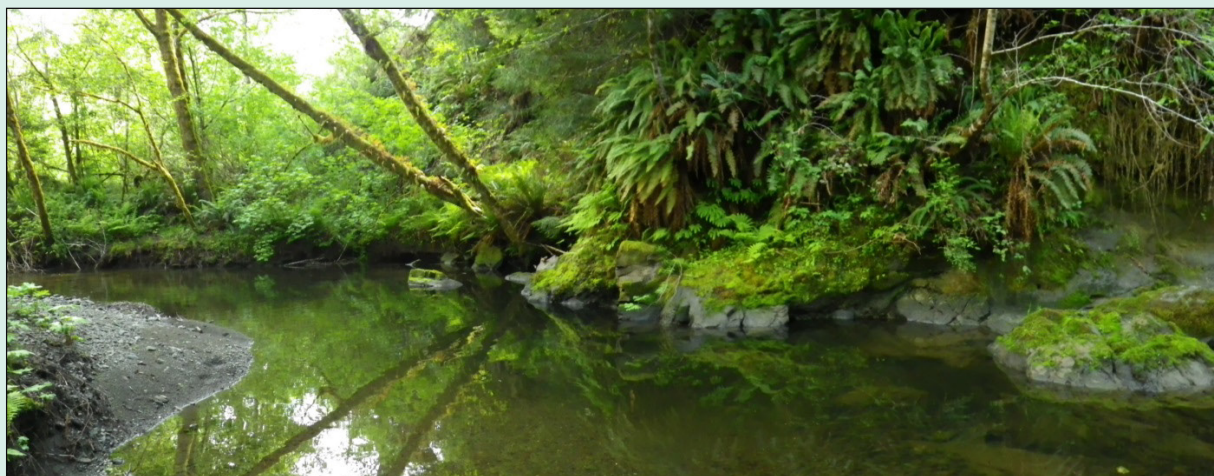


FINAL REPORT • MARCH 2016

Adult Life History of Pacific Lamprey in Freshwater Creek, a Tributary to Humboldt Bay, California



PREPARED FOR
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Cover photos: Upper left, sexually mature and immature Pacific lampreys captured in Freshwater Creek. Upper right, spawning adult Pacific lampreys. Bottom, Pool in Middle Mainstem Freshwater Creek.

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1 INTRODUCTION

1.1 Background and Objectives

Little information on Pacific lamprey (*Entosphenus tridentatus*) adult migration and spawning timing, population status, and basic biology is available for small Northern California streams or tributaries to large estuarine habitats like Humboldt Bay. To help address these data gaps, in recent years California Department of Fish and Wildlife (CDFW) along with Humboldt State University's Sponsored Program Foundation (SPF) has collected data on lamprey migration and spawning in Freshwater Creek, a significant tributary to Humboldt Bay (Ricker et al. 2014). Adult lamprey data was collected incidentally to long-term monitoring of salmon and steelhead as part of the California Coastal Monitoring Program (CMP). Freshwater Creek is also an intensively monitored Life Cycle Monitoring Station (LCS) within the regional sample frame of the CMP. The primary objectives of LCS salmonid studies are to (1) define the relationship between spawning survey data and adult escapement; (2) estimate juvenile and adult abundance, and freshwater and marine survival rates; (3) provide a study framework to investigate habitat-productivity relationships; and (4) characterize the diversity of life history patterns (Anderson et al. 2015, Rebenack et al. 2015). To achieve these objectives, CDFW/SPF operates a weir with adult and juvenile outmigrant traps on lower Freshwater Creek to capture adult salmonids migrating from the ocean, outmigrating juveniles, and post-spawn adults (Ricker et al. 2014, Anderson et al. 2015). CDFW/SPF also maintains an array of passive integrated transponder (PIT) tag antennas (Radio-Frequency Identification [RFID] stations) throughout the mainstem and at key tributary junctions, and conducts various tagging studies to monitor survival and movement of juvenile salmonids (Anderson et al. 2015). Additionally, CDFW/SPF conducts annual spawning surveys for coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) in Freshwater Creek. Focused lamprey research and monitoring and detailed data synthesis of the resulting data are generally outside the scope of the CMP and LCS studies. Therefore, Stillwater Sciences and the Wiyot Tribe Natural Resources Department (WNRD) collaborated with biologists from CDFW's Arcata office to help collect and synthesize available

adult Pacific lamprey data from Freshwater Creek. This was an excellent opportunity to utilize local expertise, land access, and the extensive existing PIT tag infrastructure to describe the life history of adult Pacific lamprey in the watershed. This work supports the larger goals of both the WNRD and the USFWS Pacific Lamprey Conservation Initiative by filling key data gaps and allowing for more effective management, conservation, and restoration of this important species.

This report synthesizes the adult lamprey data collected by CDFW/SPF, and more recently with assistance from the WNRD and Stillwater Sciences, to address the following objectives:

- Describe time of adult Pacific lamprey entry into Freshwater Creek and collect basic biological data on adult migrants;
- Describe adult Pacific lamprey movement patterns from time-of-entry into fresh water until spawning; and
- Describe abundance, timing, and spatial distribution of spawning adult Pacific lamprey.

1.2 Pacific Lamprey Life History Overview

The Pacific lamprey is a large, widely distributed anadromous species that rears in fresh water before outmigrating to the ocean, where it grows to full size (approximately 400–700 mm [16–28 in]) prior to returning to freshwater streams to spawn and ultimately die (Figure 1). The species is distributed across the northern margin of the Pacific Ocean, from central Baja California north along the west coast of North America to the Bering Sea in Alaska and off the coast of Japan (Ruiz-Campos and Gonzales-Guzman 1996, Lin et al. 2008). Adults migrate into and spawn in a wide range of river systems, from short coastal streams to tributaries of the Snake River in Idaho, where individuals may migrate over 1,450 km (900 mi) (Claire 2004).

Pacific lampreys typically spawn from March through July depending on water temperatures and local conditions such as seasonal flow regimes (Kan 1975, Brumo et al. 2009, Gunckel et al. 2009). More inland, high-elevation, and northerly populations generally initiate spawning considerably later than southerly populations (Kan 1975, Beamish 1980, Farlinger and Beamish 1984, Chase 2001, Brumo et al. 2009), presumably due to cooler water temperatures. Spawning generally occurs at daily mean water temperatures from 10–18°C (50–64°F), with peak spawning around 14–15°C (57–59°F) (Stone 2006, Brumo 2006). Redds are typically constructed by both males and females in gravel and cobble substrates within pool and run tailouts and low gradient riffles (Stone 2006, Brumo et al. 2009, Gunckel et al. 2009). During spawning, eggs are deposited into the redd and hatch after approximately 15 days, depending on water temperatures (Meeuwig et al. 2005, Brumo 2006). Pacific lampreys are highly fecund: depending on their size, females lay between 30,000 and 240,000 eggs (Kan 1975). Adults typically die within a few days to two weeks after spawning (Pletcher 1963, Kan 1975, Brumo 2006).

After hatching, the egg-sac larval stage, known as prolarvae, spend another 15 days in the redd gravels, during which time they absorb the remaining egg sac, until they emerge at night and drift downstream (Brumo 2006). After drifting downstream, the eyeless larvae, known as ammocoetes, settle out of the water column and burrow into fine silt and sand substrates that often contain organic matter. Within the stream network they are generally found in low-velocity, depositional areas such as pools, alcoves, and side channels (Torgensen and Close 2004). Depending on factors influencing growth rates, they rear in these habitats from 4 to 10 years, filter-feeding on algae and detrital matter prior to metamorphosing into the adult form (Pletcher 1963, Moore and Mallatt 1980, van de Wetering 1998). During metamorphosis, Pacific lampreys develop eyes, a suckoral disc, sharp teeth, and more-defined fins (McGree et al. 2008). After metamorphosis,

smolt-like individuals known as macrophthalmia migrate to the ocean—typically in conjunction with high-flow events between fall and spring (van de Wetering 1998, Goodman et al. 2015). In the ocean, Pacific lampreys feed parasitically on a variety of marine fishes (Richards and Beamish 1981, Beamish and Levings 1991, Murauskas et al. 2013). They are thought to remain in the ocean, feeding for approximately 18–40 months before returning to fresh water as sexually immature adults, typically from winter to early summer (Kan 1975, Beamish 1980, Starcevich et al. 2014, Stillwater Sciences and WNRD 2016). In the Klamath and Columbia rivers, they have been reported to enter fresh water year-round (Kan 1975, Larson and Belchik 1998, Petersen Lewis 2009). Notably, recent research suggests that two distinct life history strategies, analogous to summer and winter steelhead, may occur in some river systems: one, an “ocean maturing” life history that likely spawns several weeks after entering fresh water, and two, a “stream-maturing” life history—the more commonly recognized life history strategy of spending one year in fresh water prior to spawning (Clemens et al. 2013). The adult freshwater residence period for the stream-maturing life history can be divided into three distinct stages: (1) initial migration from the ocean to holding areas, (2) pre-spawning holding, and (3) secondary migration to spawning sites (Robinson and Bayer 2005, Clemens et al. 2010, Starcevich et al. 2014).



Figure 1. Generalized Pacific lamprey life cycle.

1.3 Study Area

The Freshwater Creek watershed is located in Humboldt County, California, between Eureka to the south and Arcata to the north (Figure 2). The entire watershed is located within the Ancestral Territory of the Wiyot Tribe. Freshwater Creek, which drains into Humboldt Bay via the Eureka Slough (also known as Freshwater Slough), is a fourth order stream with a drainage area of approximately 160 km² (62 mi²), counting the Ryan Creek [38 km² (15 mi²)] and Fay Slough [32 km² (12 mi²)] drainages and small streams draining the north and west sides of Eureka (Figure 2). Other significant tributaries to Freshwater Creek include, McCready Gulch [9.6 km² (3.6 mi²)], Little Freshwater Creek [11.9 km² (4.6 mi²)], Clone y Gulch [12.2 km² (4.7 mi²)], Graham Gulch [6.5 km² (2.5 mi²)], and South Fork Freshwater Creek [8.3 km² (3.2 mi²)]. Elevations in the watershed range from 823 m (2,700 ft) at the headwaters to near sea level at the mouth.

Levees confine the channel in the lower 6 km (3.7 mi) of Freshwater Creek and the surrounding land is primarily used for cattle grazing. The stream continues at low gradient from river kilometer (RK) 6 to RK 9.7 (river mile 6.0) of the main-stem where landownership it is mainly small-parcel residential. Upstream of RK 9.7, approximately 71 km² (27 mi²) of the upper watershed, encompassing 13 km (8 mi) of channel suitable for anadromous fish spawning, is owned and managed for timber production by the Humboldt Redwoods Company. The riparian community is dominated by willow (*Salix* spp.), red alder (*Alnus rubra*), and blackberry (*Rubus ursinus*), with a few black cottonwoods (*Populus trichocarpa*) in the lower reaches, and transitions to a complex of red alder, willow, redwood (*Sequoia sempervirens*), Douglas-fir (*Pseudotsuga menziesii*), salmonberry (*Rubus spectabilis*), big leaf maple (*Acer macrophyllum*) and various herbaceous plants in the upper reaches.

The climate of the Humboldt Bay region is characterized by distinct wet and dry periods. The vast majority of the rainfall occurs in late fall through early spring fall, with little rain occurring otherwise. Mean annual precipitation data from 1981 to 2010 ranged from approximately 102 cm (40 in) at Eureka to over 179 cm (70 in) in Kneeland (PRISM Climate Group, Oregon State University, <http://prismmap.nacse.org/nn/>), with significant annual variability. Air and water temperatures generally remain moderate year round due to the marine influence on the region. The mean monthly temperature at Eureka varies by only 10 degrees Fahrenheit throughout the year, with the low mean in January [8°C (47 °F)] and the high mean in August [14°C (57 °F)] (Barnhart et al. 1992). Water temperatures generally range from 8°C (47 °F) in winter to 15°C (59 °F) degrees in the late summer and early fall (C. Anderson, CDFW, unpubl. data). Refer to Barnhart et al. (1992) and (HBWAC and RCAA 2005) for a more detailed description of the Humboldt Bay watershed, including Freshwater Creek.

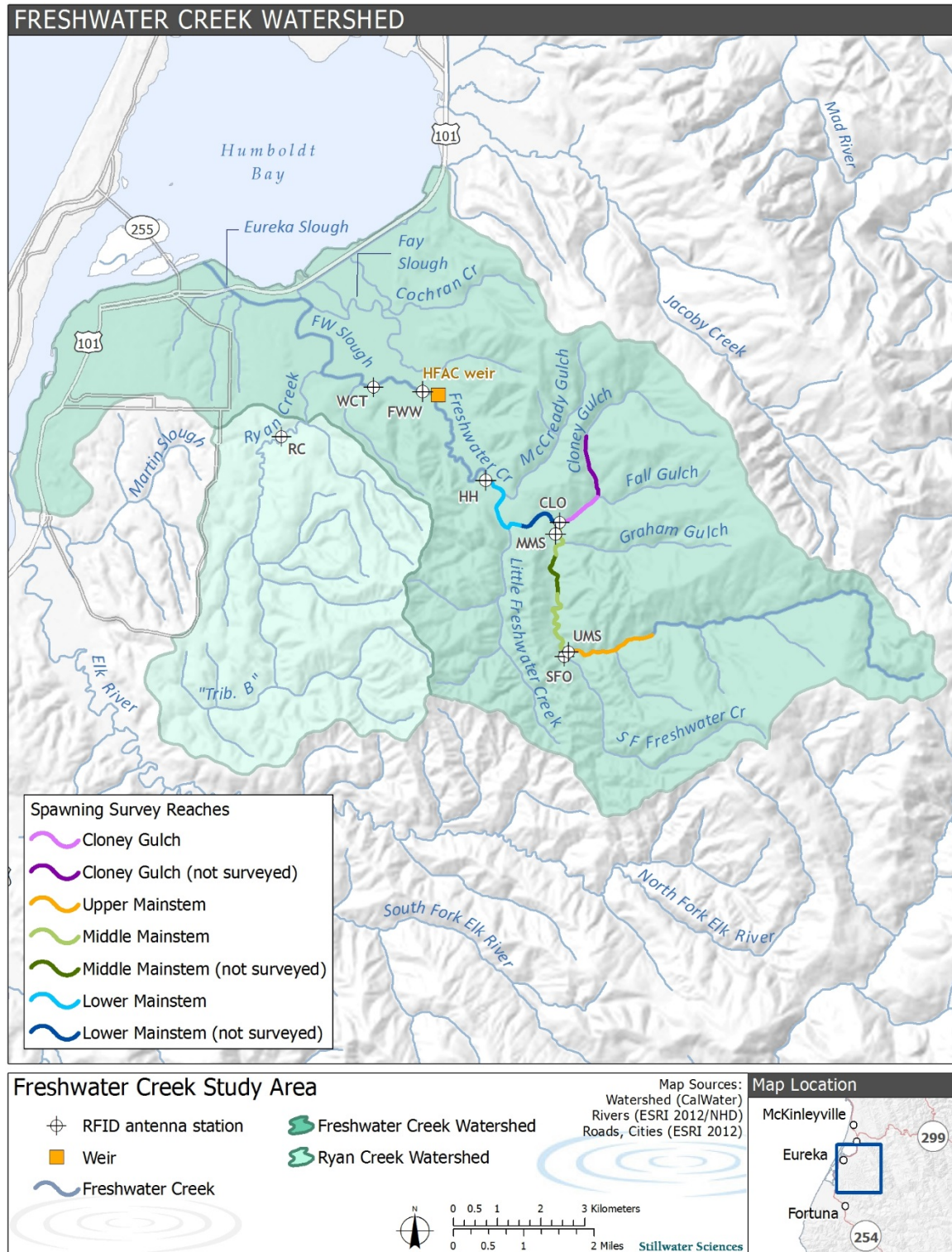


Figure 2. Freshwater Creek watershed overview map, showing major tributaries, the HFAC weir and RFID stations, and reaches where spawning surveys were conducted.

2 METHODS

2.1 Adult Lamprey Capture

Annual abundance, timing of entry into fresh water, and biological characteristics were assessed by capturing adult Pacific lampreys moving past a permanent, channel-spanning weir facility located at RK 8.6 in lower Freshwater Creek near the upper extent of tidal influence (hereafter referred to as “HFAC weir,” Figure 2, Figure 3). Beginning in 2007, in tandem with ongoing efforts to monitor outmigrating salmonids, CDFW/SPF began counting adult Pacific lampreys captured in a downstream migrant trap operated at the weir. From 2012–2015, lampreys encountered moving both downstream and upstream at the weir were captured and counted and data on size, sex, and relative level of maturity were recorded. In 2014, after receiving training from CDFW/SPF in weir operation and capture techniques, the Wiyot Natural Resources Department (WNRD) and Stillwater Sciences put in additional capture effort at the weir during the peak migration period, with the intent of increasing the sample size of tagged fish to allow more intensive study of timing of adult entry and movement patterns.

The downstream migrant trap was consistently operated from March through mid-June in each year, providing a reasonably robust annual measure of relative abundance of lampreys moving in the downstream direction during the trapping period. The downstream migrant trap consisted of a pipe and box trap connected to the weir. To operate the weir as a downstream migration trap, a 4 ft X 5 ft X 3 ft plywood entrance cone was fixed to the upstream end of the center weir panel. The entrance cone then connected to a 10 in diameter PVC pipe that extended 20 ft downstream before terminating in a 4 ft X 4 ft X 8 ft aluminum live cart (Figure 3). Fish encountering the weir were directed to the entrance cone with screened fence with $\frac{1}{4}$ in openings angled approximately 60° to the direction of flow. The downstream migrant trap is expected to capture the majority of the fish moving downstream, but each year it is generally not operated for short periods when high flow events overtop the weir panels.

Lampreys moving upstream at the weir were captured opportunistically using one of three methods: (1) actively with a long-handled dip net; (2) passively with a custom-built plywood box trap designed to fit within the adult salmonid trap in the weir (Figure 4); or (3) when “cranking-up” the floor of the adult salmonid trap out of the water to capture lampreys holding the trap (Figures 3 and 4). Dip-netting was generally attempted after high water events when the lampreys were attempting to pass the fish screen at the upstream end of the adult salmonid trap. Notably, prior to installation of the fish screen in March (for operating the downstream migrant trap), adult lampreys could more readily pass through the weir and were less likely to be caught by dip-netting or in the adult trap. The box trap was generally fished during periods of low to moderate discharge when a relatively large tidal change occurred at night. These opportunistic efforts to capture lampreys moving upstream typically occurred during March–June, the suspected period of lamprey peak migration, though in some years sporadic capture effort occurred as early as December. Relative effort devoted to each of the capture approaches varied within and between years depending on conditions and in response to new insight into capture strategies gained each year. Due to the varying effort and sporadic periods when little or no effort occurred, data on upstream migrating lampreys captured with these approaches contributes little to understanding annual relative abundance, but does add to the understanding of migration timing and basic biology.



Figure 3. Photos of the HFAC weir and associated traps in lower Freshwater Creek. Top left: looking at upstream edge of weir in foreground and adult salmonid trap in background. Top right: looking at downstream edge of weir and pipe leading to downstream migrant trap. Bottom left: downstream migrant trap below weir. Bottom right: looking downstream at adult salmonid trap with the plywood lamprey box trap in place (Photos by C. Anderson).



Figure 4. Plywood box trap designed to capture upstream migrating adult Pacific lampreys at the HFAC weir on Freshwater Creek during lower stream flows (Photo by C. Anderson).

Beginning in winter 2012, captured lampreys—moving both downstream and upstream—were placed in a tagging cradle for biological sampling and tagging. Each fish was measured to the nearest 10 mm (0.4 in) for total length and nearest 5 mm (0.2 in) for inter-dorsal length (IDL) (to inform sexual maturity level; see Clemens et al. 2009), interrogated for previous PIT tags, examined for predator marks and other wounds, and sexed when possible (sexually mature individuals only). The direction each fish was moving when trapped was also recorded. Prior to release, all lamprey in good health were implanted with a unique 32 mm HDX passive integrated transponder (PIT) tag to identify individuals. PIT tags were inserted into the body cavity following methods described by Keefer et al. (2009). After sampling and tagging, fish were released above or below the weir depending on the direction in which they were moving when captured (e.g., upstream of the weir if moving upstream). Gravid females were not PIT-tagged to avoid the potential for damaging or spilling their eggs during tagging. PIT tags were used to track individual adult lamprey throughout their freshwater residency as described in Section 2.2.

For analysis and reporting, level of sexual maturity was assigned to each captured adult lamprey, either based on inter-dorsal length (2013–2015) or visual assessment of body color, condition, fin size and shape, eye clarity, and whether females were gravid (2012) (Figure 5). Adult lampreys with IDL ≥ 20 mm were categorized as immature, whereas fish with IDL < 20 mm were categorized as sexually mature. The latter category included both fish that were mature but in pre-spawn condition (good condition and/or gravid females) and fish that were clearly in post-spawn condition (e.g., mature females without eggs or individuals with fungus, scars, or worn fins from redd construction and spawning). Categorizations based on IDL were largely confirmed by visual observations of color, condition, fish size and shape, and fish that were clearly gravid or spawned-out.



Figure 5. Example of Pacific lampreys categorized as sexually mature (top) and immature (bottom) (Photo: by CDFW staff).

In addition to capture at the HFAC weir, CDFW incidentally captured adult Pacific lampreys during 7 years (2001–2007) of spring outmigrant trapping for salmonids in mainstem Freshwater Creek, Cloney Gulch, Graham Gulch, Little Freshwater Creek, and South Fork Freshwater Creek (C. Anderson, CDFW, unpubl. data; Stillwater Sciences 2016). These fish were presumably mostly spawning or post-spawn individuals. Green Diamond Resource Company also provided 11 years (2004–2014) of incidental adult lamprey capture data collected during salmonid outmigrant trapping in lower Ryan Creek (Green Diamond Resource Company, unpub. data; Stillwater Sciences 2016). Results from these annual trapping efforts shed additional light on the movement and distribution of adult Pacific lamprey in the watershed and are discussed in that context below.

2.2 Movement Patterns

Beginning in winter 2012, migration timing and movement patterns of tagged adult Pacific lampreys were monitored using paired, stream-width PIT tag detection stations (RFID stations) located in mainstem Freshwater Creek and at key tributary junctions (Figure 2, Table 1). Design and orientation of RFID stations closely followed antennas described as “swim through” by Zydlewski et al. (2006). Detection of tags occurred in the entire “swim through” area when antennas were properly tuned. Direction of movement was determined by analysis of the time stamps associated with the PIT tag number of recaptured fish at the paired antennas. An additional RFID station was installed 150 meters downstream of the HFAC weir in November 2013. This antenna was placed on a large spanning log and fished continuously through high flow events. In October 2014, a second antenna was added to allow detection of directional movement below the weir. This second antenna was installed as a flat plane and was fished through all high flow events. In February 2013, a RFID station was installed by CDFW for another project near the upper extent of tidal influence in Ryan Creek, a major tributary to Freshwater Slough.

Each year, RFID stations were generally operated from the first rains in the fall until summer low flow began in late-June or early-July. During periods of very high stream flow, some of the RFID stations could not be operated. In general, the Howard Heights (HH) station becomes inoperable when 1.5–2.0 inches of rain falls in a 24 hr period. The Cloney Gulch (CLO) and Middle Mainstem (MMS) stations can withstand more rainfall and are inoperable less often, while the Upper Mainstem (UMS) and South Fork Freshwater Creek (SFO) stations are inoperable even less. The Freshwater Creek below weir (FWW) RFID station is operated continuously, throughout all high flow events.

Movement patterns of fish captured and tagged in 2012–2014 were continuously monitored using the RFID stations, except from early-July until the first fall rains (typically late-October) when fish movement was expected to be minimal due to low stream flows and presence of shallow or sub-surface riffles. RFID detection data for fish tagged in 2015 were only available through 18 June 2015 at the time this report was produced. Additional analysis of movement patterns of fish tagged in 2015 will be presented in a forthcoming report. Rates of movement of individual fish between RFID stations were calculated as distance between stations divided by time between detections. Finally, stream flow data were not available for Freshwater Creek, so discharge data from the nearby Little River (USGS gage 11481200) was used as a proxy for assessing effects of flow on lamprey movements.

Table 1. RFID stations in the Freshwater Creek watershed and their abbreviations and locations.

| RFID station | Station abbreviation | Location description | River kilometer from Humboldt Bay |
|-----------------------------|----------------------|--|-----------------------------------|
| Ryan Creek | RC ¹ | Near upper extent of tidal influence in Ryan Creek (2.6 km u/s of Freshwater Slough) | 7.9 ² |
| Wood Creek tide gate | WCT ³ | On tide gate at entrance to Wood Cr; on river left edge of Freshwater Slough. | 6.8 |
| Below HFAC weir | FWW ⁴ | 150 m downstream of HFAC weir | 8.5 |
| Howard Heights | HH | Lower FWC (3.8 km u/s of weir) | 12.4 |
| Cloney Gulch | CLO | Cloney Gulch just u/s of mainstem confluence | 15.4 |
| Middle Mainstem | MMS | Middle FWC just u/s of Cloney Gulch | 15.4 |
| Upper Mainstem | UMS | Upper FWC just u/s of South Fork | 19.2 |
| South Fork Freshwater Creek | SFO | South Fork FWC just u/s of mainstream confluence | 19.3 |

¹ Station installed in February 2013.

² The Ryan Creek confluence with Freshwater Creek is 5.3 river kilometers from Humboldt Bay.

³ Antennas at this station do not span the lower Freshwater Creek channel.

⁴ Station installed in November 2013.

2.3 Spawning Surveys

From 2011–2015 CDFW collected data on Pacific lamprey redds, live adults, and carcasses encountered during and after their annual salmonid spawning surveys in Freshwater Creek. Lamprey-focused surveys were generally conducted once per month in each study reach from March through June; although counts were less frequent in 2011 (not conducted in April) and 2012 (not conducted in May). In 2015, surveys were conducted in July to investigate whether later spawning occurred. WNRD and Stillwater Sciences staff assisted with lamprey-focused spawning surveys in May and June of 2014.

Four study reaches, ranging from 2.1 km to 5.0 km in length, were identified for conducting lamprey redd surveys (Table 2, Figure 2). Due to access restrictions, 1.5 km and 1.2 km sections of the Lower Mainstem (LMS) and Middle Mainstem (MMS) reaches, respectively, could not be surveyed. In Cloney Gulch (CLO), lamprey focused surveys were ceased at Falls Gulch, approximately 1.7 km upstream from Freshwater Creek. In 2011, the lower 1 km of South Fork Freshwater Creek was surveyed, but this reach was not surveyed in subsequent years due to the absence of redds and lack of adult lamprey activity at the SFO RFID station.

Table 2. Freshwater Creek watershed study reaches where lamprey spawning surveys were conducted from 2011–2015.

| Study reach | Reach code | Study reach length (km) | Surveyed length (km) |
|----------------------------------|------------|-------------------------|----------------------|
| Lower Mainstem Freshwater Creek | LMS | 3.26 | 1.83 |
| Middle Mainstem Freshwater Creek | MMS | 4.97 | 3.77 |
| Upper Mainstem Freshwater Creek | UMS | 2.06 | 2.06 |
| Cloney Gulch | CLO | 3.20 | 1.67 |
| Total length | | 13.49 | 9.33 |

During each spawning survey, all visible Pacific lamprey redds, adults, and carcasses were counted. Two observers surveyed the entire channel visually by wading or walking the stream margin in the downstream to upstream direction. All areas of disturbed substrate encountered were carefully examined to determine whether they were created by Pacific lamprey based on shape and area of disturbance and substrate size and sorting patterns. Redd dimensions were measured and recorded for each redd. Locations of each redd counted were recorded using a handheld GPS unit and marked with colored flagging at the nearest downstream tree branch or vegetation above the high water mark. The distance and compass bearing from the flagging to each redd were recorded to allow the exact location to be determined on subsequent surveys to avoid recounting and help evaluate detectability over time. When encountering flagging denoting presence of a previously-counted redd during a subsequent survey, the redd's condition was noted to help assess the duration redds remain detectible. All data were entered into handheld data recorder (Rugged Digital Assistant) or recorded on hardcopy datasheets.

Subjectivity exists in redd identification. For these surveys, lamprey redds were generally defined as roughly circular depressions (~0.5 x 0.5 m) in the streambed substrate where most substrate larger than pea-gravel (~6–10 mm) is piled on one or more sides of the depression and not well-sorted by size. Inexperienced observers can misidentify steelhead redds as lamprey redds (and vice versa). However, unlike lamprey redds, steelhead redds typically have larger substrates remaining in the redd depression and a more defined tail-spill that is found only on the downstream edge of the depression and sorted by size (with smaller particles further downstream). During surveys, only redds deemed to be complete were counted. It was assumed that partially complete redds would be counted during a later survey if they appeared to be complete at that time.

3 RESULTS

3.1 Adult Lamprey Capture

3.1.1 Annual abundance

From 2007–2015, the total numbers of adult Pacific lamprey captured at the HFAC weir varied substantially between years, from a low of 10 in 2009 to a high of 197 in 2015 (143 captured in downstream migrant trap) (Figure 6). Since individuals moving in the upstream direction were not targeted for capture until 2011 and effort to capture them varied by year, annual trends in abundance are best evaluated through comparison of captures in the downstream migrant trap, which was operated consistently each year. Over the 9 years, there was no apparent trend in the relative abundance of adult lampreys captured in the downstream migrant trap (Figure 6).

In years where sexual maturity level was assigned (2012–2015), both sexually mature and immature fish were captured at the weir during the trapping period. Sexually immature fish were captured both moving downstream and moving upstream. With the exception of 2015, when 14 sexually mature fish were captured moving upstream, sexually mature fish were only captured moving downstream. Notably, from 2012–2015, when more detailed notes were recorded, several adult female lampreys described as gravid, and numerous others described as “spawned-out” were captured moving downstream at the weir.

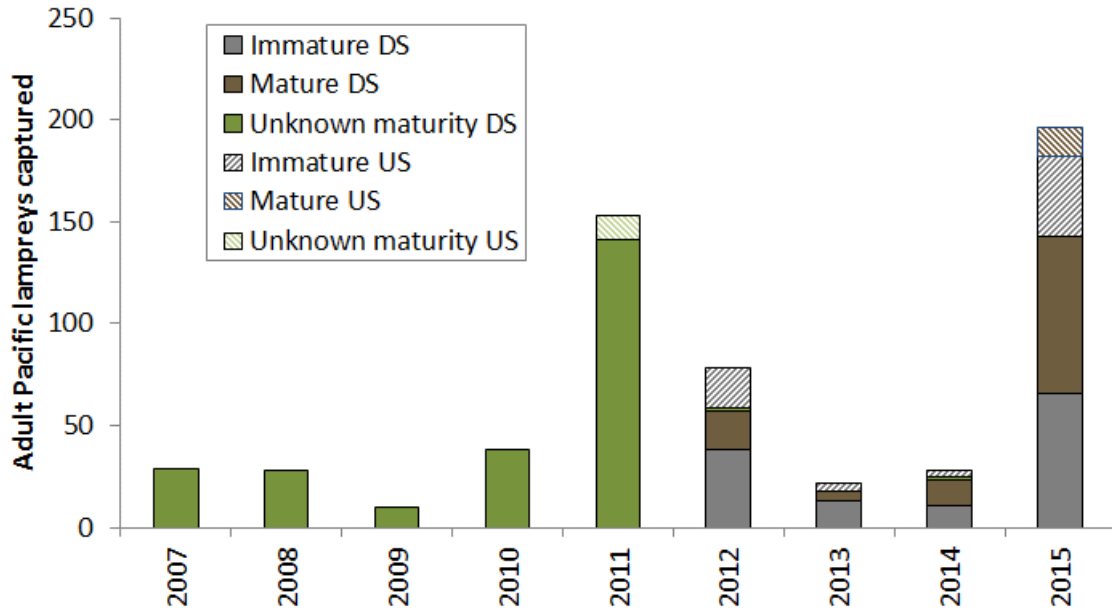


Figure 6. Numbers of mature and immature adult Pacific lampreys captured moving downstream (DS) and upstream (US) at the HFAC weir from 2007-2015. Maturity level was not recorded from 2007-2011. Fish moving upstream were not targeted for capture until 2011.

3.1.2 Timing of entry into fresh water

Pacific lampreys categorized as immature have been captured at the HFAC weir from as early as late-December through June, with earliest and peak capture varying by trapping year (Figure 7). Peak capture of immature lampreys was generally from March through May. Capture of sexually mature fish has occurred from February through June, generally peaking in April and May, coincident with the peak spawning period in Freshwater Creek (Section 3.3).

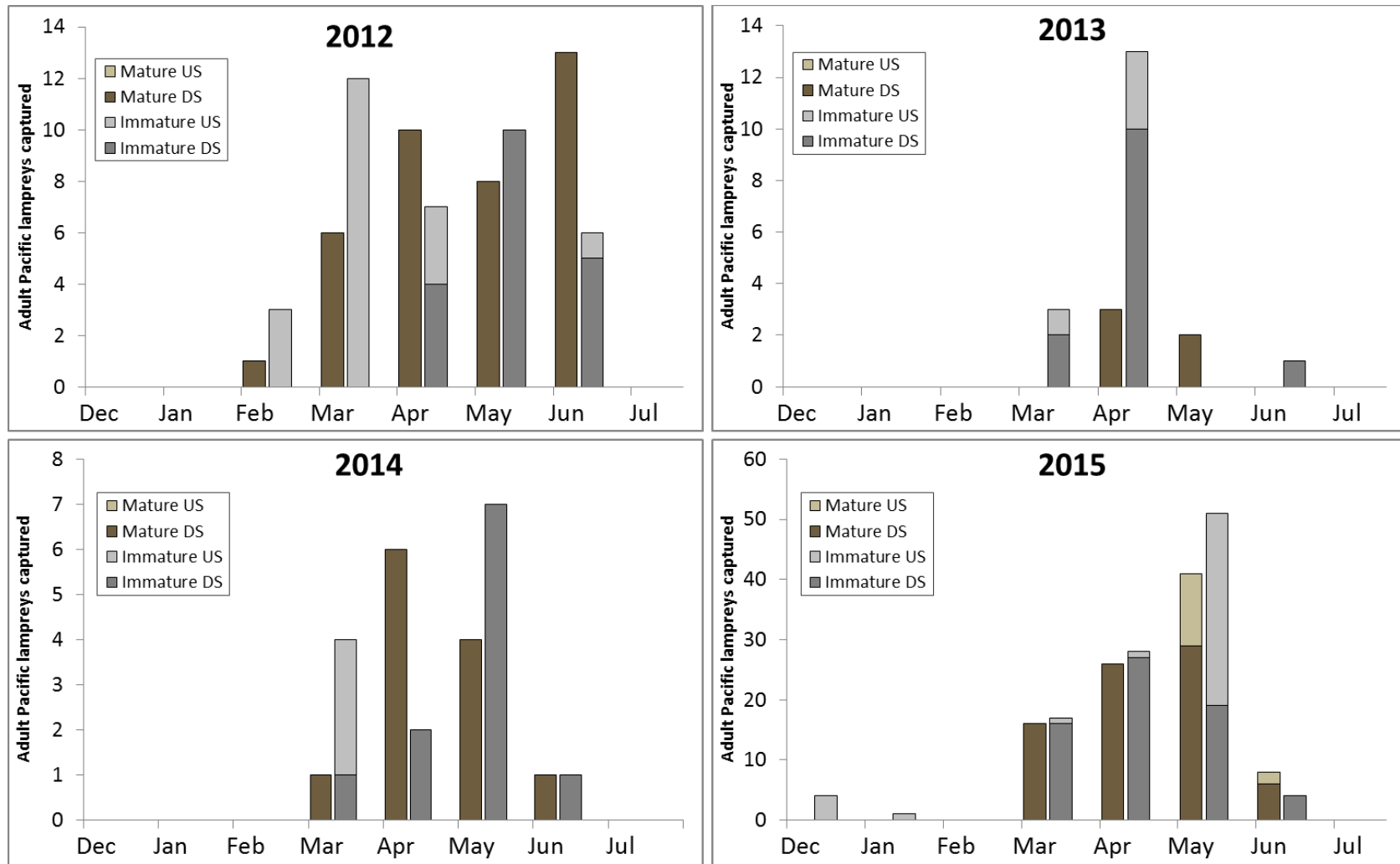


Figure 7. Numbers of mature and immature adult Pacific lampreys captured moving downstream (DS) and upstream (US) at the HFAC weir in each month of trapping years 2012-2015. Note the differences in the y-axis scales.

3.1.3 Biological characteristics

Total length for the 313 adult Pacific lampreys measured at the HFAC weir from 2012–2015 ranged from 390 mm to 660 mm, averaging 532 mm (Table 3). Mean length of fish categorized as immature (566 mm) was significantly greater than mature fish (481 mm) (two-sample t-test; $df = 306$, $P < 0.0001$). Sex could only be reliably determined for 120 of the 313 adult lampreys measured, most of which were sexually mature fish. Of these 120 fish, 83 were female and 37 were male. Since sex of females was more likely to be confidently assigned by the various staff working at the weir, this sex ratio is unlikely representative of the population. Mean length of males [511.5±9.2 mm (SE)] was significantly longer than females [461.0±4.9 mm (SE)] (two-sample t-test; $df = 221$, $P < 0.0001$).

Table 3. Total lengths of immature and mature adult Pacific lampreys captured at the HFAC weir from 2012–2015. Note lengths were not taken for all captured fish and lengths of fish with unknown maturity are not included here.

| Year | Maturity | N | Total length (mm) | | | |
|-----------|----------|-----|-------------------|-----|-------|------|
| | | | Min | Max | Mean | SE |
| 2012 | Immature | 57 | 390 | 660 | 551.1 | 7.6 |
| | Mature | 19 | 390 | 520 | 444.4 | 9.1 |
| | All Fish | 76 | 390 | 660 | 527.7 | 8.0 |
| 2013 | Immature | 17 | 460 | 610 | 550.3 | 9.9 |
| | Mature | 5 | 410 | 520 | 440.0 | 20.2 |
| | All Fish | 22 | 410 | 610 | 524.0 | 13.5 |
| 2014 | Immature | 15 | 500 | 640 | 568.6 | 13.2 |
| | Mature | 12 | 390 | 530 | 460.8 | 13.2 |
| | All Fish | 27 | 390 | 640 | 518.8 | 14.0 |
| 2015 | Immature | 99 | 460 | 650 | 576.3 | 3.5 |
| | Mature | 89 | 400 | 630 | 492.8 | 6.1 |
| | All Fish | 188 | 400 | 650 | 536.8 | 4.6 |
| All Years | Immature | 188 | 390 | 660 | 565.7 | 3.4 |
| | Mature | 125 | 390 | 630 | 481.1 | 5.1 |
| | All Fish | 313 | 390 | 660 | 532.2 | 3.7 |

Inter-dorsal length was measured for 225 fish from 2013–2015 (Table 4). Mean IDL for adult Pacific lampreys captured at the HFAC weir was 19.1±0.7 (SE) (Table 4). Mean IDL was significantly greater for immature fish compared with mature fish (two-sample t-test; $df = 223$, $P < 0.0001$).

Table 4. Inter-dorsal lengths of immature and mature adult Pacific lampreys captured at the HFAC weir from 2013–2015.

| Maturity | N | Inter-dorsal length (mm) | | | |
|----------|-----|--------------------------|-----------------|------|-----|
| | | Min | Max | Mean | SE |
| Immature | 121 | 20 | 40 | 28.0 | 0.4 |
| Mature | 104 | 0 | 30 ¹ | 8.9 | 0.6 |
| All Fish | 225 | 0 | 40 | 19.1 | 0.7 |

¹ Two fish with an IDL >20 mm were categorized as sexually mature due to their apparent gravid state.

3.2 Movement Patterns

From 2012–2015, a total of 217 adult Pacific lampreys were PIT-tagged at the HFAC weir (Table 5). Numbers of fish tagged varied substantially between years, with a low of 20 in 2013 and a high of 109 in 2015. In each year, considerably more fish classified as immature were tagged compared with mature fish. Percentage of tagged fish that were recaptured at least once after tagging (either through detection at RFID stations or physically, at the weir) ranged from 40% in 2013 to 86% in 2014. The much higher recapture percentage in 2014 and 2015 reflects the installation of the FWW RFID station just downstream of the weir in late fall 2013 (Figure 2).

Table 5. Number of adult Pacific lampreys tagged and recaptured in Freshwater Creek from 2012-2015.

| Tagging year | Number of lampreys tagged | | | Number recaptured ¹ | | | Recapture percentage ¹ | | |
|-------------------|---------------------------|------------|------------|--------------------------------|------------|------------|-----------------------------------|------------|------------|
| | Mature | Immature | Total | Mature | Immature | Total | Mature | Immature | Total |
| 2012 | 8 | 58 | 66 | 2 | 27 | 29 | 25% | 47% | 44% |
| 2013 | 4 | 16 | 20 | 0 | 8 | 8 | 0% | 50% | 40% |
| 2014 | 10 | 12 | 22 | 9 | 10 | 19 | 90% | 83% | 86% |
| 2015 ² | 32 | 77 | 109 | 24 | 59 | 83 | 75% | 77% | 76% |
| Total | 54 | 163 | 217 | 35 | 104 | 139 | 65% | 64% | 64% |

¹ Recapture includes individuals detected at least once via RFID stations or physically recaptured at the HFAC weir on a subsequent date.

² 2015 recapture data is only for the period from tagging through 18 June 2015.

Movement patterns of tagged fish varied considerably between and within years, with individual fish displaying a diversity of migratory behaviors (Table 6). More detailed summaries of movement patterns for each year are presented in the sections that follow.

Table 6. Numbers of individual adult Pacific lampreys recaptured at the HFAC weir or detected at RFID stations downstream and upstream of the weir in tagging years 2012-2015.

| Tagging year | Downstream RFID stations | | | | HFAC weir | Upstream RFID stations | | | | | |
|-------------------|--------------------------|-----|-----|---------------------------|-----------|------------------------|-----|-----|-----|-----|---------------------------|
| | RC | WCT | FWW | DS all sites ¹ | | HH | CLO | MMS | SFO | UMS | US all sites ² |
| 2012 | n/o | 6 | n/o | 6 | 3 | 13 | 16 | 2 | 2 | 4 | 27 |
| 2013 | 0 | 3 | n/o | 3 | 0 | 5 | 4 | 1 | 0 | 0 | 5 |
| 2014 | 0 | 1 | 17 | 17 | 1 | 2 | 0 | 0 | 0 | 0 | 2 |
| 2015 ³ | 2 | 7 | 49 | 49 | 7 | 39 | 1 | 8 | 3 | 2 | 40 |

¹ Number of individual fish detected at least once downstream of HFAC weir.

² Number of individual fish detected at least once upstream of HFAC weir.

³ 2015 recapture data is only for the period from tagging through 18 June 2015.

3.2.1 2012 tagging year

In 2012, 29 of the 66 tagged fish were recaptured (Table 5). Of these fish, 27 were detected at least once upstream of the HFAC weir tagging location (Table 6, Figure 8). A considerable number of fish were detected moving upstream relatively rapidly in the spring. This movement which was generally coincident with increased stream flow. Thirteen fish were detected at the HH

station. Notably, eight individuals that were detected at one of the stations upstream of the HH station (CLO, MMS, SFO, or UMS) were not detected passing HH, which indicates these fish likely moved upstream during a high flow event when HH was inoperable. Sixteen fish were detected at the CLO station, suggesting that a high percentage of fish at least briefly entered lower Cloney Gulch and may have attempted to ascend the culvert, which is a significant obstacle to lamprey migration. Two fish were detected moving into lower South Fork Freshwater Creek.

Most of the fish that moved upstream in the spring or early summer of 2012, were not detected again after the summer (Figure 8). However, three individuals, all immature when tagged, were detected the subsequent fall or spring. One fish tagged in mid-April 2012 was first detected at CLO in late-June 2012 and re-detected moving downstream past HH in early April 2013. This fish, presumably post-spawn, was then detected at the Wood Creek tide gate (WCT) station for 13 days in a row in mid-April. A second fish, tagged in late-June 2012 was detected moving upstream past HH and again at CLO in late-November after the first fall rains of 2012 (Figure 8). A third fish, tagged in early-June 2012 was not detected again until early-April 2013, at CLO.

Six fish were recaptured at the WCT station in the Freshwater Creek estuary in the spring or summer after tagging. Four of these fish were detected within 1–5 days after being tagged, but the other two were detected there 43 and 52 days later, respectively. Of these fish, three were detected at WCT for multiple days (ranging from 2–7) in a row. Only two fish classified as mature, both described as “spawned-out” on the date of tagging, were recaptured in 2012: one at the HFAC weir 17 days after tagging and one at the WCT station 3 days after tagging.

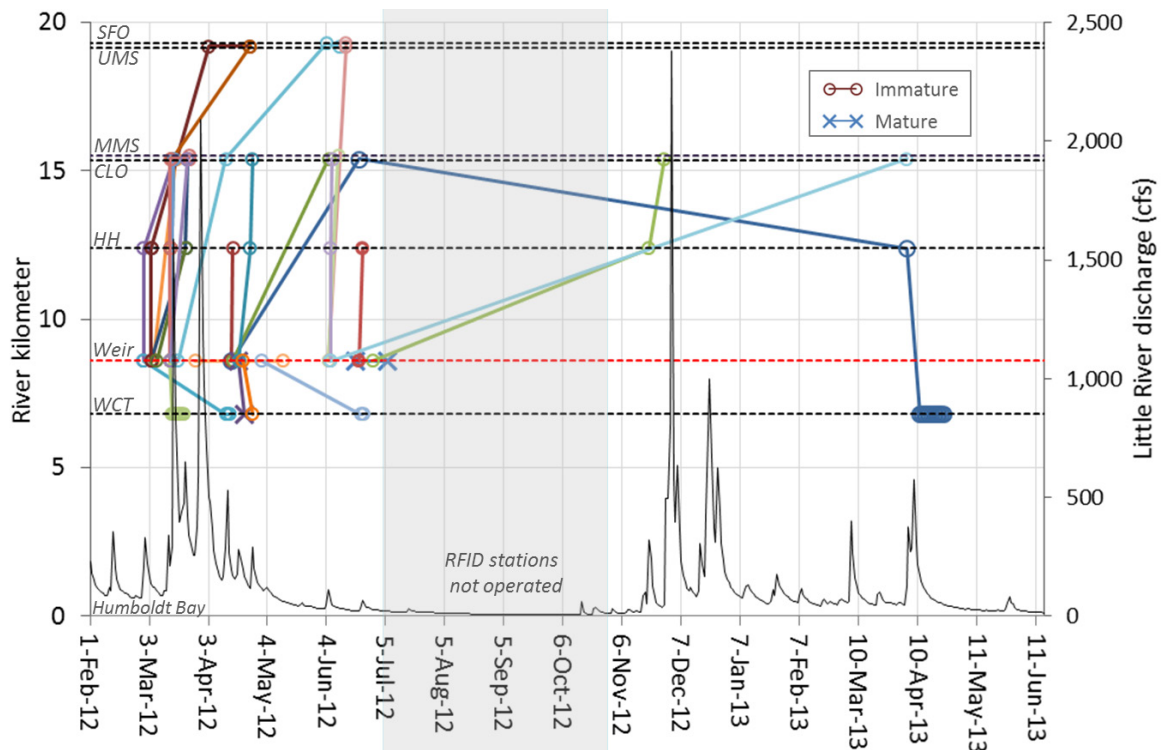


Figure 8. Movement patterns of adult Pacific lampreys recaptured after 2012 tagging at HFAC weir. Each line represents an individual fish with points indicating date and location of initial tagging and subsequent detections. Horizontal dashed lines represent river kilometer locations of RFID stations (black) and the HFAC weir tagging location (red). Discharge of the nearby Little River (USGS gage 11481200) is shown on the secondary Y-axis as a proxy for discharge of Freshwater Creek.

3.2.2 2013 tagging year

Only eight of the 20 fish tagged in spring 2013 were subsequently detected at RFID stations (Tables 5 and 6, Figure 9). All of these individuals were classified as immature during tagging. Five of the eight detected fish were recaptured at RFID stations upstream of the weir soon after tagging (within 1 to 6 days). All five fish that moved upstream were detected at HH and four of the five were detected at CLO, but only one was detected at MMS (Table 6). Three fish were detected downstream of the HFAC weir, at the WCT station. Each of these fish was detected for multiple (12–15) days in a row at the WCT and not detected thereafter. None of the tagged fish were detected again after the spring in which they were tagged (Figure 9). Stream flow peaks were less frequent and of lower magnitude in winter and spring 2013 compared with 2012 (Figures 8 and 9).

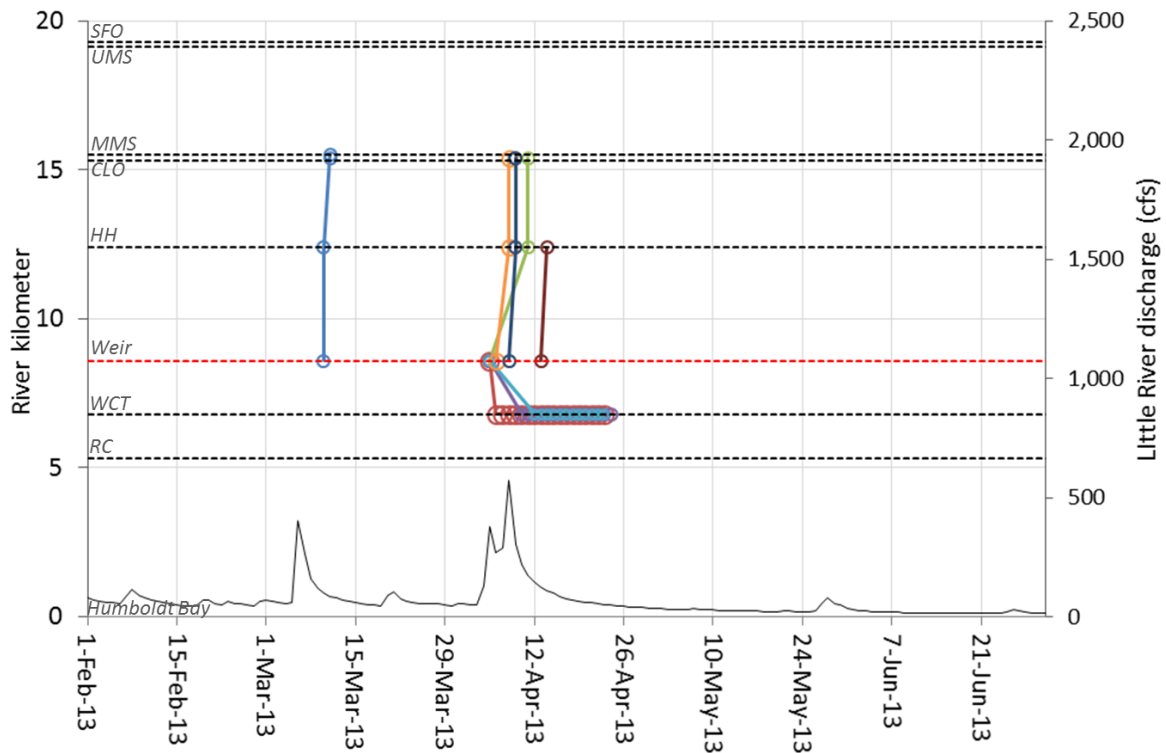


Figure 9. Movement patterns of adult Pacific lampreys recaptured after 2013 tagging at HFAC weir. Each line represents an individual fish with points indicating date and location of initial tagging and subsequent detections. Horizontal dashed lines represent river kilometer locations of RFID stations (black) and the HFAC weir tagging location (red). The X-axis was restricted to show only March–June, since no detections occurred after this period. Discharge of the nearby Little River (USGS gage 11481200) is shown on the secondary Y-axis as a proxy for discharge of Freshwater Creek.

3.2.3 2014 tagging year

In 2014, 19 of 22 tagged fish were detected (Table 5). Nine of the recaptured fish were classified as mature and ten were classified as immature. Only two fish moved upstream of the HH station; both were detected during a small flow increase in May, but were not detected again (Figure 10). The remaining 17 fish were detected at the FWW station just downstream of the weir, soon after being tagged in the spring (Table 6, Figure 10). Only one of these downstream-moving fish was

detected again at another station, WCT. This individual made repeated movements—all presumably at night based on detection times—back and forth between FWW and WCT over a 16 day period between 22 April and 8 May, but was never detected again thereafter. Also notable, several individuals were detected multiple times (on 2 to 10 different days) at the FWW station over the course of 4 to 60 days after being tagged. As with 2013, none of the fish tagged in 2014 were detected again after the spring in which they were tagged (Figure 10).

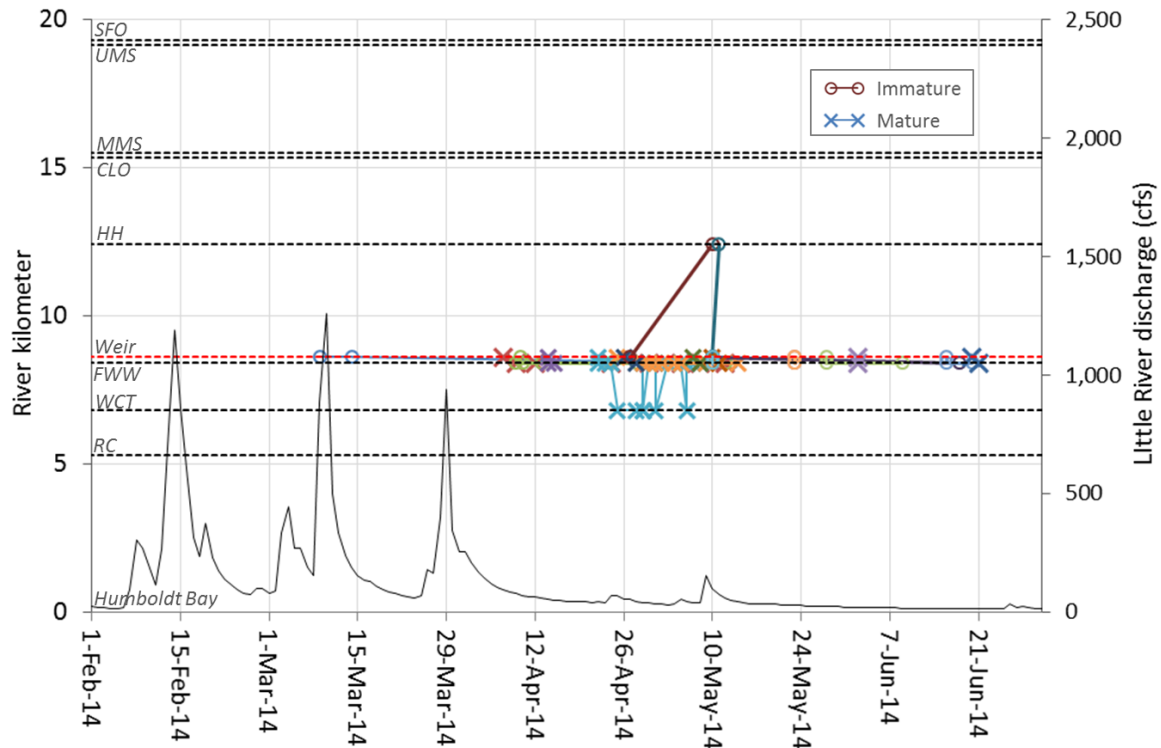


Figure 10. Movement patterns of adult Pacific lampreys recaptured after 2014 tagging at HFAC weir. Each line represents an individual fish with points indicating date and location of initial tagging and subsequent detections. Horizontal dashed lines represent river kilometer locations of RFID stations (black) and the HFAC weir tagging location (red). The X-axis was restricted to show only March-June, since no detections occurred after this period. Discharge of the nearby Little River (USGS gage 11481200) is shown on the secondary Y-axis as a proxy for discharge of Freshwater Creek.

3.2.4 2015 tagging year

As of 18 June 2015, 83 of the 109 fish tagged in winter and spring 2015 had been detected by at least one RFID station (Table 5). Forty-nine tagged individuals were detected at one or more stations downstream of their tagging location, with the majority of these being detected at FWW just downstream of the weir (Table 6). Forty individuals were detected at one or more upstream stations. Seven fish were recaptured at the HFAC weir. Remarkably, two individuals were detected at the RFID station in Ryan Creek (RC). Reaching this station from the weir requires moving approximately 3 km downstream through the tidally influenced reach of lower Freshwater Creek, then moving another 3 km upstream in Ryan Creek (Figure 2). One of the fish recaptured at RC was categorized as mature during tagging and the other was categorized as

immature. The mature fish was first detected at RC on 23 March 2015, 2 days after being tagged at the weir, and was also detected at RC on the 24–26 March. The immature fish was detected at RC only once, on 30 April 2015, 15 days after being tagged at the weir. As of 18 June, both fish were not detected again. Movement of fish tagged during 2015 was still being monitored at the time this report was produced, and a complete description of detection and movement patterns will be presented in a future report.

3.2.5 Movement rates and general movement observations

Rates of upstream movement of adult Pacific lampreys between RFID stations varied within and between years (Table 7). In general, movement through lower Freshwater Creek (from HH to MMS or CLO) was much more rapid than movement in more upstream reaches (MMS or CLO to UMS). Across all years, the median rate of movement between HH and MMS or CLO was 651 m/hr (15.6 km/day), ranging from 8 m/hr to 1,498 m/hr (0.2–35.5 km/day). In contrast, the median rate of movement from MMS or CLO to UMS was 4.5 m/hr (0.11 km/day), ranging from 3 m/hr to 9 m/hr (0.09–0.22 km/day). Rapid upstream movements often coincided with increased stream flows, even in some cases when the magnitude of the flow increase was relatively small (Figures 8–10). Slower movement rates generally occurred during periods of relatively low and constant streams flows, such as spring in 2015. Rate of movement from the FWW station (just below the weir) to the Ryan Creek (RC) station (6,010 meters) was calculated for two individuals: one moved at a rate of 131 m/hr (3.1 km/day) and the other at 16 m/hr (0.4 km/day).

With very few exceptions, detections of PIT-tagged adult Pacific lampreys at RFID stations occurred exclusively at night, suggesting that most movement occurred after dark. Most exceptions to this finding were detections of fish moving upstream during the day with high stream flow events. Additionally, inspection of tagging data indicated individuals were sometimes detected repeatedly at certain RFID stations (namely FWW and WCT) over the course of several days.

Table 7. Upstream movement rates of individually tagged adult Pacific lampreys between specified RFID antenna stations in Freshwater Creek.

| RFID stations detected | | Distance between stations (m) | Tagging year | Fish ID | Time between detections (hr) | Movement rate ¹ | |
|------------------------|------------|-------------------------------|--------------|---------|------------------------------|----------------------------|--------|
| From | To | | | | | m/hr | km/day |
| HH | MMS or CLO | 3,257 | 2012 | #72 | 2.5 | 1,303 | 31.3 |
| | | | | #35 | 3.2 | 1,018 | 24.4 |
| | | | | #33 | 3.4 | 958 | 23.0 |
| | | | | #31 | 3.8 | 857 | 20.6 |
| | | | | #49 | 12 | 263 | 6.3 |
| | | | | #27 | 41 | 79 | 1.9 |
| | | | 2013 | #01 | 3.0 | 1,086 | 26.1 |
| | | | | #06 | 3.9 | 835 | 20.0 |
| | | | | #13 | 5 | 651 | 15.6 |
| | | | | #14 | 19 | 171 | 4.1 |
| | | | 2015 | #55 | 2.2 | 1,480 | 35.5 |
| | | | | #46 | 4.5 | 724 | 17.4 |
| | | | | #3 | 27 | 121 | 2.9 |
| | | | | #15 | 27 | 121 | 2.9 |
| | | | | #61 | 86 | 38 | 0.9 |
| | | | | #32 | 163 | 20 | 0.5 |
| | | | | #30 | 426 | 8 | 0.2 |

| RFID stations detected | | Distance between stations (m) | Tagging year | Fish ID | Time between detections (hr) | Movement rate ¹ | |
|------------------------|-----|-------------------------------|--------------|---------|------------------------------|----------------------------|--------|
| From | To | | | | | m/hr | km/day |
| HH | UMS | 8,229 | 2012 | #28 | 720 | 11 | 0.3 |
| | | | 2015 | #48 | 162 | 51 | 1.2 |
| | | | | #15 | 579 | 14 | 0.3 |
| MMS or CLO | UMS | 4,972 | 2012 | #33 | 984 | 5 | 0.1 |
| | | | | #40 | 1,272 | 4 | 0.1 |
| | | | 2015 | #15 | 552 | 9 | 0.2 |
| | | | | #55 | 1,656 | 3 | 0.1 |

¹ These values represent the average movement rate based on detection times at RFID stations. Values were converted to km/day to allow comparison with other studies

3.3 Spawning Surveys

3.3.1 Annual abundance

Across all Freshwater Creek surveyed reaches combined (approximately 8 km), annual redd counts ranged from 56 in 2014 to 250 in 2015 (Table 8). For reaches in which redds were documented, total linear densities of redds ranged from 7.3 redds/km in 2014 to 32.6 redds/km in 2015 (Table 8). Very few live adults and carcasses were counted in each year, with a peak of three adults and six whole carcasses in 2015. In most years surveyors noted one or more partial carcasses that appeared to be partially consumed by scavengers or predators. Also, evidence of pre-spawn mortality (dead immature female with eggs intact) was documented in 2014 and 2015.

Table 8. Number and density of Pacific lamprey redds and numbers of live adults and carcasses counted in surveyed reaches of Freshwater Creek during 2011-2015 spawning surveys.

| Year | Redds counted | Linear density (redds/km) | Live adults | Carcasses ¹ |
|------|---------------|---------------------------|-------------|------------------------|
| 2011 | 218 | 28.4 | 1 | 0 |
| 2012 | 105 | 13.7 | 2 | 1 |
| 2013 | 66 | 8.6 | 1 | 1 |
| 2014 | 56 | 7.3 | 3 | 4 ² |
| 2015 | 250 | 32.6 | 3 | 6 |

¹ Numbers include whole carcasses only.

² Two carcasses were pre-spawn females with immature eggs.

3.3.2 Spawning locations and spatial patterns

Over the 5 years of spawning surveys, Pacific lamprey redds were documented throughout the surveyed portions of the LMS, MMS, and UMS study reaches (C. Anderson, SPF, unpubl. data). Lamprey redds have not been documented in mainstem Freshwater Creek upstream of the UMS study reach during past exploratory surveys conducted there (C. Anderson, SPF, pers. comm., 28 January 2016). A massive log jam and natural constriction point at the upper end of the UMS reach likely impedes upstream fish migration in most years. No redds, adults, or carcasses were counted in Cloney Gulch in any year. Additionally, no lamprey redds were detected during an exploratory survey of the lower kilometer of South Fork Freshwater Creek in 2011. Captures of adult Pacific lampreys during spring outmigrant trapping in tributaries to Freshwater Creek (2001–2007), suggest that, in some years, the species enters and likely spawns in Cloney Gulch,

Graham Gulch, Little Freshwater Creek, and South Fork Freshwater Creek (C. Anderson, SPF, unpubl. data; Stillwater Sciences 2016). Adult Pacific lampreys were not captured during 7 years of outmigrant trapping in lower McCreedy Gulch from 2001–2008. Numerous, presumably mostly post-spawn adult Pacific lamprey have also been captured during annual outmigrant trapping in lower Ryan Creek, suggesting spawning occurs there (Green Diamond Resource Company, unpub. data; Stillwater Sciences 2016).

In each year spawning surveys were conducted, the highest numbers of redds were counted in the MMS study reach, which is in the mainstem between Cloney Gulch and South Fork Freshwater Creek (Figures 2 and 11). The fraction of the total annual counts from within each reach, however, varied between years.

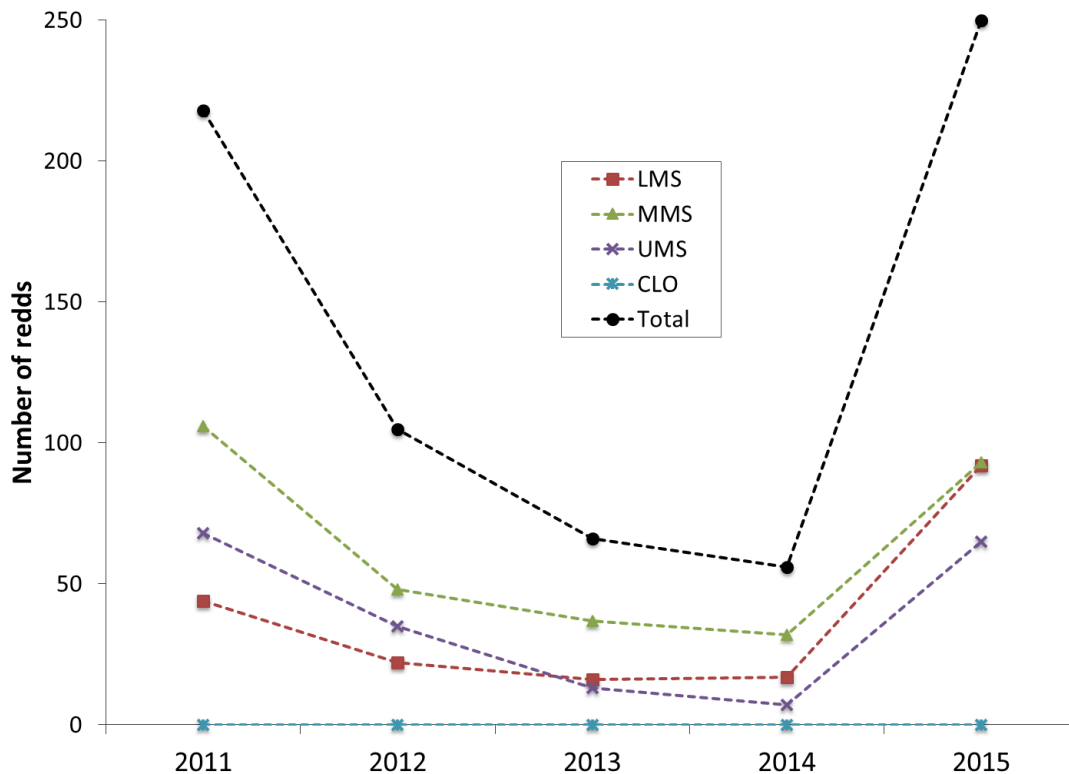


Figure 11. Number of Pacific lamprey redds counted by study reach in Freshwater Creek during 2011-2015 spawning surveys.

Linear density of redds was highest in the UMS survey reach in 2011 and 2012, highest in MMS during 2014, and highest in LMS during 2015 (Figure 12). From 2011–2013, redd densities did not differ considerably between survey reaches, but in 2014, density in LMS was nearly three times that observed in UMS. Likewise in 2015, redd density in LMS was over twice the density of MMS and nearly twice the density of UMS (Figure 12).

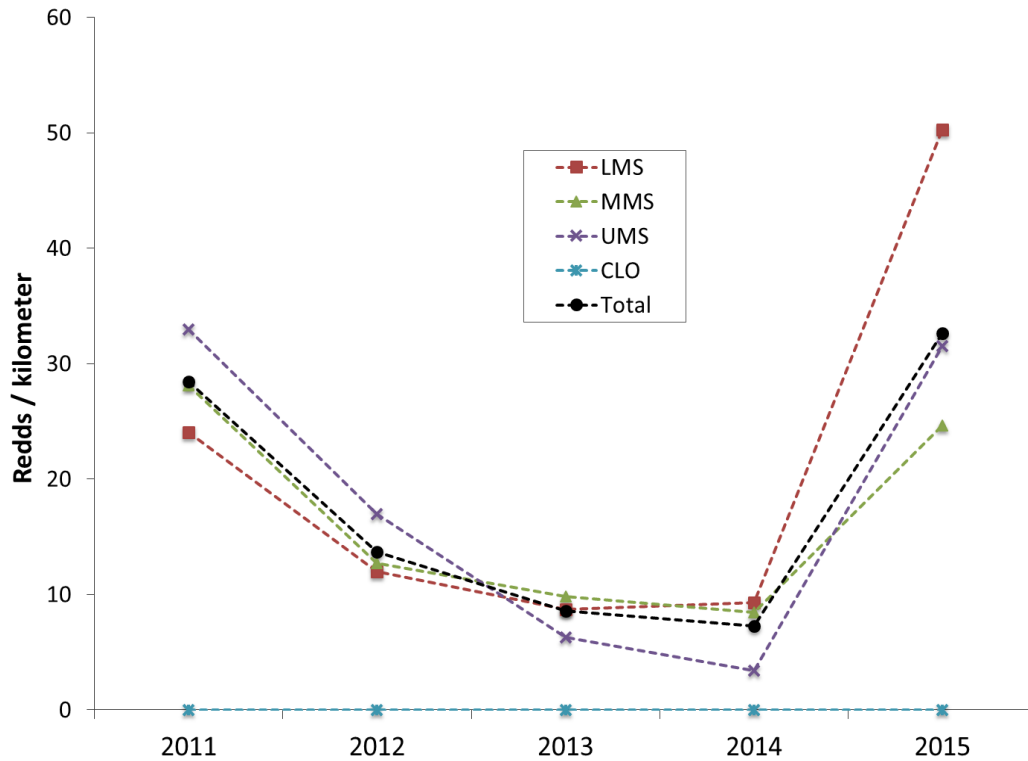


Figure 12. Linear density of Pacific lamprey redds by study reach in Freshwater Creek from 2011-2015 spawning surveys. Calculations of total density over all reaches do not include Cloney Gulch (CLO).

Over the 5 years of spawning surveys, annual redd densities were generally correlated among the 2–3 km study reaches (Table 9, Figure 12). However, only the relationship between redd densities in MMS and UMS was statistically significant at the 95% confidence level ($P = 0.005$). Excluding 2015 data, redd densities were highly and significantly correlated among all three study reaches. From 2011–2015, redd densities from all three study reaches were significantly correlated with densities over the entire 10-km survey area (all sub-reaches combined) (Table 9).

Table 9. Matrix of r^2 statistics and associated P-values for relationships between linear densities of redds in each mainstem Freshwater Creek study reach and the entire 10-km survey area (all sub-reaches combined) over five years of surveys (2011-2015).

| Study reach | LMS | | MMS | | UMS | | Total | |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------|---------|
| | r^2 | P-value | r^2 | P-value | r^2 | P-value | r^2 | P-value |
| LMS | -- | -- | 0.586 | 0.132 | 0.626 | 0.111 | 0.813 | 0.036 |
| MMS | 0.586 | 0.132 | -- | -- | 0.949 | 0.005 | 0.931 | 0.008 |
| UMS | 0.626 | 0.111 | 0.949 | 0.005 | -- | -- | 0.946 | 0.005 |
| Total | 0.813 | 0.036 | 0.931 | 0.008 | 0.946 | 0.005 | -- | -- |

3.3.3 Spawning timing

From 2013 through 2015, when spawning surveys were consistently conducted monthly from March through June, redd counts peaked during April in 2014 and during May in 2013 and 2015 (Figure 13). A small number of redds were documented during the March survey in 2015, but not in other years. No new redds were documented in July surveys in 2015 (July surveys were not conducted in other years).

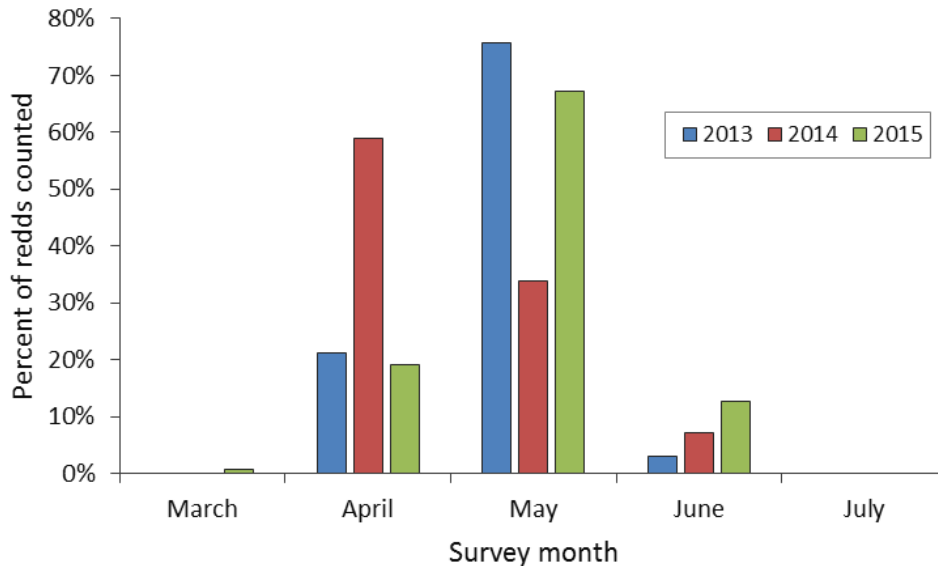


Figure 13. Percent of total annual Pacific lamprey redds counted during each survey month in Freshwater Creek, 2013–2015. July surveys were not conducted in 2013 and 2014.

3.3.4 Redd counts versus weir counts

From 2011–2015, there was a significant linear relationship between the number of redds counted in Freshwater Creek during spring spawning surveys and the total number of adults captured at the HFAC weir in the winter and spring of same year ($r^2 = 0.983$; $P = 0.001$) (Figure 14). Notably, from 2012–2015 (when data on sexual maturity were available), redd counts were highly correlated and statistically significantly with weir counts of both fish categorized as mature ($r^2 = 0.975$; $P = 0.013$) and fish categorized as immature ($r^2 = 0.942$; $P = 0.030$). Contrary to what might be expected based on the general understanding that Pacific lamprey adults usually hold in fresh water for a year prior to spawning, the number of redds counted in a year was not significantly correlated with the number of sexually immature adults counted at the weir the previous year ($r^2 = 0.265$; $P = 0.656$)—albeit only three years of data were available to evaluate this relationship (2013–2015).

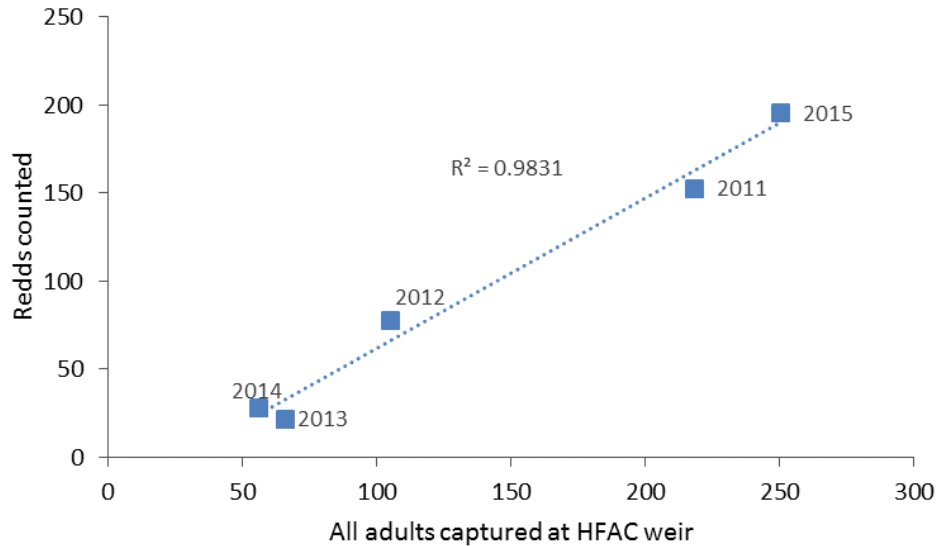


Figure 14. Relationship between redds counted during annual spawning surveys in Freshwater Creek and the number of adults captured at the HFAC weir (both mature and immature) in the same year from 2011-2015.

4 DISCUSSION

4.1 Adult Lamprey Capture

Data from opportunistic capture of lampreys migrating upstream at the HFAC weir cannot be used as a reliable indicator of annual adult abundance, since annual and monthly capture effort varied. However, these captures expanded our understanding of run timing (e.g., by catching fish as early as December) and increased the number of fish available for tagging and evaluating movement patterns.

Numbers of adult Pacific lamprey captured in the downstream migrant trap at the weir serve as a useful measure of annual relative abundance of lampreys moving in the downstream direction in lower Freshwater Creek during the period the trap was operated (March through June). The trap presumably captures the majority of the fish moving downstream, but in most years it is generally not fished for short periods during high flow events when the weir panels overtop. For this reason, an unknown number of individuals pass downstream without being captured. As reported above, adult counts at the weir were highly correlated with upstream redd counts in the same year, confirming that they are likely a viable metric of annual adult abundance. Importantly, data from detections of tagged fish suggest that many of the fish captured in the downstream migrant trap ultimately migrate back upstream to hold and or spawn in Freshwater Creek.

Since both sexually mature and sexually immature fish are captured in the downstream migrant trap, these data potentially represent to two different spawning cohorts: one that spawned in the spring before or soon after capture at the weir and one expected to spawn the following spring after reaching sexual maturity. Detections of a small number of tagged fish moving in the spring approximately 1 year after tagging (in addition to clearly mature individuals) verified the presence of these two cohorts. As discussed below, however, it is also possible that some

percentage of individuals classified as immature at time of capture, actually spawned in the spring they were captured.

The finding of a significant linear relationship between the number of redds counted in Freshwater Creek during spring spawning surveys and the number of adults captured (including both fish classified as mature and immature) at the weir in the winter and spring of same year is notable. You would expect such a relationship between counts of redds and sexually mature fish, but given the scientific evidence and commonly held belief that Pacific lamprey generally spend approximately one year in fresh water prior to reaching sexual maturity and spawning (Robinson and Bayer 2005, Clemens et al. 2010, Starceovich et al. 2014), it is surprising that this relationship was significant for fish categorized as immature. One explanation for this finding is that a considerable number of the “immature” individuals spawned in the spring soon after they were captured at the weir. Recent research from the Willamette and Klamath rivers indicates the presence of two distinct Pacific lamprey life history strategies, analogous to summer and winter steelhead, may occur in some river systems: one, an “ocean-maturing” life history that likely spawns soon after entering fresh water, and the other, a “stream-maturing” life history—the more commonly recognized life history strategy of spending one year in fresh water prior to spawning (Clemens et al. 2013). The relative prevalence of these two life history types in Freshwater Creek (and other small coastal streams) needs further investigation since most studies describing the dominance of the stream-maturing life history come from large river systems, where spending a year in fresh water prior to spawning may infer a greater selective advantage (i.e., evolutionarily) due to the longer migration distances required to reach suitable spawning areas.

Another possible explanation for the significant relationship between redds and immature adults captured at the weir is that the characteristics used to categorize sexual maturity for this study (mainly IDL, color, and apparent condition) are not closely correlated with actual sexual maturity or readiness to spawn (conditions of ova and sperm as described by Clemens et al. 2013). Furthermore, the actual amount of time that immature individuals spent in fresh water prior to capture at the weir is unknown. Since most immature fish were captured moving downstream (and there is limited effort to catch fish moving upstream and early in the presumed migration period), it is likely that many of the fish entered fresh water earlier than indicated by the weir. The weir is located at the approximate upper extent of tidal influence, but the reach below the weir is generally fresh water dominated in the fall and winter, and thus it is also possible that some individuals held for an unknown amount of time below the weir before capture.

Timing of capture of immature adult Pacific lamprey at the weir is presumably indicative of timing of adult entry into fresh water; however inconsistent and sometimes low effort to capture fish in the early part of the season (prior to installation of the downstream migrant trap and associated fish screen) may have resulted in not documenting the early part of the run (December through February) in some years. The early capture of immature fish (all migrating upstream) in 2015 likely resulted from the relatively large run and more effort to capture fish early in the run compared with previous years. Further effort to document earlier migration into Freshwater Creek would be valuable. In the nearby Klamath River, both historical (Petersen-Lewis 2009) and recent (D. Goodman, USFWS, pers. comm., 28 September 2015) accounts indicate that, in some years, migratory adults may enter fresh water in as early as summer or early fall.

Mean length of sexually immature adult Pacific lampreys measured in Freshwater Creek was larger than mean length of sexually mature individuals, a finding explained by a decrease in size that occurs prior to spawning, during the migration and sexual maturation periods (Clemens et al. 2010). Mean length of immature adult Pacific lampreys measured in Freshwater Creek (566 mm) was considerably less than that reported for larger river systems, including the Eel River (619

mm; Stillwater Sciences and WNRD 2016), the Umatilla River (678 mm; Jackson and Moser 2012), and the Columbia River (648–679 mm; Keefer et al. 2009). One possible explanation for this discrepancy is the greater size and lipid reserves needed to undertake a long migration required in these larger rivers compared with the short migration in Freshwater Creek. As discussed above, it is also possible that some individuals classified as immature had been in fresh water for some time prior to capture at the HFAC weir and had already begun to the sexual maturation and shrinking process.

4.2 Movement Patterns

Detections of adult Pacific lampreys tagged from 2012–2015 revealed some generalities about movement patterns in Freshwater Creek, while also highlighting the existence of a wide range of behaviors amongst individual fish and inter-annual variation in migration patterns.

Only a small percentage of tagged fish were detected after the spring in which they were tagged. Fish categorized as mature presumably spawned and died soon after tagging. However, only 3 of the 86 immature fish tagged from 2012–2014 were documented making secondary migrations in the fall or winter after tagging. These three individuals indicate that, following the initial migration from the ocean, at least some percentage of the population holds in fresh water through the summer and winter prior to spawning (“stream-maturing”). The lack of post-spring detection of the remaining 83 fish can likely be explained by some combination of the following: (1) high summer mortality related to predation or other factors; (2) selection of spawning locations within the reach that they originally migrated to (i.e., no movement past RFID stations after initial spring migration); or (3) poor detection efficiency at RFID stations, or movement during periods when RFID stations were not operating. As discussed above, it is also appears likely that some fraction of fish categorized as immature were “ocean-maturing” and spawned and died during the spring in which they were tagged.

Our finding that tagged Pacific lamprey moved almost exclusively at night is consistent with general understand that most lampreys are photophobic (Hardisty and Potter 1971) and findings from other studies that have documented night-dominated movements of pre-spawning adult lampreys (Robinson and Bayer 2005, Fox et al. 2010, Clemens et al. 2012).

The detection of two tagged fish (one mature and one immature) in Ryan Creek, a downstream tributary to the Freshwater Creek estuary, indicates some individuals that enter lower Freshwater Creek move back downstream and may ultimately hold or spawn in other nearby streams. The prevalence of this inter-watershed movement warrants continued investigation. In addition, in some years, several clearly spawned-out female lampreys were captured at the weir, apparently moving downstream into the tidally influenced reaches of Freshwater Creek. Tag detections indicate some of these fish moved downstream at least into the estuary at least as far as Wood Creek. Existing evidence suggests that Pacific lampreys typically die within a few days to weeks after spawning (Pletcher 1963, Kan 1975, Beamish 1980, Brumo 2006), but the possibility of out-migration to the ocean and repeat spawning has been reported for a stream on the Olympic Peninsula (Michael 1980, Michael 1984); though this finding was based on arguably ambiguous marking data. Since Pacific lampreys do not necessarily home to natal spawning streams (Moyle et al. 2009, Spice et al. 2012), documenting repeat spawning in Freshwater Creek based on relatively small numbers of PIT-tagged fish is unlikely. Use of acoustic tags or radio tags to further investigate movement of post-spawn Pacific lamprey in the Freshwater Creek estuary, Humboldt Bay, and possibly the ocean may be worth pursuing.

Repeated detection of tagged individuals at the WCT and FWW RFID stations suggests that fish may be attracted to either natural features or infrastructures at these locations. The WCT RFID station is located adjacent to the main Freshwater Creek channel on a tide gate at the entrance to Wood Creek (a tidally influenced slough and pond system with no spawning habitat). Repeated detection here, suggests individuals may be attracted to the concrete or other tide gate infrastructure, which is one of the few locations where fish can seek cover in the relatively simple channel in the tidally influenced reaches of lower Freshwater Creek. Repeated detection at the FWW station, which is in close proximity to the HFAC weir, suggests that upstream movement of these fish may have been impeded by the weir. Additionally, the FWW antenna is built around a large, channel-spanning log with a root wad, which may offer good habitat for lamprey resting or holding. Several individuals were detected for multiple days in a row at FWW, but only after dark, when multiple detections occurred over the course of several hours. These individuals were likely resting prior to increasing activity at night. The relatively high number of detections at the CLO RFID station, particularly in 2012, may have been related to fish attempting unsuccessfully to enter Cloney Gulch by approaching the culvert and associated downstream boulder weirs. It is also possible that some tagged fish used the boulder weirs near the RFID station for holding or resting cover.

Average movement rates (converted to km/day for comparison to other studies) of most tagged lampreys between the HH and MMS RFID stations in Freshwater Creek were generally within the range reported for migrating Pacific lampreys in other river systems. In Oregon, individual movement rates ranged from approximately 1–19 km/day in the Willamette River (Clemens et al. 2012), 1–13 km/day in the Smith River (Starcevich et al. 2014), and 1–21 km/day in the John Day River (Robinson and Bayer 2005). Some individuals in Freshwater Creek were documented moving at considerably faster (35.5 km/day) and slower (0.2 km/day) rates over the relatively short distances between RFID stations. Higher rates of movement were generally associated with high water events, suggesting that migrating lampreys take advantage of high water levels and associated increased turbidity to rapidly move upstream. The lower average movement rates may have been associated with individuals waiting for storm events to move upstream. Movement rates between the MMS and UMS RFID stations were generally much slower. The initial rapid movement into the Middle Mainstem reach (between Cloney Gulch and South Fork Freshwater) and reduced detections upstream suggests that many individuals may be moving into over-summer holding or spawning areas in the reach.

Overall, the use of an array of existing RFID stations to detect PIT-tagged fish was a cost-effective and efficient means for describing movement patterns from time-of-entry into fresh water until spawning in mainstem Freshwater Creek. However, several factors may have limited our ability to detect movement and lessened the strength of conclusions about behavior and movement. First, the 3–4 km spacing between RFID stations only allowed detection of relatively large-scale movements and did not allow exact locations of fish to be pinpointed, limiting inferences about smaller scale movements and habitat use. Second, although tag detection efficiency at RFID antennas is expected to be high due to a high read range for the tags used (~1 m), most antennas could not be operated for several days during high flow events in each year, resulting in missed detections (e.g., HH station in 2012). Finally, RFID stations were not operated from July until the first fall rains each year (typically late October). However we expect little to no lamprey movement during this period due to low stream flows and presence of very shallow, often-sub surface riffles in some reaches of Freshwater Creek. Moreover, a seasonal recreational dam that blocks adult lamprey passage is erected annually at Freshwater Park just upstream of Cloney Gulch from mid-June until early-September. A radio telemetry study in a coastal Oregon stream documented relatively little movement during the summer and early fall (Starcevich et al. 2014). This stream was substantially larger (525 km² versus 160 km² contributing drainage area)

and presumably had more summer base flow than Freshwater Creek. In future years, a handheld PIT tag wand (and possibly radio telemetry) will be used to help pinpoint holding locations of tagged lamprey and verify the assumption of limited summer movement.

4.3 Spawning Surveys

Based on redd counts, the Pacific lamprey spawning population in Freshwater Creek was most abundant in 2015 and least abundant in 2014. Redd densities documented in Freshwater Creek during 2011–2015 spawning surveys (7.3–32.6 redds/km) were higher than redd densities from surveys (using similar methods) of similarly-sized streams in the Eel River basin conducted in 2014. In Lawrence Creek, a tributary to Yager Creek in the Van Duzen watershed redd densities averaged 7.2 redds/km, while densities in Bull Creek, a tributary to the South Fork Eel River, averaged 2.9 redds/km (Stillwater Sciences and WNRD 2016).

Over the 5 years of spawning surveys, annual redd densities were generally correlated amongst the 2–3 km study reaches, suggesting counts from individual reaches are generally representative of the overall annual population trend for Freshwater Creek.

The finding of a strong and significant correlation between the number of redds counted in Freshwater Creek during spring spawning surveys and the number of adults captured at the HFAC weir during the same year further supports the assertion that monthly redd counts are a viable method for assessing relative abundance of the adult Pacific lamprey population in Freshwater Creek. However, it is likely that some redds constructed immediately after a given survey date were missed on the subsequent survey due to the monthly survey interval. A recent analysis of redd detectability based on a single year of data collected in similar streams in the Eel River basin indicated ability to detect redds decreased over time and only about 60% of redds were still detectable after 30 days (Stillwater Sciences and WNRD 2016).

The rarity of live adult Pacific lampreys and carcasses detected during spawning surveys is noteworthy and is in contrast to results reported from some other river systems. Over five years of spawning surveys in Freshwater Creek, nearly 700 redds were documented, but only 10 live adults and 12 whole carcasses were observed. In comparison, during two seasons of spawning surveys on the South Fork Coquille River in Oregon, Brumo et al. (2009) counted approximately one live adult for every five redds observed and one carcass for every eight redds observed. Similarly, on Cedar Creek in Southern Washington, Stone (2006) counted at least one live adult for every four redds counted. In the nearby Eel River basin, however, very few adults and carcasses were observed during 2014 spawning surveys (Stillwater Sciences and WNRD 2016). Nocturnal spawning of Pacific lampreys has been observed (e.g., Brumo 2006), and it is possible that much of the spawning activity in Freshwater Creek occurred at night, while most lampreys remained hidden during daytime surveys. The relative lack of live adults and carcasses observed in Freshwater Creek could also be due to greater predation on spawning and post-spawn adults, as well as scavenging of carcasses. Several observations of partial lamprey carcasses within and outside of the wetted stream channel, add to evidence of the importance of Pacific lamprey as a food source for scavengers and predators. Marine derived nutrients from adult lampreys and their eggs likely play an important role in the productivity of the freshwater and riparian environments during the spring and summer (Nislow and Kynard 2009, Weaver et al. 2015).

Based on monthly redd counts from 2013–2015, spawning activity in Freshwater Creek peaked in April or May. This finding should be viewed generally due to error associated with the one month survey periodicity (e.g., some of the redds counted in May could have been constructed in late-

April); however, April and May peak spawning is generally consistent with that documented in other river systems in the region (Brumo et al. 2009, Gunckel et al. 2009, Stillwater Sciences and WNRD 2016). The apparent annual variation in peak spawning time (e.g., earlier in 2014) may be related to the effects of timing and magnitude of stream flows on migration and spawning behaviors, the impact of water temperature on spawning activity, or the impact of water temperature during the pre-spawning period on rate of sexual maturation (Clemens et al. 2009).

Surveys documented widespread spawning in mainstream Freshwater Creek, but no redds were found in Cloney Gulch, its largest tributary. Small numbers presumably post-spawn adult Pacific lamprey have been captured in an outmigrant trap in Cloney Gulch, but they were not detected there until after a box culvert under Freshwater Road at the confluence with Freshwater Creek was modified in 2002 (C. Anderson, SPF, unpubl. data; Stillwater Sciences 2016). Even with this modification (added baffles and downstream rock weir aimed at improving salmonid passage) the culvert appears to impede lamprey passage under most stream flows and likely explains the lack of redds observed in the stream, which otherwise has a considerable amount of suitable spawning habitat. The site has a vertical 0.5 meter drop between the culvert outlet and the outlet pool, as well as a ninety degree angle at the top of a vertical concrete wall. Notably, the CLO RFID station, located just below the culvert outlet, has detected several tagged lampreys that moved upstream then back downstream.

5 RECOMMENDATIONS AND FUTURE RESEARCH

Recent data collected by CDFW/SPF and summarized in this collaborative synthesis report have begun to shed light on Pacific lamprey annual abundance, migration and spawning timing, movement patterns, and basic biology of adult Pacific lamprey in Freshwater Creek. However, numerous questions and important data gaps remain and we recommend additional investigation and analysis to address them, with the ultimate goals of understanding the factors limiting lamprey population size in the watershed and developing effective restoration and conservation actions.

Many data gaps can be addressed in part by analyzing additional years of data from existing activities, including capture and tagging lamprey at the HFAC weir, tag detection at existing RFID stations, and monthly spawning surveys. For example, continued tracking and analysis of the large number of fish tagged in 2015 is expected to improve our understanding of movement patterns and general holding locations. In general, we recommend continued use of existing RFID stations to track PIT-tagged fish, since it allows passive detection and tracking with minimal staff time. However, where feasible, we recommend considering the following additional steps to improve understanding of adult Pacific lamprey life history in Freshwater Creek:

- Increased effort to capture and tag adult lampreys at the HFAC weir in late fall and early winter to document early migration and potentially unique life history strategies and to improve understanding of migration timing.
- Expanded investigation of variation in sexual maturity of adult lampreys captured at the weir, including documenting the presence and prevalence of ocean-maturing and stream-maturing life history types using methods described in Clemens et al. (2013).
- Further analyses to describe differences in movement and behavior between sexes and maturity levels.
- Additional RFID stations in other Humboldt Bay tributaries to help document and describe inter-watershed movements. Possible locations include North Fork Elk River, South Fork

Elk River, Janes Creek, and Jacoby Creek. These stations would also support existing studies of juvenile coho salmon movement.

- Survey the entire mainstem of Freshwater Creek upstream of the weir with a backpack PIT-tag wand to pinpoint locations of tagged individuals during the summer, document habitat characteristics of holding locations (e.g., substrates used, association with instream wood, water depth and velocity, water temperature, and dissolved oxygen level) and verify the assumption that movement is limited during the summer.
- More thoroughly describe (1) distribution of adult lampreys during the pre-spawning summer holding period and (2) factors affecting seasonal and annual variation in holding locations as more data become available from PIT-tag wand surveys and existing RFID stations.
- Collect physical and chemical information in locations where holding adults are found.
- If PIT-tag wand surveys do not detect sufficient holding individuals, consider using radio tags and telemetry to pinpoint holding fish.
- Investigate the use of tidally influenced and brackish portions of lower Freshwater Creek (below the HFAC weir) during pre-spawning migration, holding, and post-spawn periods.
- Describe range of salinity levels tolerated by adult lampreys of different sexual maturity levels in lower Freshwater Creek.
- Use acoustic tags or radio tags to further investigate movement of post-spawn Pacific lampreys into the Freshwater Creek estuary, Humboldt Bay, and possibly the ocean.
- Install a pressure transducer to measure stream stage and help understand the effects of both annual and shorter-term variations in stream flows on lamprey migration timing, movement behaviors, and distribution within the watershed. This gage would also contribute to understanding stream flow effects on salmonid biology in this intensively monitored watershed. If gage installation is not possible, use data from the nearby gaging station on Little River (USGS gage 11481200) as a proxy to for analyses of stream flow effects.
- Collect stream temperature data to improve understanding of the impacts of water temperature on timing of migration and spawning.
- Perform spawning surveys more frequently, ideally on a bi-weekly basis to minimize missed redds (especially during wet years) and provide a better understanding on spawning timing.
- Further investigate the lack of live adults and carcasses observed during spawning surveys. Describe level of predation during the pre-spawning holding and spawning periods and its impact on the adult population.

Additionally, analogous to the intensively monitored Life Cycle Monitoring Station approach being applied to studying salmonids in the Freshwater Creek watershed, we recommend expanding Pacific lamprey studies to include other parts of the life cycle (e.g., embryonic development, ammocoete rearing, and macrophthalmia outmigration). Intensive life-cycle monitoring of Pacific lamprey in Freshwater Creek will greatly improve our understanding of the overall life history, population dynamics, and factors impacting survival in the watershed and the Humboldt Bay region. Specifically, we recommend:

- Conducting species-specific ammocoete distribution surveys throughout Freshwater Creek and its tributaries to document both presence and upper distribution of Pacific lampreys and *Lampetra* species. These surveys should be done using lamprey-specific electrofishing protocols (e.g., Reid and Goodman 2015, Stillwater Sciences and WNRD 2016).

- Alternatively (or in tandem with electrofishing surveys), presence/absence of lamprey species could be rapidly assessed by testing for environmental DNA (eDNA) in water samples collected from each tributary. This technology shows promise for improving detection of rare freshwater species (Thomsen et al. 2012, Taberlet et al. 2012, Rees et al. 2014) and has been used successfully to monitor various fishes (e.g., Jerde et al. 2011, Thomsen et al. 2012), including lampreys (Docker et al. 2014, Gustavson et al. 2015).
- Document the extent to which Pacific lamprey ammocoetes and macrophthalmia use of tidally influenced areas and sloughs in the estuaries of Humboldt Bay tributaries (including the Wood Creek slough and pond).
- Describing the range of salinities tolerated. It would also be valuable to compare length-frequency and relative abundance of ammocoetes collected in tidally influenced areas with upstream reaches to improve understanding of the roles these areas play in population dynamics.

Finally, additional recommendations aimed at restoration and conservation in Freshwater Creek include:

- Assess potential barriers to adult migration and remediate problem sites. The process developed to assess and prioritize barriers in the Eel River basin (Stillwater Sciences 2014) as well as information gained by other recent lamprey passage studies can be used to guide evaluation and remediation of passage barriers in Humboldt Bay tributaries.
- Continue to educate biologists working in the Humboldt Bay watershed on lamprey identification and methods for consistent collection of biological data (e.g., measurement of IDL). Encourage them to include lamprey data and observations in published reports or share data with the Wiyot Tribe or the USFWS Pacific Lamprey Conservation Initiative.
- Expanded coordination with biologists from local timber companies, such as Green Diamond Resource Company, to summarize lamprey data collected through their ongoing fish and habitat monitoring in the Humboldt Bay watershed.
- Encourage local biologists and restoration practitioners to consider lamprey-specific habitat requirements when designing and implementing restoration projects.

In general, to addresses remaining data gaps and work toward restoration and conservation of this important species, we recommend continued coordination between the WNRD, CDFW, SPF, and other stakeholders in the Freshwater Creek watershed and Northern California in general. In all cases, it is important to work cooperatively and share information amongst other researchers, Tribes, and agencies to encourage collaboration and avoid duplication of effort. Where possible, we recommend working within the structure of the ongoing USFWS Pacific Lamprey Conservation Initiative (Luzier et al. 2011, Goodman and Reid 2012) and its regional implementation plan for the northern California coast (Goodman and Reid 2015).

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