Technical Report 2020-3

EVALUATING SWIMMING BEHAVIOR AND PERFORMANCE OF UPSTREAM MIGRATING PACIFIC LAMPREY USING EXPERIMENTAL FLUMES AND ACCELEROMETER BIOTELEMETRY, 2019

A Report for Study Code: LMP-P-17-1

by

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Department of Fish and Wildlife Sciences
University of Idaho, Moscow, ID 83844-1136

for

U.S Army Corps of Engineers
Portland District, Portland, OR

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Executive Summary

Stream connectivity is necessary for the persistence and viability of migratory fish populations. However, many of the world's rivers are dammed, creating barriers to movement. Fishways have been constructed to provide passage for migratory fishes, but traditional designs target fish with strong burst swimming capabilities, such as subcarangiform salmonids (*Oncorhynchus* spp), and do not consider species with alternate swimming modes (i.e. anguilliform). Additionally, high velocities and turbulent conditions within fishways can force fish to swim at prolonged high swimming speeds, potentially resulting in fatigue or delayed passage. The Federal Columbia River Power System (FCRPS) has served as a model system for fish passage designs worldwide. In particular, Bonneville Dam, the lowest mainstem dam on the Columbia River, is a complex facility comprised of three channels separated by islands and has two primary fishways. Each fishway begins with low gradient collection sections that lead to steeper pool and weir ladders, and then transition to vertical slot weirs before exiting into the forebay. These fishways provides passage for multiple species, though passage rates are low for some. Pacific lamprey (*Entosphenus tridentatus*) passage efficiency is typically ~50%. Previous research has revealed approximately one quarter to one third of lampreys that reach the serpentine weir section of both fishways fail to pass the dam. Past studies have demonstrated that lamprey can pass a single, high velocity vertical slot weir. We hypothesized fatigue may occur during passage of the 12 weirs present in the serpentine weir section. We tested this hypothesis using experimental behavioral observations and a novel application of acceleration biotelemetry in *at-liberty* animals. We manipulated exercise histories of adult Pacific lamprey prior to a weir passage challenge in an experimental flume (11.6-m long × 1.2-m wide × 2.4-m high). Treatments were applied using a 2 × 2 factorial design with two exercise velocities (1.0 m/s and 1.4 m/s) and two exercise durations (1 x 20 min and 2 x 20 min with a 10 min recovery interval) in addition to an unexercised control. After the exercise treatment, lamprey were presented with a high velocity passage challenge at a single vertical slot weir. The results indicate that the exercise history of lamprey and water velocity affect passage success. In the experimental flume experiment, lamprey passage success was lowest (53%) during the high-velocity, long-duration treatment as compared to the control (89%) ($\chi^2 = 22.3$, $P < 0.001$). Dorsal distance, after accounting for body length, was the only morphological covariate associated with passage success; dorsal distance increased the odds of successful passage. We used accelerometer biotelemetry to identify activity and behavioral responses of adult Pacific lamprey to local passage conditions at a previously identified passage bottleneck (the upper Washington-shore fishway of Bonneville Dam), with an emphasis on the serpentine weir section. Accelerometer biotelemetry results revealed that lamprey exhibited high intraspecific variability in duration and timing of attached and burst movements among sections of the fishway. Within the serpentine weirs, lamprey that were successful at passing spent more time bursting compared to lamprey that did not pass. Unsuccessful fish spent a longer duration attached and had higher turn-around rates, leading to longer residence times in the upper fishway. There appears to be a threshold fatigue associated with changes in behavior or motivation. Collectively, these results reveal that cumulative effects of passage are important to consider when designing or evaluating fishways. Velocities that require high activity levels over a prolonged period of time may induce fatigue, indicating the need for refuge areas to allow recovery.

Chapter 1: Evaluating the Influence of Past Experience on Swimming Behavior and Passage Success in Adult Pacific Lamprey

Abstract

Radio-telemetry studies have revealed that bottlenecks and high turn-around rates in fishways contribute to low passage success of Pacific lamprey at Columbia River dams. Structural and operational modifications to fishways and installation of lamprey-specific passage structures have improved fishway entrance and dam passage efficiencies, but conditions in the upper fishways continue to be passage obstacles. In particular, lamprey turn-around rates are high at Bonneville Dam serpentine weirs (n = 12), which are characterized by high water velocity and hydraulic complexity. Approximately one quarter to one third of lampreys that reach the serpentine weirs fail to pass the dam. While previous research has demonstrated that most lamprey readily pass a single challenge with high water velocity (2.4 m/sec), fatigue during passage of multiple weirs may be problematic. We manipulated exercise histories of adult Pacific lamprey prior to a weir passage challenge in an experimental flume (11.6-m long × 1.2-m wide × 2.4-m high). Treatments were applied using a 2 × 2 factorial design with two exercise velocities (1.0 m/s and 1.4 m/s) and two exercise durations (1 x 20 min and 2 x 20 min with a 10 min recovery interval) in addition to an unexercised control. Six replicates were run of each treatment with six lamprey/replicate. Passage success was then evaluated at a 1-m long vertical weir having 2.2 m/sec water velocity. We found that passage success declined with increasing velocity and duration of exercise. Lamprey passage success was lowest (53%) during the high-velocity, long-duration treatment as compared to the control (89%) ($\chi^2 = 22.3$, $P < 0.001$). Dorsal distance, after accounting for body length, was the only morphological covariate associated with passage success; dorsal distance increased the odds of successful passage. Lamprey that were characterized as ‘late’ run were more likely to fail compared to those earlier in the season. This effect was particularly noticeable for the high-velocity treatments. The results indicate that the exercise history of lamprey affects their passage success. This is consistent with the hypothesis that lamprey reach an endurance threshold or lose motivation when passing multiple high velocity obstacles in succession.

Introduction

Stream network connectivity is vital for migratory fishes. However, habitat fragmentation is ubiquitous among watersheds around the world, often resulting in declining or extinct fish populations (Liermann et al. 2012). One of the major sources of fragmentation includes dam construction for hydroelectric generation and/or irrigation (Hall et al. 2011). Over 50% of the world's rivers are used for hydroelectric production (Rosenberg et al. 2000). To mitigate, fishways have been constructed to aid fish migration (Clay 1995). However, most fish passage designs including vertical slot, pool and weir, and Denil designs (Bell 1990) target relatively few species of economic importance. Consequently, many passage systems have velocities and gradients that exceed the swimming ability of non-salmonids (Noonan et al. 2012), or do not fully consider migration strategies, swimming modes, or behaviors of non-salmonid fishes.

Fish passage guidelines have largely been developed from swimming performance trials conducted in swim tunnels or highly modified environments that can impair swimming abilities and alter natural behavior (Haro et al. 2004). During upstream passage, fishways may require fish to swim at prolonged or burst swim speeds in areas with homogenous flow fields. This does not always take into account the effect of exhaustion (particularly for poor swimmers), or that fish may not exhibit swim speeds that are optimum for passage (Castro-Santos 2004a; Cai et al. 2015). Morphological differences among species (e.g., anguilliform vs fusiform) further contribute to variable fishway effectiveness. This is especially true for species whose swimming modes do not match those of fish targeted by traditional passage designs.

Traditionally, salmonids have served as a model organism for understanding migration behavior during dam passage. They exhibit strong homing and minimal meandering, thereby potentially saving energy relative to less-directed species (Bernatchez and Dodson 1987). Other strategies, such as those exhibited by lamprey and other non-philopatric species, are poorly understood. In Pacific lamprey, movement patterns can vary widely between individuals (Kirk and Caudill 2017), resulting in differences in the duration and intensity of swimming experience. The effect of past experience, particularly if it creates fatigue, could affect the physiological status of an individual and its response to environmental conditions.

There can be high intra- and inter-species variability in behavioral responses to conditions and cues received during migration (Farwell and McLaughlin 2009). Fish determined to have a higher level of motivation were deemed to be more successful in passing compared to their less-motivated counterparts (Goerig and Castro-Santos 2017). The underlying factors that affect motivation, particularly exhaustive exercise, have been poorly characterized. While these factors can modulate behavior and influence passage success (Ackerman et al. 2019), there are relatively few behavioral studies in fish passage research (Silva et al. 2018). Cumulative effects of exhaustive exercise, manifesting in slowed or delayed movement through multiple dams, can have negative consequences on spawning success (Caudill et al. 2007). Therefore, we need a better mechanistic understanding of how conditions experienced during passage affect behavior, and consequently motivation.

The Federal Columbia River Power System (FCRPS) has served as a model passage system for other hydropower systems worldwide (Silva et al. 2018). Bonneville Dam, the lowermost dam on the Columbia River, provides passage for a multitude of migratory species, including Chinook salmon (*Oncorhynchus tshawytscha)*, Coho salmon (*Oncorhynchus kisutch*), Steelhead trout (*Oncorhynchus mykiss*), American shad (*Alosa sapidissima*), and Pacific lamprey (*Entosphenus tridentatus*). Fishways at Bonneville Dam generally have low gradient collection sections that lead to steeper pool and weir ladders, and then transition to vertical slot weirs before exiting into the forebay. There are many possible routes, with numerous fishway entrances that conjoin to form two primary exits. Current passage rate estimates for adult salmonids are approximately 90%, while passage rates for adult Pacific lamprey are ~50% (Keefer et al. 2015; Keefer et al. 2019).

Pacific lamprey is a native, anadromous species found in the Pacific Northwest that has experienced major population declines (Beamish 1980; Clemens et al. 2017). They spend 1- 4 years in the ocean as an ectoparasite before migrating upstream to spawn in freshwater. Interior Columbia River populations generally enter freshwater in spring/summer and overwinter before spawning in the following spring. Similar to salmonids, they cease feeding after freshwater entry and must conserve energetic reserves for the development and maturation of gonads. Unlike salmonids, they do not home to natal streams, instead relying on pheromones from juvenile lamprey as a guide to spawning habitat (Yun et al. 2011; Spice et al. 2012). Hence, the extirpation of interior lamprey populations tributaries has serious implications for cues needed by upstream migrating adults. The vital role Pacific lamprey play in freshwater ecosystems, as well as their importance as a harvestable food source and icon in Native American culture, has prompted efforts to improve lamprey passage at dams (Close et al. 2002).

Poor passage at dams has been implicated in Pacific lamprey declines (Moser et al. 2002b). Lamprey swimming behavior, morphology, and migration strategy all contribute to low success rates at fishways relative to salmonids (e.g., Mesa et al. 2003; Kemp et al. 2009; Moser et al. 2008; 2011; Kirk et al. 2017). High water velocities in fishways (>2 m/s) can exceed the critical swimming speed of Pacific lamprey (~0.86 m/s) (Mesa et al. 2003). Furthermore, lamprey do not have the endurance and burst speed capabilities of salmonids, instead relying on their oral disk to attach and then burst forward in high velocity and/or turbulent conditions (Reinhardt et al. 2008). Gratings and other surfaces present in fishways prevent oral disk attachment, further contributing to unfavorable passage conditions (Moser et al. 2002a).

Efforts have been made at Bonneville and John Day dams to improve passage conditions for lamprey (Moser et al. 2002b, 2011), including implementation of structures specifically suited for lamprey passage. These structures, referred to as lamprey passage structures (LPS), take advantage of the natural climbing ability of lamprey and reroute them through difficult passage areas (Moser et al. 2002b; 2011). Once lamprey enter the structures, passage success rates are generally greater than 90% (Moser et al. 2006, 2008, 2011). However, many lamprey only use LPSs after failing to pass upstream through the traditional fishways (Keefer et al. 2019). Energetic costs associated with climbing are largely unknown, as are effects of size selection that may be occurring in lamprey that successfully use these structures. Qualitative observations of lamprey climbing up ramps has revealed that they can make several attempts to pass after falling back, indicating that climbing is energetically costly.

Despite the promising use of lamprey-specific passageways, local areas of poor passage persist (i.e., bottlenecks, Keefer et al. 2013a). In particular, the serpentine weir section near the exits of both fishways at Bonneville Dam are areas with ~25-30% turn-around rates for lamprey, despite having similar velocities and turbulence to obstacles encountered further downstream in the fishways (Keefer et al. 2013a, 2014b). Moreover, recent laboratory experiments have demonstrated high passage rates through high velocity obstacles (e.g., Kirk et al. 2016). These patterns highlight the need for a mechanistic understanding of the factors affecting turnaround behaviors.

The primary objective of this study was to investigate the effects of exhaustive exercise on adult Pacific lamprey swimming performance and passage using an experimental flume in the Bonneville Dam adult fish facility (AFF). We hypothesized that lamprey subjected to longer swim durations and strenuous velocities prior to a passage challenge would exhibit decreased passage success relative to unexercised lamprey. Additionally, we hypothesized that physiological, and/or morphological traits would affect endurance thresholds.

Our second objective was to estimate upstream escapement of experimental lamprey past dams on the mainstem and tributaries of the Columbia River and to evaluate possible correlates with upstream migration distance. These included passage of the experimental flume and morphological measurements (i.e., body size). We hypothesized that upstream migration distance would be positively related to successful passage of the experimental flume. We expected that larger body size would be positively correlated with migration distance, given previous work in the same system (Keefer et al. 2009; Hess et al. 2014).

Methods

Lamprey collection and tagging:

Adult Pacific lamprey (*n* = 360) were collected at night from June 20 to August 5, 2018 (*n*=180*)* and May 28 to July 12, 2019 (*n*=180*)* from a lamprey flume structure (LFS) located at the adult fish facility (AFF) at Bonneville Dam (45.6°N, 121.9°W) on the lower Columbia River. Fish were removed from the collection box the following morning and were held in large aluminum tanks (92-cm wide × 152-cm long × 122-cm high) that received constantly aerated river water. Prior to tagging, lamprey were anesthetized in a 60-ppm (3 mL/50 L) solution of eugenol (AQUI-S 20E). An approximate 12-mm ventral incision was made directly below the anterior insertion of the first dorsal fin and a half-duplex passive integrated transponder tag (HD-PIT tag; 4 × 32 mm) was inserted into the body cavity. Fish were weighed (g), measured for total length (cm), body girth (cm), and distance between dorsal fins (dorsal distance, cm). Dorsal distance is used to indicate maturation status in lamprey (Clemens et al. 2010) and decreases as lamprey mature. Dorsal distance was calculated as dorsal distance /total length to account for variation in body size among individuals. Percent body fat (lipid) was estimated using a fish fat meter (Assurant Innovations, San Jose, CA) and a fin clip was removed for future genetic studies

A blood sample was taken from 50 fish late in the run (7/2/2018 – 8/5/2018). After insertion of a PIT tag, a 5 ml, 1-inch, 23-gauge needle was inserted ~2 cm posterior to the vent at a slight angle (angled towards caudal fin, away from vent). Approximately 40 µl of blood was removed from caudal vasculature. The blood sample was processed using a VetScan i-STAT® 1 handheld analyzer (Abaxis products, Union City, CA) for hematocrit, hemoglobin, ionized calcium, glucose, sodium, potassium, pH, $p\mathrm{CO_2}$, $\mathrm{HCO_3}$, $\mathrm{TCO_2}$, base excess, $P\mathrm{O_2}$, and $\mathrm{sO_2}$. A Lactate Plus meter (Sports Resource Group Inc., U.S.A) was used to measure plasma lactate. Tagged fish were allowed to recover for 8-12 hours prior to the experiment.

Experiments were conducted in an experimental flume (11.6-m long × 1.2-m wide × 2.4-m high). The flume consisted of a 9-m long experimental section with a 10% slope. A chamber at the upstream end of the flume had fyke nets that limited downstream movements after lamprey had ascended the flume. Acclimation and pre-trial exercise treatments were conducted in an aluminum frame structure (1.1-m long × 0.91-m wide × 0.61-m high) lined with mesh to prevent attachment at the downstream end of the flume (Figure 1.1). Passage performance was tested using a single vertical slot weir (1-m long), which was situated upstream from the mesh chamber (Figure 1.2). The weir slot was 46 cm wide, replicating the dimensions of vertical slot weirs present in fishways at Bonneville Dam. The flume was supplied with river water via two pipes at flow rates up to 835 L/s. Velocity was manipulated by altering the discharge through the pipes. Additional details of the flume can be found in Keefer et al. (2011) and Kirk et al. (2016, 2017).

Figure 1.1 Mesh exercise chamber located in the downstream portion of the experimental flume

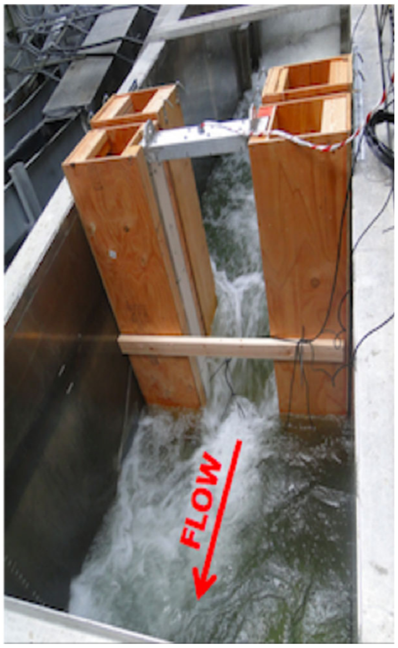

Figure 1.2 Experimental flume with vertical slot weir. The downstream PIT antenna is visible as the metal frame at the downstream end of the weir. Weir slot length was 1.0-m rather than 0.66-m as pictured.

All experiments were conducted at night (1900-0700) because Pacific lamprey are primarily nocturnal during their migration. In 2019, experiments were restricted between the hours of 0200 – 0700 due to differences in activity level during night hours revealed during 2018 experiments (see Results). The experiment consisted of a 2 × 2 factorial design with a control that manipulated two treatment variables (Table 1.1). The design had two pre-challenge exercise velocities (1.0 m/s and 1.4 m/s) and two pre-challenge exercise durations (20 min and 2 × 20 min with a 10 min recovery interval in between repetitions). Lamprey in control treatments were not exercised. Hereafter, the treatments will be referred to as C (control), L1 (1.0 m/s, 20 min), L2 (1.0 m/s, 2 x 20 min), H1 (1.4 m/s, 20 min) and H2 (1.4 m/s, 2x20 min). The experimental design was a randomized block design consisting of three blocks per year, representing "early," "mid," and "late" time periods, for a total of six blocks. The blocks were used to account for seasonal variation in river temperature and lamprey traits. Variation in lamprey trapping rates within blocks and between years created differences in the timing and duration of each block (see Results). Each treatment was replicated twice within each block.

Each exercise treatment consisted of an acclimation period and an exercise period. All lamprey were acclimated in the mesh chamber for a minimum of 20 minutes prior to exercise or the passage trial, including the control treatment. In 2018, lamprey acclimated for 20 minutes and released for the passage trial (control) or immediately exercised and released (treatments), resulting in variable durations in the mesh chamber among treatments (total time 20-70 minutes). In 2019, all lamprey spent 70 minutes in the chamber, with 70, 50, or 20 minutes of acclimation for control, 1 x 20 and 2 x 20 minute exercise treatments, respectively. Velocity at the weir was 2.2 m/s for all treatments and lamprey were given two hours to pass. Treatment velocities were measured at three equidistant points spanning the flume channel directly in front of the mesh covered cage at 60% of the depth and averaged to determine exercise velocity. Three additional measurements were made at the vertical slot weir at 20% and 80% depth and averaged to determine velocity during the passage trial. All measurements were made using a Marsh-McBirney flow meter (Hach Company, Loveland, CO, USA). After the conclusion of each experiment, lamprey were removed from the flume, scanned for HD-PIT tags, and allowed at least 25 minutes recovery before being released upstream from Bonneville Dam at the Stevenson, WA boat launch.

After release, lamprey movements were monitored using an array of PIT antenna sites located inside dam fishways and/or at top-of-ladder exits at Bonneville, The Dalles, John Day, and McNary dams. Additional PIT antennas were located at top-of-ladder exits at upper Columbia River and Snake River dams, including Priest Rapids, Wanapum, Rock Island, Rocky Reach, Ice Harbor, Lower Monumental, Little Goose, and Lower Granite dams. Additionally, several lower Columbia River tributaries had PIT antennas installed, including the Klickitat River, Hood River, Fifteenmile Creek, and the Deschutes River. Final location was assigned according to the site of last detection. Lamprey recorded at top-of-ladder exits were considered to have passed the dam.

Table 1.1. Number of replicates by treatment pre-trial exercise velocity and duration of exercise treatments used in both 2018 and 2019. Each replicate included 6 adult lamprey with a total of 180 lamprey across all replicates per year (total n = 360).

Exercise Velocity (m/s)	Exercise Duration (min)		
	0	20	2x20
0	6		
1		6	6
1.4		6	6

Lamprey movements and behaviors were recorded using two underwater cameras (Speco Technologies; Model #CVC320WP8) placed on the upstream end and two cameras on the downstream end of the vertical slot weir. Two cameras were placed in the mesh chamber for behavioral observations. HD-PIT antennas were placed at the downstream and upstream ends of the vertical slot weir, as well as at the entrance to the fyke section to record approach and finishing times. PIT tag records for individual lamprey were used to score each individual as having passed the weir or not, and to calculate passage times. When passing through the vertical slot weir, lamprey employed saltatory behavior; attaching for a period of time below the weir before bursting forward through the slot and re-attaching at the upstream end. In some instances, lamprey would attach below the weirs but not swim through them, instead swimming back down after a period of time and remaining attached for the duration of the experiment. This event was scored as a ‘failed attempt’ using video and PIT tag records.

Data Analysis

Passage success was defined as the number of fish that successfully passed the vertical slot weir divided by the total number of fish in each trial. We used logistic regression to test for treatment effects with ‘passed’ vs. ‘did not pass’ as the binary response variable. The logistic regression included all treatment variables, run date (block), fish length, and dorsal distance relative to body size (length of lamprey). Physiological data was fitted to a multiple regression model with pass/fail as the binary response. Analysis of variance (ANOVA) was used to evaluate passage time in the experimental flume across treatment levels. In order to meet assumptions of normality and equal variance, data were $\log_e$ transformed. Tukey's HSD multiple comparison tests were used to compare mean passage times between each treatment. Hosmer-Lemenshow tests were used to evaluate goodness of fit for logistic regression models.

We tested for factors associated with upstream escapement past dams with a multinomial logistic regression model that evaluated passage at five upstream dams: The Dalles, John Dam, McNary, Ice Harbor, and Priest Rapids dams. Individual lamprey were binned into one of the following classes: (1) final record between release and below The Dalles Dam; (2) passing The Dalles Dam; (3) passing John Day Dam; (4) passing McNary Dam, (5) passing Ice Harbor Dam or (6) passing Priest Rapids Dam. Predictor variables included run date, dorsal distance, and length. The significance level for all tests was $\alpha = 0.05$.

All analysis was conducted using R (R Core Team, 2017).

Results

The timing and magnitude of the lamprey run differed considerably between 2018 and 2019 (Figure 1.3). The total run size in 2019 was ~53% of the 2018 run and the 2018 run arrived 16 days earlier (on median) than the 10-year average, resulting in collection of the adults from relatively late in the run compared to 2019. Flow was higher than the 10-year average during May 2018 and lower than average during summer months in both years (Figure 1.4). Temperature was similar across both sample periods and the 10-year average (Figure 1.4). Pairwise comparisons among blocks revealed some significant differences ($P < 0.04$), e.g., lamprey in blocks 1 and 2 were longer and heavier than other blocks, but there were not consistent trends in metrics among blocks or between years (Table 1.2).

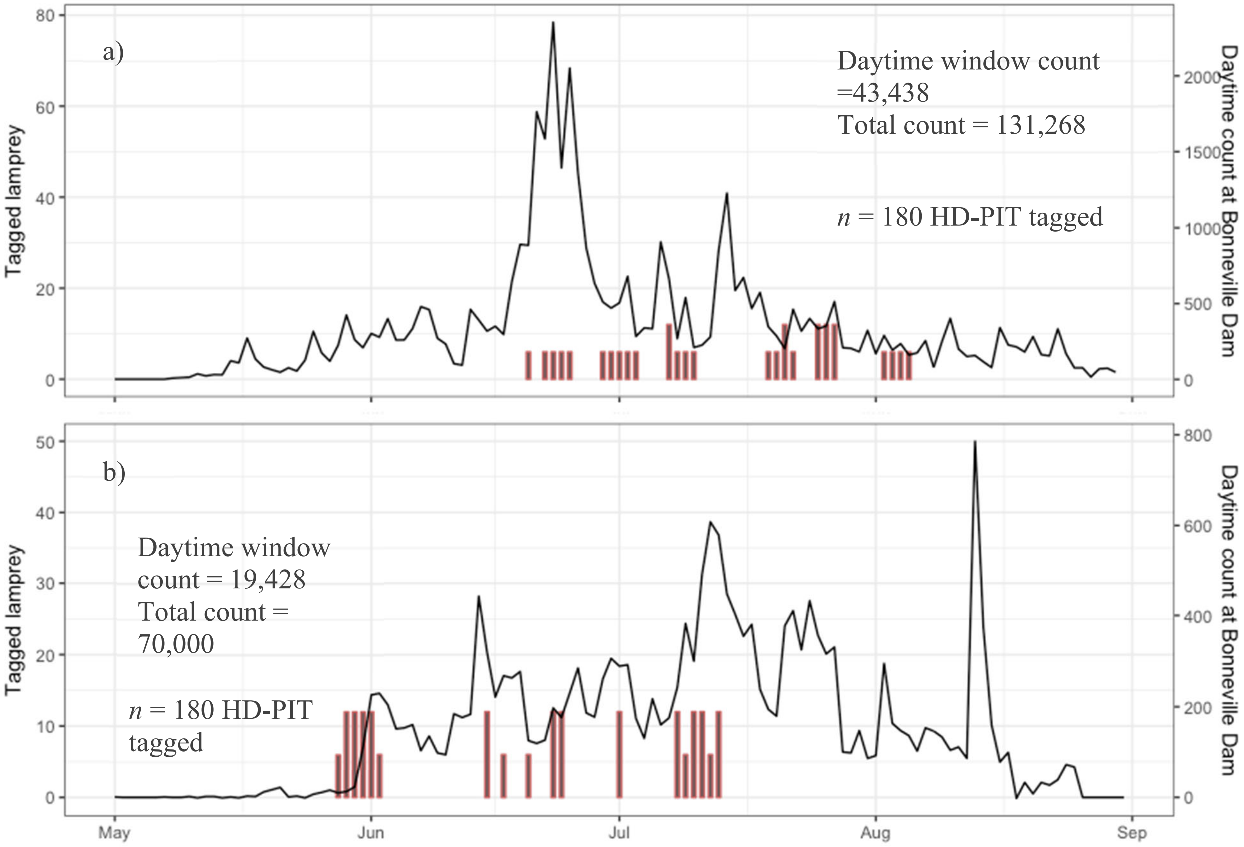

Figure 1.3 Daytime (line) and total count (including night estimates) of adult Pacific lamprey passing Bonneville dam and the number of fish collected and tagged for flume trials (bar) in 2018 (a) and 2019 (b). Passage estimates are from May 1 to December 31.

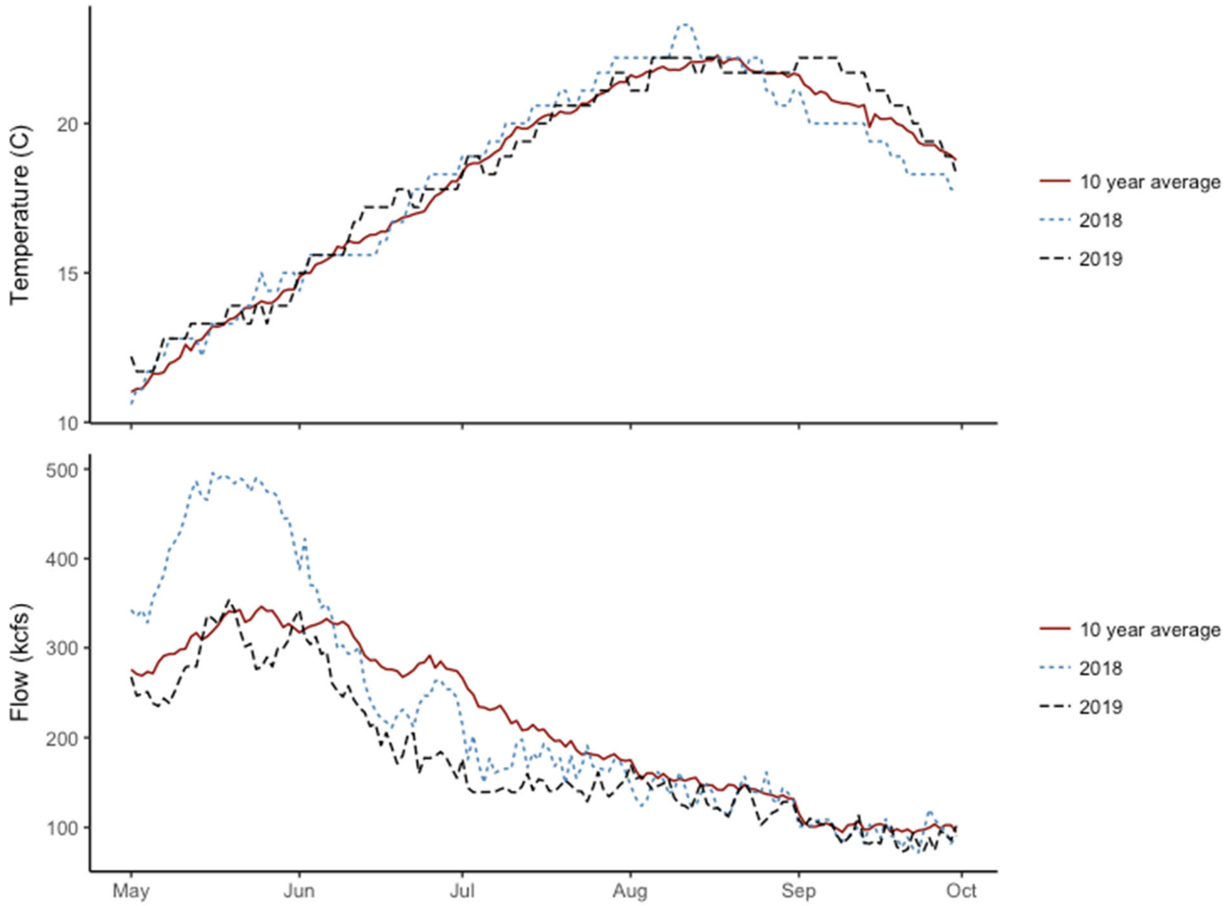

Figure 1.4 Mean daily Columbia River water temperature (°C) and discharge (flow, kcfs) at Bonneville Dam in 2018, 2019, and the ten-year average (source: http://www.cbr.washington.edu /dart/query/river_daily).

Table 1.2 Summary of number of blocks and corresponding dates, mean (SD) weight, and mean (SD) dorsal distance of lamprey. Mean values that were significantly different from other blocks within metric are indicated by superscripts with different values.

Block	Dates	N	Mean Length (cm)	Mean Weight (g)	Mean Dorsal Distance (cm)
			2018		
1	6/20 – 7/03	60	68.2 (5.06)^1	516 (112)^3	3.75 (0.630)
2	7/07 – 7/22	60	68.8 (4.76)^1	512 (98.2)^3	3.91 (0.793)^5
3	7/25 – 8/05	60	65.7 (4.83)^2	457 (104)^4	3.69 (0.548)
			2019		
4	5/28 – 6/02	60	65.3 (4.95)^2	466 (103)	3.46 (0.692)^6
5	6/15 – 7/01	60	67.5 (4.59)	493 (105)	3.83 (0.638)^5
6	7/08 – 7/13	60	67.8 (3.66)	509 (81.4)	3.96 (0.599)^5

Overall, passage success declined with increasing pre-challenge velocity and duration of exercise (Figure 1.5). Point estimates of mean passage success were lower in the high pre-challenge velocity treatments (53-67%) than the control treatment (89%) regardless of the pre-challenge exercise duration (Figure 1.5). Mean passage success in the 1 × 20 min low pre-challenge velocity treatment (94%) was similar to the control and the 2 × 20 min low velocity mean passage success (70%) was intermediate between control and high velocity treatments. Passage success in the low velocity, low duration exercise treatment was significantly different from the low velocity, high duration treatment ($\chi^2 = 13.90$, $P < 0.001$) and the high velocity, low duration treatment ($\chi^2 = 14.6$, $P < 0.001$) (Table 1.3). Additionally, across the high velocity treatments, passage success was significantly lower after the longer duration compared to the shorter duration treatment ($\chi^2 = 4.3$, $P = 0.04$). When including the time-of-night effect for 2018 trials, passage probability was higher for fish that were tested post-midnight ($\chi^2 = 6.25$, $P = 0.01$); there was no treatment by trial time interaction ($P>0.05$).

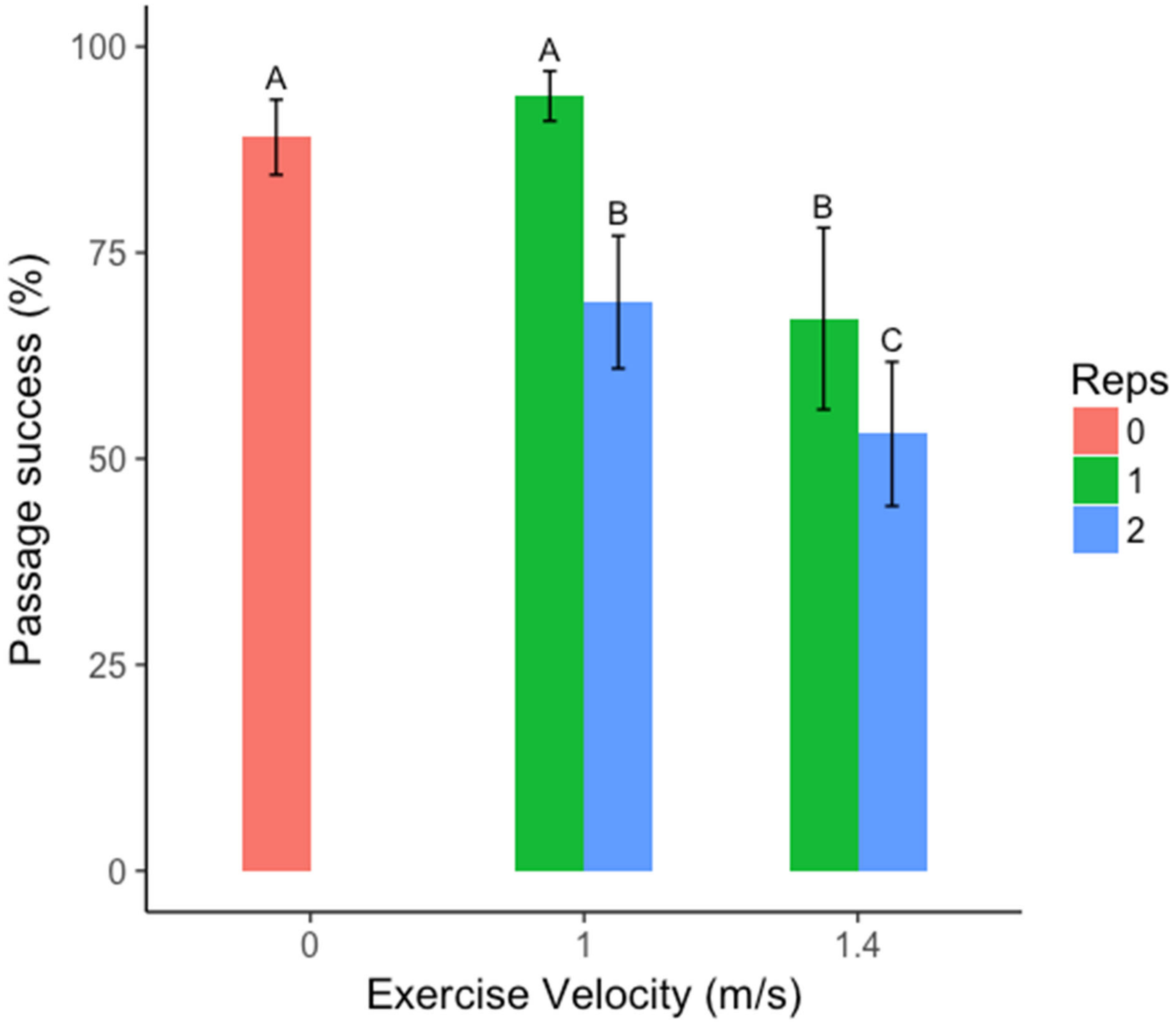

Figure 1.5 Percentage of lamprey that successfully passed the experimental flume by treatment. Reps refers to the duration of exercise (Control [0 min], 1 x 20 min or 2 x 20 min). Means with different letters indicate significant differences between treatments.

Table 1.3. Logistic regression results testing for the effect of treatment and morphometric measurements on passage success. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration. Contrasts that are significant are indicated in bold/italicized.

Factor	χ^2	*P*	Odds Ratio	95% CI
L1 - C	1.6	0.20	2.30	0.66 – 9.22
H1 - C	***9.2***	***0.002***	***0.24***	***0.09 – 0.59***
L2 – C	***8.4***	***0.004***	***0.25***	***0.09 – 0.62***
H2 - C	***22.3***	***<0.001***	***0.10***	***0.04 – 0.25***
L2 – L1	***13.9***	***<0.001***	***0.11***	***0.03 – 0.35***
H1 – L1	***14.6***	***<0.001***	***0.10***	***0.03 – 0.33***
H2 – L2	***5.0***	***0.03***	***0.42***	***0.19 – 0.90***
L2 – H1	0.02	0.88	1.06	0.49 – 2.30
H2 – H1	***4.3***	***0.04***	***0.44***	***0.20 – 0.96***
Dorsal Distance/TL	***4.4***	***0.03***	***1.14***	***1.01 – 1.30***
Length	<0.001	0.98	1.03	0.96 – 1.12
Block 1 – Block 2	***17.13***	***<0.0001***	***16.41***	***4.29 – 62.74***
Block 1 – Block 3	***17.71***	***<0.0001***	***17.89***	***4.67 - 68.61***
Block 2 – Block 3	0.04	0.84	1.09	0.47 – 2.55
Block 4 – Block 5	***11.37***	***<0.001***	***6.96***	***2.25 – 21.49***
Block 4 – Block 6	3.33	0.07	2.97	0.92 – 9.55
Block 5 – Block 6	3.55	0.06	0.43	0.18 – 1.03

Dorsal distance, after accounting for body length, was the only morphological covariate associated with passage success (Table 1.3). The odds of success increased by a factor of 1.14 according to a one unit increase in DD/TL (Figure 1.6). Interestingly, across both years, lamprey in early season trials were more likely to pass compared to trials later in the season (Figure 1.7). Lamprey categorized as “mid” or “late” had lower odds of passing as compared to those classified as “early” in 2018. In 2019, lamprey classified as “early” had higher odds of passing compared to those considered “late.” This effect was magnified for the high velocity treatments. Season and temperature covaried (Figure 1.4), so temperature was negatively associated with the probability of passage (Figure 1.8).

At the warmer temperatures occurring in the later season, the odds of successful passage were considerably lower in the high velocity treatments relative to the control and low velocity, low duration treatment (Figure 1.8). Lamprey in the high velocity, long duration treatment were 90% less likely to pass when compared to the control group and 58% less likely compared to the low velocity, long duration group. Within the high velocity treatments there was a 56% decrease in odds of successful passage as the duration of exercise increased. Though in most comparisons, an increase in exercise velocity and duration resulted in a corresponding decrease in the probability of passage, fish in the low velocity, low duration treatment were more likely to pass than the control group by a factor of 2.3. The odds of successful passage were similar for lamprey in the low velocity, long duration treatment and the high velocity, low duration group.

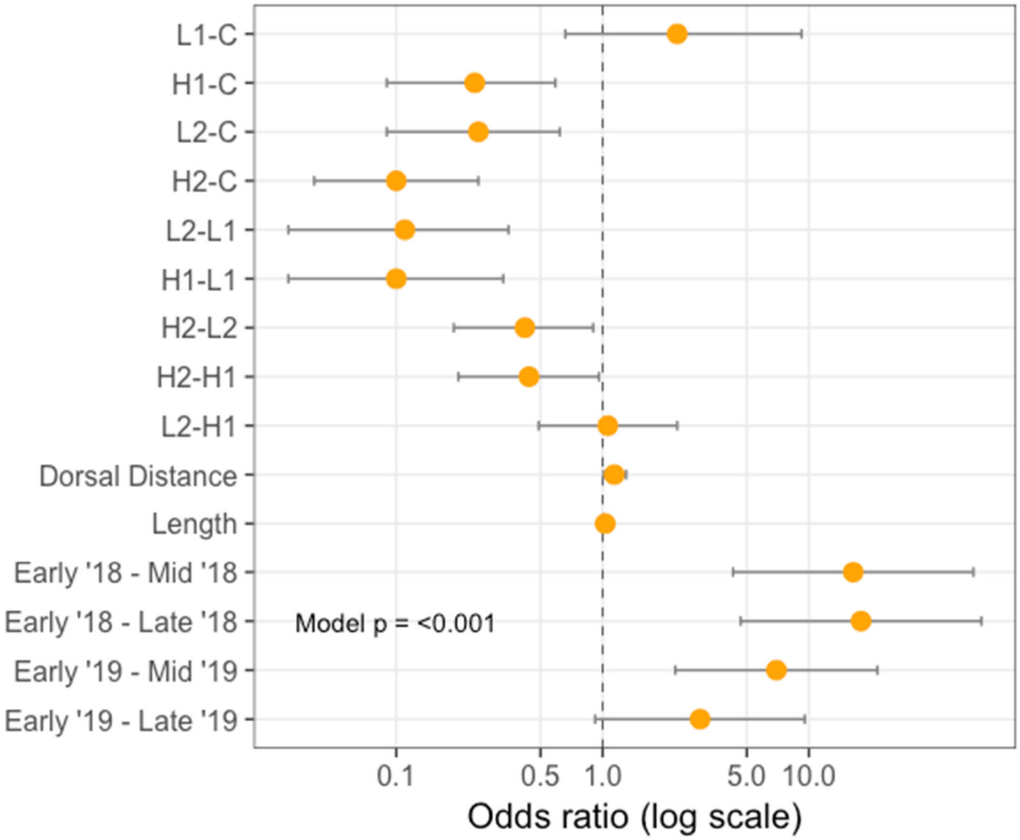

Figure 1.6. Multiple logistic regression results testing for the effect of treatment evaluating successful passage of a vertical slot weir in an experimental flume. Predictor variables included treatment, dorsal distance, length, and block date. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

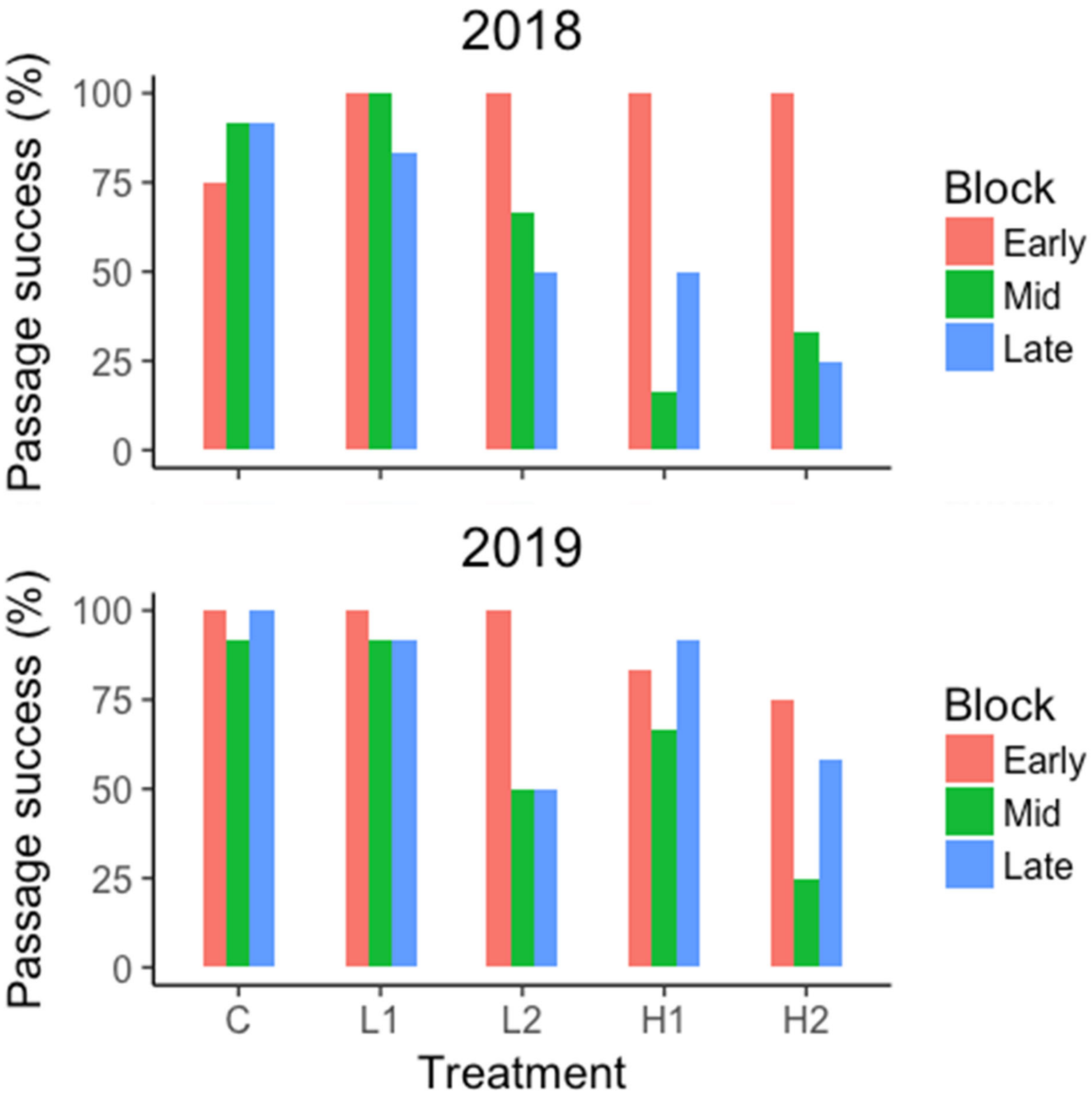

Figure 1.7. Percentage of lamprey that successfully passed the experimental flume by treatment and block in 2018 (top) and 2019 (bottom). Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration

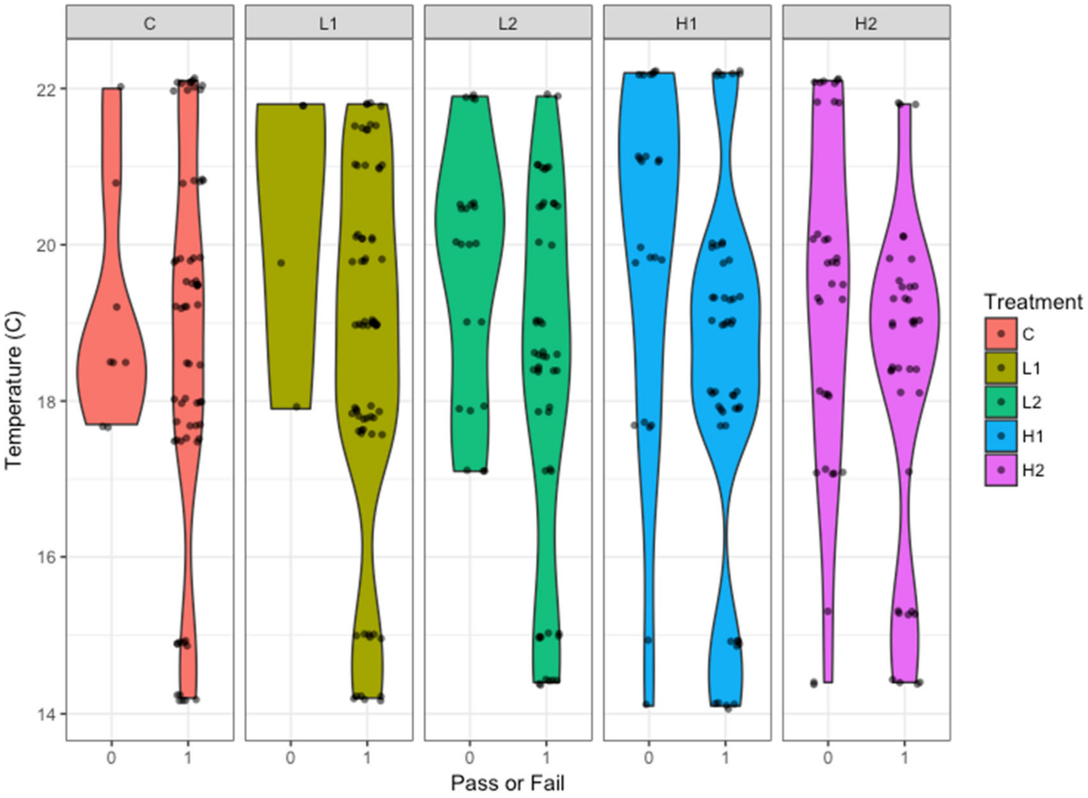

Figure 1.8. Water temperature (°C) plotted against the number of lamprey within each treatment that passed or failed to pass the experimental flume. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

Failed passed attempts were disproportionately exhibited in the higher velocity and longer duration treatments (Figure 1.9). In most instances, lamprey made only one attempt before falling back and did not re-initiate upstream movement. Passage time records were incomplete in some cases and observations with incomplete time records were censored from passage time analysis (Table 1.4). Passage time among successful lamprey did not differ significantly across treatments (*F*=1.12, *P*=0.17, df=4; Figure 1.10) when data was combined across both years. However, in 2018, we found that passage times were significantly longer in all treatments and in trials that were run prior to midnight compared to trials run after midnight (*F*=93.13, *P*=<0.0001, df=1; Figure 1.12). There was no interaction between treatment and trial time (*F*=1.12, *P*=0.17, df=4), however there was a significant difference in passage times between treatments when comparing overall passage time, regardless of trial time (*F*=5.12, *P*=0.0008, df=4; Figure 1.11). Counter to expectations, the control had longer passage times compared to L1 and L2 (*P*=0.008, *P*=0.008, respectively). In 2019, trials were conducted only after midnight in order to address this contrast; as in 2018, significant differences in passage times between treatments occurred (*F*=3.40, *P*=0.01, df=4; Figure 1.13). Fish in treatment L2 took longer to pass than both the control and L1 (*P*=0.04, *P*=0.01, respectively).

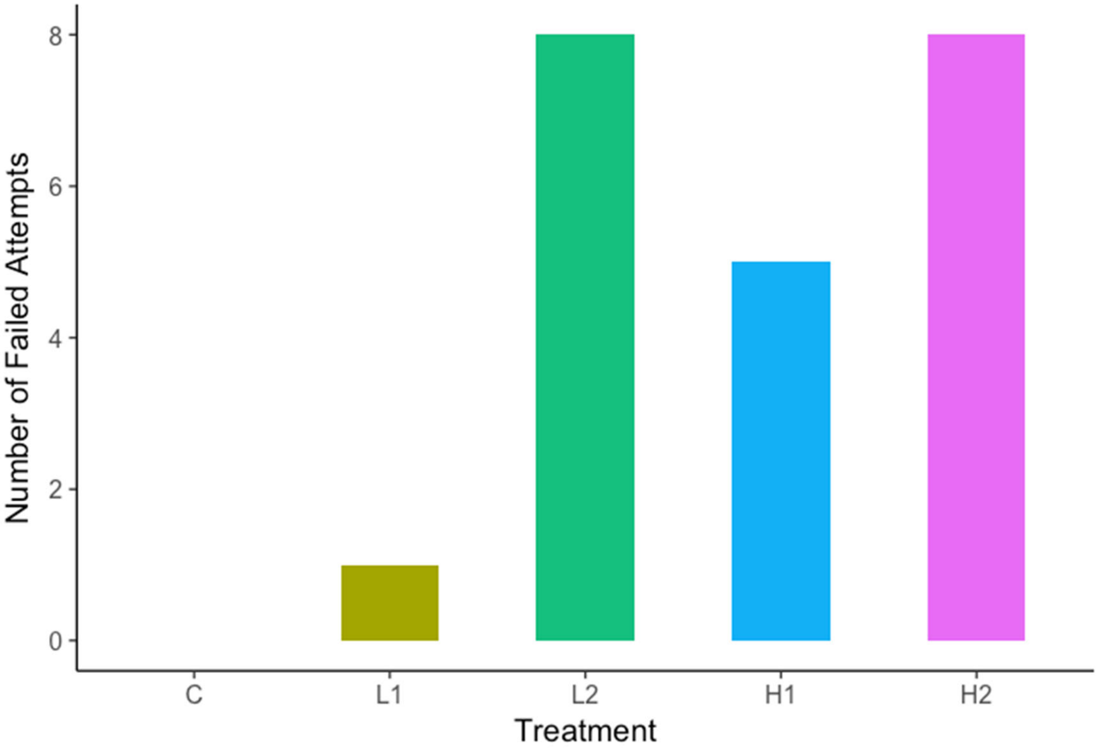

Figure 1.9. Number of lamprey that turned around after approaching a vertical slot weir in an experimental flume according to treatment level, 2018 and 2019. Each turn-around event represents one unique lamprey. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

Table 1.4. Number of observations censored from passage time analysis by treatment level and year.

Treatment	# Censored 2018	# Censored 2019
C	5	7
L1	7	10
L2	4	6
H1	1	5
H2	4	2
Total	21	30

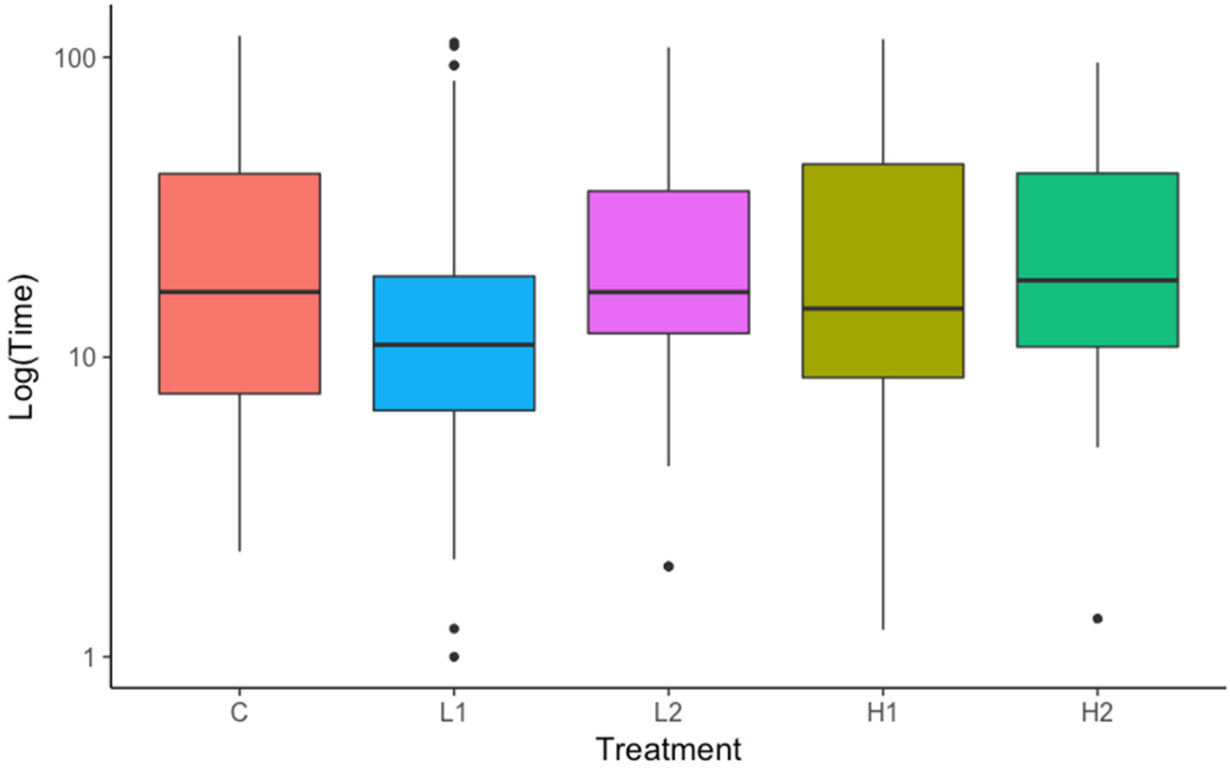

Figure 1.10. Passage times (log transformed) of a vertical slot weir in an experimental flume by treatment for 2018/2019 data combined. Median passage times for each treatment are as follows: C=16.5 min, L1=11 min, L2 = 16.5 min, H1 = 14.5 min, and H2 = 18.2 min. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

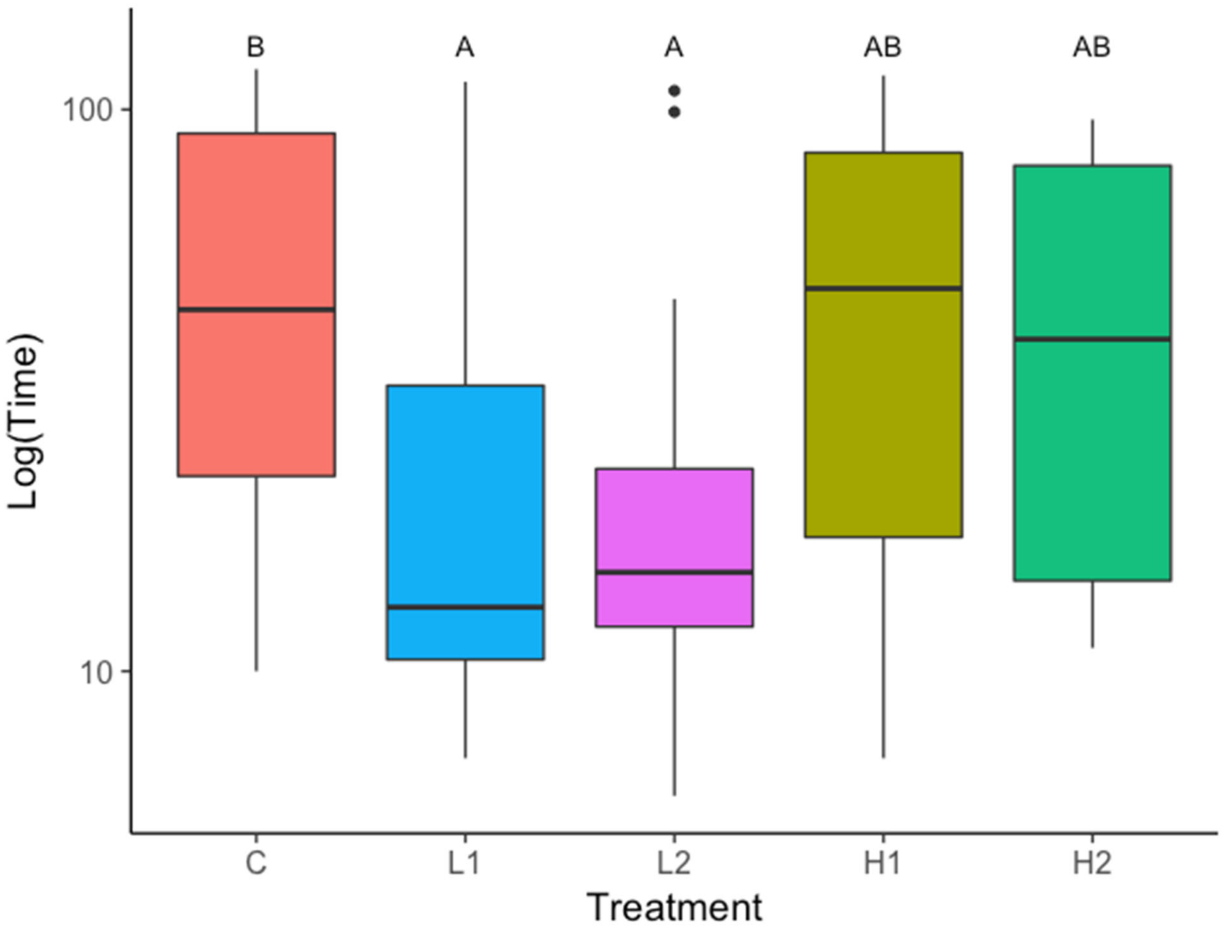

Figure 1.11. Passage times (log transformed) of a vertical slot weir in an experimental flume by treatment level, 2018. Median passage times for each treatment are as follows: C=44.5 min, L1=13 min, L2=15 min, H1=48 min, H2 = 39 min. Boxes with different letters indicate significant differences between treatments. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

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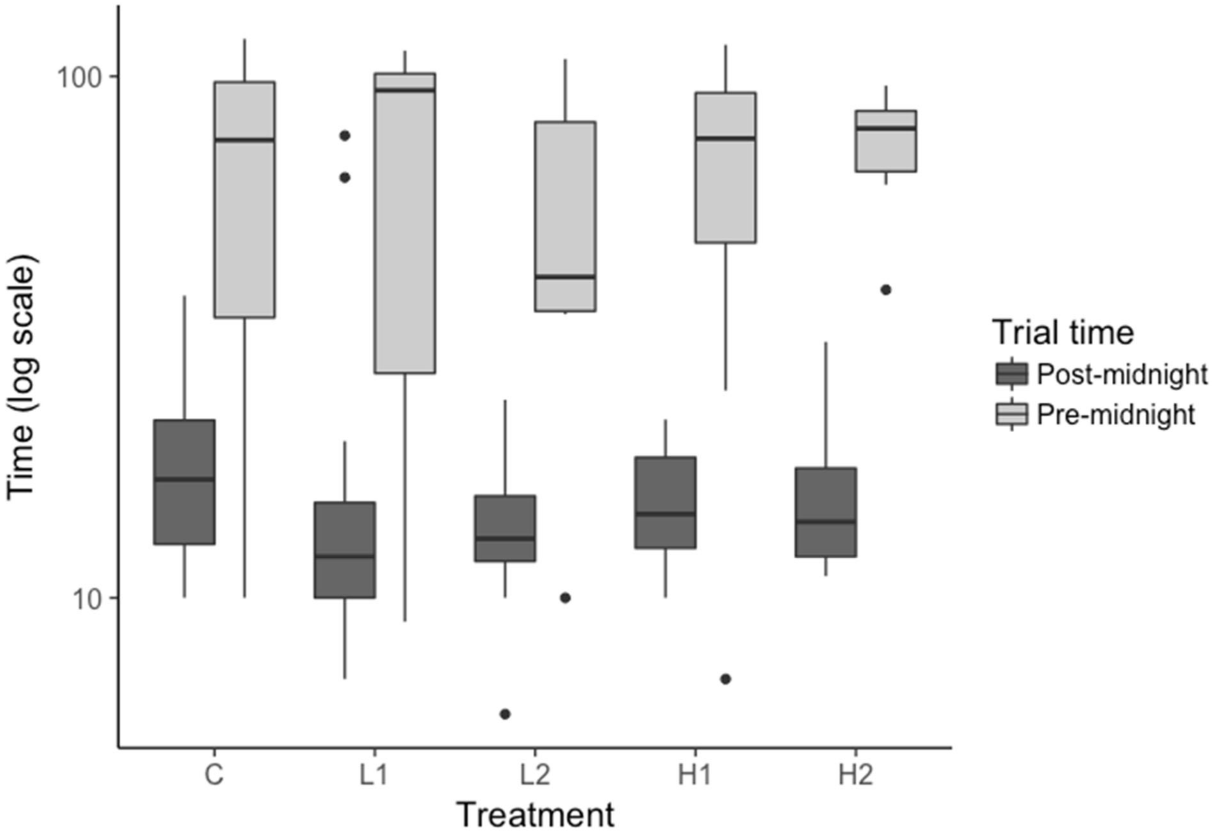

Figure 1.12. Passage times (log transformed) of a vertical slot weir in an experimental flume by treatment level and trial time, 2018. Median passage times of pre-midnight trials are as follows: C=75.5 min, L1=94 min, L2=41.5 min, H1=76 min, H2=79.5 min versus median passage times of post-midnight trials C=17 min, L1=12 min, L2=13 min, H1=14.5 min, H2=14 min. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

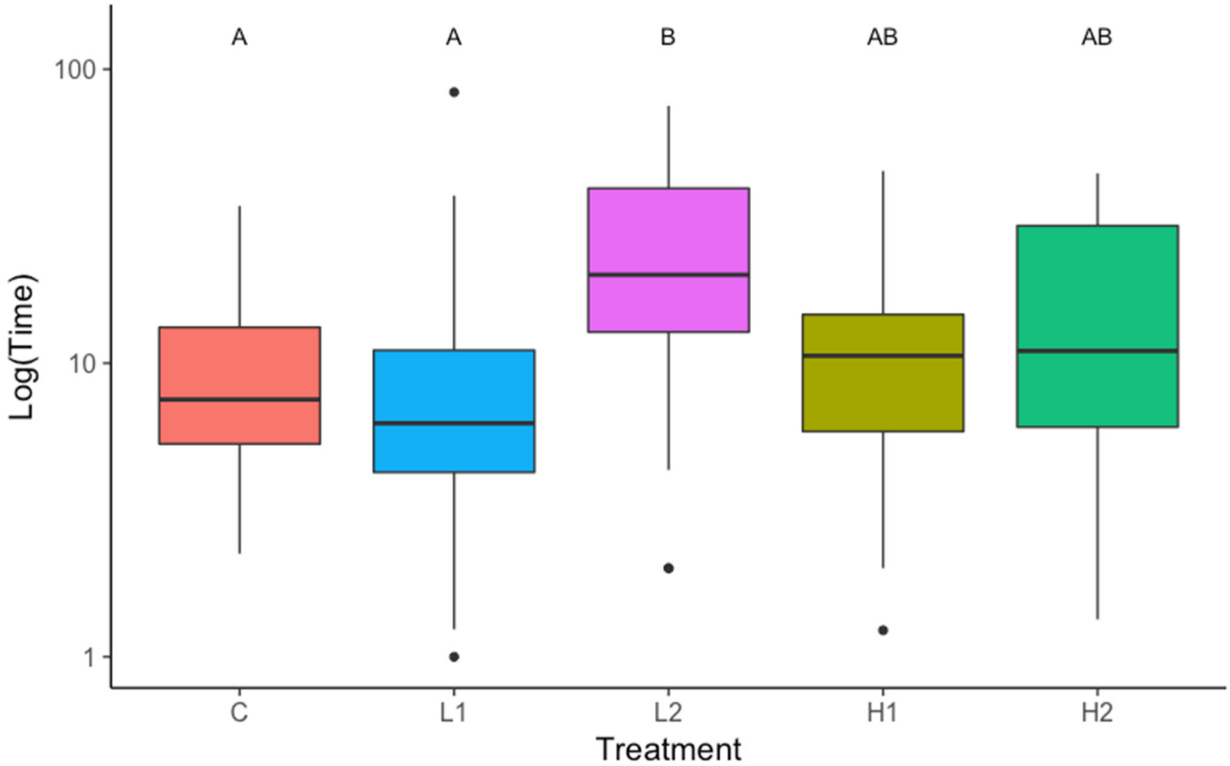

Figure 1.13. Passage times (log transformed) of a vertical slot weir in an experimental flume by treatment level, 2019. Median passage times for each treatment are as follows: C= 7.5 min, L1=6.25 min, L2=20 min, H1=10.5 min, H2=11 min. Boxes with different letters indicate significant differences between treatments. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

No blood physiological parameter was associated with passage success in preliminary models. Values for blood lactate ($\chi^2 = 2.24$, $P = 0.13$), glucose ($\chi^2 = 0.285$, $P = 0.59$, hematocrit ($\chi^2 = 0.65$, $P = 0.42$), and pH ($\chi^2 = 0.39$, $P = 0.53$) were similar across individuals (Figure 1.14).

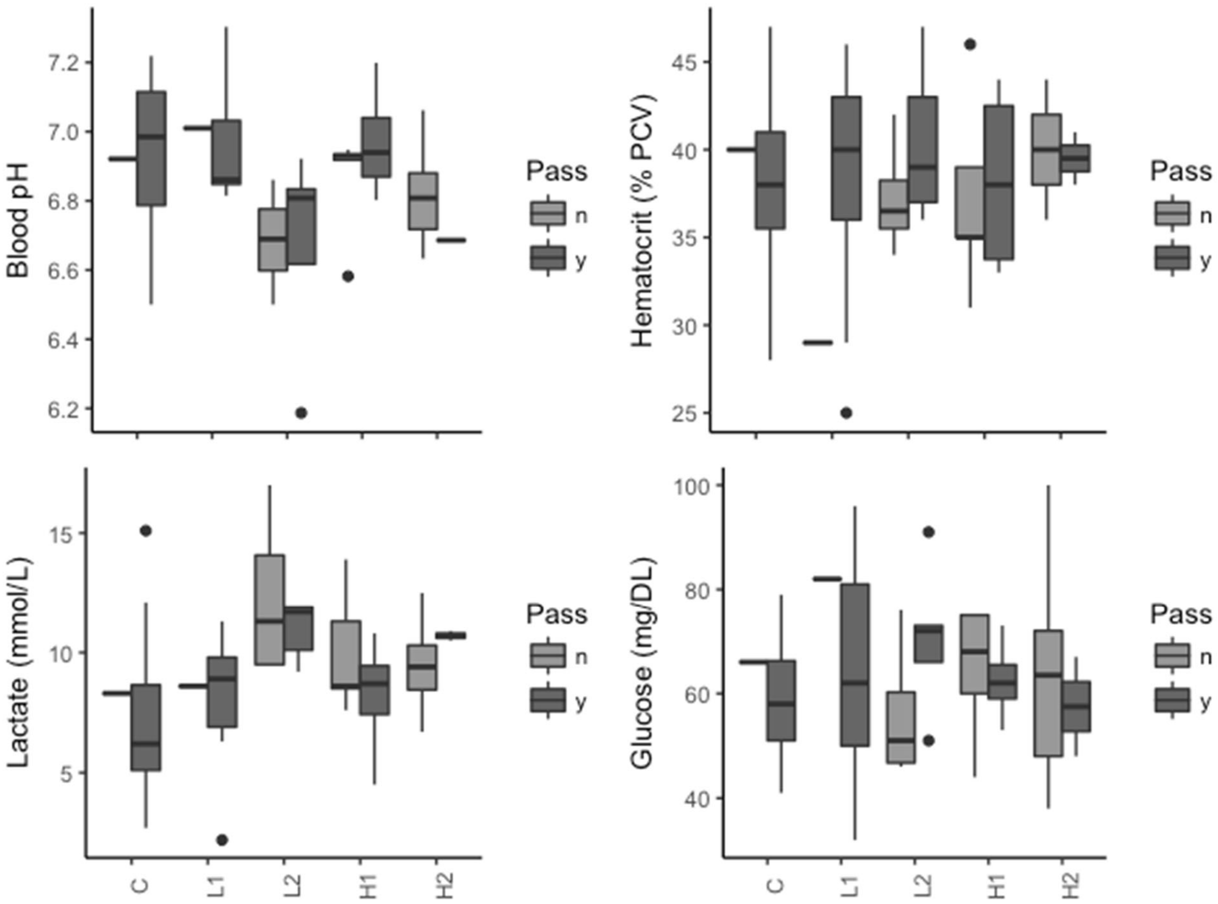

Figure 1.14. Blood pH, hematocrit (%PCV), lactate (mmol/L), and glucose (mg/DL) levels according to treatment among lamprey (n=50) who passed and failed to pass the experimental flume. Treatment factors refer to the following: C= control; L1 = low velocity, low duration; L2 = low velocity, high duration; H1 = high velocity, low duration; H2 = high velocity, high duration.

When evaluating upstream post-experiment escapement past upstream dams, multinomial logistic regression analysis indicated that lamprey length was the only significant predictor of migration distance for fish passing The Dalles (χ^2= 6.29, *P*=0.01), John Day (χ^2=19.71, *P*= <0.0001), Ice Harbor (χ^2=4.52, *P*=0.03), and Priest Rapids (χ^2=14.90, *P*=0.0001) dams as compared to Bonneville Dam (Table 1.5). In all cases, the odds of passing increased with every 1 cm increase in length. The majority of fish had final telemetry records in the lower Columbia River (Figure 1.15). Due to small sample size of fish detected in tributaries and upper segments of the Columbia and Snake rivers, analysis was restricted to passage of major dams. Nonetheless, a small number of fish had last detections at major

Columbia River tributaries and further upstream on the upper Snake and upper Columbia rivers (Figure 1.16).

Table 1.5. Multinomial logistic regression results testing for the effect of experimental flume passage success and morphometric measurements on upstream escapement past dams. Reference level is Bonneville Dam. Factors that are significant are indicated in bold.

Logit	Variable	χ^2	P	Odds Ratio	95% CI
The Dalles	Passed Weir	0.002	0.97	1.01	0.55-1.87
	Dorsal Distance/TL	2.02	0.15	0.93	0.83-1.03
	Length	**6.29**	**0.01**	**1.08**	**1.02-1.15**
John Day	Passed Weir	0.04	0.84	0.93	0.48-1.81
	Dorsal Distance/TL	0.67	0.41	0.95	0.85-1.07
	Length	**19.70**	**<0.0001**	**1.17**	**1.09-1.25**
McNary	Passed Weir	0.001	0.97	1.03	0.19-5.42
	Dorsal Distance/TL	0.04	0.84	0.97	0.73-1.29
	Length	2.52	0.11	1.14	0.97-1.35
Ice Harbor	Passed Weir	0.16	0.69	1.60	0.16-15.85
	Dorsal Distance/TL	1.08	0.30	0.80	0.53-1.22
	Length	**4.52**	**0.03**	**1.29**	**1.02-1.64**
Priest Rapids	Passed Weir	0.14	0.71	0.82	0.29-2.33
	Dorsal Distance/TL	0.0004	0.98	1.00	0.83-1.21
	Length	**14.90**	**0.0001**	**1.26**	**1.12-1.41**

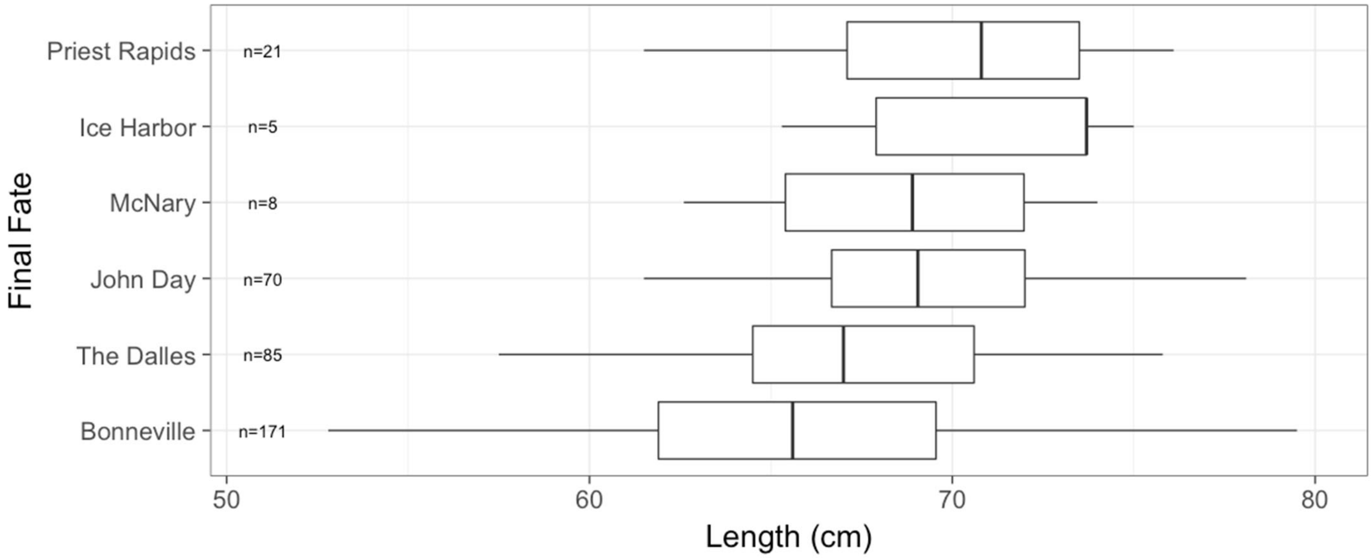

Figure 1.15. Distributions of tagged lamprey length (cm) by the final recorded location for each fish for 2018-2019. Data shown are for lamprey released upstream of Bonneville Dam. Fishway locations include fish last recorded inside fishways without known passage.

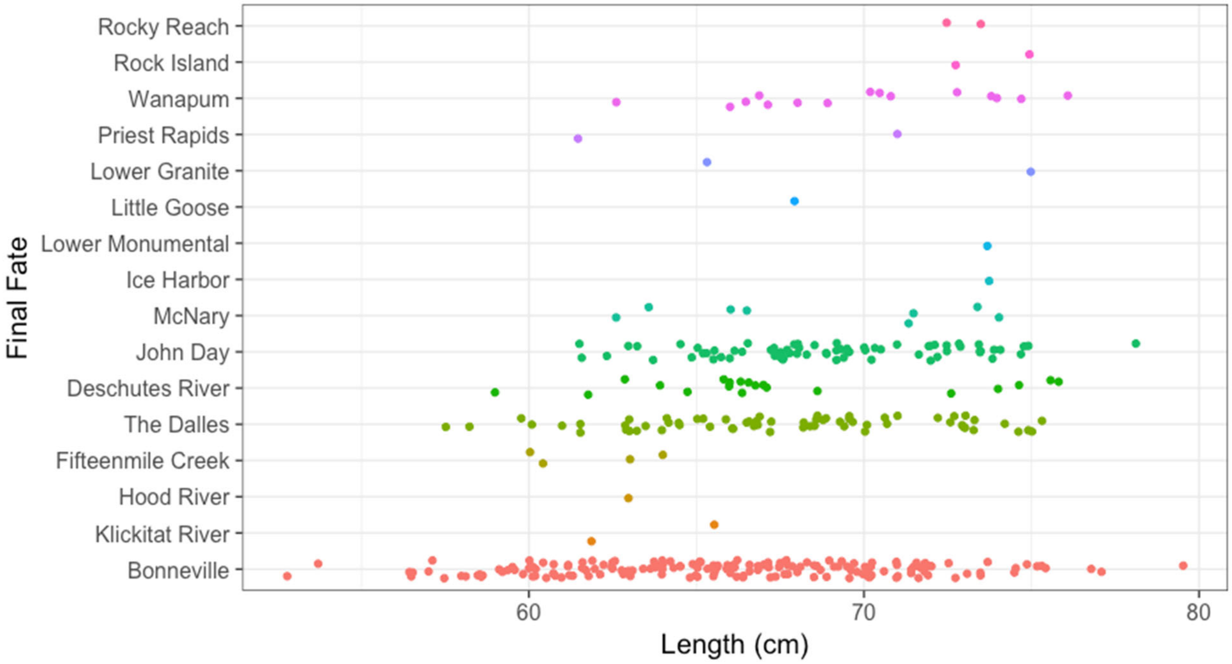

Figure 1.16. Distributions of tagged lamprey lengths (cm) by the final recorded locations for each fish for 2018-2019. Data shown are for lamprey released upstream of Bonneville Dam.

Discussion

Bonneville Dam features complex, multiple fishways that pass adult Pacific lamprey at rates of ~49% (Keefer et al. 2013a, 2014b, 2019). Extensive telemetry research has revealed passage bottlenecks that shift temporally and spatially, generally with high turn-around rates exhibited late in the season at higher elevation fishways (Keefer et al. 2013a, 2019). This study has yielded valuable insights linking behaviors observed in experimental laboratory conditions to behaviors that occur in fishways at the large, dam-wide scale.

Lamprey at Bonneville Dam must traverse a series of pool-and-weir fishways before entering a section with vertical slot (serpentine) weirs prior to exiting the dam. Hence, they may experience fatigue by the time that they reach higher elevation fishways. Exhaustive exercise has been shown to result in decreased swimming capability (Cai et al. 2015) and may have important implications for lamprey passage at Bonneville Dam. We sought to characterize behavior and swimming performance under varying levels of exhaustive exercise to evaluate possible mechanism(s) behind passage failures at a known bottleneck to passage.

Overall, we found that the duration of prior exercise significantly lowered passage success rates through a vertical slot in the laboratory. High water velocity produced the same effect, with significantly lower passage success rates as velocity increased (Figure 4). These results indicate that lamprey may be able to pass through high velocities at a single challenge but may reach an endurance threshold when faced with multiple obstacles. Sockeye salmon that continuously swam for extended periods at speeds greater than critical swimming speed were unsuccessful in passing a fishway in a reach characterized by high velocities and turbulent flows as compared to successful migrants that had been swimming at lower speeds (Hinch and Bratty 2000). This variability in behavior ultimately translated to unsuccessful migrants having longer residence times while swimming at speeds that have been known to cause exhaustion.

An alternative hypothesis is that Pacific lamprey adults lose motivation when faced with multiple passage barriers. Lamprey are not philopatric (e.g., Hess et al. 2013), unlike salmonids (Keefer and Caudill 2014a), and thus factors leading to the decision to turn-around in a fishway or cease upstream migration likely differ between the taxa. Since lamprey do not attempt to return to a natal destination, they may respond differently to suboptimal or difficult conditions as compared to salmonids that may result in lamprey pursuing spawning locations further downstream after turning around. Approaching a physiological or motivational threshold may be a factor in a decision to cease upstream migration.

Notably, these data may help reconcile conflicting conclusions of previous studies. While recovery time after exhaustion is relatively rapid in lampreys compared to salmonids (Paton et al. 2011; Mesa et al. 2003), Pacific lamprey passage through the energetically demanding serpentine weir sections at Bonneville Dam has been consistently poor (Keefer 2013a, 2019). Kirk et al. (2016) found that lamprey readily passed an experimental slot similar to a single serpentine weir, but lamprey attachment time significantly increased at velocities of 2.4 m/s in the presence of a turbulence-inducing wall as compared to lower velocity and less turbulent conditions. They suggested exhaustion after multiple weirs could explain the difference in passage success in the single-weir experiment and field observations in the serpentine weirs and our results are consistent with this hypothesis. Specifically, high velocity alone does not pose a barrier, but the cumulative effect of multiple challenges might be an obstacle to lamprey passage. Lamprey attachment and repeated burst swimming movements over a prolonged period has been considered to indicate exhaustion (Quintella et al. 2004). Given that lamprey pass through 12 vertical slot weirs prior to exiting the fishway and the high turbulence in the serpentine weir section, anaerobic metabolism and exhaustion may be a contributing factor to passage failure. Furthermore, lamprey frequently turn-around in the serpentine weir section after passing through the majority of the weirs, indicating that they may be approaching some threshold (Keefer et al. 2019). Notably, lamprey passage rates through pool-and-weir sections of the fish ladder, with similar high velocity slots between weirs, are high, suggesting the pool-and-weir sections may be less turbulent and/or allow lamprey to rest between challenges.

Maturation status may affect passage outcomes. We found that lamprey with a smaller dorsal gap relative to body size were less likely to pass. Maturation status is associated with a decrease in dorsal distance, and lamprey that are closer to spawning may have lower energetic reserves compared to less mature individuals (Clemens et al. 2009). A similar trend has been observed in other studies, where dorsal distance was positively related to passage success of a vertical slot weir (Kirk et al. 2016). Interestingly, within fishways at Bonneville Dam, lamprey with shorter dorsal distances are more likely to use refuge boxes (Moser et al. 2019). Despite delaying passage, individuals who seek out refugia have the same probability of passing as those who do not utilize them. This was true even though some individuals would hold in rest boxes for several days. Based on these findings and related research, the relatively poorer swimming capabilities and apparent lower exhaustive threshold for fish that are closer to maturation further supports the need for fishways that contain velocity refugia.

Behavioral observations of lamprey attempting to pass through the weir revealed that lamprey rarely made multiple attempts during the duration of the trial (2 hours). If they failed to pass the first time, they would attach below the weirs and remain there for the duration of the experiment. Failed attempts ("turnarounds") also occurred more frequently in the high velocity and long duration treatment when compared to the control and low velocity, low duration treatment (Figure 8). Rate of attempts has been suggested to be a proxy for motivation (Castro-Santos 2004; Goerig and Castro-Santos 2017), with fish attempting at higher rates being categorized as 'more motivated'. As lamprey rarely attempted multiple times, motivation may be influenced by fatigue at the time scale of the experimental trials. Perhaps some physiological threshold is reached and fish cease activity in order to pursue other options before reaching complete exhaustion (Castro-Santos 2004b). While lamprey in the control and lower velocity/duration treatments had few or no failed attempts, the relatively high passage rates may mean that motivated individuals were disproportionally successful as compared to fish who underwent more strenuous exercise.

Telemetry studies have revealed lamprey often mill the top of fishways and some subsequently moved downstream to the tailrace and were very unlikely to progress upstream again (Keefer et al. 2013a). In a species that is non-philopatric, such as lamprey, the decision to cease upstream movement and turn-around may be a behavioral adaptation when faced with conditions that demand undue metabolic or energetic costs. Turn-arounds at the top of fishways occurred at higher rates later in the season (Keefer et al. 2013a), possibly indicating that migration timing may be an important factor in this decision. A similar increase in failure later in the season was observed in laboratory experiments reported here as well. It is unclear to what degree lamprey orient to exogenous cues such as pheromones from larval lamprey during upstream migration in main stem rivers, though the concentration of larval cues is not expected to vary strongly through the season given larval populations consist of multiple cohorts. The relative influence that exogenous and endogenous factors have on movement decisions by lamprey deserves further attention, and it is likely that temperature, discharge, and maturation status of the individuals are key components.

Qualitatively we noted that lamprey also appeared to attach in close proximity to one another and would appear to "cue" off of each other – as one lamprey initiated movement others would follow shortly thereafter. Commonly, lamprey would attach in groups of two or three at the lateral margins of the flume, just downstream from the vertical slot weir. An individual would depart, attach directly downstream from the weir for a varied period of time, then burst forward. After this event, other individuals appeared to follow shortly thereafter. Similar behavior was observed by Kirk et al. (2016), but it is difficult to discern whether lamprey were responding to hydrologic stimuli, conspecifics, or a combination of both. In sockeye, the timing of upstream migration was influenced by social interactions that were largely independent of environmental conditions (Berdahl et al. 2017). The role that social behavior plays in a species such as Pacific lamprey may be an important factor in determining route use and discovery. Given that lamprey migrate in low-light conditions and frequently exhibit demersal behavior when moving upstream, social interactions, both olfactory and tactile, could be pivotal in finding paths that demand lower energetic costs, particularly at "bottleneck" locations where lamprey densities are high.

Pacific lamprey primarily migrate at night (Robinson and Bayer 2005). The influence photophobia has on passage times and behavior within a fishway has been documented (Keefer et al. 2013b; Kirk et al. 2015), but evaluating how activity levels differ throughout during the night remains less understood. During the first year of this study, we found that passage times across all treatments were substantially faster for fish that underwent trials conducted in the hours directly preceding and following dawn as compared to those in the earlier evening. Time of arrival is an important predictor of passage success at Bonneville Dam fishways (Keefer at al. 2013b); fish who entered during the night had higher efficiency than those that entered during the day, though this trend was influenced by route and season. A similar phenomenon could occur at a smaller scale during the night, whereby fish may be more motivated to pass as dawn approaches in order to avoid light, particularly if there are no refuges available. Motivation, or the propensity to initiate movement can be greatly influenced by diel patterns (Goerig and Castro-Santos 2017). Further research evaluating activity patterns during the active migration period (i.e. during night hours) could help elucidate these patterns at the dam-wide scale. Regardless, we recommend that future studies consider diel patterns of lamprey behavior because time-of-day effects could easily obscure important biological patterns and/or compromise study outcomes.

Contrary to expectation, passage times were not any longer in the high velocity, high duration treatments than in the less strenuous treatments. Passage times were similar across all treatment levels; most fish either initiated movement through the vertical slot weir shortly after being released into the flume or remained attached just below it. This was particularly true in the high and long duration treatments, as most of the fish subjected to the control and lower velocity/duration treatment were successful. Though the cause for this behavior remains unclear, there are a few possible explanations. Notably, the variation was caused by differences in recent experience among treatments and/or inter-individual differences in condition or intrinsic traits because all lamprey experienced the same high velocity conditions during the passage challenge. Differences in personality have been linked to variations in risk-taking behavior and mobility (Farwell and McLaughlin 2009). Indeed, intraspecific variation in temperament was linked to consistent differences in swim speeds for upstream migrating lamprey after taking a host of environmental, morphological, and biological factors into account (Moser et al. 2013). In addition, experimental outcomes may have been influenced by an individuals' metabolic rate. The effect that metabolic rate has on temperament has only recently been considered (Metcalfe et al. 2016). Individual variation in metabolic rate has been linked to differences in aerobic scope – a determining factor in swimming performance and capacity as well as physiological traits (Metcalfe et al. 2016). Furthermore, individuals with a higher aerobic scope have been shown to recover faster after exhaustive exercise (Marras et al. 2010). The degree of intraspecific variation in metabolic rate for Pacific lamprey is unknown. Due to the variability in ocean experience, such as time since last feeding or time spent in freshwater, there may be significant variability. This variability could translate to marked intraspecies differences in swimming performance and behavior, potentially influencing passage capabilities.

One of the most consistent observations has been the increasing average size of lamprey reaching upstream sites during migration through the lower Columbia River (Keefer et al. 2009, 2019; Hess et al. 2014). However, this study and Kirk et al. (2016) found no evidence of size selection at the smaller scale of single and cumulative velocity challenges. While we did not observe statistical support for size selection after release and passage of McNary Dam, our sample size was smaller than previous studies and point estimates were consistent with size selection (Table 1.5). Thus, we speculate that a larger sample would have produced statistically significant effects. Regardless, fish that had a larger body size had higher rates of passage at The Dalles, John Day, and Priest Rapids Dam on the mid and upper Columbia and Ice Harbor Dam on the Snake River. Previous research has shown that there is a genetic link to body size and migration distance (Hess et al. 2014), with larger bodied-fish possibly having greater energetic reserves and possessing stronger swimming abilities. Thus, our findings are consistent with previously identified trends but the mechanism(s) causing size selection at larger scales remain unclear.

Conclusion

We found evidence that the exercise trials induced physiological stress and physical "exhaustion" that contributed to passage failure beyond the effects of high water velocity alone. Lamprey species are in decline worldwide (Baras and Lucas 2001), and poor passage has been implicated as one of the major contributors. Traditional fishways, while efficient for the passage of salmonids and other economically important species, often can be obstacles to lamprey and other species that are sensitive to high velocities and turbulence. As shown in this study, the recent prior experience of lamprey and cumulative effects are a potentially important consideration when designing and implementing passage criteria. As construction of impoundments continues to grow worldwide, understanding the life-history and migratory behavior of non-commercial diadromous and potamodromous fishes is increasingly relevant (Mallen-Cooper and Brand 2007). For a species like Pacific lamprey, the lack of strong homing behavior coupled with differences in morphology and swimming style are key characteristics that influence migratory behavior. When designing future fishways, an understanding of how species ecology drives migratory behavior is needed. In addition, measuring physiological responses, such as stress and fatigue, could provide insights into how behavior and physiology interplay with the elements of the fishway environment.

Chapter 2: Evaluating Swimming Behavior During Passage of Migrating Adult Pacific Lamprey Using Accelerometer Biotelemetry

Abstract

Dams can impede connectivity for migratory fish species. Despite increasing attention to fishway design, fish passage estimates still remain low for many non-target (i.e., non-salmonid) species. Non-target species such as Pacific lamprey (*Entosphenus tridentatus*) typically do not possess the burst swimming capabilities and strong homing drive present in salmonids. We used accelerometer biotelemetry to identify activity and behavioral responses of adult Pacific lamprey to local passage conditions at a previously identified passage bottleneck (the upper Washington-shore fishway of Bonneville Dam, serpentine weir section). Lamprey exhibited high intraspecific variability in duration and timing of attached and burst movements among sections of the fishway. Clear diel behavioral patterns were present, with longer durations of burst movement during the night hours. Within the serpentine weirs, lamprey that were successful at passing spent more time bursting compared to lamprey that did not pass. Unsuccessful fish spent a longer duration as attached and had higher turn-around rates, overall leading to longer residence times in the upper fishway. However, there appeared to be a threshold fatigue level with turnaround and failure because neither recent activity or activity prior to entry of the serpentine weirs was clearly associated with passage success. Regardless, the biotelemetry results indicated that the serpentine weirs, as compared to the rest of the fishway, require extended periods of burst swimming.

Introduction

A majority of the world's large rivers are highly regulated and impounded by dams, which serve as partial or complete barriers to fish migration (Rosenberg et al. 2000). Though increasing attention has been focused on improving fishway designs, passage estimates remain low for many non-target species (Mallen-Cooper and Brand 2007; Noonan et al. 2012; Silva et al. 2018). In many modified river systems, including the Columbia River, fishways were primarily constructed for salmonids and designed to accommodate their biological traits (migration strategy, morphology, swimming style, behavior, etc.). Use of fishways by other species can result in a mismatch between species requirements and fishway design (Peake et al. 1997; Katopodis 2005; Roscoe and Hinch 2010). Inappropriate attraction cues, challenging hydraulic features, and/or structural features may slow or block passage of non-salmonids (e.g., Kemp 2016; Haro and Kynard 1997; Keefer et al. 2010).

Identifying the mechanisms responsible for problematic passage areas is a challenge. Past telemetry research has revealed large-scale (~100m – 1,000 km) trends in fish movement behaviors (Moser et al. 2002; Caudill et al. 2007; Johnson et al. 2012; Caudill et al. 2013; Keefer et al. 2013a, 2014b; Kirk et al. 2015b), but elucidating the behavioral responses at a local-scales (<10 m) is more difficult. Accelerometers provides a method to monitor fine-scale behavioral responses to hydraulic conditions (Broell et al. 2013). Accelerometers measure acceleration induced by fish movement (primarily swimming) in units of gravity (*g)*. Radio transmitters (tags) equipped with accelerometers summarize these measurements and transmit them to radio receiving stations where unique fish identifiers, acceleration summaries, and time stamps are logged. Accelerometer tags have previously been used to quantify behavioral responses in wild fish, including spawning (Fuchs and Caudill 2019), foraging (Brownscombe et al. 2014), diel-specific activity levels (Whitney et al. 2007), passage (Silva et al. 2015), and sex-specific responses to modified flow (Burnett et al. 2014b). Furthermore, accelerometer biotelemetry provides a method to obtain acceleration data remotely via telemetry, providing the opportunity to study behavior in *at-liberty* animals in environments where recovery of a tag would be infeasible or impossible (e.g., Fuchs and Caudill 2019).

Pacific lamprey (*Entosphenus tridentatus*) is an anadromous species native to the Columbia River basin that has experienced dramatic population declines (Clemens et al.

2017b). Lamprey spend 1-4 years in the ocean before migrating upstream to spawn in freshwater rivers and are non-philopatric, responding to pheromones from ammocoetes in headwater streams to direct them towards spawning locations within spawning tributaries (Close et al. 2002; Yun et al. 2011; Spice et al. 2012). Bonneville Dam, the lowermost dam on the Columbia River, is the first major anthropogenic obstacle Pacific lamprey face when migrating upstream past as many as nine mainstem dams to spawn (Keefer et al. 2013a). Bonneville Dam has a complex system of fishways in three river channels converging into two primary exits. Multiple fishway entrances lead into low gradient collection sections to steeper pool and weir ladders before transitioning to vertical slot weirs at the tops of both fishways. Extensive radio-telemetry research has identified areas of poor passage (i.e., bottlenecks) characterized by milling and high-turn around rates (Moser et al. 2002; Keefer et al. 2009; Johnson et al. 2012; Keefer et al. 2013a). The vertical slot weir sections in the upper Bonneville Dam fishways (a.k.a. serpentine weir sections) feature relatively high velocities and turbulent conditions, and have been identified as lamprey bottlenecks (e.g., ~25-30% failure rates, Keefer et al. 2013a, 2014). Consequently, lamprey-specific passage structures (LPS) have been installed to bypass lamprey around such problematic fishway sections (Moser et al. 2011). Despite overall high success rates after LPS entry, many individuals initially pass by LPSs when moving upstream (Keefer et al. 2019), furthering the need to evaluate movement patterns in the upper fishway to identify mechanisms responsible for poor passage.

Upstream migrating Pacific lamprey are characterized by high intraspecific variability in movement patterns (Kirk and Caudill 2017), resulting in differences in route use and duration of time spent in specific fishway sections. Previous research has documented a wide range of behaviors when navigating through serpentine weirs (Kirk et al. 2015b), and fish can spend as little as one hour to several days navigating upstream (Keefer et al. 2013a). In addition to environmental conditions, swimming ability and migration distance are impacted by the physiology, behavior, and genomics of an organism (Cooke et al. 2008; Hanson et al. 2008; Hatry et al. 2014). The relative importance of each component, and the interactions between them can determine the cues and constraints encountered during migration.

The relative influence of proximate and ultimate mechanisms leading to a decision to cease migratory activity are not always clear. For Pacific lamprey, which cease feeding for a prolonged period of time before spawning, the consequences of behavioral decisions may have important implications for energy budgets. Shorter term influence of physiology on behavior may also occur. Lamprey commonly use a saltatory swimming mode (burst-and-attach swimming) to move upstream through challenging flow conditions (Keefer et al. 2010). This can result in anaerobic metabolism and potentially influence swimming performance and/or behavioral decisions. Costs associated with burst swimming can result in delayed deleterious effects in salmon (Burnett et al. 2014a). Motivation to overcome obstacles during passage is likely a result of all of these factors, and intraspecific variability can create differences in performance (Goerig and Castro-Santos 2017). In non-philopatric species such as lamprey, these factors may collectively affect migration decisions such as a shift from directed upstream migration to tributary-seeking behavior. Accelerometer telemetry can be used to understand how environmental conditions influence behavior during fishway passage and associations between behavior and passage attempt outcome, particularly for sections with high hydraulic complexity, while simultaneously documenting variability amongst individuals (Cooke et al. 2004; Burnett et al. 2014b; Silva et al. 2015).

The goal of this study was to link acceleration records with swimming behaviors in Pacific lamprey during upstream passage. The objectives were to 1) classify and validate acceleration telemetry records with behaviors using an experimental flume and 2) release and monitor accelerometer-tagged lamprey into the Washington-shore fishway at Bonneville Dam. With this information we aimed to a) identify general trends and activity levels associated with fishway use and b) evaluate associations between fine scale behaviors in the serpentine weir section by passage outcome (turnaround vs. fishway exit).

Methods

Lamprey collection and tagging:

Adult Pacific lamprey (*n* = 44) were collected at night from May 20 to August 11, 2019 from a lamprey flume structure (LFS) located at the adult fish facility (AFF) at Bonneville Dam (45.6°N, 121.9°W) on the lower Columbia River. Fish were removed from the collection box the following morning and were held in large aluminum tanks (92-cm wide × 152-cm long × 122-cm high) that received aerated river water. Prior to tagging, lamprey with a girth circumference ≥11 cm (to reduce tag burden effects; measured at the insertion of the first dorsal) were anesthetized in a 60-ppm (3 mL/50 L) solution of eugenol (AQUI-S 20E). An approximate 12-mm ventral incision was made directly below the anterior insertion of the first dorsal fin and a half-duplex passive integrated transponder tag (HD-PIT tag; 4 × 32 mm) was inserted into the body cavity. Following this, a 14-cm long sterile catheter was placed inside the body cavity and pushed through the body wall approximately 3-5 cm posterior to the incision. An accelerometer tag (9 × 42 mm, 6.8 g, tag life 39 d; MCFT3-SO-A, Lotek Wireless, Newmarket, ON) antenna was threaded through the catheter and the tag was inserted into the body cavity. Three sutures were used to close the incision. Fish were weighed (g), measured for total length (cm), body girth (cm), and distance between dorsal fins (dorsal distance, cm). Dorsal distance is used to indicate maturation status in lamprey (Clemens et al. 2009) and decreases as lamprey mature. Dorsal distance was calculated as dorsal distance/total length to account for variation in body size among individuals. Percent body fat (lipid) was estimated using a fish fat meter (Assurant Innovations, San Jose, CA) and a fin clip was removed for future genetic studies. Fish were allowed to recover for a minimum of 8 hours before release.

Accelerometer tags:

The accelerometer tag measures acceleration at 12.5 Hz and reports maximum acceleration in any vector every 5 seconds (referred to as the burst interval, BI), with a maximum value of 1.5 g (gravity, m/s^2). We used Lotek SRX 800 receivers and a combination of 3- and 4- element aerial antennas and an underwater antenna to monitor behaviors using standard telemetry methods; locations are shown in Figure 2.1. An underwater test tag was used to establish detection range for each aerial and underwater antenna. Based on these findings, the fishway was divided into three primary sections for the purpose of differentiating behaviors by location. All detections downstream of an HD-PIT (half-duplex passive integrated transponder) antenna located in the lower ladder (downstream of the AFF) were categorized as "lower." All detections above this point were considered "upper." A series of four dual-reading (full and half duplex) PIT antennas in the serpentine weir section was used to classify a "serpentine" location.

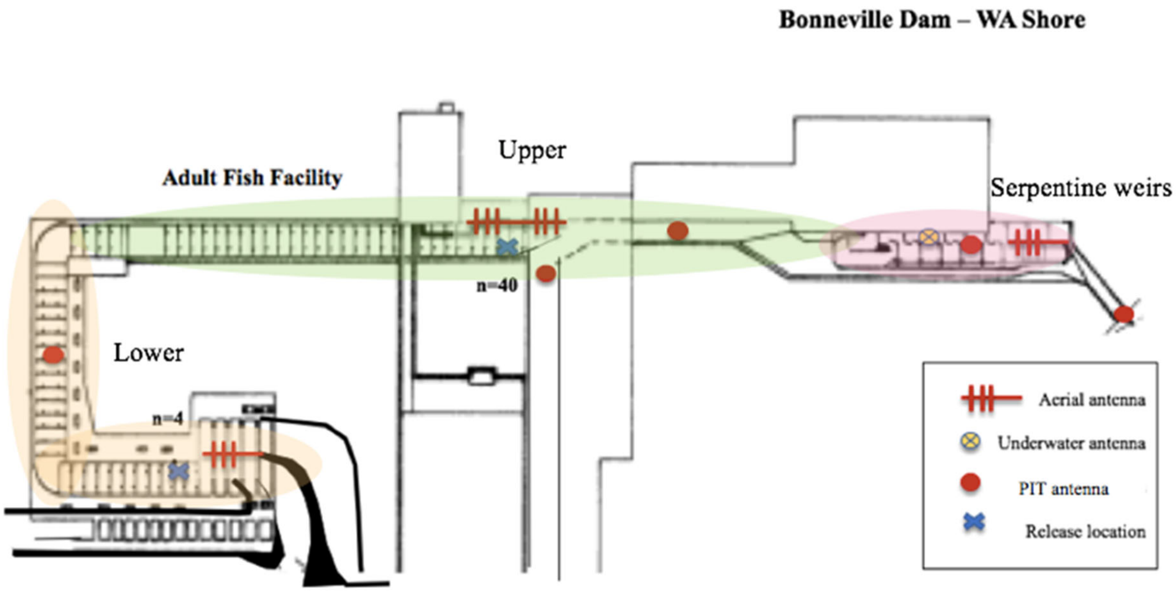

Figure 2.1. Washington-shore fishway at Bonneville Dam, with locations of monitoring locations and the release sites for lamprey tagged with accelerometer radio-tags (n = 44). Aerial antennas consisted of two 3-element and one 4-element yagi antennas. One underwater antenna was positioned between weir one and two in the serpentine weirs. PIT antennas in the serpentine weirs represented four dual-reading PIT antennas at weirs 3,5,7, and 11.

Observations in experimental flume:

A subset of lamprey (n = 6) were placed in an experimental flume to observe behaviors under velocities ranging from 0.8 m/s to 2.2 m/s in order to associate behaviors with the tag output prior to release in the fishway. Lamprey were initially placed in a mesh-lined chamber that prevented attachment and allowed observation of free-swimming behavior. They were allowed to acclimate at a velocity of 0.8 m/s for 10 min, velocity was increased to 1.2 m/s for 5 min, and then increased to 1.4 m/s for 5 min. Lamprey were released from the chamber into the flume at a velocity of 2.2 m/s for 1 hour. Movements and behaviors were recorded using underwater cameras (Speco Technologies; Model #CVC320WP8). Based on previous video observations, lamprey behavior was classified as attached (oral disk attached to substrate), sustained swimming, and burst swimming (including saltatory movements).

Start and end times for behaviors were recorded and aligned to telemetry records. Acceleration records aligned to behaviors were qualitatively examined to identify threshold acceleration values separating each behavior. A subset of acceleration telemetry records were independently scored using accelerometer criteria without accompanying video data and using video. The two sets of scores were then compared to validate telemetry criteria. Lamprey tagged with accelerometer radio-tags (including those used for validation work) were released into the Washington-shore fishway at two locations in a block design. There were three blocks of releases (Figure 2.2), with fish tagged in approximate proportion to run size in order to account for seasonal variation in river temperature and lamprey traits.

Data Analysis:

In order to assign behaviors from accelerometer output, one viewer watched and scored behaviors based on video records within the experimental flume. Average duration of footage reviewed per lamprey was 32 minutes (range 15 – 46 minutes). Each behavior (attached, swimming, bursting [including saltatory bursts]) was noted and given a time duration. Swimming and attached behaviors were categorized separately with respect to water velocity levels. The frequency, variation, and amplitude of acceleration records was evaluated graphically for each behavior to identify classification criteria. Additionally, mixed discriminant analysis was used to confirm independence of behavioral groups based on the acceleration records. The analysis was used to predict behavior groups (attached, mixed, burst) based on acceleration values. All acceleration data obtained from flume records were split into a training (80%) and test (20%) set to test model accuracy. The resulting acceleration criteria were then used to assign behaviors to the time series of acceleration records for individual *at-liberty* fish released at the WA-shore fishway.

In the serpentine weir section, approach times were calculated as the time from release until fish were detected on an HD-PIT antenna located in the lower serpentine weir section. We used logistic regression to test for difference in approach times, with passed vs. did not pass as the binary response variable. A chi-square test was performed to determine if the proportion of fish lamprey released that approached the serpentine weir section was constant across blocks of date. Passage times were calculated for fish that successfully passed either by entering and exiting via the serpentine weir section or through LPSs in the Washington-shore fishway. To assess whether passage was associated with morphometric measurements and run timing, we used logistic regression. Predictor variables included length, dorsal distance relative to body size, and release date. Minimum number of turn-arounds in the serpentine weirs were determined from detections on the four dual-reading PIT antennas. We qualitatively compared behavior event rates during the first hour and last hour between successful and unsuccessful passers because the duration of time spent in the serpentine weir section differed among groups.

All analysis was conducted using R (R Core Team 2017).

Results

Sampling and tagging of lamprey were approximately proportional to the run, with the exception of the earliest tagged group (Figure 2.2). Pairwise comparisons among blocks revealed no significant differences in body size or maturation status as estimated by dorsal distance (Table 2.1).

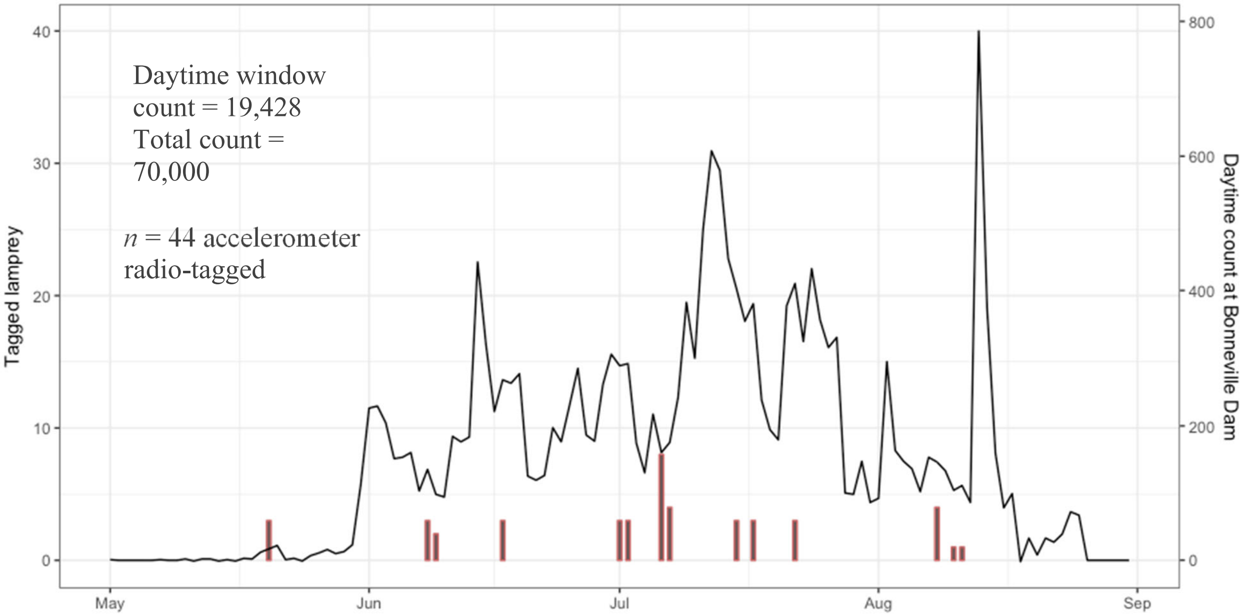

Figure 2.2. Daytime (line) and total count (including night estimates) of adult Pacific lamprey passing Bonneville dam and the number of fish collected and tagged for accelerometer release in WA-Shore fishway (bar). Passage estimates are from May 1 to September 31.

Table 2.1 Summary of number of blocks and corresponding dates of mean (SD) length, mean (SD) weight, and mean (SD) dorsal distance of lamprey.

Block	Dates	n	Mean Length (cm)	Mean Weight (cm)	Mean Dorsal Distance (cm)
Early	5/20 – 6/18	11	69.8 (±3.09)	565 (±85.8)	3.67 (±0.58)
Mid	7/01-7/22	27	70.8 (±3.11)	575 (±58.9)	4.00 (±0.56)
Late	8/08 – 8/11	6	67.6 (±4.36)	524 (±82.1)	3.50 (±0.99)
Total	5/20 – 8/11	44	70.1 (±3.39)	566 (±69.9)	3.85 (±0.65)

Based on video observations in the experimental flume, three behavior categories were identified for classification of acceleration records: attached, mixed, and bursting (Figure 2.3). Attached behavior occurred below a 0.225 g threshold and was marked by low frequency, low amplitude, and low variability through time. Free-swimming behavior in low to high velocities (0.8 – 2.2 m/s) observed in video did not have acceleration records that were distinct from records during videoed attached behavior in moderate/high water velocities (>1.4 m/s), and thus these two categories were combined and considered 'Mixed'. This category was characterized by high variation, high amplitude, and moderate frequency. The threshold acceleration levels were determined to be > 0.225 and <1.2. Burst movements were identifiable as a saltatory action, whereby the fish would be attached and burst forward for several seconds before attaching again. Bursting behavior had high amplitude, low frequency (i.e., short duration), and moderate variation. The threshold levels were >1.2 – 1.5 *g* (1.5 *g* = maximum recordable value). Mixture discriminant analysis classified groups with an 86% accuracy rate (Figure 2.4), with most misclassification occurring between mixed and attached. Behavioral groups identified in the flume corresponded to frequency of behaviors of *at-liberty* fish (Figure 2.5). Distribution of acceleration values by behavioral category was similar between fishway sections but had slightly higher median values for the serpentine weirs (Figure 2.6).

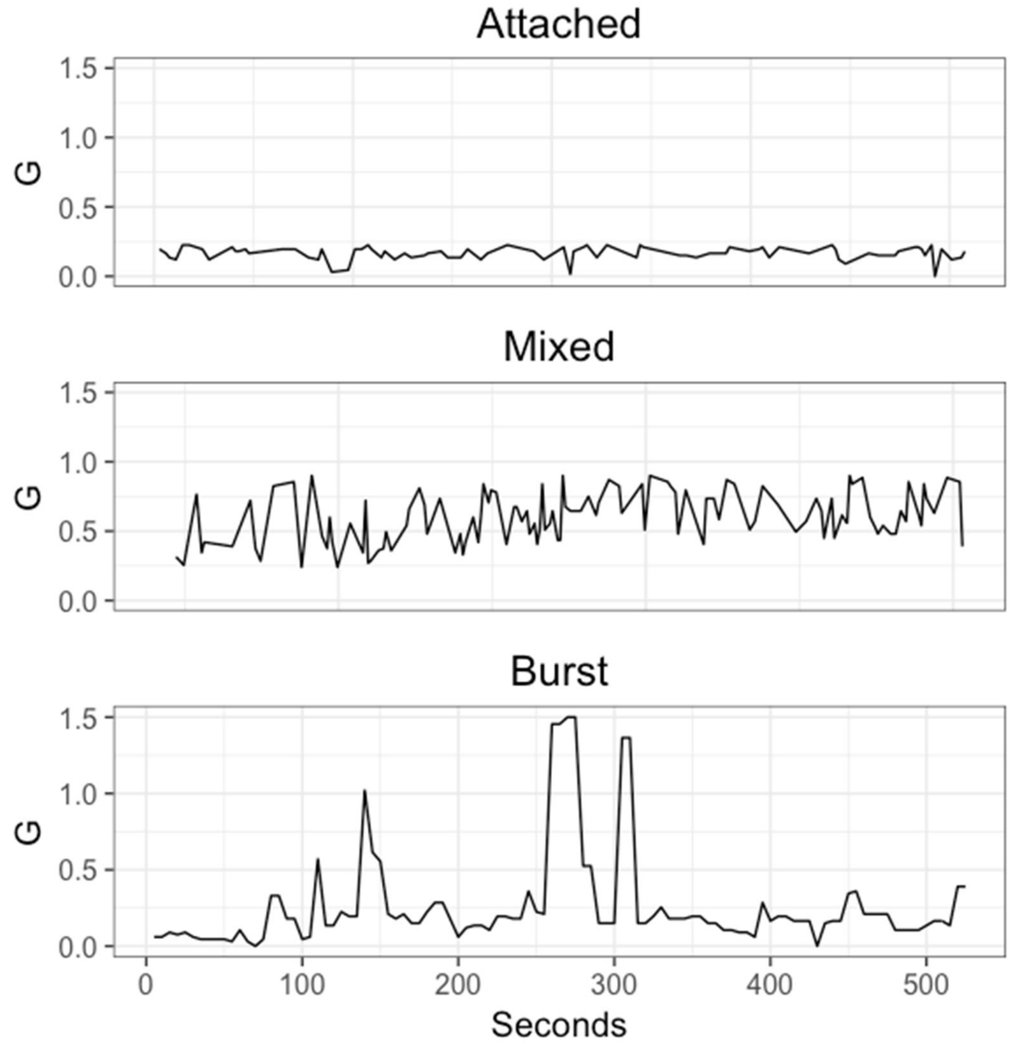

Figure 2.3. Acceleration time series for behaviors identified during video observation in the experimental flume. Behavioral events consisted of a continuously attached lamprey at 1.6 m/s (top), attached lamprey at 2.0 m/s (middle), and saltatory swimming with two distinct burst movements at 2.4 m/s (bottom). Time series represents approximately 8 min; tag burst interval was 5s.

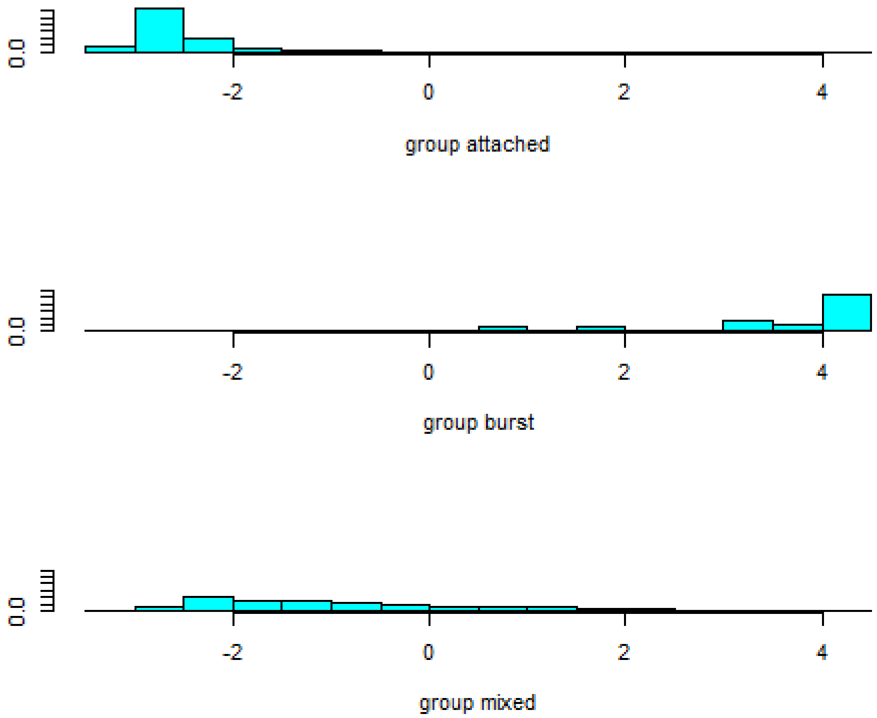

Figure 2.4. Histogram of discriminant function values for three different behavior groups

All Fish:

Overall, the majority of acceleration records for all fish released were classified as “attached”, with the average fish spending 18.9 hours attached (Table 2.2; Figure 2.7). Lamprey spent the least amount of time bursting, with just under an average of two hours per fish. However, time spent for each behavior was highly variable among lamprey, particularly attached. Gaps in detections occurred across most fish, with time intervals lacking acceleration data ranging from 10 s to several days. However, the majority of these gaps were less than one minute and only a few occurrences were greater than 12 hours (Figure 2.8). In several cases telemetry records indicated detection gaps resulted from fish moving downstream of the study area and returning at a later time. Clear diel behavioral patterns were visible, with fish exhibiting bursting behavior at a greater frequency during the ‘night’ hours (Table 2.3; Figure 2.9 with nighttime classified as 2100-0700 hours). On average, fish spent

88.7 minutes bursting during night hours versus 7.8 minutes during the day. In contrast, mean attached time was 11.85 hours during the day compared to 8.87 hours during the night.

Behavior varied between the lower and upper fishway segments. Lamprey in the "lower" section spent a greater amount of time attached compared to either burst or mixed behaviors (Table 2.4; Figure 2.10). It is noted that since the majority of fish were released upstream of the "lower" section, comparatively fewer records exist for this section (Figure 2.11 & 2.12). In contrast, lamprey in the upper section on average spent more time in the mixed behavior category. In the serpentine weir section, which had the fewest number of detections overall, mixed or burst had the longest duration, based on the average or median, respectively. Overall, mean and median duration of behaviors occurred at different proportions across fishway sections, with a general increase in activity level higher in the fishway.

Table 2.2. Summary of range, median (%) and mean (SD) number of hours for each behavior for all lamprey (n=44) released at the Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (hour)	Median duration (hour) (%)	Range (hour)
Attached	18.9 (31.5)	4.85 (23.6)	0.001-137
Burst	1.47 (1.77)	0.917 (4.5)	0.003-7.57
Mixed	2.69 (3.25)	1.82 (8.8)	0.013-14.1
Unknown	78.6 (137)	13 (63.1)	0-581.29

Table 2.3. Summary of range, median (%) and mean (SD) number of hours for each behavior by night and day time periods for all fish (n=44) released at the Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (min)	Median duration (min) (%)	Range (min)
Day			
Attached	711 (1147)	136 (90.0)	0.083-4841
Burst	7.81 (14.1)	0.792 (0.5)	0.083-60.3
Mixed	37.3 (61.2)	14.4 (9.5)	0.083-304
Night			
Attached	532 (838)	197 (56.6)	0.167-3388
Burst	887 (103)	58.3 (16.7)	0.333-451
Mixed	128 (151)	92.9 (26.7)	0.083-668

Table 2.4. Summary of range, median (%) and mean (SD) number of hours for each behavior by fishway section for all lamprey (n=44) released at the Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (min)	Median duration (min) (%)	Range (min)
Lower			
Attached	448 (1765)	1 (27.9)	0.083-7067
Burst	2.01 (3.75)	0.5 (14.0)	0.083-13.6
Mixed	16.1 (45.7)	2.08 (58.1)	0.417-185
Upper			
Attached	1063 (1570)	314 (67.5)	0.667-6171
Burst	81.6 (98.2)	53 (11.4)	0.083-399
Mixed	150 (174)	98.2 (21.1)	0.333-755
Serpentine Weirs			
Attached	0.939 (1.22)	0.333 (1.8)	0.083-4
Burst	14.2 (12.6)	10.2 (54.3)	1.33-52.1
Mixed	15 (18.6)	8.25 (43.9)	1.17-84.1

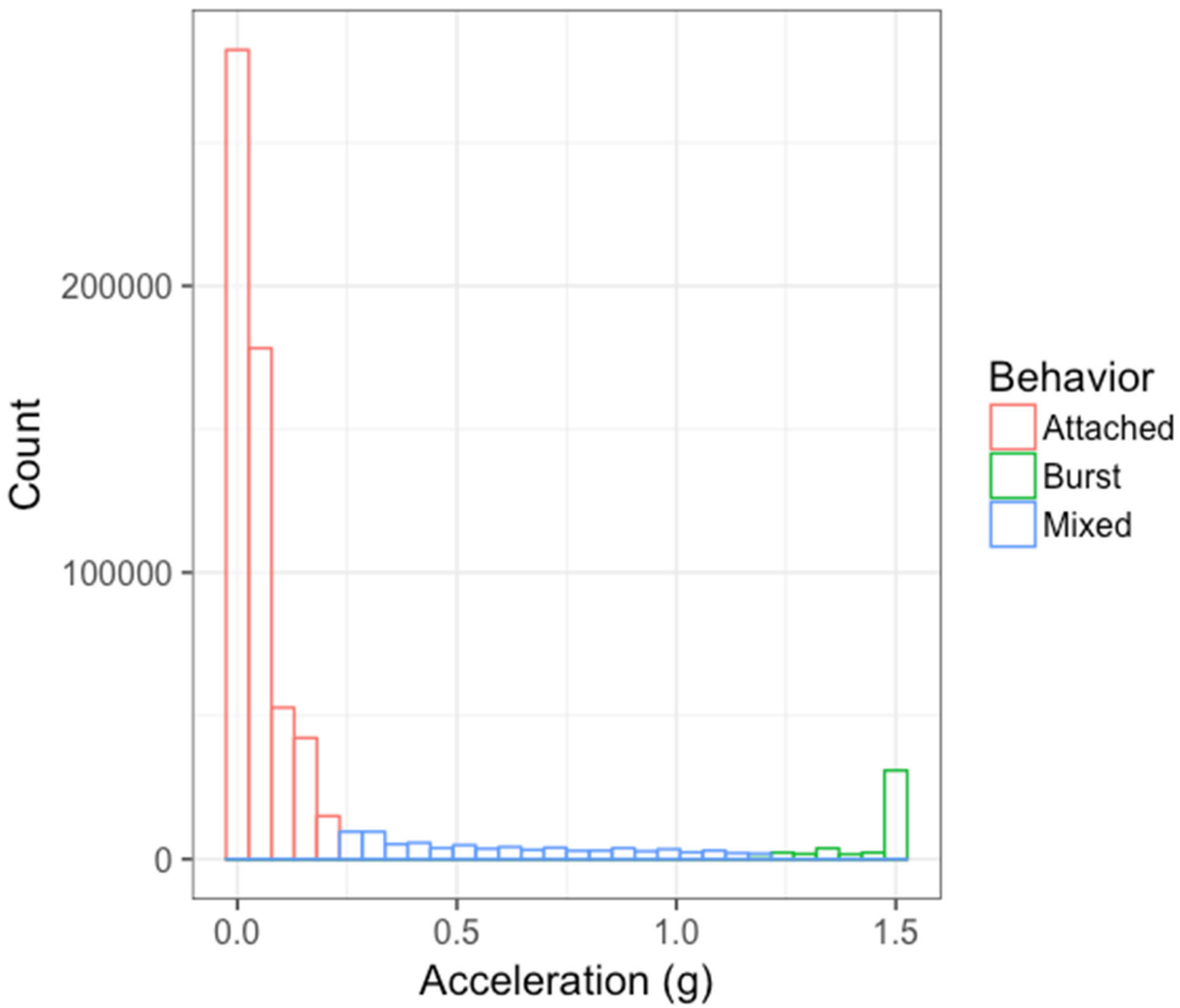

Figure 2.5. Histogram of all acceleration detections for lamprey (n=44) released in the Washington-shore fishway by behavior. Each record represents one burst interval (BI) at 5s.

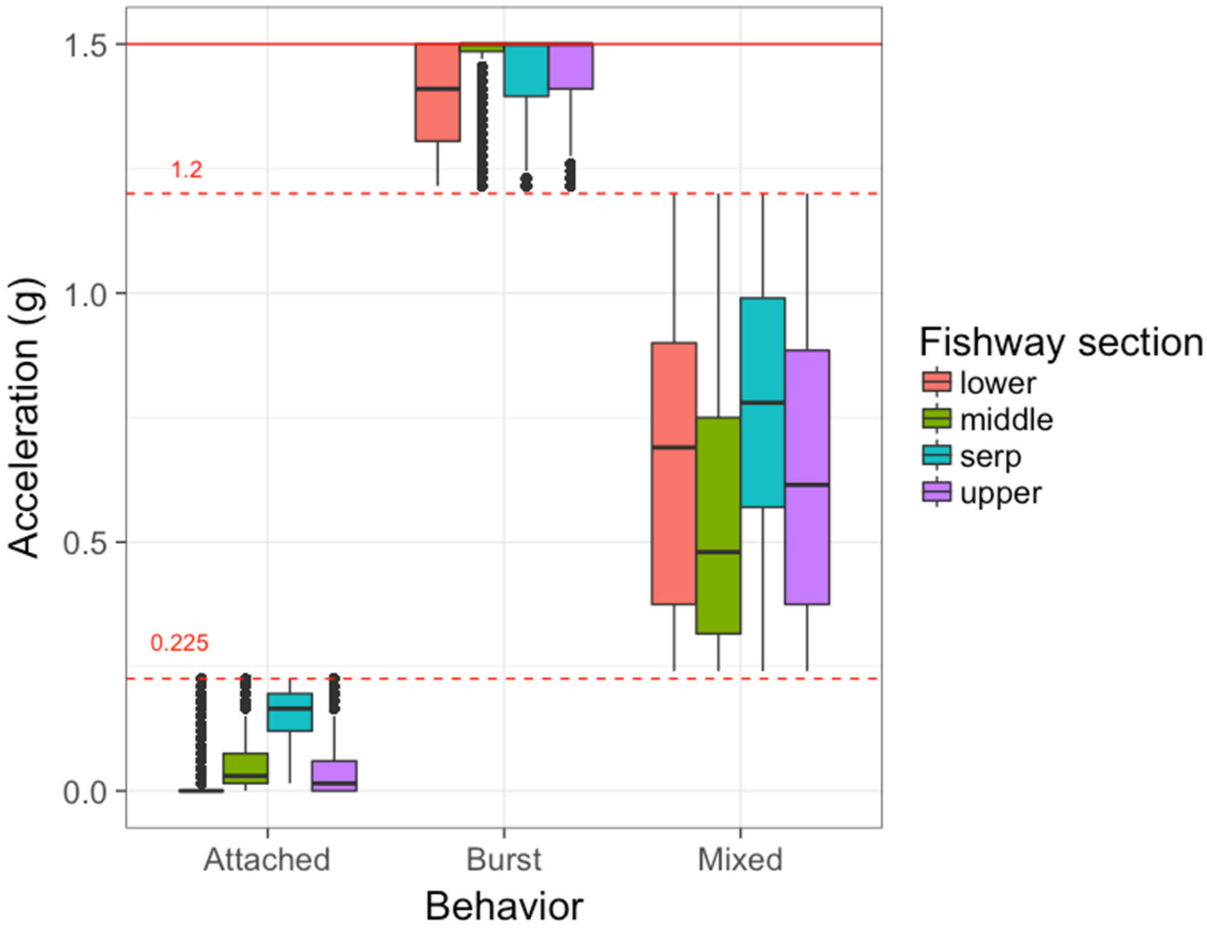

Figure 2.6. Distribution of all acceleration (g) records by each behavior within each fishway section for all fish (n=44). Red dashed line indicates threshold for attached and mixed behavior categories. Red solid line indicates upper threshold for burst behavior category.

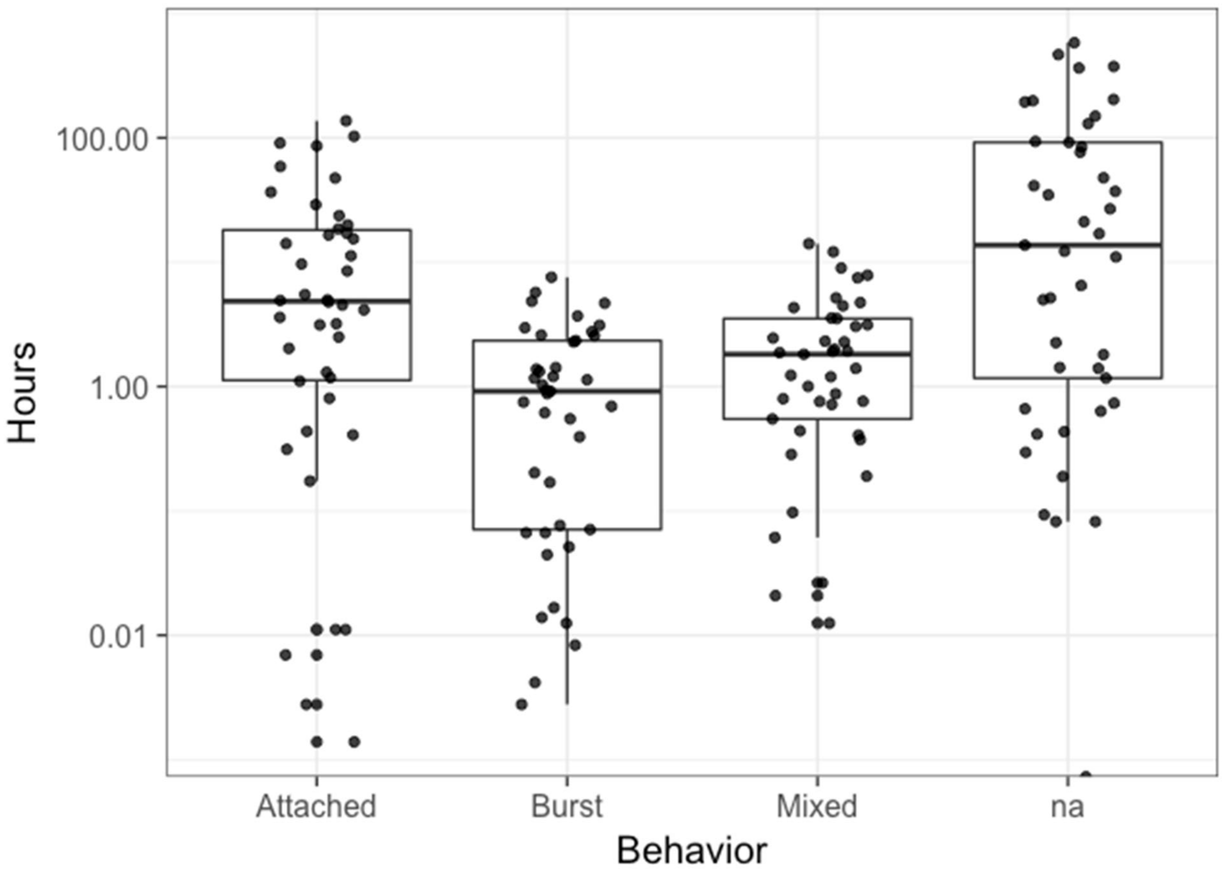

Figure 2.7. Duration of attached, burst, and mixed behaviors by lamprey (n=44) released at Washington-shore fishway at Bonneville Dam. NA refers to the duration of time that lamprey had missing acceleration data while in the study area. Median duration for each behavior and na are as follows: Attached=4.85 hours, Burst=0.917 hours, Mixed=1.82 hours, na=13 hours.

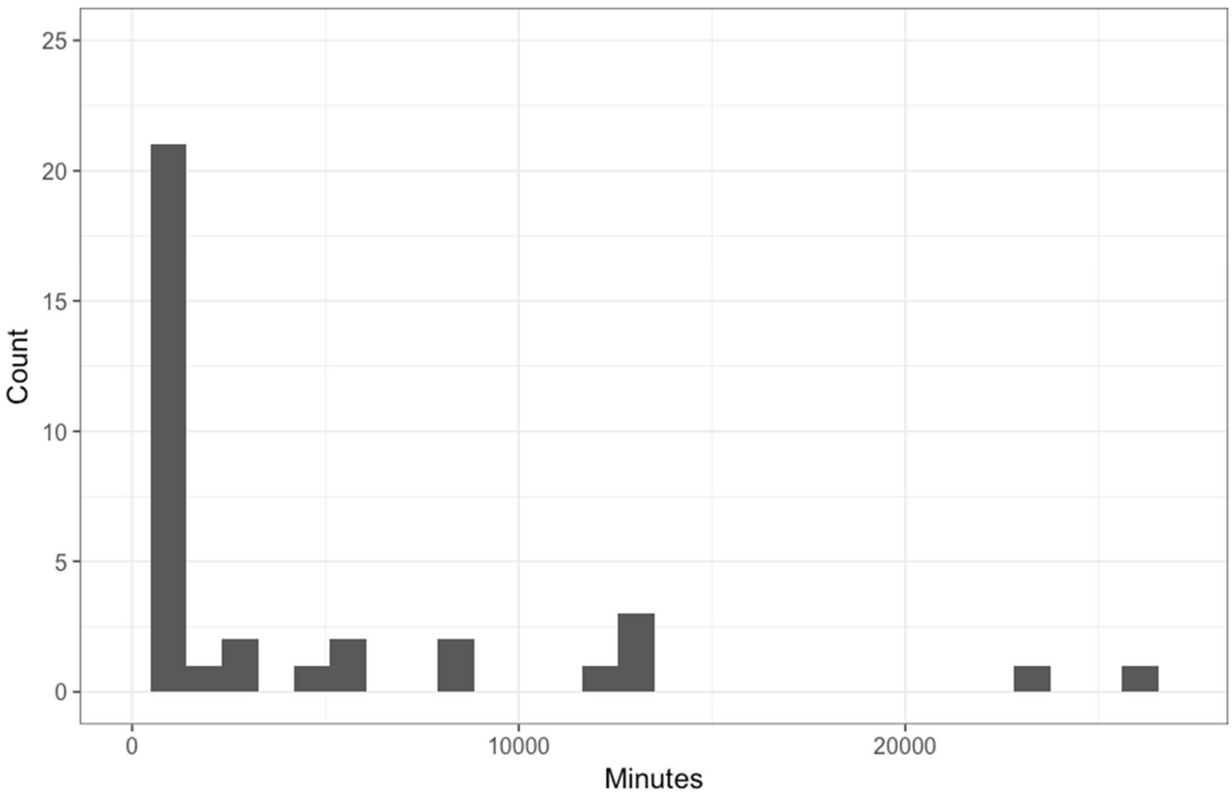

Figure 2.8. Histogram displaying the count of missing records by duration of time gap for all lamprey (n=44) released at Washington-shore fishway at Bonneville Dam.

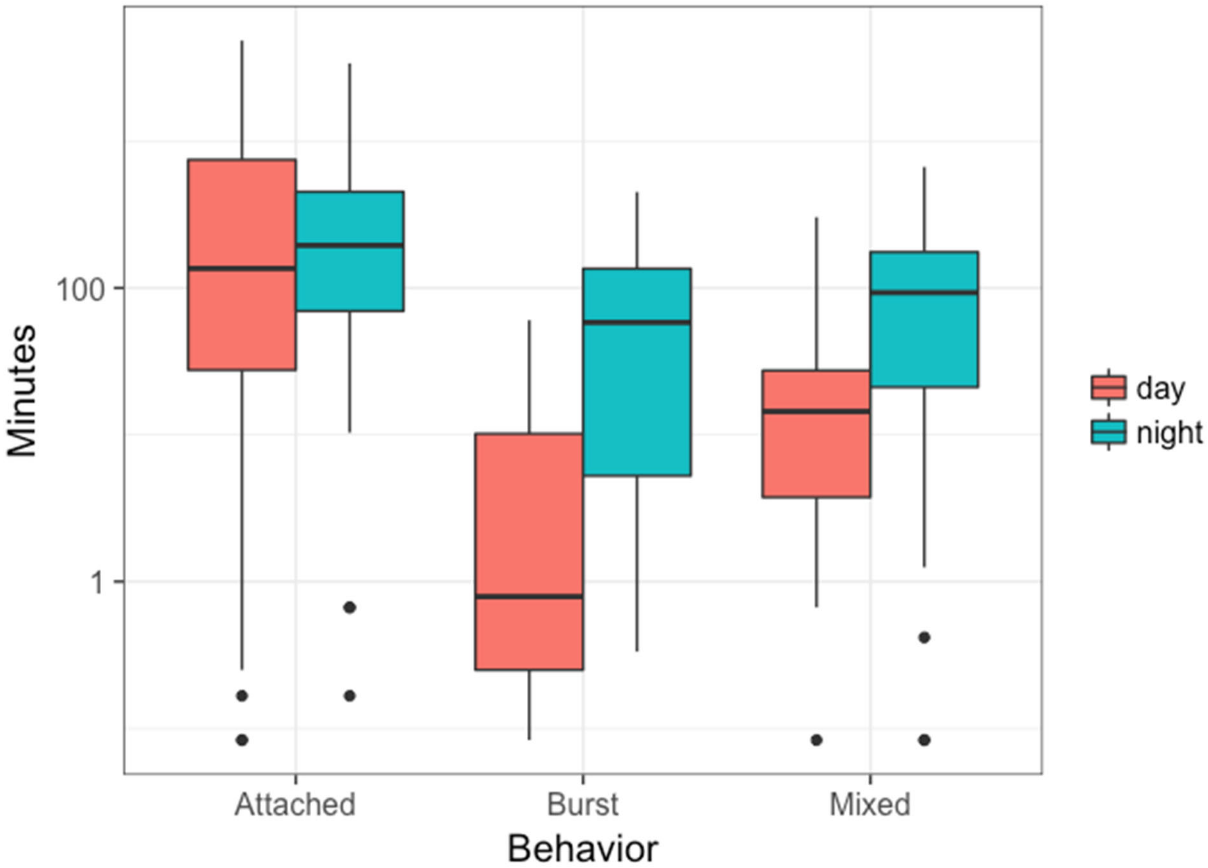

Figure 2.9. Duration of attached, burst, and mixed behaviors for all lamprey (n=44) by day and night periods released at Washington-shore fishway at Bonneville Dam. Median duration for each behavior by day are: Attached=136 min, Burst=0.792 min, Mixed=14.4 min. Median duration for each behavior by night are: Attached=197 min, Burst=58.3 min, Mixed=92.9 min.

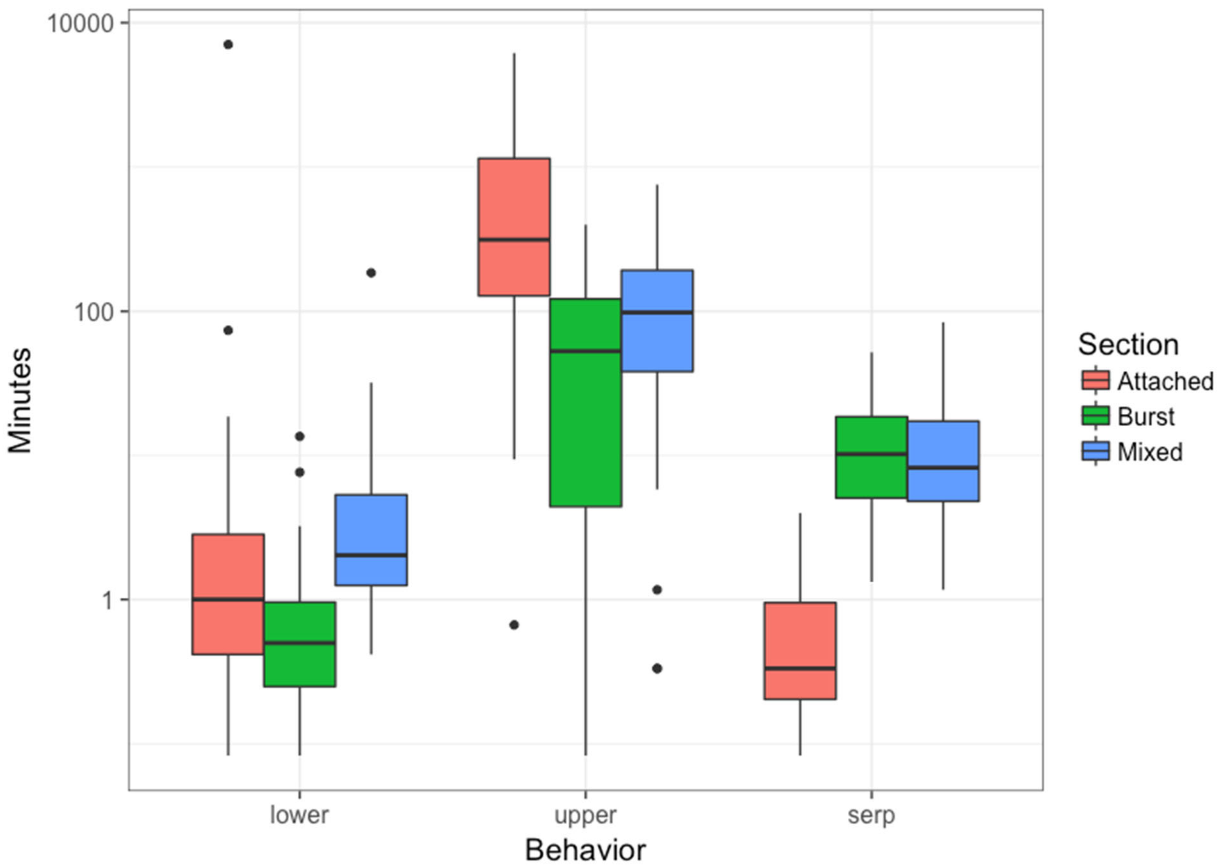

Figure 2.10. Duration of attached, burst, and mixed behaviors for all lamprey (n=44) by fishway section released at Washington-shore fishway at Bonneville Dam. Median duration for each behavior by lower are: Attached=1 min, Burst=0.5 min, Mixed=2.08 min. Median duration for each behavior by upper are: Attached=314 min, Burst=53 min, Mixed=98.2 min. Median duration for each behavior by serpentine weir (serp) are: Attached=0.33 min, Burst=10.2 min, Mixed=8.25 min.

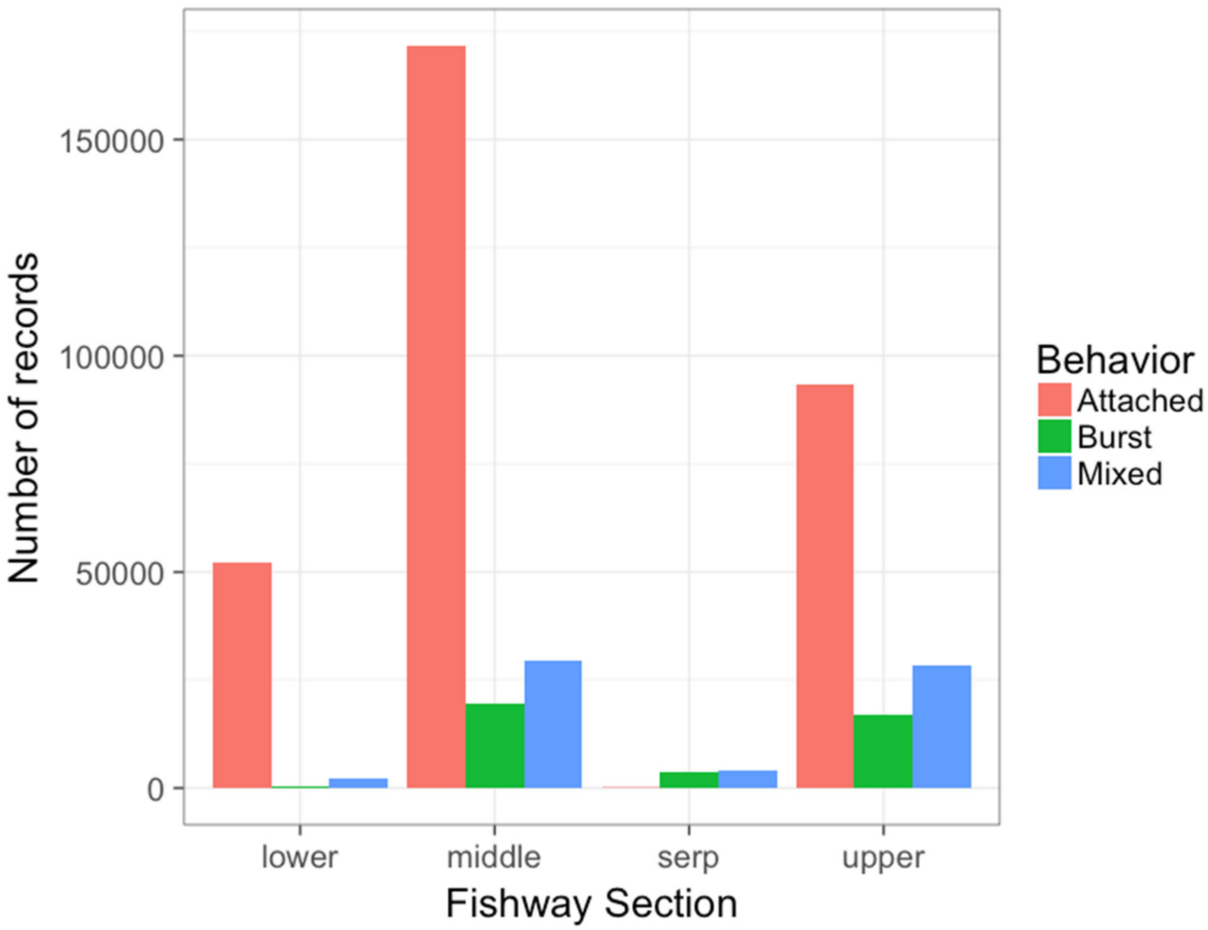

Figure 2.11. Total number of detections by fishway for attached, burst, and mixed behaviors for all lamprey released at Washington-shore fishway at Bonneville Dam. Each detection represents one burst interval (BI).

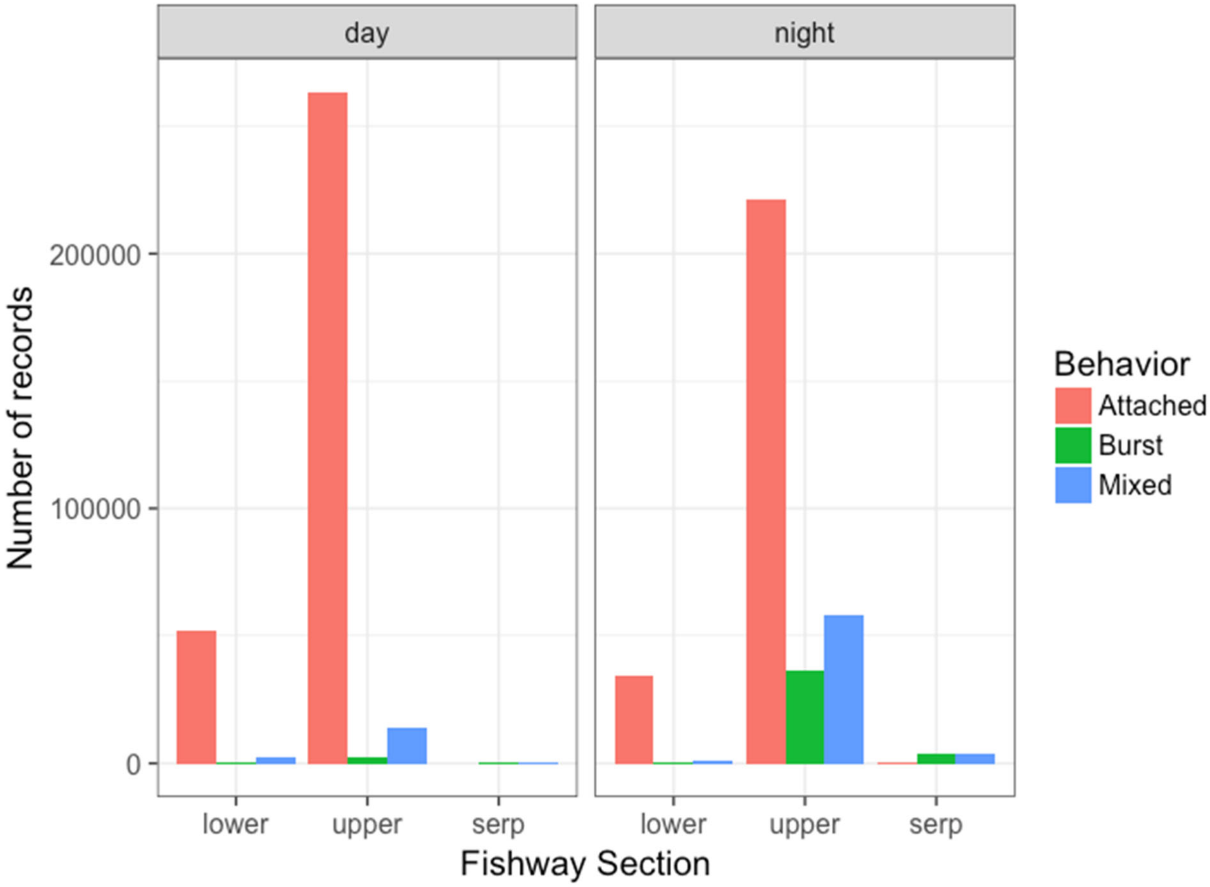

Figure 2.12. Total number of detections by fishway and time of day for attached, burst, and mixed behaviors for all lamprey released at Washington-shore fishway at Bonneville Dam. Each detection represents one burst interval (BI).

Fish that did not approach serpentine weirs:

Of the 44 lamprey released, 19 did not approach the serpentine weir section. This included the four fish that were released at the downstream site- these fish had no recorded acceleration values in either the upper or serpentine weir sections. Similar to the trend observed across all fish, there was a great deal of variability among the duration of behaviors by section. In the upper section, on average, fish that did not approach spent the majority of time attached, with an average of 15.4 hours, though this differed widely between individuals (Table 2.5; Figure 2.13). Lamprey that did not approach spent the least amount of time bursting across both sections. They spent relatively little time in the lower section, exhibiting on average a duration of less than a minute for attached and burst behaviors, and just over a minute for mixed behaviors. Generally, these fish spent the majority of the time attached in the upper section before leaving the Washington-shore fishway.

Table 2.5 Summary of range, median (%) and mean (SD) number of hours for each behavior by fishway section for lamprey (n=19) that did not approach the serpentine weirs in the Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (min)	Median duration (min) (%)	Range (min)
Lower			
Attached	0.821(0.849)	0.417 (21.8)	0.083-2.33
Burst	0.357 (0.249)	0.25 (13.0)	0.083-0.667
Mixed	1.83 (1.67)	1.25 (65.2)	0.417-5.33
Upper			
Attached	926 (1990)	271 (90.8)	0.667-6171
Burst	38.1 (102)	3.08 (1.0)	0.083-342
Mixed	64.8 (136)	24.4 (8.2)	0.333-471

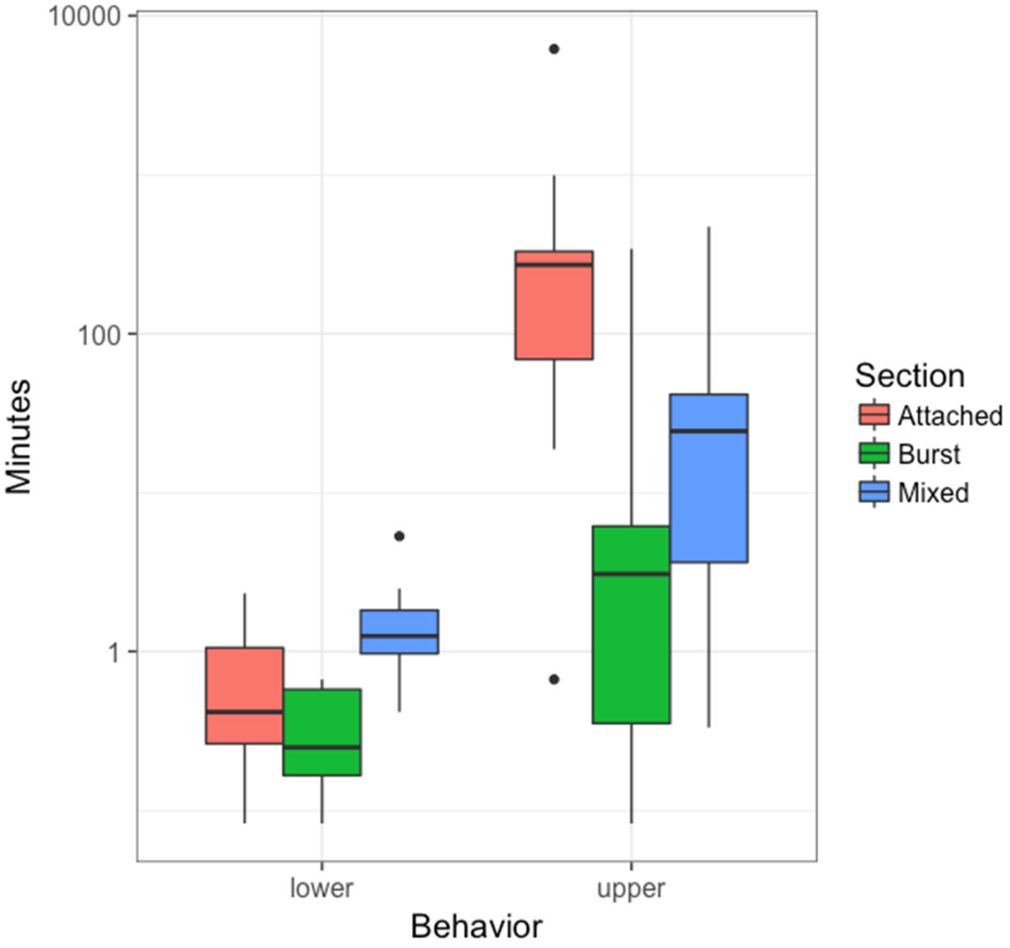

Figure 2.13. Duration of attached, burst, and mixed behaviors by fishway section for lamprey released at Washington-shore fishway that did not approach the serpentine weirs (n=19) at Bonneville Dam. Median duration for each behavior by lower section are: Attached=0.417 min, Burst=0.25 min, Mixed=1.25 min. Median duration for each behavior by upper section are: Attached=271 min, Burst=3.08 min, Mixed=24.40 min.

Fish that passed using lamprey passage structures:

Four fish passed and exited into the forebay through lamprey passage structures (LPS) located in the Washington-shore fishway: one fish passed via the upstream migrant tunnel junction (UMTJ) LPS and the three other fish passed using the auxiliary water supply (AWS) LPS. While behaviors on the LPS were not verified with video observations, in the majority of cases, lamprey appeared to exhibit intermittent mixed behavior, presumably while climbing and/or free swimming in traversing ducts, reflected as short bouts of movement accompanied by periods of rest (Figure 2.14). Travel time in the LPS averaged ~70 minutes from approach to exit, with a minimum time of 35 minutes and maximum time of 2 hours, 2 minutes. The majority of acceleration values were below the 1.5 threshold for all fish when passing through LPSs, with an average acceleration of 0.55 (±0.41) g (Figure 2.15). From release to approach of an LPS, lamprey spent the majority of the preceding time attached (average = 380 ±170 minutes) (Figure 2.16). This group spent the least amount of time bursting, with an average of 46.9 (±71.4) minutes.

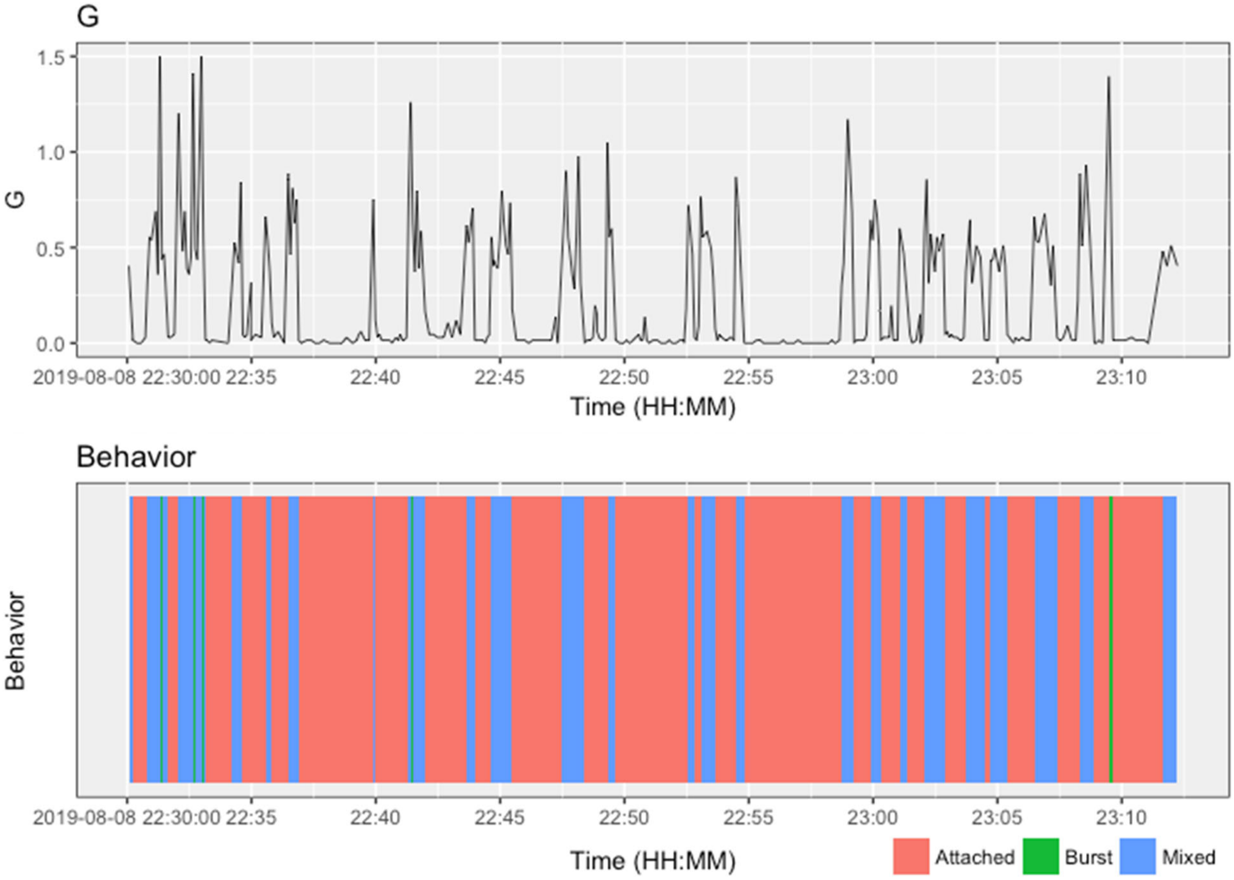

Figure 2.14. Example time series of acceleration and behavior of an individual lamprey that passed though the auxiliary water supply lamprey passage structure (AWS LPS) at the Washington-shore fishway at Bonneville Dam. Time series includes detections from approach to last record within the LPS.

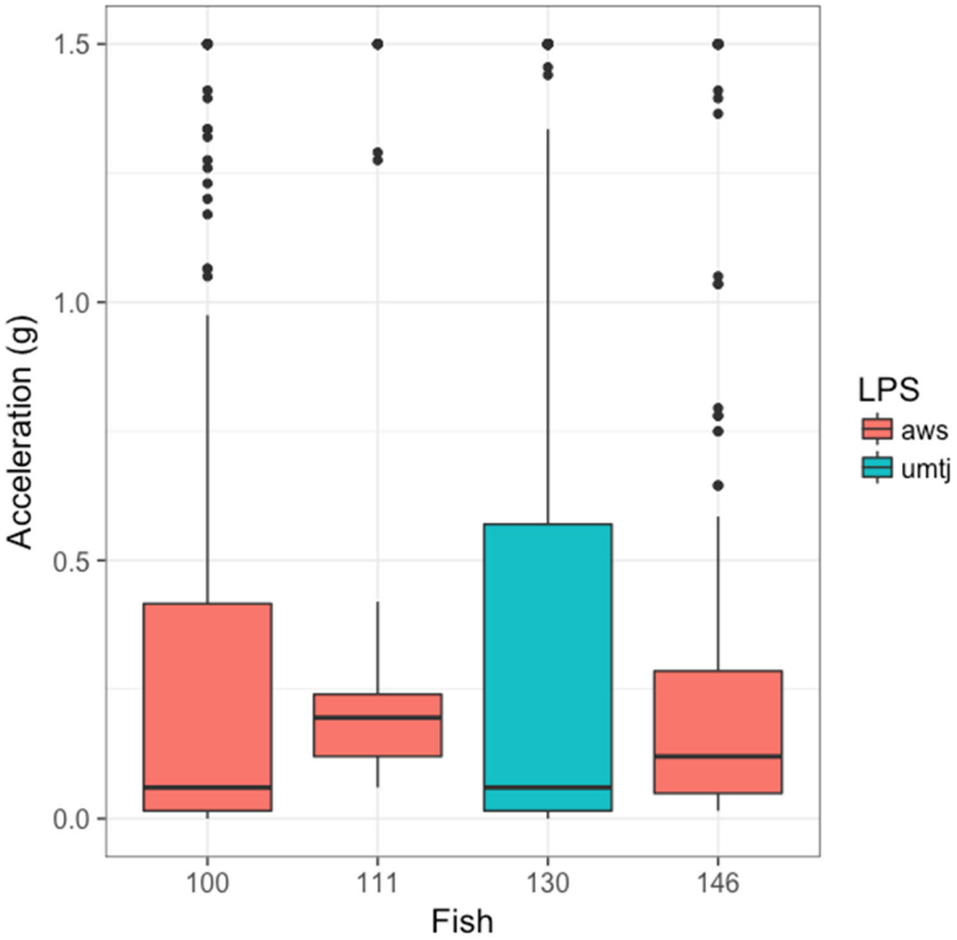

Figure 2.15. Distribution of acceleration (g) records by lamprey (n=4) that passed Bonneville Dam via lamprey passage structure (LPS). Lamprey either passed using the Auxiliary Water Supply (AWS) LPS or the Upstream Migration Tunnel (UMTJ) LPS. Total record duration spans from approach to exit in each LPS.

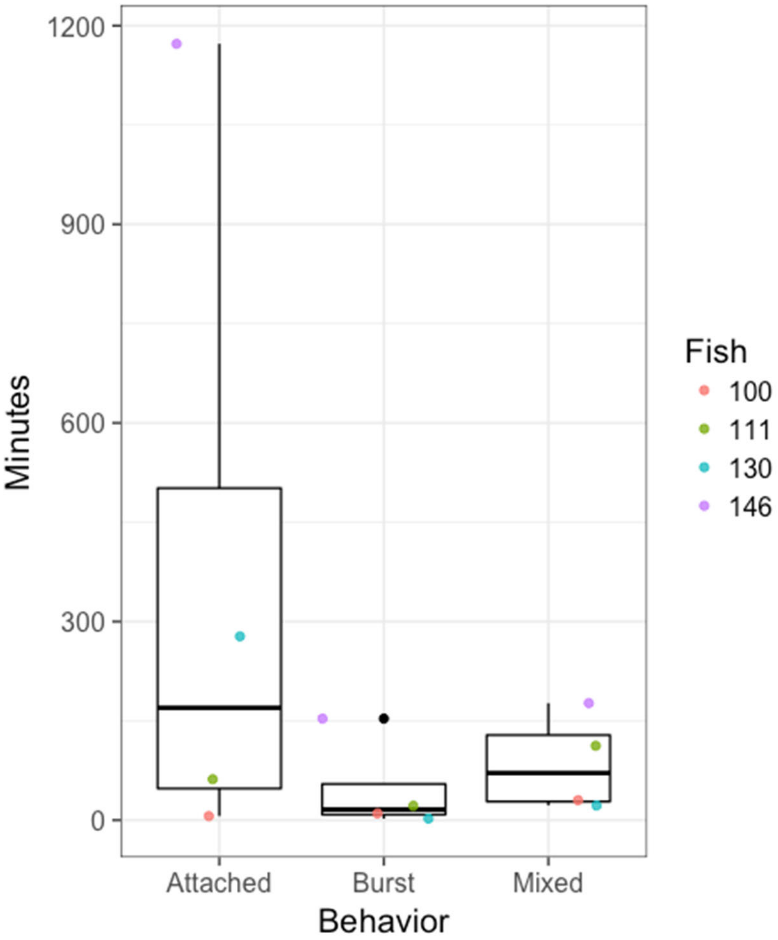

Figure 2.16. Duration of attached, burst, and mixed behaviors for lamprey (n=4) released at Washington-shore fishway that passed via lamprey passage structure (LPS) at Bonneville Dam. Points represent individual fish. Median duration for each behavior are as follows: Attached=170 min, Burst= 16.0 min, Mixed=71.2 min. Total record duration spans from time of release to approach in LPS.

Fish that approached serpentine weirs:

Of the 44 fish released at Washington-shore, 25 approached the serpentine weir section, as determined by a detection on the lowermost HD-PIT antenna within the weirs. A total of six fish successfully passed. Passage success through Washington-shore via the serpentine weir section was not associated with any morphological covariates or release date (Table 2.6) in this small sample. Additionally, there was no association between release date and the number of fish who approached (χ^2=0.88, df=2, *P*=0.64). Furthermore, approach times did not differ significantly between individuals who passed versus those who did not pass ($\chi2$ = 0.17, *P* = 0.68; Table 7). Mean approach time for fish that did not pass was 17.8 (±22.0) hours as compared to 13.7 (±20.7) for lamprey that passed.

Table 2.6 Logistic regression results testing for the effect of morphometric measurements and release date on passage success of fish (n=25) who approached the serpentine weir section in Washington-shore fishway, Bonneville Dam.

Factor	χ^2	*P*	Odds Ratio	95% CI
Length	1.18	0.28	0.80	0.50 – 1.17
TL/DD	0.15	0.70	0.93	0.58 – 1.34
Early - Mid	0.70	0.40	0.37	0.04 – 4.46
Early - Late	0.20	0.66	2.22	0.05 – 107.81
Mid - Late	1.13	0.29	5.94	0.17 – 260.54

Table 2.7 Logistic regression results testing for the effect of approach time on passage success of fish (n=25) who approached the serpentine weir section in Washington-shore fishway, Bonneville Dam.

Factor	χ^2	*P*	Odds Ratio	95% CI
Approach time	0.17	0.68	0.99	0.93 - 1.03

Activity levels through the serpentine weirs mainly consisted of burst movements preceded and followed by punctuated durations of mixed behavior. In a time-series reflecting the activity of one fish that successfully passed, attached behavior did not occur until near the exit of the weirs (Figure 2.17) and this pattern was characteristic of other lamprey that successfully passed. In contrast, lamprey that spent a longer duration in the weir section but were ultimately unsuccessful in passing had longer periods of attached behavior (generally over the course of several hours) after prolonged periods of burst and mixed behavior (e.g., Figure 2.18). In some instances, lamprey would spend several days in the serpentine weirs, and, in addition to alternating burst and mixed behaviors, would have attached behaviors ranging from relatively brief to extended periods during passage attempts (Figure 2.19).

From time of release to approach, successful fish spent a similar amount of time bursting compared to those that did not pass (Table 2.8; Figure 2.22). Approximate duration for attached and mixed behaviors was fairly similar across passers and non-passers as well, though non-passers displayed a higher degree of variation among individuals. Lamprey that passed took an average (±SD) of 19.9 (±44.2) hours to pass after the first approach, however this was skewed by one fish that took 110 hours (after approaching and turning around), thus the median time to pass was 1.93 hours (Figure 2.22). Lamprey that did not pass spent an average of 84.5 (±116) hours attempting to pass after the first approach, with a median of 53 hours, overall spending a longer time in the weir section compared to fish that were successful. In almost all cases, lamprey that passed appeared to move relatively quickly, with a limited number of turn-arounds compared to fish that did not pass (Figure 2.24). Turn-arounds are a minimum estimate of the likely total number of turn-arounds due to limited number of PIT antennas located in the serpentine weirs. Relative duration of behaviors in the weirs was similar between passers and non-passers for burst and mixed behaviors, though non-passers spent a longer time being attached (Table 9; Figure 2.23). In fact, those that did not pass spent on average 39.5% (±36.8) of their total time budget in the serpentine weir section attached compared to 15.4% (±31.4) for those that passed (Figure 2.25). In contrast, fish that passed spent a greater proportion of their time budget bursting and exhibiting mixed behaviors compared to non-passers.

The initial behavior of passers and non-passers in the serpentine weirs was similar. For example, the average number of burst movements during the first hour in the serpentine weir following the initial approach did not differ substantially between fish that passed and those that failed to pass (Figure 2.26). Rate of attachment was also similar between groups, though the rate decreased with time for the fish that passed while increasing for the fish that did not pass. A similar trend was observed for mixed rates; rates were generally similar, with fish that did not pass exhibited a slightly increased rate as time progressed while fish that passed exhibited a decrease. During the last hour of activity within the serpentine weirs, fish that did not pass initially exhibited a marked increase in attachment rate, which then decreased with a corresponding increase in burst and mixed rates (Figure 2.27). This was in contrast to patterns exhibited by fish that passed; bursting and mixed rates decreased over time.

Table 2.8. Summary of range, median (%) and mean (SD) number of hours for each behavior by passage outcome (pass vs no pass) lamprey (n=25) from time of release to approach at the serpentine weirs in the Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (min)	Median duration (min) (%)	Range (min)
	Pass		
Attached	248 (341)	154 (79)	16.8-925
Burst	35.6 (60.9)	11.2 (5.8)	4.5-159
Mixed	42.1 (33.8)	29.7 (15.2)	19.7-109
	No Pass		
Attached	522 (904)	136 (76)	1.42-3528
Burst	20.7 (34.8)	11.4 (6.4)	0.417-148
Mixed	73.9 (87)	31.3 (17.6)	0.917-276

Table 2.9. Summary of range, median and mean (SD) number of hours for each behavior by passage outcome (pass vs no pass) for lamprey (n=25) from approach to last detection in the serpentine weirs at Washington-shore fishway, Bonneville Dam.

Behavior	Mean (SD) duration (min)	Median duration (min) (%)	Range (min)
	Pass		
Attached	168 (371)	1.5 (1.6)	0.75-831
Burst	62.1 (43.2)	53.7 (55.6)	2.77-145
Mixed	67.4 (59.1)	41.3 (42.8)	26.5-180
	No Pass		
Attached	738 (991)	110 (48.3)	0.167-2800
Burst	103 (96.3)	64.9 (28.5)	1.25-302
Mixed	135 (154)	52.8 (23.2)	0.167-542

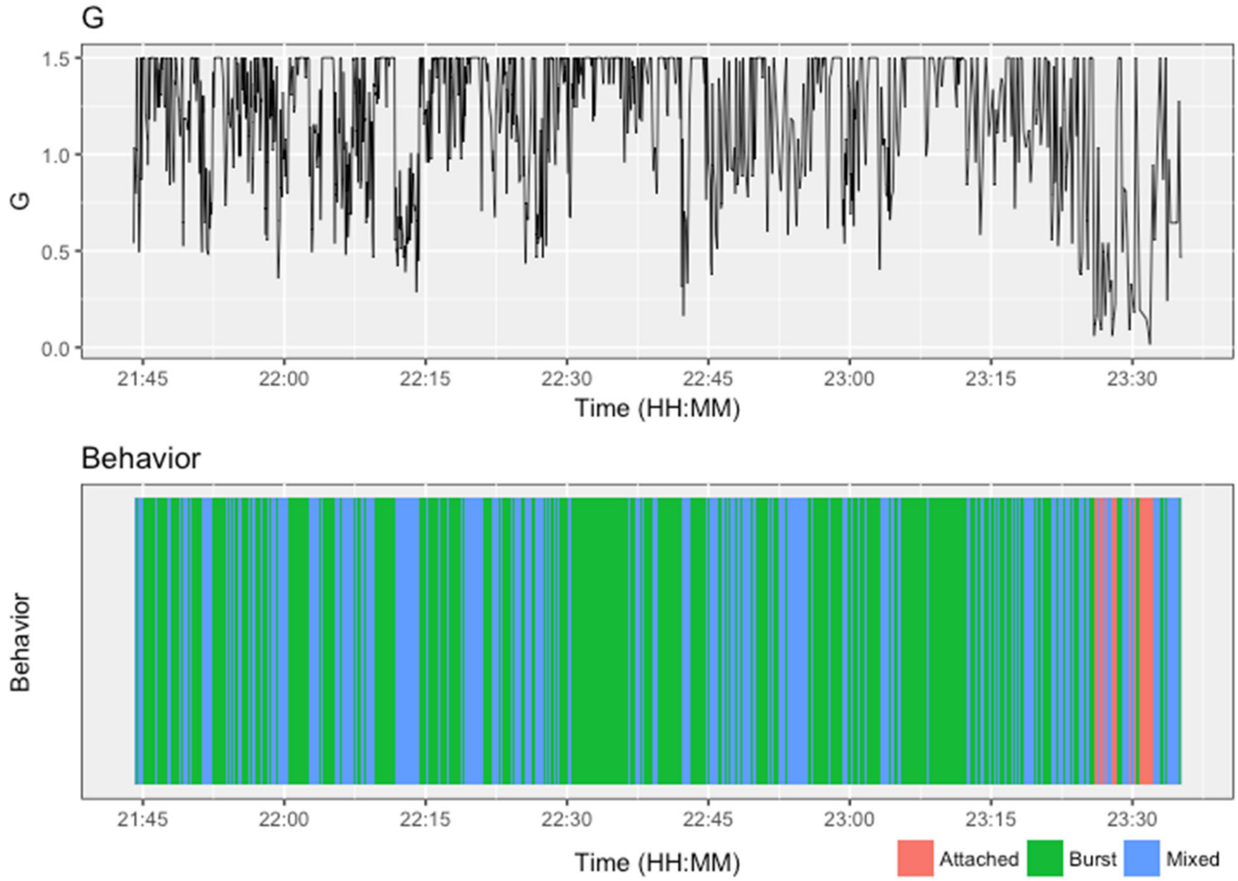

Figure 2.17. Example time series of acceleration and behavior of an individual lamprey that successfully passed through the serpentine weir section of Washington-shore fishway at Bonneville Dam. Time series includes detections from approach to exit within the serpentine weirs.

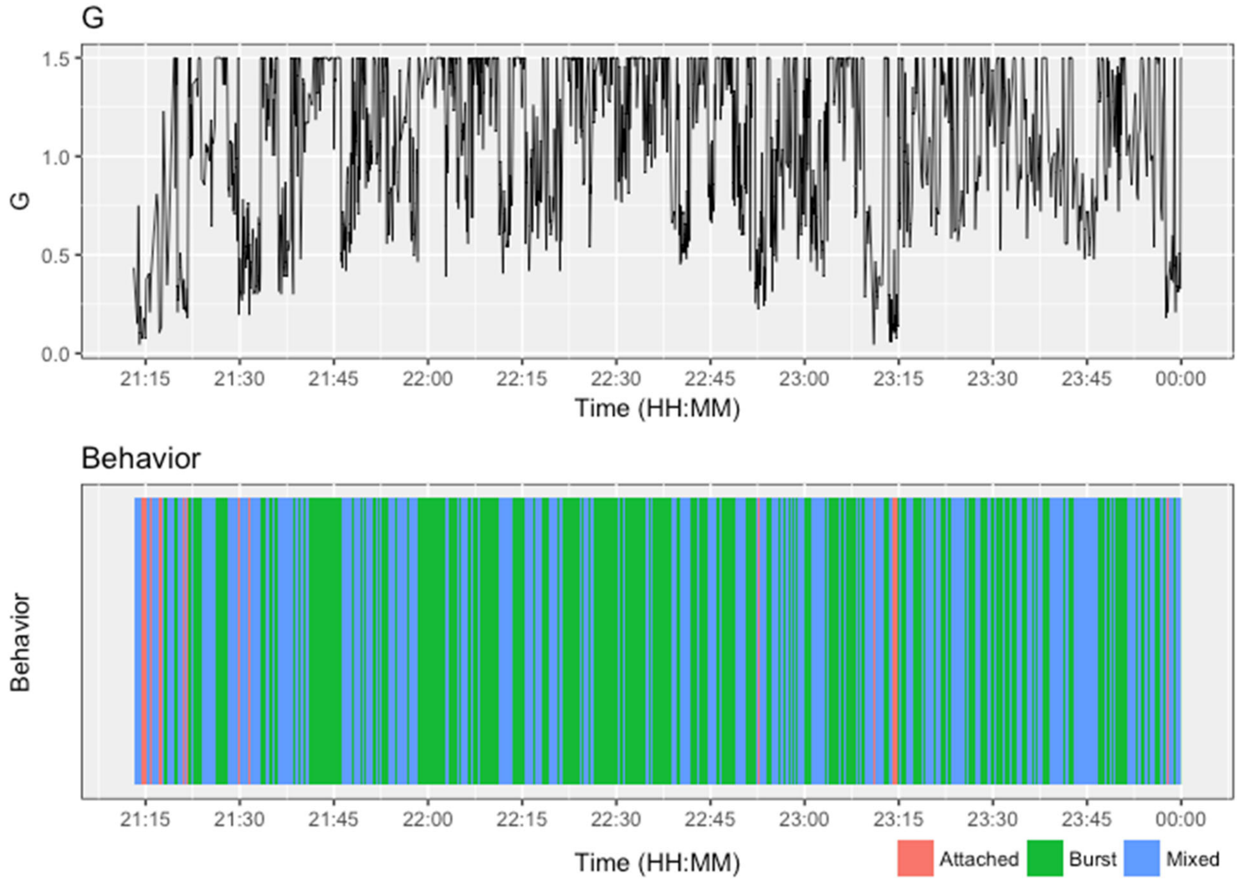

Figure 2.18. Example time series of acceleration and behavior of an individual lamprey that was unsuccessful in passing through the serpentine weir section of Washington-shore fishway at Bonneville Dam. Time series includes values from approach to last recorded detection within the serpentine weirs.

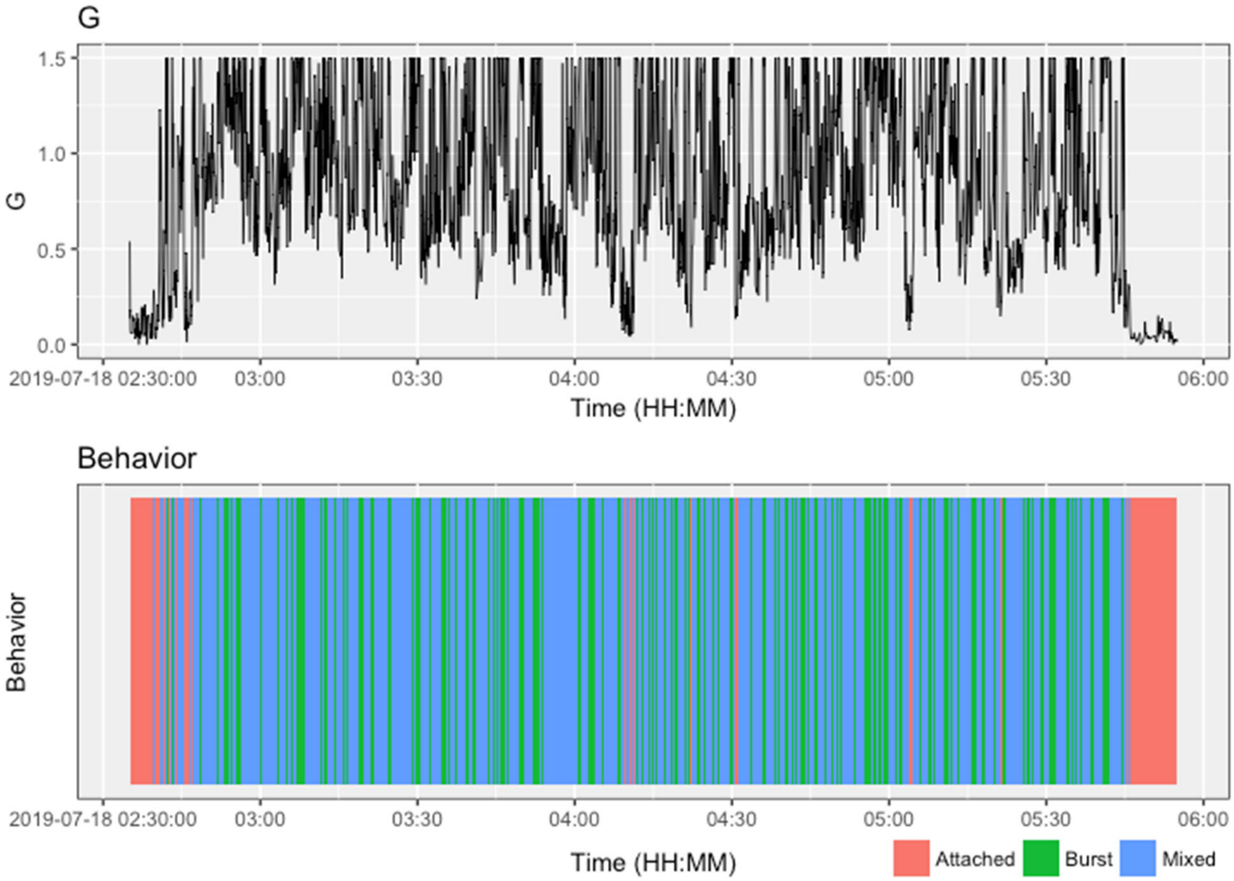

Figure 2.19. Example time series of acceleration and behavior of first attempt of an individual lamprey (Fish 102) that was unsuccessful in passing through the serpentine weir section of Washington-shore fishway at Bonneville Dam. Time series includes values from approach to last record detection within the serpentine weirs.

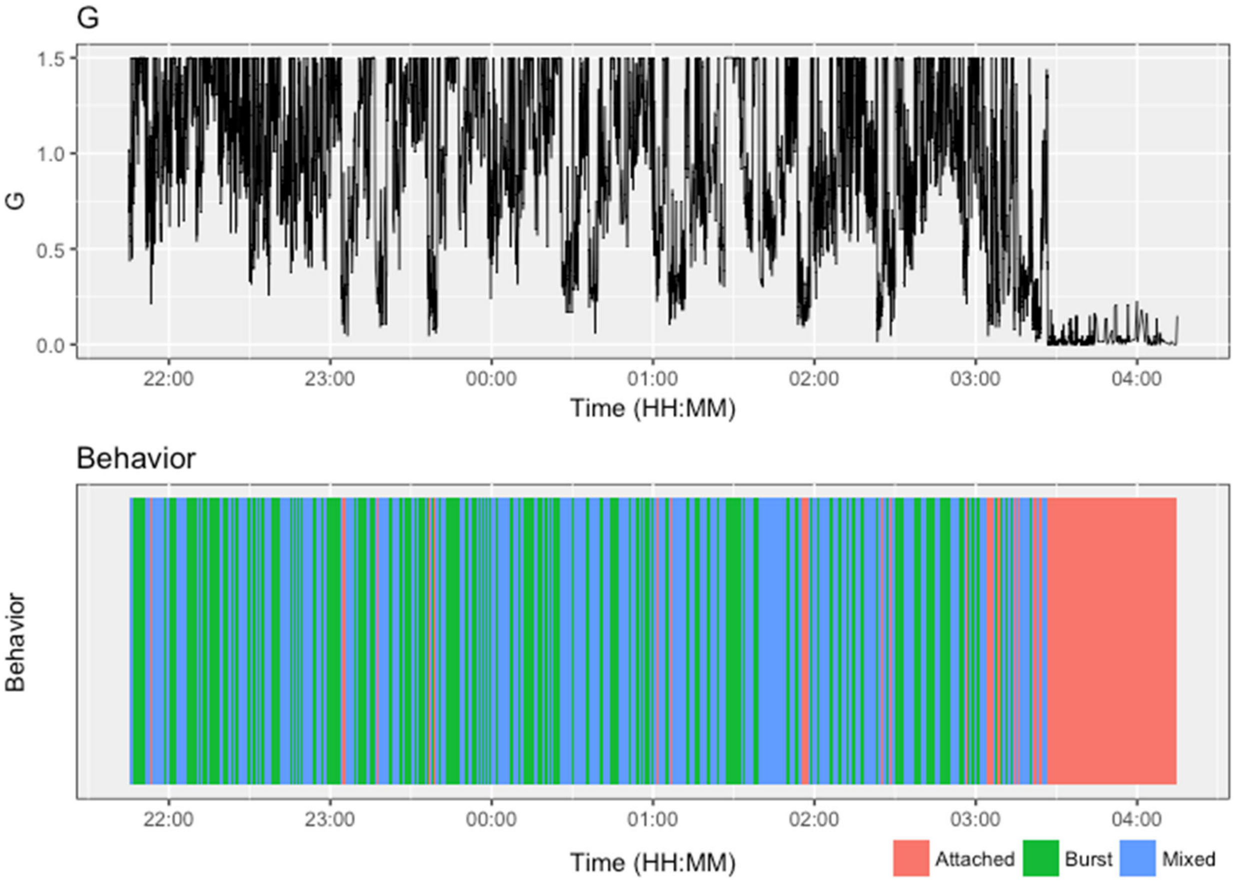

Figure 2.20. Example time series of acceleration and behavior of second attempt of an individual lamprey (Fish 102) that was unsuccessful in passing through the serpentine weir section of Washington-shore fishway at Bonneville Dam. Time series includes values from approach to last recorded detection within the serpentine weirs.

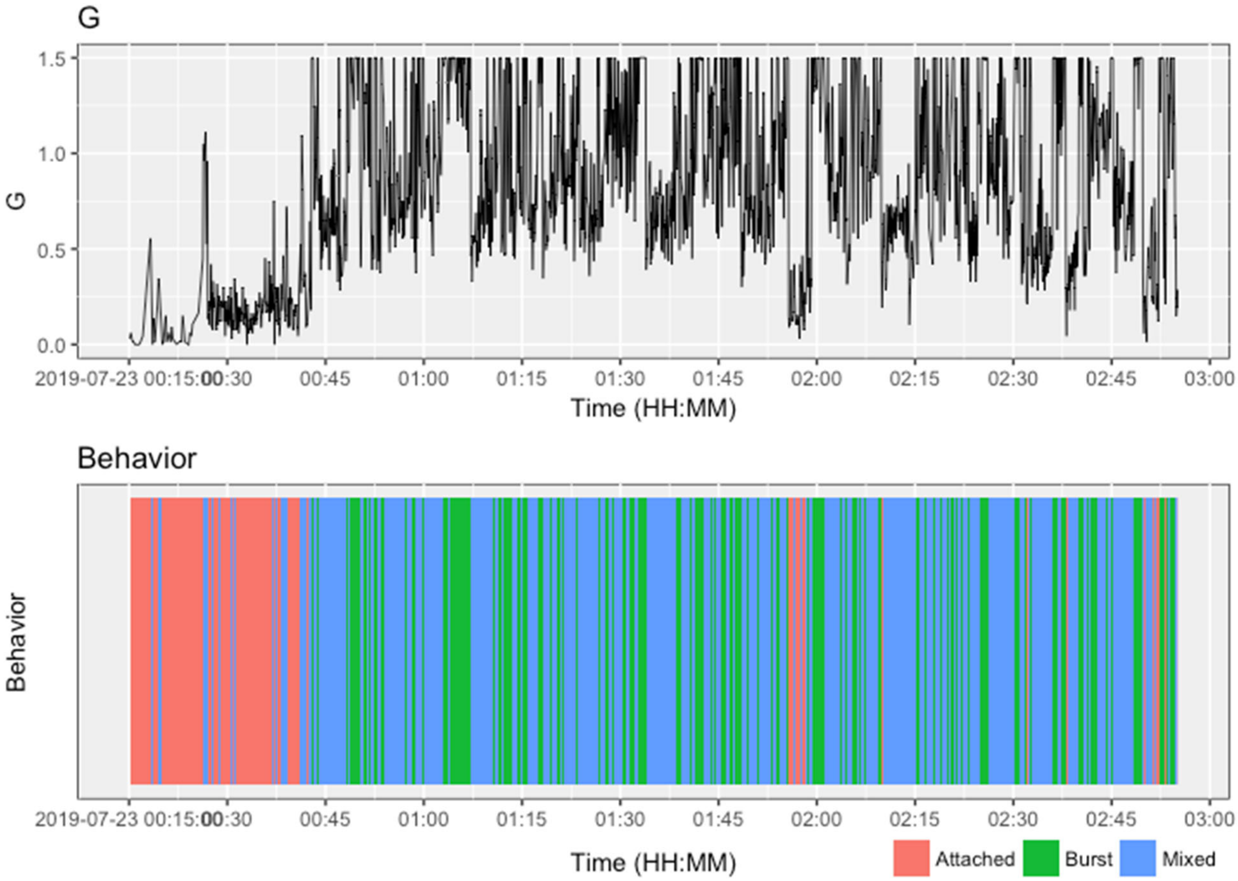

Figure 2.21. Example time series of acceleration and behavior of third and final attempt of an individual lamprey (Fish 102) that was unsuccessful in passing through the serpentine weir section of Washington-shore fishway at Bonneville Dam. Time series includes values from approach to last recorded detection within the serpentine weirs.

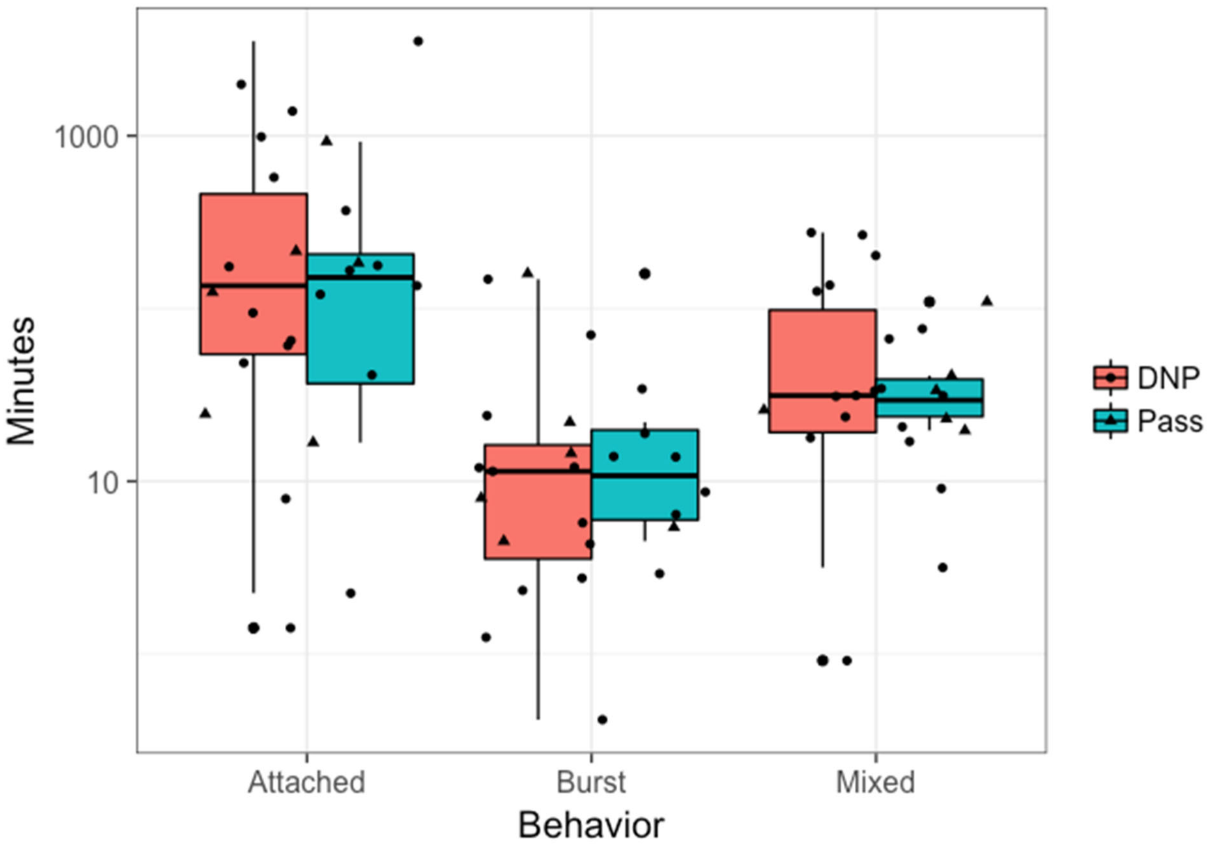

Figure 2.22. Duration from release to serpentine weir approach for each behavior (attached, burst, and mixed) by passage outcome (passed vs did not pass (DNP)) among lamprey that approached the serpentine weir section (n=25) at Washington-shore fishway. Median duration for each behavior for fish that passed was: Attached=154 min, Burst=11.2 min, Mixed=29.7 min. Median duration for each behavior for fish that did not pass was: Attached=136 min, Burst=11.4 min, Mixed=31.3 min.

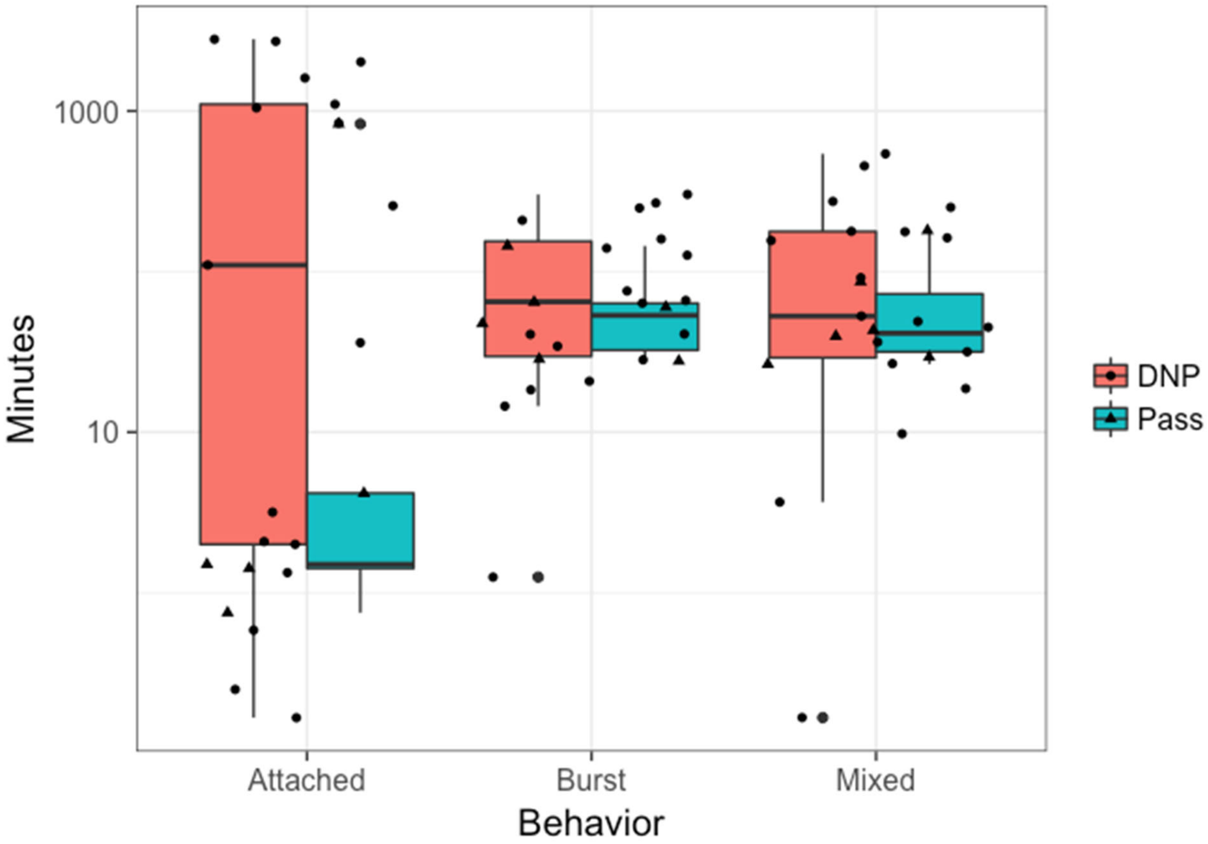

Figure 2.23. Duration from approach to last detection in the serpentine weir section for each behavior (attached, burst, and mixed) by passage outcome (passed vs did not pass (DNP)) among lamprey that approached the serpentine weir section (n=25) at Washington-shore fishway. Median duration for each behavior for fish that passed was: Attached=1.5 min, Burst=53.7 min, Mixed=41.3 min. Median duration for each behavior for fish that did not pass was: Attached=110 min, Burst=64.9 min, Mixed=52.8 min.

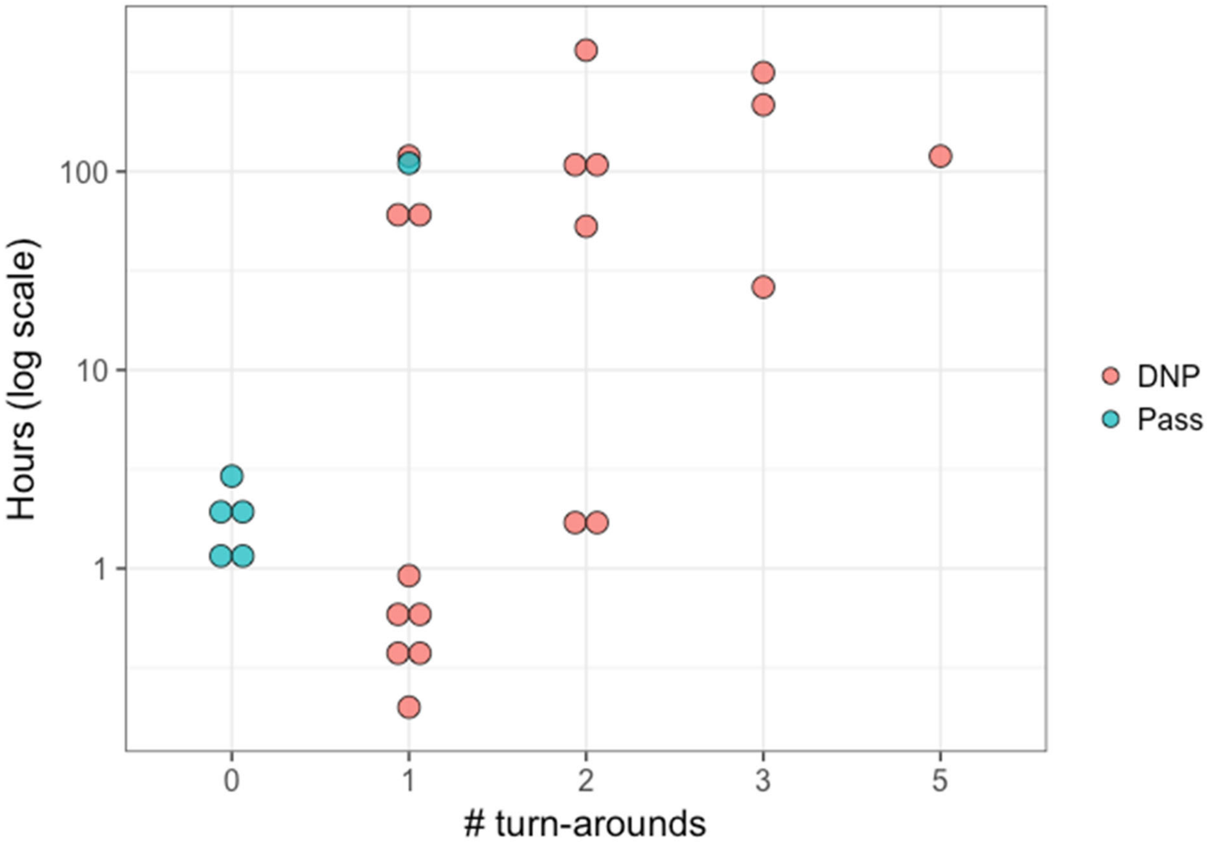

Figure 2.24. Duration of time spent in serpentine weirs and number of known turn-arounds by lamprey that passed vs did not pass (DNP) that approached the serpentine weir section (n=25) at Washington-shore fishway. Median time spent in serpentine weirs for lamprey that passed was 1.93 hours and for lamprey that did not pass was 53 hours. Turnarounds represent a minimum estimate.

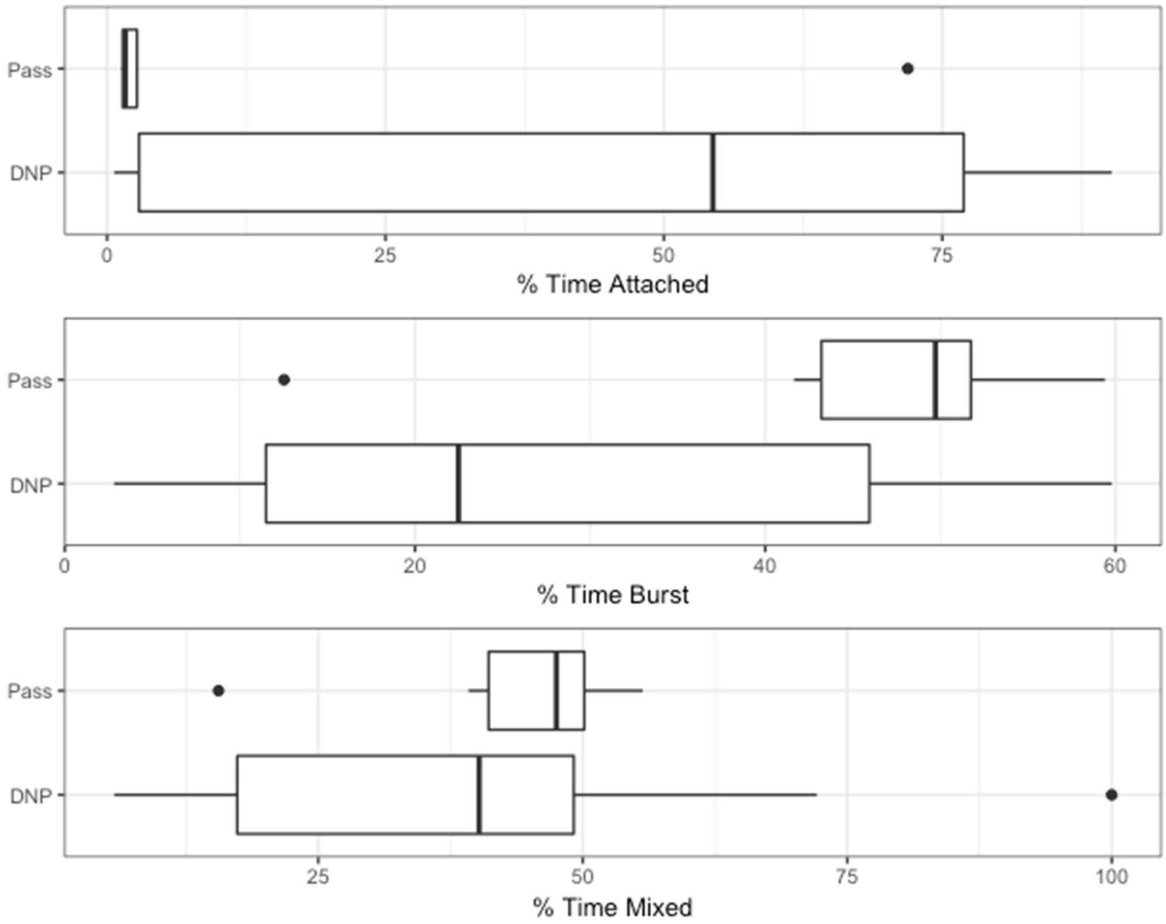

Figure 2.25. Percentage of total time budget spent for each behavior by passage outcome (passed and did not pass (DNP)) for lamprey that approached the serpentine weirs (n=25) at the Washington-shore fishway. Time duration spans from approach to last detection in the serpentine weir section.

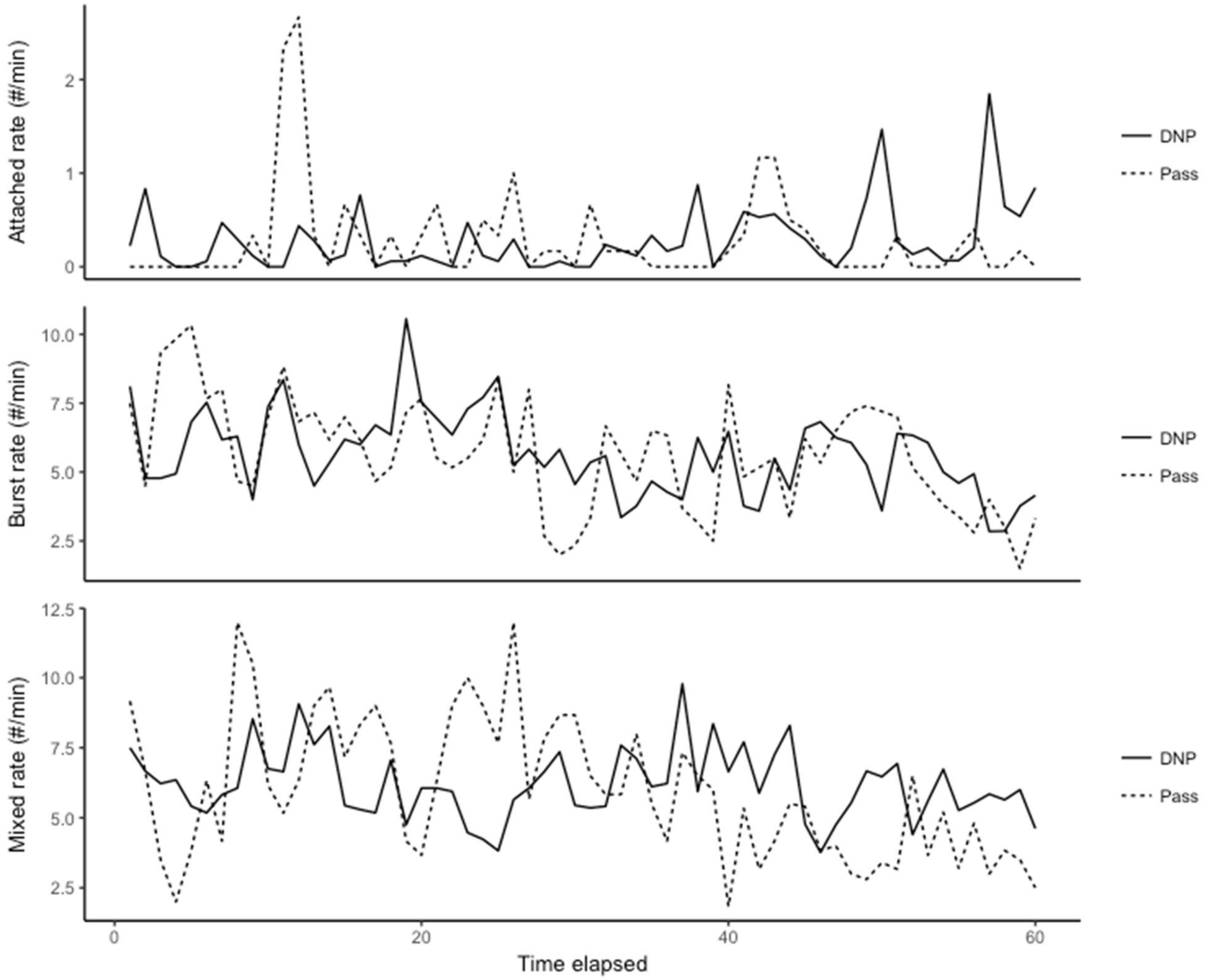

Figure 2.26. Mean number of behavioral events per minute for each behavior (attached- top panel; burst-middle panel; mixed-bottom panel) by passage outcome (pass vs did not pass (DNP)) during the first hour after approaching the serpentine weir section for all lamprey that approached and entered the serpentine weir section (n=25) at Washington-shore fishway.

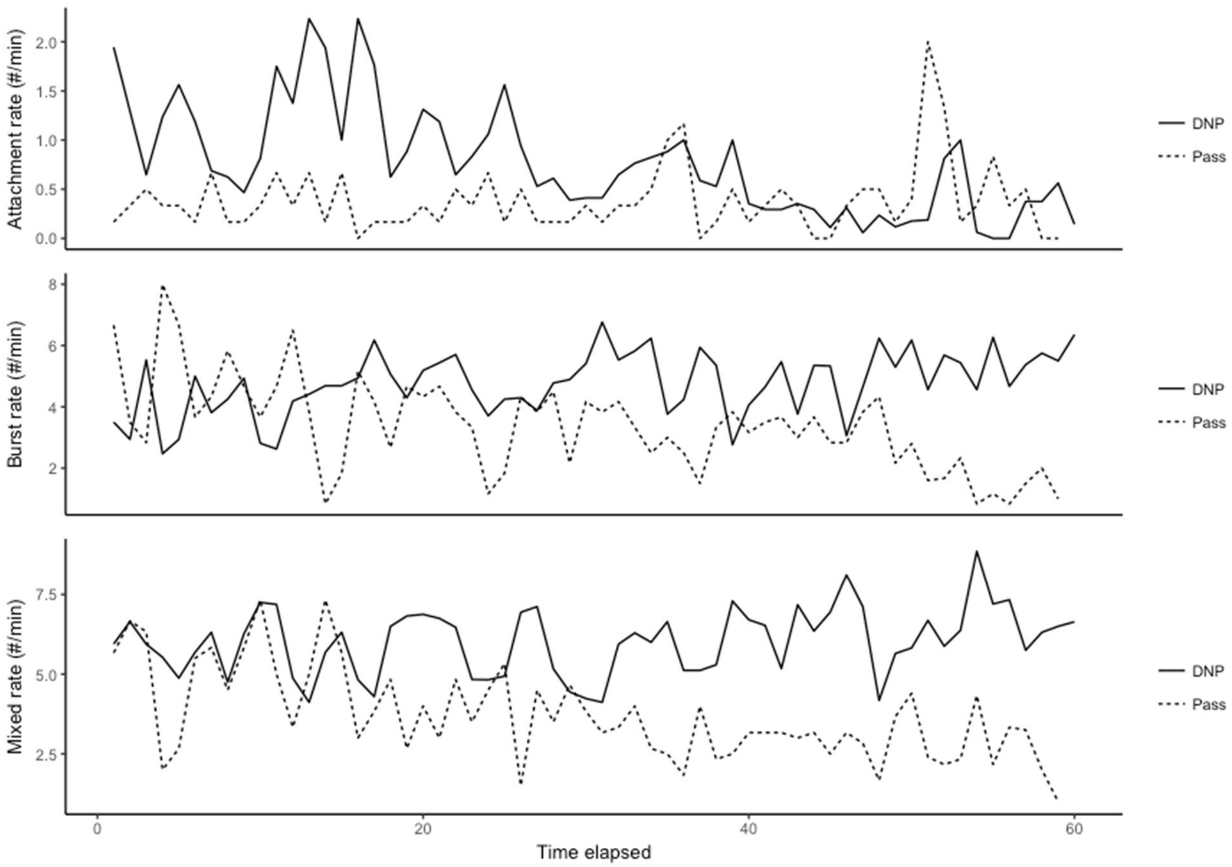

Figure 2.27. Mean number of behavioral events per minute for each behavior (attached- top panel; burst-middle panel; mixed-bottom panel) by passage outcome (pass vs did not pass (DNP)) during the last hour of detections for all lamprey that approached and entered the serpentine weir section (n=25) at WA-shore fishway.

Discussion

Measuring movement and behavior of free-swimming fishes is a challenging task. Accelerometer biotelemetry offers a method to study fine-scale behavioral movements that are not captured at the scale of most traditional telemetry studies. We evaluated behavioral patterns and trends associated with a portion of a fishway containing distinct sections identified by high-turn around rates and local bottlenecks (Moser et al. 2002; Johnson et al. 2012; Keefer et al. 2013a). Across all areas of the Washington-shore fishway, the duration and frequency of attached and swimming behaviors exhibited high intraspecific variability among lamprey. Passage obstacles characterized by high velocities and turbulence were associated with an increase in the number of burst movements, potentially reflecting a transition into an anaerobic state or periods of anaerobiosis. Lower elevation segments saw a combination of free-swimming and attached behaviors with relatively fewer burst movements.

Though we were able to discern between attached and burst movements, we were not able to differentiate free-swimming at low to moderate velocities and attachment at comparably higher velocities. Thus, this category of behaviors was combined and may underestimate or overestimate energetic costs associated with each behavior. At lower segments ‘mixed’ behavior is likely to reflect free-swimming behavior, as lamprey have been reported to employ prolonged swimming speeds at velocities present in this portion of the fishway (Quintella et al. 2009; Kirk et al. 2015b). In higher elevation fishway segments, such as the serpentine weirs, mixed category likely reflects attached and free-swimming behavior. Incidences of mixed behavior were regularly preceded and followed by burst movements, possibly representing attachment in a turbulent zone while resting/recovering or as part of saltatory swimming.

Another important consideration to take into account was that fish were released in the middle of the ladder rather than further downstream, potentially giving rise to behavioral differences relative to fish at large. Due to sample size restrictions and low entrance rates of fish released downstream from the dam (Keefer et al. 2013a, 2019), the release site was chosen in an effort to have sufficient numbers of lamprey approaching the area of interest in the Washington-shore fishway. Consequently, behavioral estimates in the lower ladder had fewer detections compared to upstream locations and may reflect a greater influence of net downstream movement.

Throughout the fishway, clear diel patterns were visible. Burst and mixed behaviors occurred in much higher proportions during the night than during the day. Burst movements only represented about one-minute median cumulative duration during the day but were approximately an hour median duration during the night. The majority of attached records occurred during the day, indicating that lamprey were more likely to seek refuge in low velocity locations in addition to finding refuge from light. Previous research has documented nocturnal migration behavior of lamprey (Keefer et al. 2013b), and timing of arrival at high-velocity obstacles may influence behavior and performance during passage. Lamprey that encounter a high velocity flow field near daylight hours may be more inclined to turn-around and seek refuge in lower velocity refuges.

Across all fish, gaps in detections of acceleration data varied from 10 seconds to several days. In some instances, this was due to fish moving out of the study area, but in other cases it may be due to attaching in rest boxes or other cryptic areas that impeded reception, as demersal movement patterns in these portions of the fishway have been observed in lamprey previously (Moser et al. 2002; Keefer et al. 2010; Kirk et al. 2015b). Therefore, our estimates may underrepresent the total duration of attachment.

The costs of attachment among areas of different water velocity are unknown. Seeking lower velocity portions of the fishway may be important for rest and recovery, particularly when lamprey face upcoming sections that contain difficult to navigate conditions or after a turnaround event. Previous studies have found that lamprey use dark, low-velocity rest boxes in fishway sections with high velocity/turbulent conditions and may spend up to several days residing in them before moving upstream (Moser et al. 2019).

Saltatory swimming represents an effective strategy for anguilliform swimmers to overcome high-velocity and turbulent conditions (Moser et al. 2002; Clemens et al. 2010; Keefer et al. 2010). However, this mode is energetically costly, requiring repeated forays into anaerobic activity that can result in physical exhaustion. Lamprey in the serpentine weir section primarily exhibited bursting behavior, reflective of the high velocity, high turbulence conditions present there, but lamprey also displayed high intraspecific variability in the frequency and duration of not only burst movements, but attached and mixed behaviors as well. Among lamprey who successfully passed, some individuals attached in low velocity areas for as little as one minute while one individual remained attached for several hours.

Intraspecific differences in behavior can be manifestations of variable swimming abilities, but the degree to which behavior and swimming performance are either the cause or result of those abilities is difficult to parse out. Genes associated with large body size have been linked to migration distance (Hess et al. 2014), with larger fish migrating farther distances, but the role that motivation has in optimizing behavioral patterns for increased performance is difficult to measure. Although we did not observe any size differences among successful and non-successful fish (possibly due to low variation in size in our sample of relatively large lamprey selected to minimize tag burden effects), we still observed substantial differences in performance and behavior. Of note, we observed relatively rapid passage with high burst rate among successful passers relative to unsuccessful lamprey.

Despite the differences between passers and non-passers in duration associated with passage after approaching the serpentine weirs, approach behavior and time between release and approach of the serpentine weirs did not differ statistically between successful and unsuccessful fish. For example, although unsuccessful fish did take longer, the median duration, ~5.7 hours, was not substantially different from a median duration of ~4.0 hours for fish who passed. Variation in approach time was high though, with some fish approaching in a few hours while others took several days. Overall, the proportion of time spent bursting, attaching, and classified as mixed was similar between groups. Thus we found no evidence that lamprey that eventually passed exhibited behaviors during the approach period that reflected increased motivation or swimming capacity, though we note sample sizes were small. Motivation has been related to the number of attempts at an obstacle (Goerig and Castro-Santos 2017). Unsuccessful lamprey attempted up to five times and in some cases spent more than 100 hours doing so, indicating that they were motivated but still unable to pass. It is possible that lamprey who were not successful may have been searching more for alternate routes, thereby spending a greater amount of time attempting to pass. Alternatively, unsuccessful lamprey may have been turning around and holding for a period of time before making additional attempts at passing.

Generally, successful fish passed more quickly than those that did not pass, averaging under two hours from approach to exit. Unsuccessful fish spent time in the serpentine weir section ranging from less than an hour before turning around and exiting downstream to making multiple attempts across several days. During the first hour that fish were navigating the serpentine weirs, there were no major distinctions between burst rate and ultimate passage outcome. That is, initial differences in behavior were not associated with passage performance. The average and median number of bursts, mixed, and attached behaviors was similar between successful and non-successful individuals. However, during the last hour unsuccessful fish increased bursting and mixed behaviors, possibly indicating longer rest intervals between bursts, as has been observed in lamprey encountering high velocities over a prolonged period of time (Quintella et al. 2004). Lamprey that did not pass also exhibited more turnaround events in the serpentine weirs and spent a longer time attempting to pass as well. Given that attachment duration increased over time and fish were frequently observed seeking out refuges in between attempts, exhaustion may have been occurring. Lamprey that did not pass spent almost 40% of their time budget as attached, while fish that passed spent only ~16%. To compare, upstream migrating sea lamprey spent ~43% of their total time budget as attached when passing through difficult passage conditions (Quintella et al. 2009). Though variation within groups that passed and failed to pass was high, fish that passed were consistently faster and spent a greater portion of their time budget bursting. Consistent differences in performance related to both swimming and climbing speed has been observed among individuals previously, with faster fish remaining faster independent of environmental or morphological variables (Kemp et al. 2009; Moser et al. 2013).

Movement patterns can vary widely between individuals, particularly in a non-philopatric species such as lamprey (Kirk and Caudill 2017). The degree to which these movement patterns are a consequence of behavioral differences or physiological limits or a combination of both is unknown. Do fish with lower fitness pursue other options due to the inability to overcome obstacles? Does this result in increased searching behavior? Or, is the propensity to meander, fallback and mill about due to something else (e.g., absence of adequate directional cues, loss of orientation, exhaustion, etc.)? McElroy et al. (2012) presented results that suggested individual migratory pallid sturgeon chose paths which minimized energetic costs by avoiding high velocity flows (McElroy et al. 2012). Lamprey may also seek pathways of lower energetic costs (Kirk et al. 2017), as bursting incurs a high energetic expense (Burnett et al. 2014a).

Overall, acceleration biotelemetry is a promising method for identifying and quantifying behaviors as they relate to hydraulic conditions encountered in a fishway environment. Increasing spatial resolution of transmitted acceleration data would allow for more robust estimates of behavioral responses at a finer-scale. Future research should also consider combining acceleration data with respirometry. This would allow direct estimation of energetic costs associated with various behaviors and, ultimately, the cost of passage.

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