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Behavioral Response of Pacific Lamprey (Entosphenus tridentatus) to Predator Odors

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TO PREDATOR ODORS

A Thesis

Presented to

The Graduate Faculty

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In Partial Fulfillment

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Master of Science

Resource Management

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by

Laurie Lynn Porter

August 2015

Graduate Studies

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BEHAVIORAL RESPONSE OF PACIFIC LAMPREY (*Entosphenus tridentatus*) TO PREDATOR ODORS

by

Laurie Lynn Porter

August 2015

Pacific lamprey (*Entosphenus tridentatus*), a species facing serious threats to their existence, experience a number of challenges in reaching their desired spawning grounds during the adult migratory phase, and predators are suspected to be one of these challenges. Understanding if Pacific lamprey respond to predator odorants may provide a management tool for use in conjunction with attractants in guiding lamprey to suitable spawning habitat and deterring them from poor habitat. Previous research has failed to explore Pacific lamprey response to predator odorants, although much research exists on attractant odorants. In our study, we tested Pacific lamprey response to 4 predator odorants: white sturgeon (*Acipenser transmontanus*), human saliva, decayed lamprey, and river otter (*Lontra canadensis*). We conducted a 2 choice maze test and measured the number of entries (count) and duration of time spent in the test arm during a control trial and odorant trial. Results showed a significant (*t*-test; $P < 0.01$) response to the river otter odorant, in both count and duration; however, fish spent more time and made more entries into the test arm with the treatment than with the control. This could be evidence of predator inspection and/or ‘hiding’ (remaining still). No significant difference (*t*-test;

using the decayed lamprey odorant (t-test; $P = 0.47$ for counts and $P = 0.16$ for duration) were indicative of a repellent response for duration. Results from this study indicate that Pacific lamprey respond to some predator odorants and suggest that future testing may be valuable.

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INTRODUCTION

SPECIES DESCRIPTION

Lamprey (Petromyzontidae) are an ancient primitive fish, which have been in existence approximately 450 million years. Often referred to as "eels" due to their elongated body and resemblance to true eels, they are of a separate class from eels (eels are jawed, bony fish in the class Actinopterygii; Nelson 2004). Lamprey are classified as Agnatha or jawless fish (class Hyperoartia), which includes hagfish. Lamprey and hagfish are the only 2 extant taxa in this class (Shimeld and Donoghue 2012). Pacific lamprey (*Entosphenus tridentatus*) are an anadromous parasitic fish, they lack jaws, have a cartilaginous skeleton, are scaleless, lack paired fins, have a single nasal opening, and seven external gill slits. The circular sucker like mouth (buccal funnel) is surrounded by dentition and functions both for feeding and helps with attaching to surfaces during locomotion as well as nest building. The tongue has small abrasive laminae or horny teeth, which are used in the parasitic stage to rasp through scales and skin in order to feed off the host. Fossils of lamprey have been found in rocks in South Africa that date back to the Devonian period in the Paleozoic era (Gess et al. 2006).

In the Pacific Northwest, the Pacific lamprey are one of the oldest fish in the Columbia River Basin and its tributaries, where they have evolved side by side with other fishes of the region-preceding most (Kostow 2002). As such, they are important to the overall ecosystem, cleaning the water through filter feeding while they are in the larval stage, providing marine nutrients to freshwater environments when spawning, dying, and prey on other fishes such as salmon, providing a buffer to commercially valuable fish (Close et al. 2002; CRITFC 2011). Studies have shown lamprey to be a preferred prey for seals, sea lions, other marine mammals, and piscivorous birds (Orlov et al. 2009). Lamprey larva may also be a food source for salmon fry in their freshwater environments (Close et al. 1995). As lamprey decline, these predators must choose other fish, including salmon, as prey thus impacting these other species.

The anadromous Pacific lamprey have a complex and interesting life cycle. Adult lamprey return to freshwater to spawn after spending approximately three years in the ocean environment, although exact length of stay varies considerably. When adults begin their upstream migration (February through June) they do not feed and can lose a substantial amount of their body weight by the time spawning occurs (Beamish 1980). Lamprey movement occurs primarily at night, possibly as a predator avoidance tactic. It is believed that they do not return to their natal stream but they are drawn to suitable environments by homing in to pheromones released by larval (ammocoetes) conspecifics (Yun et al. 2011). Once they reach their chosen streams, they will remain for a season before spawning the following spring (May through June). They may migrate 100s of kilometers upstream to suitable spawning habitat during these migrations. Males and females participate in building nests (redds), and choose gravel substrates similar to salmonids where the female lays upwards of 10,000 to 100,000 eggs (Kan 1975) and is highly correlated to water temperatures between 10 degrees to 15 degrees Celsius (Clemens et al. 2009; Mayfield et al. 2014). Adults die after spawning, and the eggs will hatch in approximately 19 days. The newly hatched juveniles then float downstream to larval (ammocoete) stage. They then begin to undergo a metamorphosis to prepare them for the saltwater environment and for the parasitic lifestyle. This metamorphic stage is the juvenile (macropthalmia) stage and they will continue their transformations as they make their way downstream (Beamish 1980). During this stage they will develop eyes, teeth, full olfactory functioning, and other changes to allow them to survive in salt water. They will begin this transformation in the fall and most outmigration has commenced by the end of winter when the cycle begins again as they spend several years in the ocean environment.

MANAGEMENT CONCERNS

Many species of lamprey are receiving scientific attention due to the recent significant declines in numbers throughout their historic ranges (Close et al. 1995; Close et al. 2009; Schultz et al. 2014). The historic range of the Pacific lamprey stretches from as far south as Baja Mexico, along the North Pacific Rim, over Alaska and to the Hokkaido Island of Japan. It includes the Coastal Pacific regions of Alaska, Canada, Washington, Oregon, and California, and major river basins including the Columbia River, Willamette River, and Snake River (Idaho) (Hardisty and Potter 1971; Beamish 1980; Kostow 2002).

The plight of Pacific lamprey got the attention of the Oregon Department of Fish and Wildlife [ODFW] in 1993 due in part to tribal fish managers and other groups who had noted declines in lamprey populations for years and had become increasingly concerned about the loss of this important natural and cultural resource (Close et al.

species in 1993 and further protected in 1996 through harvest restrictions (ODFW, Oregon Native Fish Status Report Vol. II). They have also been listed as "at risk" by the USFWS in 2003 after reviewing a petition by 11 environmental groups to list them (and three other lamprey species) as endangered in the states of Oregon, Washington, and California. The USFWS concluded they did not have sufficient information on the species to list it as endangered (USFWS 2004). Therefore it is imperative to expand scientific research into the species in order to provide accurate assessments of past and present populations, their habitat needs (both freshwater and ocean), and issues that may be contributing to the decline and/or ways to improve the restoration efforts of native species into their traditional ranges and historical numbers.

Pacific lamprey have experienced unprecedented declines in their populations in the past 50 years and have been extirpated from the upper reaches of many rivers and tributaries in Washington, Oregon, California, and Idaho, including the Umatilla River (Close et al. 1995; Wang and Schaller 2015; Lampman 2011). Counts at Columbia River dams and tributaries have dropped from historical highs in the 1,000,000s at the lower mainstem dams (Kostow 2002) to lows in the 100s at Lower Granite Dam on the Snake River in recent years (Ward et al. 2012). Historical tribal accounts reveal that during migrations Celilo Falls was 'black with eels' prior to the The Dalles Dam construction (CRITFC 2011). Lamprey were commercially harvested in the Willamette River beginning in the early 1900s and harvests reached 816 tons which was described as only 1/10th or 1/20th of the total number of fish present (CRITFC 2011). Tribal harvests of lamprey (*ksuyas*) have been going on since time immemorial, and historically they were a replace other highly valued food sources such as salmon (Close et al 2002). These fish are an important cultural and nutritional resource to Native Americans of the Pacific Northwest. Lamprey hold a place of high cultural and natural significance to many Pacific Northwest tribes and are used for ceremonial, medicinal, spiritual, and subsistence purposes (Larson and Belchick 1998; Close et al. 1995; Close et al. 2004). The Columbia River tribes gathered together and developed the ‘Tribal Pacific Lamprey Restoration Plan’ to provide guidance for the conservation, restoration, and reintroduction efforts and to highlight where there is need for more research.

In the 1990s the Confederated Bands and Tribes of the Umatilla Indian Reservation (CTUIR) began a formal lamprey restoration program focusing on returning lamprey to their traditional habitats and at historical numbers (Close et al. 1995; Close et al. 2004). During the springtime tribal fisherman historically have fished for adult lamprey at falls where they are harvested from rocks using nets, by hand, or long poles to capture them as they attempt to migrate past the falls (Close et al. 2002; CRITFC 2011). A limited tribal harvest continues on the Willamette River at Willamette falls (Kostow 2002). Tribal fisherman and biologists were some of the first persons to note the decline in lamprey and express concern to state and federal fishery biologists regarding the plight of the lamprey (Close et al. 1995; Yun et al. 2011) and to take action to protect them.

LITERATURE REVIEW

Pacific lamprey are an anadromous fish, and similar to salmonids they return from the ocean environment to freshwater beginning in the spring time for their spawning appears they do not home to their natal streams and what guides their migrations is still relatively unknown (Hatch and Whiteaker 2009). What is well known is that migration is highly attuned to water temperatures and water flow (Hardisty and Potter 1971; Binder and McDonald 2010).

Pacific lamprey undergo extensive migrations during juvenile and adult phases and encounter numerous natural and man-made obstacles, which threaten their survival (Beamish 1980; Robinson and Bayer 2005). Navigational hazards, dewatering, water pollution, intentional poisonings, culverts, and stream channelization have all been identified as contributing factors to their decline (Beamish and Northcote 1989; Close et al. 1995; Ward et al. 2012). Lamprey experience high failure rates navigating the dams on the mainstem Columbia River and traditional counting methods may underestimate this trend (Moser and Close 2003). During the juvenile (macropthalmia) phase, fish drift downstream as they undergo transformations that prepare them for the saltwater environment and may be especially susceptible to changes in water velocity and water quantity at this time (Close et al. 1995; Torgersen and Close 2004). The ocean phase (ectoparasitic) lasts several years and lamprey are known to travel over 62 miles offshore in depths of up to 2600 feet parasitizing a variety of fish during this time (Beamish 1980). Sea lamprey (*Petromyzon marinus*) are guided to freshwater spawning habitats by pheromones released from larval conspecifics and it is likely the same holds true for Pacific lamprey (Li et al. 1995; Johnson et al. 2009). Upon returning to freshwater, adult migratory phase lamprey are known to migrate 100s of km inland to suitable spawning habitat if there are no barriers to that migration (Torgersen and Close 2004). In the before they pause to overwinter prior to spawning the following spring (Robinson and Bayer 2005). During this migration lamprey face even more challenges as they encounter dams on their upstream migration. Pacific lamprey migration is unique in that they overwinter in the freshwater environment prior to spawning the following spring. During this time they cease eating and lose approximately 20% of their body weight. Female Pacific lamprey may also be guided by a pheromone released by spermiating males similar to sea lamprey.

Dams have been identified as a significant contributor to lamprey decline, as they are barriers to lamprey reaching traditional spawning grounds and fish passage structures designed for salmonids are not suitable for the swimming capabilities of lamprey (Moser and Close 2003; Keefer 2009; Jackson and Moser 2012). While many studies have looked at the passage problem through a structural lens (Jackson and Moser 2012; Keefer et al. 2014) less is known about natural impediments to passage, such as the presence of predators.

Pacific lamprey are preyed upon by a number of species of fish, marine mammals, pinnipeds, piscivorous birds, and humans in both their freshwater and saltwater life stages (Close 1995; Orlov 2009). The ability to recognize predators in the aquatic environment and identify and respond to alarm cues from injured conspecifics is critical to fish fitness (Korpi and Wisenden 2001; Mathuru et al. 2012) and is an important evolutionary adaptive trait. It is suspected that predators may impact lamprey during the upstream migrations through the dams by delaying their progress and/or predating on them while they paused to seek a suitable passage route. Sea lions and seals also congregate at the

2002).

Fish use a wide variety of sensory adaptations to monitor their environment. One survival mechanism for many fish may be the ability to detect predators through olfactory cues and use this information to avoid risk. Lamprey face predation at every stage in the life cycle and from both native fish and introduced exotic species such as catfish and smallmouth bass (Close et al. 1995). They are predated upon by juvenile rainbow trout as eggs and larvae, catfish as juveniles, terns and gulls as ammocoetes, and seals, sea lions, whales and herons as adults (Close et al. 1995). Lamprey parasitize a variety of freshwater and saltwater fish during the adult parasitic phase including halibut, pollock, flounder, herring, and cod (Orlov 2009).

Lamprey are known to use chemosensory cues such as natural pheromones and alarm cues for communication and several studies indicate that lamprey may show a strong behavioral response to odorants (Li et al. 1995; Wagner et al. 2011; Imre et al. 2014). In electro-olfactogram and laboratory studies it was found that sea lamprey (*Petromyzon marinus*) were attracted to bile acids released by larvae and these bile acids -allocholic acid (ACA) and petromyzonol sulfate (PS) may guide migrating adults to suitable spawning habitat (Li et al. 1995). Ovulating females were lured into traps baited with spermiating males in a natural spawning stream and the experiment was duplicated using a synthesized component of the male hormone 3kPZS with similar results (Johnson et al. 2005; Johnson et al. 2009). These experiments were conducted to investigate alternatives to using lampricides in controlling the invasive sea lamprey in the Laurentian Great Lakes. Studies on sea lamprey and Pacific lamprey behavior have been conducted been studying the use of repellents to manipulate the behavior of the invasive sea lamprey as a management tool in controlling their populations with some success (Wagner et al. 2011; Di Rocco et al. 2014; Imre et al. 2014). Imre et al. (2014) tested a variety of predator odorants in a laboratory semi natural stream channel in which they released stimuli in either the right or left side of the channel and observed fish response to the stimuli. They found that adult sea lamprey had a strong avoidance response towards both predator odorants and towards damage released alarm cues from conspecifics and sympatric heterospecifics. This supports the idea that lamprey use olfaction not only in reproduction but also to avoid predation. Less is known about Pacific lamprey response to predator odorants as no similar studies have been conducted.

In our study, we examined the response of adult migrating phase Pacific lamprey (*Entosphenus tridentatus*) to a suite of repellent odorants; human saliva-- (mammalian predator cue) a repellent to sea lamprey and anecdotally found to repel Pacific lamprey, river otter-- predator of lamprey, white sturgeon-- predator of lamprey, and decayed adult Pacific lamprey-- a conspecific alarm cue. Fish behavior was tested using a two choice maze test to compare the amount of time spent and total number of entrances in a control arm and in the arm with odorant. Information gained from this experiment will fill a data gap in understanding Pacific lamprey behavior. The goal to our study was to determine if repellents altered lamprey behavior and to understand whether repellents could provide a tool that will enhance current management efforts at increasing lamprey numbers and access to historical spawning areas during the migratory phase.

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to Predator Odors.

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Pacific lamprey (*Entosphenus tridentatus*), a species facing serious threats to their existence, experience a number of challenges in reaching their desired spawning grounds during the adult migratory phase, and predators are suspected to be one of these challenges. Understanding if Pacific lamprey respond to predator odorants may provide a management tool for use in conjunction with attractants in guiding lamprey to suitable spawning habitat and deterring them from poor habitat. Previous research has failed to explore Pacific lamprey response to predator odorants, although much research exists on attractant odorants. In our study, we tested Pacific lamprey response to 4 predator odorants: white sturgeon (*Acipenser transmontanus*), human saliva, dead lamprey, and river otter (*Lontra canadensis*). We conducted a 2 choice maze test and measured the number of entries (count) and duration of time spent in the test arm during a control trial and odorant trial. Results showed a significant ($P < 0.01$; t-test) response to the river otter odorant, in both count and duration; however, fish spent more time and made more entries into the test arm with the treatment than with the control. This could be evidence of predator inspection and/or ‘hiding’ (remaining still). No significant difference ($P > 0.05$; t-test) was found in the response of lamprey to the other three odors. However, tests using the decayed lamprey odorant ($P = 0.47$ for entries and $P = 0.14$ for duration; t-test) were indicative of a repellent response for duration. Results from this study indicate that Pacific lamprey respond to some predator odorants and suggest that future testing may be valuable.

During the past 50 years, Pacific lamprey (*Entosphenus tridentatus*) have experienced unprecedented population declines and have been extirpated from the upper reaches of many rivers and tributaries in Washington, Oregon, California, and Idaho, including the Umatilla River (Close et al. 1995; Lampman 2011). In recent years, counts of individuals at Columbia River dams and tributaries have dropped from historical highs of 1,000,000s at the lower mainstem dams (CRITFC 2011) to lows in the 100s at Lower Granite Dam on the Snake River (Ward et al. 2012).

Multiple factors have been suggested to have contributed to the decline of lamprey including navigational hazards, dewatering, water pollution, intentional poisonings, culverts, and stream channelization (Beamish and Northcote 1989; Close et al. 1995; Clemens et al. 2012). Historical tribal accounts describe that during lamprey migrations, Celilo Falls was “black with eels” (CRITFC 2011). Despite their decline in abundance, lamprey continue to hold a place of high cultural and natural significance among many Pacific Northwest tribes and are used for ceremonial, medicinal, spiritual, and subsistence purposes (Close et al. 1995; Larson and Belchick 1998; Close et al. 2004). Further, a limited harvest of adult lampreys still occurs in some locations during upriver migration.

Pacific lamprey are anadromous and undergo extensive migrations during juvenile and adult phases (Beamish 1980; Robinson and Bayer 2005). Upon returning to freshwater, adult fish are known to migrate 100s of km inland to suitable spawning their natal streams, the factors that guide their migration is still relatively unknown (Hatch and Whiteaker 2009). Studies suggest migration of lamprey are highly attuned to water temperatures and water flow (Hardisty and Potter 1971; Binder et al. 2010). However, other factors such as olfactory cues are thought to play a role in adult navigation (Johnson et al. 2009; Clemens et al. 2012). For example, sea lamprey (*Petromyzon marinus*) are guided to freshwater spawning habitats by pheromones released from larval conspecifics and further guided from sex pheromones released by spermiating males (Li et al. 1995; Johnson et al. 2005) and it is likely the same holds true for Pacific lamprey (Yun et al. 2011). Olfactory cues may also function in predator recognition and may be especially important during migrations, which exposes them to increased risk of predation (Close et al. 1995; Kirk et al. 2013). While many studies have investigated fish passage problems and anthropogenic structural barriers and solutions, less is known about natural impediments to passage such as the presence of predators (Jackson and Moser 2012; Keefer et al. 2014).

Pacific lamprey are preyed upon by a number of species of fish, marine mammals, piscivorous birds, and humans in their freshwater and saltwater life stages (Close 1995; Brown et al. 2002; Orlov 2009). The presence of predators at dams may impact lamprey during upstream migrations by delaying their passage (Close et al. 1995; Close et al. 2002). Similarly, slowed passage of salmonids has been linked to failed passage (Caudill et al. 2007). Thus, adaptive traits such as the ability to respond to alarm cues (“Schreckstoff”response; Mathuru et al. 2012) from injured conspecifics or the ability to

2010; Korpi and Wisenden 2001).

Fish like many animals use a wide variety of sensory adaptations to monitor their environment and to improve their survival (Li et al. 2002; Munoz and Blumstein 2012). Several studies indicate that lamprey may show a strong behavioral response to odorants and some predator odors appear to repel lamprey (Li et al. 1995; Wagner et al. 2011; Imre et al. 2014). Imre et al. (2014) tested a variety of predator odorants in a semi-natural stream channel and observed that sea lamprey had a strong avoidance response towards both predator odorants and towards damage released alarm cues from conspecifics and sympatric heterospecifics. Strong responses to repellent odors could be used to influence the behavior of migrating fish. For example, manipulation of invasive sea lamprey in the Laurentian Great Lakes using odors has met with some success (Wagner et al. 2011; Di Rocco et al. 2014; Imre et al. 2014). Similar strong responses by Pacific lamprey could be an important management tool; however, to our knowledge no studies have been conducted on the response of Pacific lamprey to repellent odors.

In this study, we examined the response of adult, migrating phase Pacific lamprey to a suite of possible repellent odorants: white sturgeon (*Acipenser transmontanus*), human saliva, river otter (*Lontra canadensis*), and decayed adult Pacific lamprey. White sturgeon and river otter represented possible predators, decayed adult lamprey were possible conspecific alarm cues and human saliva represents a mammalian predator cue. Fish behavior was tested using a two choice maze to compare the duration of time and the control odor. This research was conducted to increase our understanding of Pacific lamprey behavior to repellent odors. The results of this study could have direct implications in using repellent odors as management tools to affect positive behaviors and increase lamprey abundance and access to historical spawning areas.

Methods

Experimental Animals

Behavioral tests were conducted on adult, migratory phase Pacific lamprey at Minthorn Springs acclimation facility, Pendleton, Oregon (Figure 1). Lamprey were collected by biologists of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) at the John Day dam on the Columbia River during their spring migration to freshwater spawning grounds. The fish were collected in July from funnel traps installed at the south fishway of the dam using dip nets. They were placed into 300-gallon tanks supplied with oxygen at 5L/min and transported directly to holding tanks at Minthorn Springs. After acclimating for several days, the fish were anesthetized with a buffered solution of 50 mg/L of tricaine methane sulfonate (MS 222) and fitted with 23mm passive integrated transponder (PIT) tags using methods from Keefer et al. (2009). The tags were inserted just off the ventral midline and in line with the anterior insertion of the first dorsal fin (Keefer et al. 2009). The fish were also measured and weighed and allowed to recover before being returned to the holding tanks. Four additional individuals were obtained for experiments in September and October from the South Fork Walla Walla hatchery. The additional fish were a conglomerate of fish that had been migration from June through August of 2014. Individuals used for experiments ranged from 610 mm to 726 mm in length and weighed 340 g to 595 g, respectively.

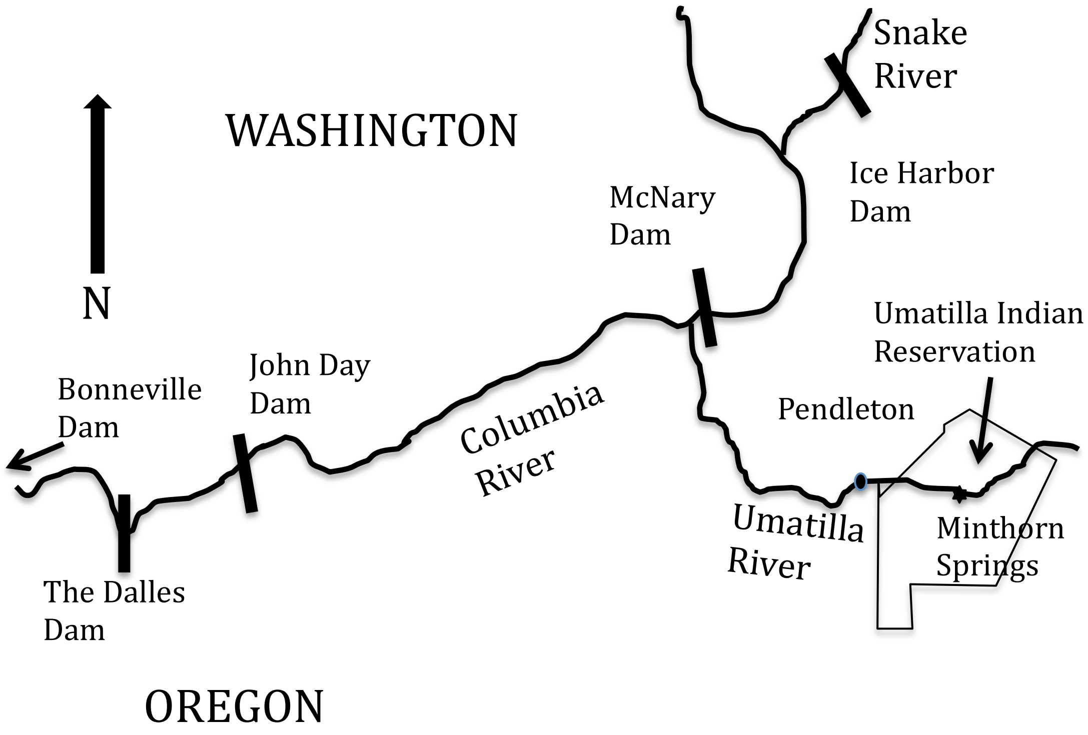

Figure 1. Location map showing Umatilla River, Minthorn Springs, and mainstem Columbia and Snake river dams.

freshwater migratory and spawning phases of their lifecycle adult lamprey do not eat and were not fed while maintained. Each fish was used only once per each odorant experiment and several fish were used in subsequent trials with different odorants. Due to mortalities, only five of the fish were used in all four odorant tests, the remaining fish were used in one or more tests.

Experimental Conditions

Trials were conducted in two 4.5 (L) x 1(W) x 0.6 (H) m fiberglass tanks. Each tank was divided in half lengthwise to make two arms of the two choice maze with a designated front and back section (Figure 2). Water was continuously pumped from Minthorn Springs into the upstream end of each arm at a rate of 6 L per min and water depth in the tank was 20 cm. Water temperature at the inflow and outflow of each tank was measured and recorded daily (Table 1). Each tank was covered with black plastic and white board to maintain a dark environment.

Odorant	Dates	Mean (range) of outflow (ºC)	Mean (range) of inflow (ºC)
Sturgeon	July 8, 2014-July 25, 2014	14.8 (12.0-18.0)	13.6 (11.5-17.5)
Saliva	July 28, 2014-August 22, 2014	15.2 (14.0-18.5)	14.1 (13.0-17.0)
Decayed lamprey	September 10, 2014-September 29, 2014	13.5 (12.0-15.0)	12.8 (11.0-15.0)
River otter	September 29, 2014-October 9, 2014	14.0 (12.0-16.0)	13.3 (11.5-15.0)

Antennas

Antennas designed to read PIT-tags were used to detect fish movement. Each tank was set up with four antennas that were placed underneath each of the arms- one at the entrance and one at the odorant end to detect the fish as they entered and exited the arms (Figure 2). These antennas were labeled A1, A2, A3, A4. Antennas were not placed under the reservoir portion of the maze; therefore, a fish was only detected as it entered one of the arms. A file that included all PIT-tag detections was downloaded at the completion of each experiment.

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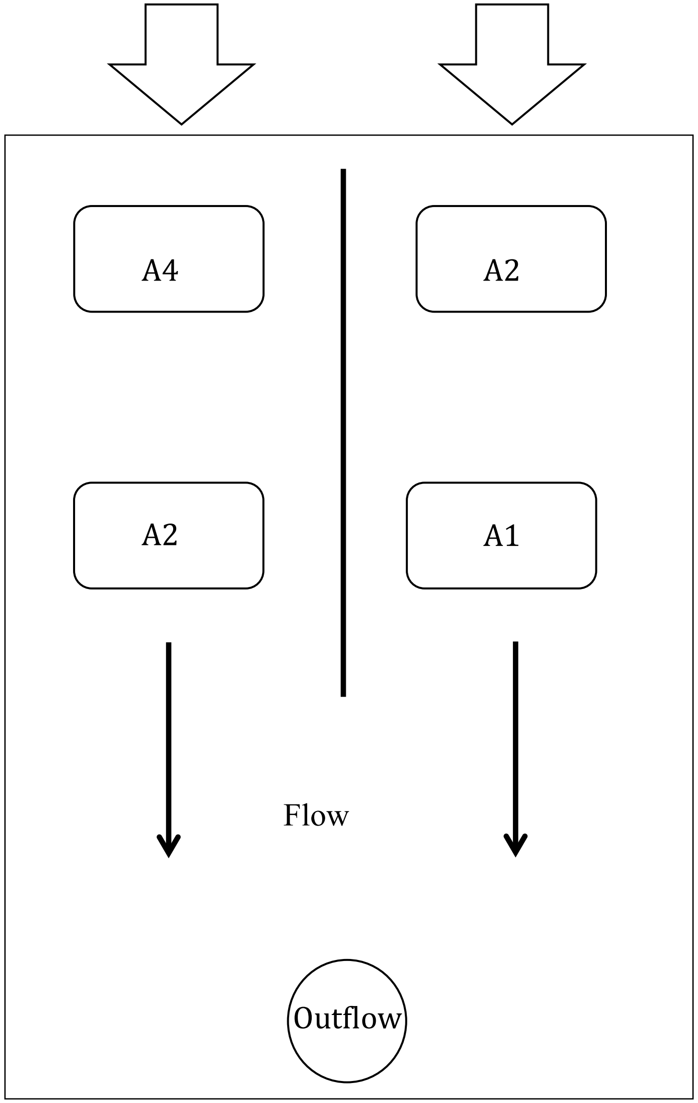


Figure 2. Diagram of the two-choice maze showing location of antennas A1, A2, A3, A4, odorant drip, and direction of flow.

Tests were conducted using four repellent odors: white sturgeon, human saliva (0.006% solution), decayed Pacific lamprey, and river otter. White sturgeon was selected for testing because they are known predators of lamprey and it is possible they may delay the travel times of lamprey during their freshwater migration up rivers and past dams as they congregate at the dams (Close et al. 1995; Orlov et al. 2009; Schultz et al. 2014). Water containing sturgeon odor was obtained at the Pacific Northwest National Laboratory lab from a 500-gallon tank holding approximately 100 juvenile sturgeon. Water flow at Bonneville dam averages 662.45 L/min and therefore we expected that the concentration of scent from the sturgeon odorant was well above the detectable level for lamprey in the mazes. The control water was taken from the same source (Columbia River) used to rear sturgeon. All samples were frozen and held until used.

Human saliva was selected because experiments showed it elicited an avoidance effect in behavioral studies of adult sea lamprey (Wagner et al. 2011; Imre et al. 2014). In addition, tribal knowledge and personal experience by one of the authors (A. Jackson) suggested that human saliva can have a repellent effect on Pacific lamprey. It is reported that saliva is used to assist in harvesting lamprey as fish will leave resting spots when saliva enters the water. We used a saliva concentration of 0.006% (6ml saliva to 6L of water) based on previous studies of concentrations that elicited a response. A graduated cylinder was filled to 94 ml with spring water and then 6 ml of saliva was added, this was then mixed in to 5.9 L of spring water. The saliva was collected no more than 24 hours prior to the testing. Saliva was collected from one of the authors (L. Porter) who did not eat or drink at least 1 hour prior to collecting the saliva. The control water was from immediately used.

Decayed Pacific lamprey were chosen as an odorant based on studies that showed that adult sea lamprey were repelled by the scent of decayed conspecifics (Wagner et al. 2011; Imre et al. 2014). We placed a previously frozen mortality into a 5-gallon bucket filled with 1 L of Minthorn Springs water and refrigerated it for 24 hours to create the decayed Pacific lamprey odorant . The water was then divided and poured into 10 glass jars filled to 100 ml and frozen. Samples were removed from the freezer 24 hours prior to each experiment and thawed. Once thawed, a 100 ml odorant sample was mixed with 4.9 L of Minthorn Springs water resulting in a 0.1% solution for testing.

River otter (*Lontra canadensis*) were chosen because they prey on juvenile lamprey and anecdotal evidence suggested they may prey on adult lamprey along with other fish during their upstream freshwater migration (Melquist and Hornocker 1983; Close et al. 1995). Fish make up a substantial proportion of river otter diets (Sample and Suter 1999). Odorant for the otter odor tests was obtained from the Woodland Park Zoo (Seattle, WA). Wearing waterproof gloves we filled 12 individual buckets each with 12 L of water from a large outdoor tank that provided habitat for two North American river otters. In addition, we filled 12 buckets each with 12 L of Seattle City water, the source water for the otter tanks to use for the controls. All odor samples were immediately transported to a freezer and stored until used in in experiments.

Each fish was tested over two consecutive nights. Fish were randomly chosen, captured using a dipnet, scanned for PIT-tag number, and then transferred to one of two randomly assigned mazes. The test fish were typically placed into the maze during the daytime, from 10 am to 2 pm and the antennas were turned on at this time; however, searching activity was not expected to occur until sunset as lamprey are not active during daytime hours (Lampman 2011; Di Rocco et al 2014). The first night served as a control test where no odorant was added and was conducted to measure any arm bias by individual fish. Odorant and control water were introduced during the second night between the hours of 1500 to 1800 hours for all fish except for two fish during the saliva experiment on one date when the odorant was not added until 2000 hours. Prior to the beginning of each experiment, we randomly determined which arm of the maze would receive the predator odorant. Thereafter, the arms receiving the odorant were alternated between experiments. The odorant was added using a peristaltic pump set to drip at a rate of 5 ml/min. Pumps were monitored after set-up for at least 10 minutes to ensure they were working properly and ran at least 12 hours subjecting the fish to a minimum of 12 hours of odorant. At the end of each experiment any remaining odorant was measured to ensure that each fish was exposed to the odorant for the full 12 hours. On completion of each experiment the fish were returned to the holding tanks. Each maze was filled and rinsed 3 times to ensure no residual odor remained before starting a new test. The spring temperature and outflow temperature of the tanks was measured upon arrival daily and condition of all fish was monitored.

Data was collected for the entire time a fish was in the two-choice maze. However, lamprey are active at night and tend to rest during daylight hours; therefore, to gain the most consistency of movement across fish and across trials we chose to analyze fish behavior only for the data collected between the hours of 1800 to 0600. The odorant drip was started before 1800 hours for the treatment night, and we measured enough odorant so that it would continue dripping until at least 0600 hours (the timeframe we chose for data analysis).

Using an R-script file, we processed the data to recognize and delete any anomalous records, thereby creating a "clean" data set. The clean data set was then analyzed for the number of entries (counts) and duration of time spent (duration) in each arm. We then compared the counts and duration between the test arm (arm receiving the predator odorant) and control arm during control trial and treatment trials. We used a conservative approach to counting entries and duration by using only the data from the A2 and A4 antennas closest to the drip end of the arms rather than at the entrance to the arm. We used the first night to test for arm bias and found no significant bias in any of the four odorant trials ($P > 0.05$; *t*-test). To determine odorant effects on fish behavior, we analyzed the data from the treatment night using a two tailed t-test of correlated samples (Whitlock and Schluter 2015) with a significance of $P < 0.05$.

We summarized the start and end times for fish activity by recording the first time the fish began movement in the evening hours after our designated 1800 hours start time and the last movement detected in the morning before 0600 hours as our designated end analysis, but in general, the majority of fish movement occurred during this time range. We used a chi-square test to analyze fish movement comparing the counts between the control trial and treatment trial. We used an ANOVA and Tukey multiple range test to compare the mean start times of activity between all four odorants for both the control night and treatment night for the five fish that completed all four trials.

Results

Duration of time and number of entries (count)

During the trial using the river otter odorant there was evidence for a significant effect on the duration of time that adult lamprey spent in each arm of the maze (*t*-test, $P < 0.05$; Figures 3). The average duration in the control arm was 2,493 seconds (687 SE) compared to the treatment arm (5,514 seconds; 1,084 SE). Trials for white sturgeon, and human saliva showed no evidence for significant differences in time spent in each arm when the predator odorant was added. In the decayed lamprey trial, the duration data suggested that adult lamprey were repelled (Figure 3); however, the *t*-test was not significant ($P > 0.05$).

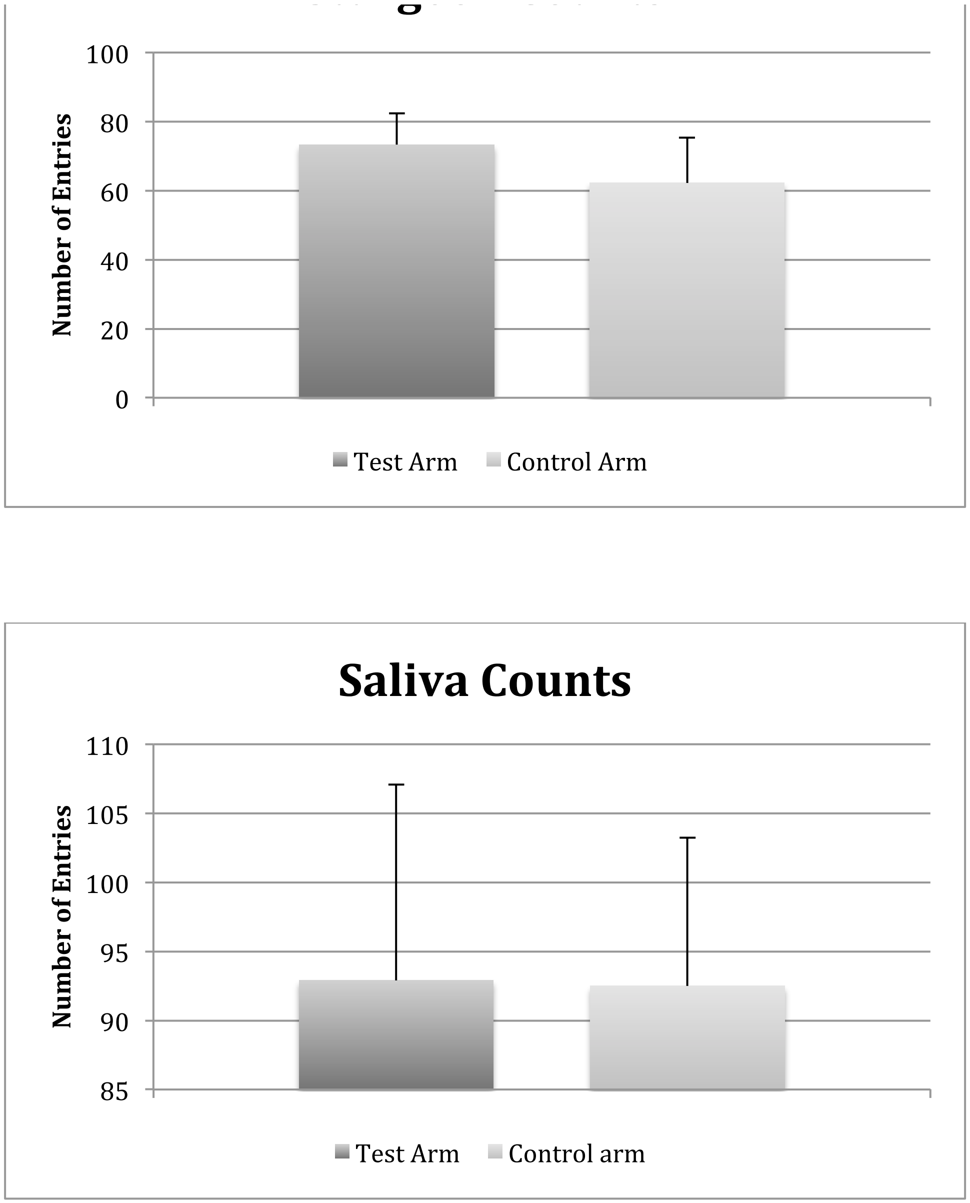
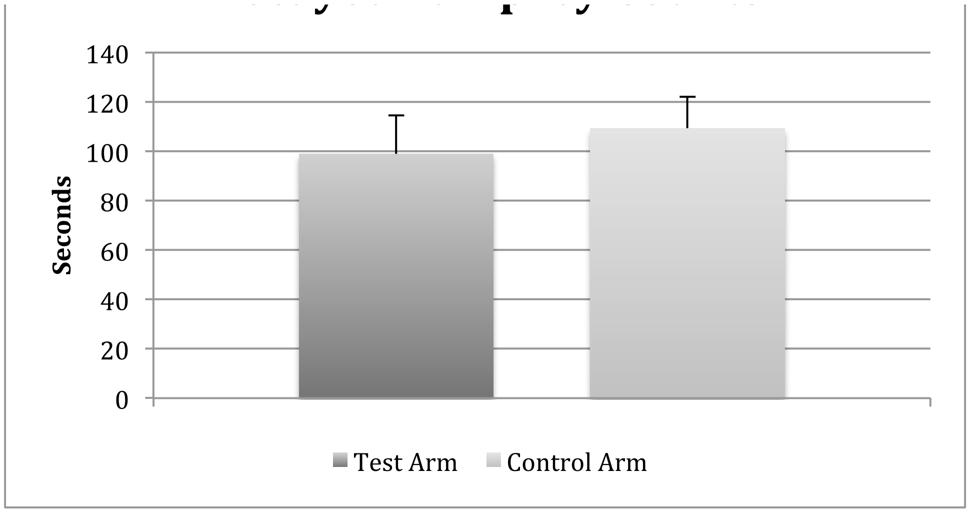


Figure 3: Mean counts during treatment trial; comparison of test arm versus control arm for all four odorants.



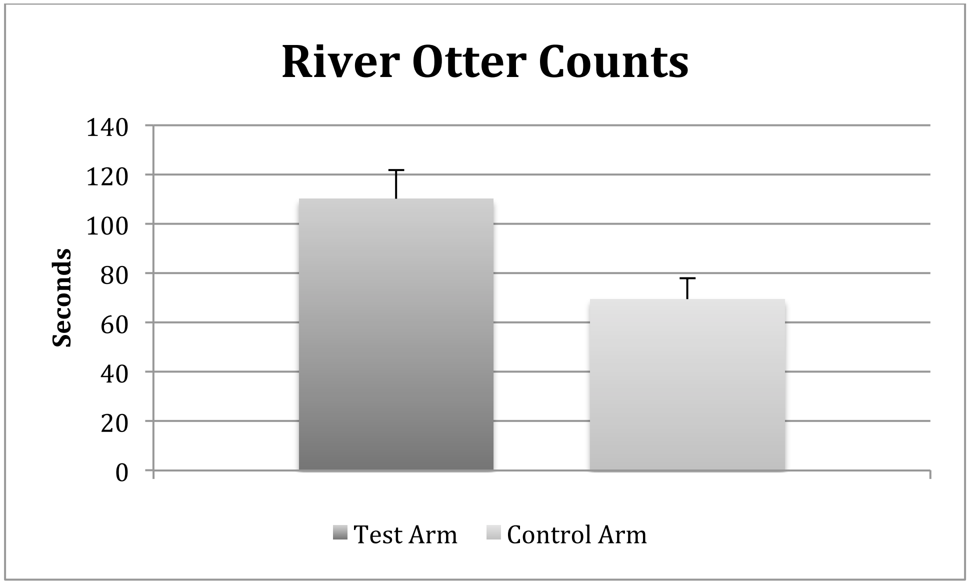


Figure 3: Mean counts during treatment trial; comparison of test arm versus control arm for all four odorants.

Results for the number of counts into each arm (control arm versus test arm) during the treatment trial (Figure 4) were similar to the results for duration. There was no evidence for significant differences in counts for white sturgeon, human saliva, and counts (8 SE) for the control arm compared to 110 counts (12 SE) for the test arm during the treatment trial (Figure 4).

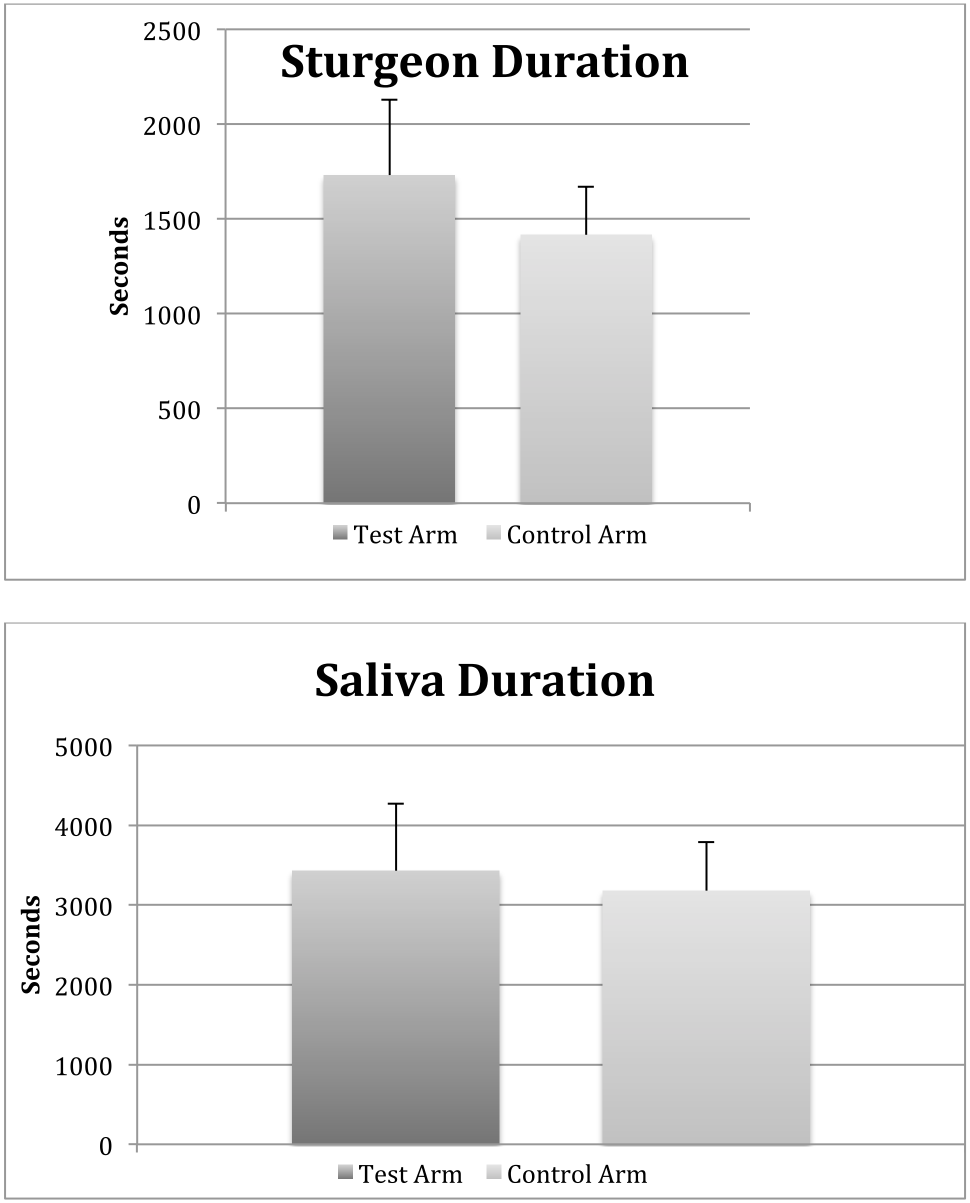


Figure 4: Mean duration during treatment trial; comparison of test arm versus control arm for all four odorants.

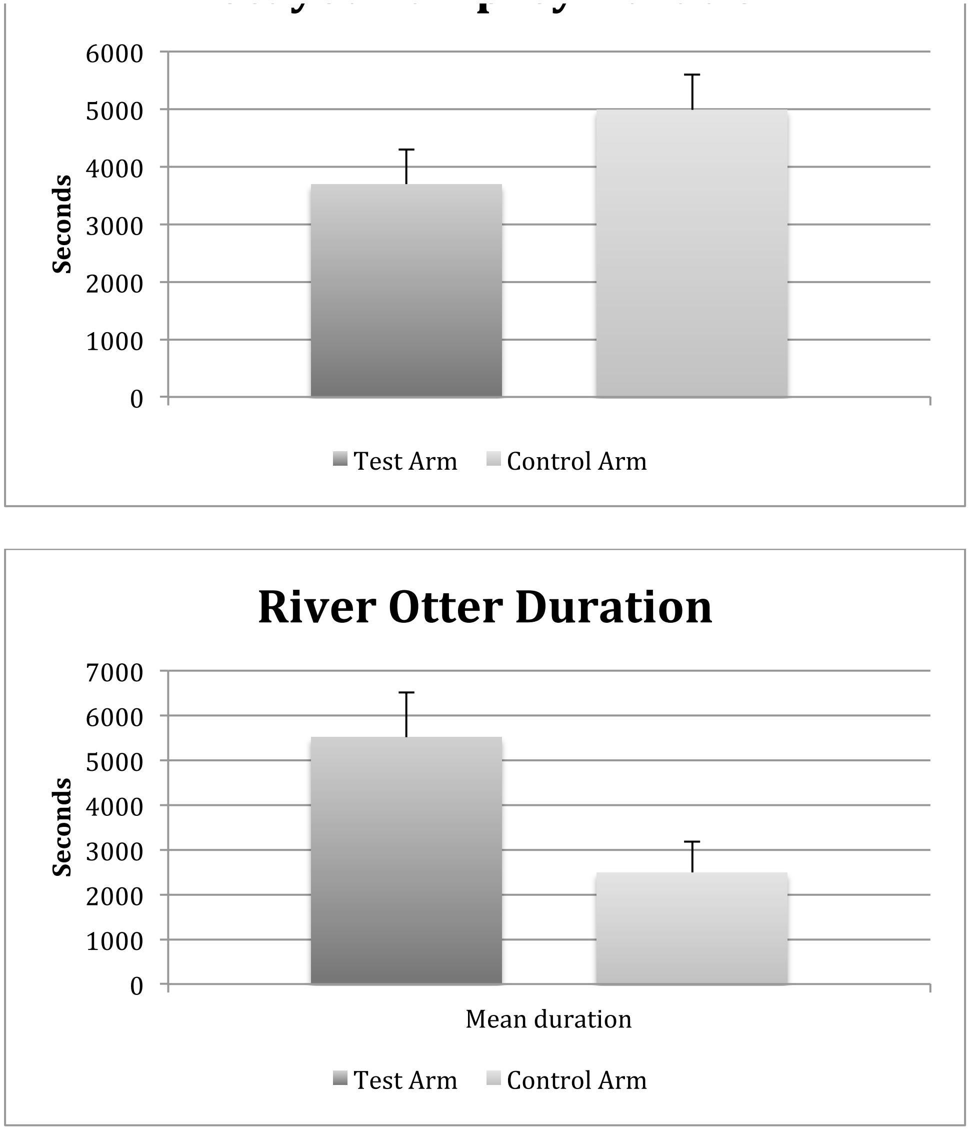


Figure 4: Mean duration during treatment trial; comparison of test arm versus control arm for all four odorants.

Fish activity

The lamprey tested in our experiments were consistently active at night. The majority of fish appeared to move little during daylight hours and typically started activity in the early evening (Figure 5). Based on the five fish used in all four odorant sturgeon odor experiment started significantly ($P < 0.05$) later (162 min after 1800 hours) than in the saliva experiment (91 min) or the decayed lamprey and river otter experiments (both 54 min). There was evidence for a significant difference in the count (numbers of entries) between tests conducted on the first night when no odorant was added and the second night when odorant was added (chi-square = 8.42, d.f. 3, $P < 0.03$) (Table 2).

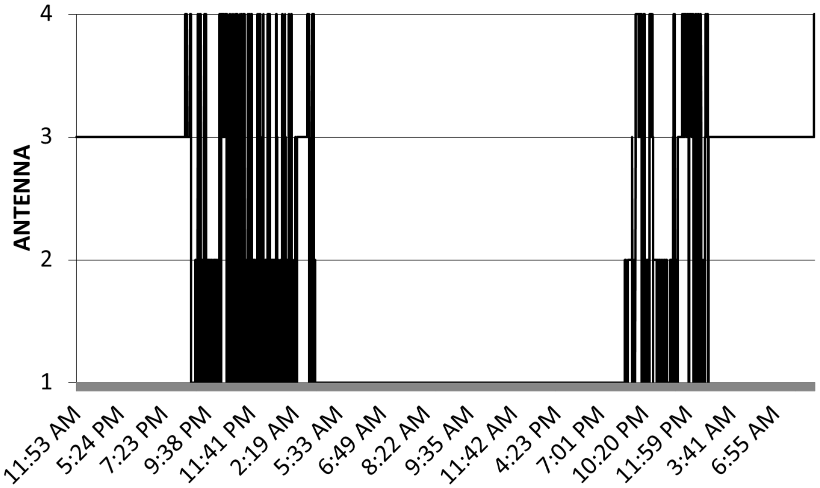


Figure 5. Bar graph showing an example of typical activity based on PIT-tag detections for one fish on each of four antennas used in a two-choice maze experiment. Bars that reach each line represent a detection at the associated antenna. Antenna numbers 1 and 3 represent the entrances to each arm of the two-choice maze while antenna numbers 2 and 4 were nearest the inflow and odorant drip in each arm of the maze.

(no odorant added) and the treatment trial (odorant added).

Odorant	Sturgeon	Saliva	Dead lamprey	River otter
Control trial	205	228	262	264
Treatment trial	112	185	208	180
Difference	93	43	54	84

The initiation of movement was likely related to calendar date and/or when sunset occurred since lamprey move primarily at night. Mean sunset time across all 4 odorant trials was; sturgeon 2041 hours, saliva 2002 hours, decayed lamprey 1926 hours, river otter 1833 hours. Analysis of sunset times across and among all 4 trials using a two tailed *t*-test of correlated samples revealed a significant difference ($P < 0.01$) in sunset across all trials

Individual fish activity

The movement of a few fish stopped earlier over several trials than the rest of the fish. During the otter treatment trial several fish stopped movement early and they stopped movement in the test arms as well as the control arm. During the saliva test, odorant was added later than normal (2039 hours) during the treatment test for two fish. These fish responded in an interesting manner. One fish moved out of the test arm 15 minutes after the odor was added and moved to the control arm where it remained from 2045 through 2234 hours. The second fish was in the test arm when odorant was added and remained there from 2032 through 2238 hours. Five of the fish completed all four combined (Figure 6).

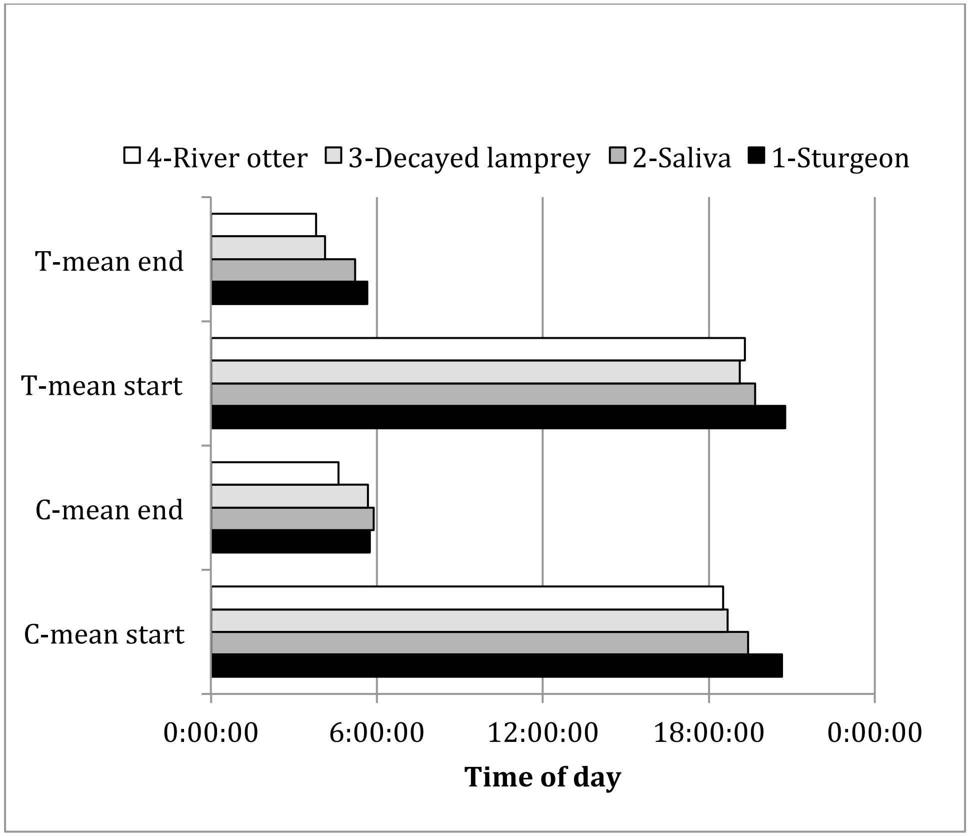


Figure 6. Mean start and end times of movement for five adult Pacific lamprey used in four repellent odorant experiments (C = control night, T = treatment night).

We tested four odors for repellent effects on the behavior of Pacific lamprey. Of the four odors tested, only one was significant-- river otter (terrestrial predator) and it showed both a significant difference in the duration of time and in the number of counts between the treatment and the control arms. However, it appeared to attract, not repel lamprey. Fish, like other species, respond by investigating, fleeing, or freezing/hiding when exposed to predator odorants and alarm cues from conspecifics (Dixson et al. 2010; Imre et al. 2014). Fish response may be dependent on what the fish is doing when exposed to the odorant (Di Rocco et al. 2014). Di Rocco (2014) tested the daytime response of sea lamprey to predator odors and found that they increased movement in response to odorant if they were swimming. However, if they were resting or hiding they made no response to any stimulus. Thus, it was possible that for our study, a fish that sat in the test arm even after odor was released may have been exhibiting anti-predator behavior by staying still as movement can alert a predator to its prey. Research on sharks has found that shark embryos will cease their gill movement in order to avoid detection when a predatory stimulus was presented (Kempster et al. 2013).

The response of adult lamprey to the other three odorants: decayed lamprey (conspecific alarm cue), sturgeon (aquatic predator) and human saliva (mammalian predator cue) did not show any significant effects We expected a repellent effect from the decayed lamprey odorant since similar studies have been conducted where sea lamprey avoided conspecific and heterospecific alarm cues by evading the laboratory stream channel where the odorant was released (Wagner et al. 2011; Imre et al. 2014). Although our test was not significant, the decayed lamprey odorant appeared to repel

We also expected a response to the sturgeon and human saliva odorants. Sturgeon are known to predate upon lamprey and they often congregate at dams (Kirk et al. 2013) and should have been recognized by adult lamprey. Lamprey are used as sturgeon bait by anglers. Based on traditional tribal knowledge, human saliva should have repelled the lamprey in our study. Imre et al. (2014) notes that sea lamprey likely recognize a component in human saliva that is present in other mammalian prey species, thus recognizing humans as predators. It may be that lamprey are responding to a combination of sensory inputs such as sight or sound as well as scent. Some studies of teleost fish found that the sight and scent of an injured conspecific combined with the scent of a novel predator produced a greater repellent response then scent of the novel predator alone (Ward and Figiel, Jr. 2013). We conclude that the response of adult Pacific lamprey to both White sturgeon and human saliva was neutral; however, it was possible that the concentrations of our extracts were not high enough to elicit a response or we may have seen a stronger response if we had combined the conspecific alarm cue with the saliva and sturgeon odorants (Lautala and Hirvonen 2008).

Pacific lamprey were consistently active during our nighttime tests. In examining the data from only 1800 to 2400 hours it was possible that we could have concluded the tests in a shorter period of time. Analysis of a subset of data showed similar results when comparing the shorter period of time to the full test period (data not presented). The cause for the reduction in the number of entries from the first test night to the second night (treatment trial) for all tests is unknown, but may have reduced our ability to determine treatment effects.

the sample size for statistical analysis. Due to the fact that lamprey populations are in a critical state, it was not possible to test larger numbers of fish as we did not want to risk harming fish in handling or transport since the numbers of lamprey in the Columbia River basin are low. Moreover, our fish were destined for use as broodstock in the Umatilla River artificial propagation program. We did not know the sex of our fish and some studies have revealed that female lamprey showed a stronger response to predator cues than males (Imre et al. 2014). With a larger sample size, we could have altered our tests to include testing multiple fish at one time to see if the behavior of the fish differed compared to a single fish. When lamprey are in their natural environment they are typically found in groups. The behavior we observed may not be typical of lamprey groups encountering predator odorants.

Possible design changes to the odorant experiment could include using video instead of or in combination with PIT-tag detections for monitoring movement. Some flaws with the PIT- tag method are that we could not see what the fish was doing while in the reservoir portion of the tank. We were also unable to see the suite of behaviors occurring underwater. Video is useful for eliciting more information about fish response, such as whether they change their swimming position either vertically or horizontally, change the speed of their swimming, or if they are exhibiting inspection behavior or just freezing (Mathuru 2012; Kirk et al. 2014). Further, video can provide us with more data for future studies of behavior besides only duration and counts.

including some that are still unknown to scientists. Further research into the biological and chemical components in natural streams may contribute to our understanding of chemical communication in lamprey. Additionally, chemical pollutants in water and changes in water chemistry including acidification can alter fishes ability to ‘smell’ or detect predators (Dixson et al. 2010). Altering the flow in the tanks may change lamprey behavior as well since lamprey swimming behavior is affected by flow and the ability to detect scents likely changes with water flow conditions. Future tests should include using a wider range of predator odors such as sea lion or seal odorant and increasing the concentration of the odorant.

Other factors that may have affected our study include the decreasing day lengths as the season progressed. Robinson and Bayer (2005) in their study of migratory behavior in the John Day River (Oregon) noted that upstream movement ceased by mid-September. In the Columbia River Basin, freshwater migrations of adult fish occur primarily from May through September and during nighttime hours (Moser and Close 2003). By mid-September their migration has slowed or ceased at which time the fish hold in place, to overwinter in freshwater for approximately six months. The spawning phase migration resumes again the following spring in March through May. Our trials using river otter odorant occurred in October. Therefore, it was possible that some of the behavior we observed (decrease in activity) was an innate biological response related to changes in day length and water temperature. It may also be interesting to conduct the same studies on spawning phase fish for comparison.

If restoration efforts are to work, lamprey must migrate to and from suitable habitats. Habitat restoration and modifications are important, as are developing ways to help outmigrationg juveniles. Behavioral solutions could be considered to keep the juveniles from becoming entrapped in irrigation screens or channels during dewatering as they drift downstream to enter the ocean. Lamprey develop their olfactory system during the metamorphosis from ammocoete to macropthalmia, therefore it is possible they could respond to specific odorants, however they are poor swimmers during this life stage (Smith 2012). For example, the recognition of predator odorants might be used to deter juvenile lamprey from entering side channels where they could be trapped during changes in water flow. Repellent chemicals or semiochemicals might also be used in tandem with pheromone attractants as a means of behavioral manipulation (push-pull method) and is a strategy that has been historically used in integrative pest management along with other stimuli (Picket 2014). When lamprey return for their upstream freshwater migration they must be able to move upstream at a reasonable rate, avoid areas at the dams that will entrap them, and navigate past predators that may be waiting for them as they attempt to pass the dams. Issues that slow lamprey at the dams are complex and may be a combination of predators, flow, and structural challenges (Kirk et al. 2014). Although the current study showed only limited effects from the repellent odors tested, future research into odorants and behavior by Pacific lamprey will be an important addition to other studies and could provide alternate techniques to manage lamprey and to improve the success of the reintroduction efforts.

We thank the CTUIR department of Natural Resources lamprey crew for their expertise and assistance with fish collection, holding facilities, and monitoring fish health. Their knowledge of Pacific lamprey was invaluable to the project. Thanks to Robert Mueller (Pacific Northwest National Laboratory) and the Woodland Park Zoo (Seattle) for providing odorants. We thank the USGS SISNAR (Student Interns in Support of Native American Relations) program for partial funding of the project. Additional funding sources included the Bonneville Power Administration, the U.S. Bureau of Reclamation, the National Oceanic and Atmospheric Administration, and the United States Geological Survey-Western Fisheries Research Center. We thank all the anonymous editors who reviewed and provided feedback to the content and structure of the article.

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