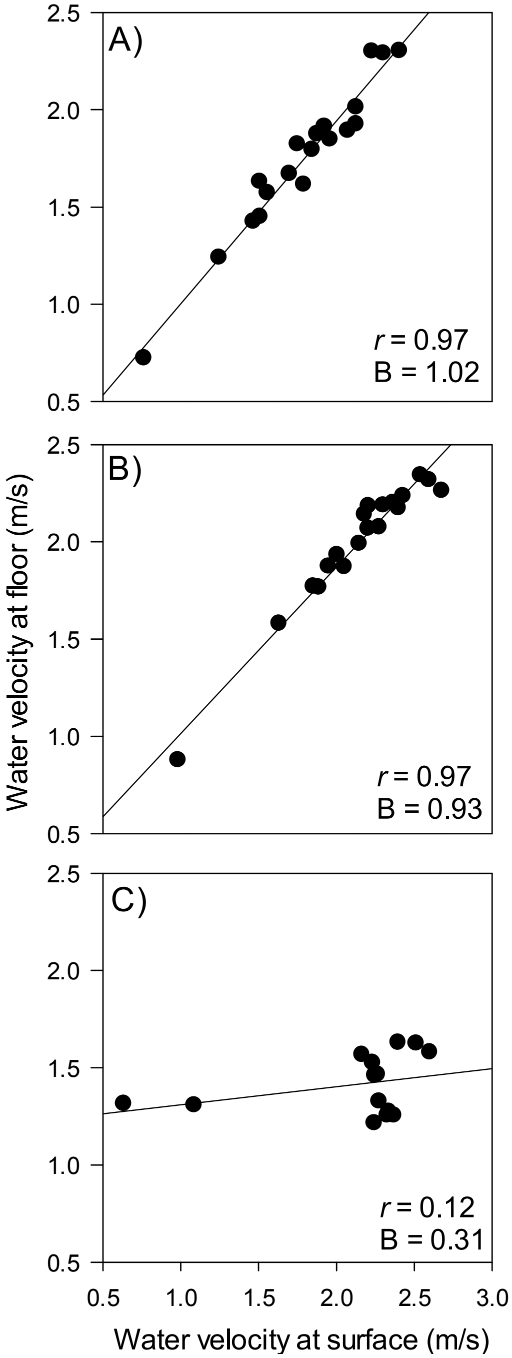
Chapter 5

Thesis Summary

The works described herein provided new and valuable insight into the migration behaviors of Pacific lamprey, which have important management implications for improving the passage of this species at hydroelectric projects on the Columbia River. Network analyses revealed substantial variation in the passage behaviors of Pacific lamprey, suggesting that the limited passage of this species is not entirely associated with poor performance and limited swimming capabilities. Instead, it is likely that there is some intrinsic difference in decision structure between Pacific lamprey and Chinook salmon that explain the passage differences observed in these two species (i.e., a lower and more variable 'giving-up' threshold in lamprey). As additional support, results from the experimental flume suggested that Pacific lamprey were capable of overcoming high velocity, high turbulence conditions and that these conditions at local barriers did not necessarily act as a barrier to passage. The measured passage conditions did not also appear to be associated with any of the processes responsible for the size-selective patterns observed for Pacific lamprey at Columbia River dams. However, these results also provided some evidence suggesting that endurance capacity plays an important role in limiting Pacific lamprey passage (e.g., Figure 4.5).

The role of endurance was further supported from *in-situ* observations with the DIDSON, which helped to link fishway locations of problematic passage with the mechanisms of problematic passage. Estimates of swimming activity in the John Day Dam collection channel provided evidence that endurance capacity has a substantial affect on the proposed 'giving-up' threshold in Pacific lamprey. However, the DIDSON also revealed that passage and behavior can be dictated by a complex set of environmental factors (predatory sturgeon, poor attraction flow, structural designs); particularly within the Bonneville Dam transition area. Taken together, these results suggest that Pacific lamprey passage success at hydropower dams is driven by not only swimming performance, but also motivation, behavior, body size, and environmental conditions. Understanding how those factors determine an individual's 'giving-up' threshold and how that threshold determines whether an individual passes a barrier will be critical for determining the most conducive passage conditions for this species.

Appendix I: Water velocity measurements taken within the experimental flume during the (A) short, (B) medium, and (C) long distances. Note that the slope for the short and medium distance treatments was ~1, indicating similar velocities between the flume floor and the water surface. In contrast, long distance treatments did not show any strong correlation, which was potentially a result of sampling error since these measurements were collected first.



Appendix II: Scatterplots of Pacific lamprey dorsal distance measurements for (A) distance, (B) velocity, and (C) turbulence treatments. Notice the greater variation in dorsal measurements for the long distance compared with short and medium distances, as well as the variation in turbulence treatments compared with turbulence controls.