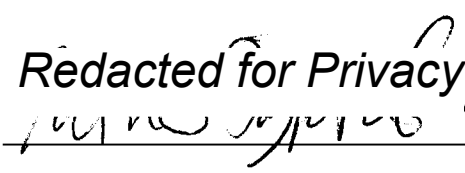
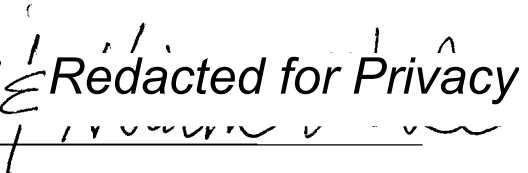


## AN ABSTRACT OF THE THESIS OF

David A. Close for the degree of Master of Science in Fisheries Science presented on January 19, 2001. Title: Effects of Acute Stress and Tagging on the Swimming Performance and Physiology of Pacific Lampreys (*Lampetra tridentata*).

Abstract approved:     
Martin S. Fitzpatrick and Hiram W. Li

Pacific lampreys (*Lampetra tridentata*) have declined in abundance in the Columbia River Basin. Although, the reasons for the decline are unclear, we suggest that development of hydroelectric dams and habitat alterations in tributaries as the main causes. The available knowledge of life history of Pacific lampreys and status from dam counts (trend data) in the Columbia River Basin and the Umpqua River along the Oregon Coast shows that populations have been declining over the last 30 years. Even though Pacific lampreys have been shown to have ecological importance both as predator and prey, the declines in their populations have been largely ignored by fisheries agencies and the public.

Recently, the National Marine Fisheries Service initiated studies on using radio-telemetry of Pacific lampreys in order to study the impact of hydroelectric dams on migration behavior. To address one of the fundamental assumptions of radio-telemetry, namely, that tagged fish are “normal,” one must be able to measure whether or not an

animal is stressed. We identified clinical indicators of stress in adult Pacific lampreys. Plasma glucose became elevated soon after acute stress and remained elevated for one week. Plasma lactate also became elevated by 30 minutes; however, it decreased to resting levels by one hour after stressor. Muscle lactate was shown to have an inverse relationship with glucose. Muscle lactate levels decreased by 4 hours and remained depressed for two days. Plasma chloride ions decreased by one hour, then returned to resting levels by 8 hours; by 24 hours, levels were again decreased with recovery occurring by 48 hours. The steroid cortisol was not found in the plasma of Pacific lampreys.

The swimming performance and physiological effects of surgical implantation of three different sized dummy radio transmitters in Pacific lampreys were assessed. Intraperitoneal implantations of 3.4 g transmitters had no significant effect on circulating levels of glucose (an indicator of stress) 4 months after surgery, while 10 gram transmitters showed a significant increase in plasma glucose. Lampreys implanted with 7.4 g transmitters recovered from surgery by day 4 based on levels of plasma glucose. Lampreys implanted intraperitoneally with 7.4 g dummy transmitters showed no significant differences in circulating glucose 30, 60, 90, and 180 days after surgery in comparison to sham-implant controls. Ventilation rate decreased significantly by 30 minutes after surgery and was stable by 60 minutes; suggesting initial recovery from surgery is rapid. Swimming performance was impaired immediately after surgery; however, swimming was not compromised at 1 and 7 days after surgery.

Tagged fish showed a significant difference in oxygen consumption when tested immediately after surgery; however, oxygen consumption was at control levels at 1 and 7 days after surgery.

Effects of Acute Stress and Tagging on the Swimming Performance and Physiology  
of Pacific Lampreys (*Lampetra tridentata*)

by

David A. Close

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submitted to  
Oregon State University

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the requirements for the  
degree of

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## CONTRIBUTION OF AUTHORS

Dr. Fitzpatrick, Dr. Li, and Dr. Schreck were involved in the design, analysis, and writing of each manuscript. Mr. Feist and Ms. Siddens assisted in the analysis of samples for steroids and participated in the discussion of the results in chapter 3. Mr. Lorion assisted in surgeries and collection of data in chapter 4.

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# Effects of Acute Stress and Tagging on the Swimming Performance and Physiology of Pacific Lampreys (*Lampetra tridentata*)

## Chapter 1

### Introduction

In the past three decades, tribal members from Columbia River Basin tribes and Oregon Coast tribes have suspected that declines have occurred in returns of adult Pacific lampreys (*Lampetra tridentata*) in Oregon and Washington and have voiced their concerns. However, these concerns have largely been ignored and declines in lamprey populations among interior subbasins and coastal streams has continued.

Tribes from the interior Columbia Basin and along the Oregon Coast regard lampreys as an important fish with cultural values. While the tribes have a positive cultural value for lampreys, fisheries agencies and the public seem to have a negative cultural value towards them. This negative attitude towards lampreys may have contributed to the lack of concern about Pacific lamprey status in the Pacific Northwest.

In order to counter this negative attitude, the following questions should be answered regarding Pacific lampreys: 1) What is their status? 2) What is their ecological value? and 3) What is their cultural value? These questions are addressed in Chapter 2, entitled “Status and Importance of Pacific lampreys (*Lampetra tridentata*) in the Columbia River Basin”.

Recently, the National Marine Fisheries Service began to study dam impacts on Pacific lampreys using radiotelemetry. However, we need to know whether the fundamental assumption of radiotelemetry is valid. The assumption is that radio-tagged fish behavior and physiology represent untagged fish. Understanding the role of stress in Pacific lampreys is an important step in evaluation of migration difficulties. Baseline data are necessary to understand or interpret impacts of surgical implants of radio transmitters in Pacific lampreys. Chapter 3, entitled “Effects of Acute Stress on the Physiology of Pacific Lampreys (*Lampetra tridentata*),” describes the physiological response in lampreys after being subjected to an acute stressor.

Chapter 4, entitled “ The Effects of Intraperitoneally Implanted Dummy Radio Transmitters on the Swimming Performance and Physiology of Pacific Lampreys (*Lampetra tridentata*),” uses the information derived in Chapter 3 to assess how radio-tagged fish respond physiologically and perform in swimming trials relative to control fish. This study is necessary to interpret radio-tagging studies currently conducted in the Columbia River with Pacific lampreys.

## Chapter 2

### Status and Importance of Pacific Lampreys (*Lampetra tridentata*) in the Columbia River Basin

by

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<sup>1</sup>To be submitted to Conservation Biology.

## Abstract

Pacific lampreys (*Lampetra tridentata*) have declined in the Columbia River Basin. Although, the reasons for the decline are unclear, we suggest that development of hydroelectric dams and habitat alterations in tributaries as the main causes. The available life history knowledge of Pacific lampreys and status from dam counts (trend data) in the Columbia River Basin and the Umpqua River along the Oregon coast shows that populations have been declining over the last 30 years. Pacific lampreys are shown to have ecological importance both as predator and prey. Native Americans continue to harvest these fish as a subsistence food, and are highly regarded for their cultural values. Even though Pacific lampreys have been shown to have ecological and cultural importance to Native Americans, fisheries agencies and the public have largely ignored the declines in their populations.



## **Introduction**

The Pacific salmon are not the only fish in trouble in the Columbia River Basin. Pacific lampreys (*Lampetra tridentata*) seem to be experiencing the same fate as other native anadromous fishes in the Columbia River. Many biologists knew about the decline; however, prevailing attitudes about the fish and the excuse of “we were too busy trying to save the salmon” have been the chorus among fisheries professionals in the Pacific Northwest. The view was much different among Native Americans throughout the Columbia River Basin. Tribal peoples of the Columbia River Basin and Oregon Coast have been worried about the declines since the early 1970s and have voiced these concerns which unfortunately were ignored. Finally in 1994, Bonneville Power Administration began funding studies through the Northwest Power Planning Council in the Columbia River Basin. The question is why did it take so long to convince people that these fish were worth saving? Many people may still believe that lampreys are just a nuisance or pest species due to the problems in the Great Lakes regarding the exotic sea lampreys (*Petromyzon marinus*). However, the argument exists that Pacific lampreys are an important part of the ecosystem and have cultural value to Native Americans.

## **Life History**

The present state of knowledge suggests that the life history of Pacific lampreys is very similar to sea lampreys. They spend the early part of their life burrowed in fine silt or sand filtering detritus and other particulate matter. After an extended time (4 to 6 years), larvae go through metamorphosis which includes major morphological and

physiological changes. The juveniles then move to the ocean or lake to feed before returning as adults for reproduction. The life history of Pacific lampreys is depicted in Figure 2.1.

### *Spawning*

Pacific lampreys along the coast of Oregon usually begin to spawn in May when water temperatures reach 10°C to 15°C and continue to spawn through July. Both spawning and pre-spawning fish were collected in the John Day River, Oregon in July (Kan 1975). Mattson (1949) described spawning activity in the Willamette River during June and July. In the Babine River system in British Columbia, Pacific lampreys were observed spawning from June through the end of July (Farlinger and Beamish 1984).

Appropriate substrate is a critical habitat feature for successful lamprey spawning. Pacific lamprey spawning occurs over gravel containing a mix of pebbles and coarse sand (Mattson 1949; Kan 1975). Pletcher (1963) found that lampreys held in aquaria divided with three inches of sand on one side and gravel substrate on the other preferred gravel. Spawning sites of Pacific lampreys generally occur in deposited gravels immediately following a pool or in gravels located in riffles (Pletcher 1963; Kan 1975).

Flow is also an important lamprey-spawning requirement. Lampreys prefer flowing water (Manion and Hanson 1980; Russell et al. 1987) for spawning. Pletcher (1963) and Kan (1975) found that spawning sites had water velocities ranging from 0.5 to 1.0 m/s in depths from 0.4 to 1.0 m. In the Babine River system, spawning depths ranged from 30 cm to 4 m, although most occurred at sites of less than 1 m (Farlinger and Beamish 1984).

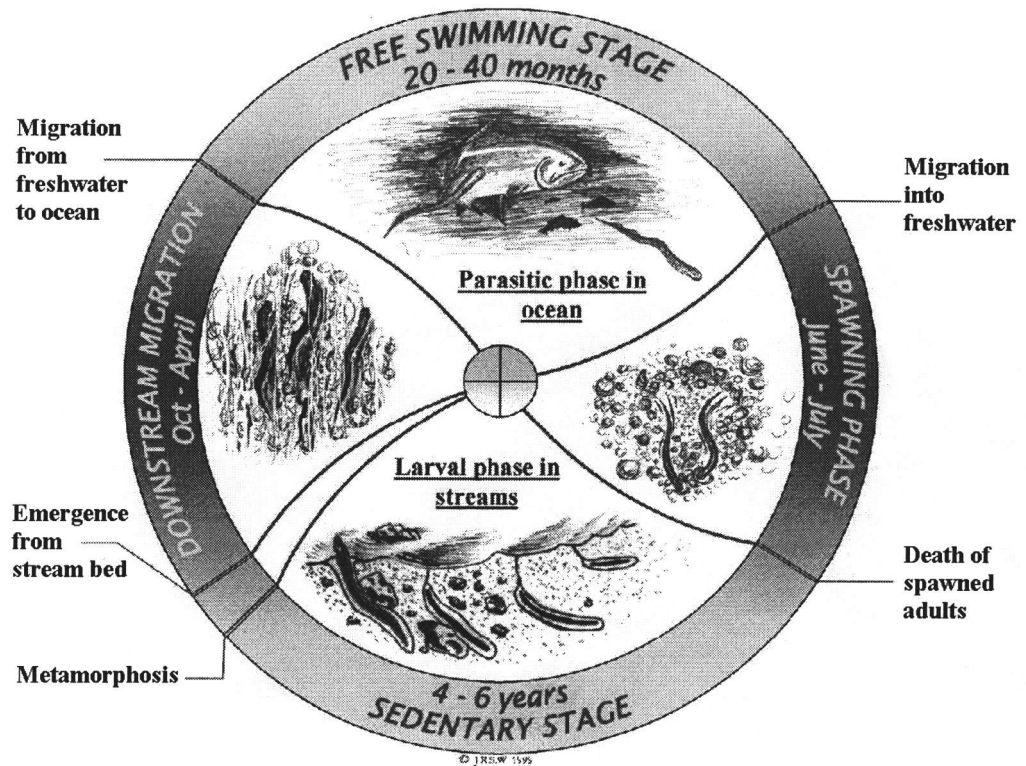


Figure 2.1 Life cycle of Pacific lampreys adapted from the Great Lakes Fishery Commission.

Although rare, Pacific lampreys have been observed spawning in lentic habitat in the Babine Lake system in Canada, where depths of the nest sites ranged from 0.5 m to 3 m. In this system, lampreys generally oriented towards the creek approximately 30 m from the mouth (Russell et al. 1987). Although lampreys will on occasion spawn in habitats that are not ideal, reproductive success can vary widely relative to the overall suitability of the habitat.

At the beginning of spawning, lampreys generally hide in the substrate or in the shade. However, as spawning proceeds lampreys are not affected by bright sunlight (Pletcher 1963; Kan 1975). Both sexes begin moving rocks with their buccal funnels to create nests in excavated depressions (Pletcher 1963). Kan (1975) observed spawning of Pacific lampreys in Oregon and found that nests were approximately 30 cm wide, 3 cm in depth, and oval in shape. In the Babine Lake system of British Columbia, nests were 20-30 cm in diameter and 4-8 cm deep (Russell et al. 1987).

Courting consists of a male approaching a female with a gliding motion to stimulate the female (Pletcher 1963). A male attaches his buccal funnel to a female's head, and then wraps his body around the female while releasing milt (Pletcher 1963; Kan 1975; Russell et al. 1987). During each spawning act, approximately 100 to 500 eggs are released and covered by sand and pebbles (Pletcher 1963).

Fecundity for Pacific lampreys in Oregon streams ranged from 98,000 to 238,400 eggs per female (Kan 1975). Fecundity was significantly different between Pacific lampreys from coastal Oregon streams, the Molalla and Umpqua Rivers, and from the John Day River (Kan 1975). Kan (1975) suggested that the lower fecundity in the John

Day lampreys was due to a higher cost of migration. After spawning, the Pacific lamprey dies within 3 to 36 days (Mattson 1949; Pletcher 1963; Kan 1975).

### *Larval Stage*

Temperature controls the hatching time of Pacific lamprey eggs. Pletcher (1963) observed eggs beginning to hatch after 19 days at 15°C. The larvae leave the gravel approximately two or three weeks after hatching and drift downstream usually at night (e.g. see Barfoot et al. 1993 concerning the Deschutes River). The larva settle in slow depositional areas such as pools and eddies (Pletcher 1963). The slow current allows the larvae to maintain position while burrowing. Under experimental conditions, emergent larvae of size 7-10 mm preferred mud (0.004 cm) to sand (0.005 cm) and gravel (1-0.5 cm) substrate (Pletcher 1963). Current greater than 30.5 cm/s prohibited burrowing by emergent larvae in all substrates. When no current was present, larvae of sizes 10-15 mm and 25-30 mm burrowed into the mud faster than larvae of size 40-50 mm. The smallest size group required the most time for burrowing in the sand. With a current of 30.5 cm/s, only the 40-50 mm larvae could burrow in the sand, but all groups burrowed into the mud substrate (Pletcher 1963). The current over lamprey larval beds in Oregon streams ranged from 10 to 50 cm/s (Kan 1975).

The density of larvae was highest in shallow areas along the banks of the Chemainus River in British Columbia (Richards 1980). Richards (1980) found that larvae less than 75 mm in length were found in the shallows but only larger larvae greater than 75 mm were found in the deeper middle portion of the river. In this study, higher densities of

larvae were also found in the lower sections of the river with low gradients as opposed to sections with steeper gradients.

Larvae are usually found in cold water but have been collected in waters ranging up to 25°C in Idaho (Mallatt 1983). In laboratory studies, larvae held at 14°C and 4°C grew 41% and 11% of body weight per month, respectively, on a variety of foods (Mallatt 1983). Larval sea lampreys preferred a summer temperature of 20.8°C and ranged from 17.8 to 21.8°C (Holmes and Lin 1994).

Larvae are blind, largely sedentary, and survive by filtering food particles. Larvae usually feed on detritus, diatoms, and algae suspended above and within the substrate (Moore and Mallatt 1980). Larvae possess high entrapment efficiency due to mucus secreted by the walls of the pharynx and goblet cells within the gill filaments. The high entrapment efficiency is coupled with low food assimilation rates. Larvae digested only 30-40% of the food taken in while passing large amounts of undigested food (Moore and Mallatt 1980).

Larval Pacific lampreys can weigh up to 5 g and grow to 20 cm in length (Mallatt 1983). The larval stage was estimated to range from 4-6 years (Richards 1980; Kan 1975; Pletcher 1963) although it may extend up to 7 years (Hammond 1979; Beamish and Northcote 1989). However, the age of lampreys is difficult to estimate. Inconsistency of length frequency data and the lack of bony structures make conventional fish aging techniques limited in value.

## *Metamorphosis*

During metamorphosis, the larvae go through morphological and physiological changes to prepare for a parasitic lifestyle in salt water. Transformation of Pacific lampreys from the larval to juvenile life stages generally occurs during July through October (Richards and Beamish 1981; Hammond 1979). However, Pletcher (1963) observed metamorphosis from July to November from the Chemainus River, British Columbia. The process occurs in seven stages according to external observations (Youson and Potter 1979). The changes occur first in the mouth, with the oral hood changing into an oval mouth. The development of the eye and the length of the oral disc increase during stage 1-4. Condition factor begins to drop after reaching stage 4 in transforming fish. After four weeks (stage 5), teeth and tongue begin to develop (Richards 1980). The teeth remain soft through stage 6, with cornification occurring near the end of stage 6. Internal changes such as development of the foregut during stage 6 coincides with the ability to osmoregulate in salt water (Richards 1980; Richards and Beamish 1981). Changes in the blood proteins also occur during metamorphosis (Richards 1980). The gallbladder and the bile duct disappear as the fish transforms to the parasitic stage (Bond 1979). The respiratory system also changes. In larvae, unidirectional water flow (over the gills and through the pharynx then out the gill pores) changes during metamorphosis to a tidal flow system in which water enters and exits the branchiopores (Lewis 1980). The transformation is associated with a new preference of habitat. Transforming fish are associated with larger substrate usually found in higher water velocity areas (Richards and Beamish 1981; Potter 1980). By stage 6, Pacific lampreys from the Qualicum River in British Columbia moved from mud and silt

areas to 1-4 cm gravels in faster flows (Beamish 1980). When the teeth harden and turn yellow, stage 7 is complete (Richards 1980).

### *Recently Metamorphosed Lampreys*

While waiting to migrate to the ocean, metamorphosed lampreys burrow in cobble and boulder substrate (Pletcher 1963). Metamorphosed lampreys from some populations may reside in freshwater for up to 10 months after metamorphosis. Different populations in British Columbia vary in their ability to survive confinement in freshwater (Beamish 1980). Confined Babine River lampreys did not survive past February, while Chemainus River fish survived until July (Clarke and Beamish 1988). The onset of mortality was associated with decrease in plasma sodium concentration and condition factor (Clarke and Beamish 1988).

Metamorphosed lampreys begin their migration to the ocean in the Fall and continue through the Spring. Time of entrance into salt water may differ among populations of Pacific lampreys due to environmental conditions (pers. comm., R.J. Beamish, Nanaimo Biological Station, Nanaimo, B.C., Canada). Kan (1975) suggested that coastal populations enter salt water in the late fall while inland populations enter in the spring. In the Nicola River of British Columbia, 99% of all metamorphosed lampreys migrated by April and May (Beamish and Levings 1991). Downstream migrants are sampled from March to June in collection facilities at John Day and Bonneville dams on the Columbia River (Hawkes et al. 1991, 1992, 1993). However, data during winter months are lacking because collection facilities do not operate during that time.



Timing of downstream migration is generally positively correlated with increased discharge (Potter 1980; Applegate 1950; Beamish and Levings 1991). In the Fraser River system, 99% of the metamorphosed lampreys left the substrate and began migration during the night with increased discharge (Beamish and Levings 1991). Long (1968) also found downstream migration occurred at night in the Columbia River. Pacific lampreys, like other species of lampreys, rely on currents to carry them downstream (Beamish and Levings 1991). Out-migrating sea lampreys do not actively swim downstream; instead, they drift downstream tail first (Applegate 1950). Long (1968) found that most migrating juvenile lampreys enter turbine intakes near the center and bottom, and therefore would not be detected by monitoring activities.

### *Ocean Life*

Although data are sparse, the ocean-phase or parasitic-phase has been estimated to last for periods of up to 3.5 years for Pacific lampreys in the Strait of Georgia, British Columbia (Beamish 1980). Off the coast of Oregon, the duration of the ocean phase was estimated to range from 20 to 40 months (Kan 1975). After entrance into salt water, Pacific lampreys can move into water greater than 70 m in depth. Parasitic-phase lampreys have been captured off the Pacific coast of Canada at depths ranging from 100 to 250 m (Beamish 1980). Pacific lampreys have been collected at distances ranging from 10 km to greater than 100 km off the Oregon Coast and up to 800 m in depth (Kan 1975).

## *Feeding*

Parasitic-phase lampreys locate their prey by means of olfaction (Kleerekoper 1958), electroreception (Kleerekoper and Sibakin 1956), and vision (Farmer 1980), which is similar to elasmobranch fishes (Kalmijn 1982). Sea lampreys, stimulated by amines from prey fish located in water added to tanks, oriented their bodies towards the source of the smell (Kleerekoper 1958). Lampreys possess electroreceptors on head and trunk regions that may be useful in finding prey (Bodznick and Preston 1983). Farmer (1980) also suggested lampreys use vision to locate prey items.

Pacific lampreys can feed in fresh water and salt water, although freshwater feeding may not be common. To feed, Pacific lampreys frequently attach to fish ventrally near the pectoral area (Roos et al. 1973; Beamish 1980). Lampreys create suction in the buccal funnel by changing the volume in the oral cavity (Hardisty and Potter 1971). The tongue contains denticles that rasp to create tissue damage and buccal glands secrete anticoagulant to assist in feeding on blood (Farmer 1980). Freshwater feeding has been documented under unusual conditions. For example, it occurred above Dworshak Dam on the North Fork of the Clearwater River in Idaho when migration was cut off in 1969 (Wallace 1978), and above dams in British Columbia that stopped migration of recently metamorphosed lampreys to the ocean (Beamish and Northcote 1989). Wallace (1978) suggested that many of the attachments were unsuccessful in Dworshak reservoir. Eventually, Pacific lampreys became extinct in both drainages above the dams.

### *Upstream Migration*

Beamish (1980) has suggested that returning adult lampreys enter fresh water between April and June and complete migration into streams by September. In the Chemainus River of British Columbia, lampreys migrated into fresh water beginning in late April and 81% of the catch occurred during two days in May (Richards 1980). It is not clear how flow affects freshwater immigration. Pacific lampreys are considered weak swimmers compared to other fish. Burst swimming speed was calculated to be approximately 2.1 m/sec for adult lampreys compared to 6.4 m/sec for adult chinook salmon (*Oncorhynchus tshawytscha*) (Bell 1990). Upstream migration rate was speculated to be 4.5 km/day in the Columbia River (Kan 1975) and 8 km/day in the Fraser River (Beamish and Levings 1991).

Pacific lampreys overwinter in fresh water and spawn the following spring (Beamish 1980). During winter, Columbia River dams remove water in fishways for maintenance and it is common for Pacific lampreys to be found and removed at this time (Starke and Dalen 1995). Pacific lampreys generally overwinter in deep pool habitat until spring (pers. comm. R.J. Beamish).

It is not known if Pacific lampreys home to natal streams; however, there is evidence that other species of lampreys do not. Bergstedt and Seelye (1995) found that sea lampreys in Lake Huron do not home to their natal streams. The experiment consisted of tagging recently metamorphosed lampreys and trapping spawning phase adults in tributaries to the lake. Marking studies with river lampreys (*Lampetra fluviatilis*) in Finland also found a lack of homing behavior. Lampreys were captured then marked with Carlin tags

from the Kalajoki and Pyhajoki Rivers. Lampreys released in the Bay of Bothnia were recaptured at rates ranging from 57% to 88% (Tuunainen et al. 1980).

Stream flow and pheromones released by larvae are important factors in selection of rivers for spawning sea lampreys (Morman et al. 1980; Li et al. 1995). Pacific lamprey larvae also contain the migratory pheromone (petromyzonol sulfate) found in sea lamprey larvae (Sorenson and Close 1998). Adult sea lampreys have an olfactory system with sensitivity ranging down to a picomolar (approximately 1 gram in 40 billion liters; Li et al. 1995; Li and Sorenson 1997), and were found to effect migratory behavior (Bjerselius et al. 2000).

Pacific lampreys do not feed during the spawning migration. The fish utilize carbohydrates, lipids, and proteins for energy (Read 1968). Beamish (1980) observed 20% shrinkage in body size from the time of freshwater entry to spawning.

## **Distribution**

Pacific lampreys are distributed in North America from the Aleutian Islands south along the Pacific coast to Baja California, Mexico, and inland to the upper reaches of most rivers draining into the Pacific ocean (Kan 1975).

## **Status**

Trends in lamprey abundance are based on lampreys counted at hydroelectric dams from 1938 until 1998. Lampreys were counted 16 hours per day at Corps of Engineer dams. Rocky Reach (mid-Columbia River) and Ice Harbor (Snake River) Dams

counted the same way until 24-hour counts started in 1996. Unfortunately, counts discontinued after 1969 at many dams, and abundance data in the Willamette River prior to the development of the first mainstem dams in the Columbia River is scarce. Oregon Department of Fish and Wildlife counted lampreys at Winchester Dam on the Umpqua River from 1965 to present (Fig. 2.2).

Two patterns are evident when the numbers of lampreys is displayed. First, a significant decrease in abundance of upstream migrant lampreys occurred in the Columbia River Basin and possibly along the Pacific coast by the early 1970's. Second, there seems to be prominent decadal fluctuations in abundance of lampreys entering the Columbia River as shown from Bonneville Dam (Fig. 2.3). The overall decline corresponded to the building of the last mainstem dams in the Columbia, Snake, and Willamette rivers, which cut off access to spawning habitat in the Columbia River basin (Vella et al. 1999a; Vella et al. 1999b), and to decreased ocean productivity (Smith 1978; Lichatowich et al. 1998). The data suggest that lampreys may have been affected by the same fluctuations in ocean productivity that affects salmon. Lichatowich et al. (1998) suggested decadal fluctuations in ocean productivity and Columbia River climate might define the decadal patterns of salmonid production. Further, human activities decreased the peaks and increased the troughs for salmonid production in the Columbia River Basin. Annual precipitation reconstructed from tree rings in the Columbia River Basin exhibited decadal fluctuations (Graumlich 1981) with lows in the 1920's through 1940's and increases in the 1950s that may have helped increase natural production for lampreys in the early and late 1960s (Fig. 2.3).

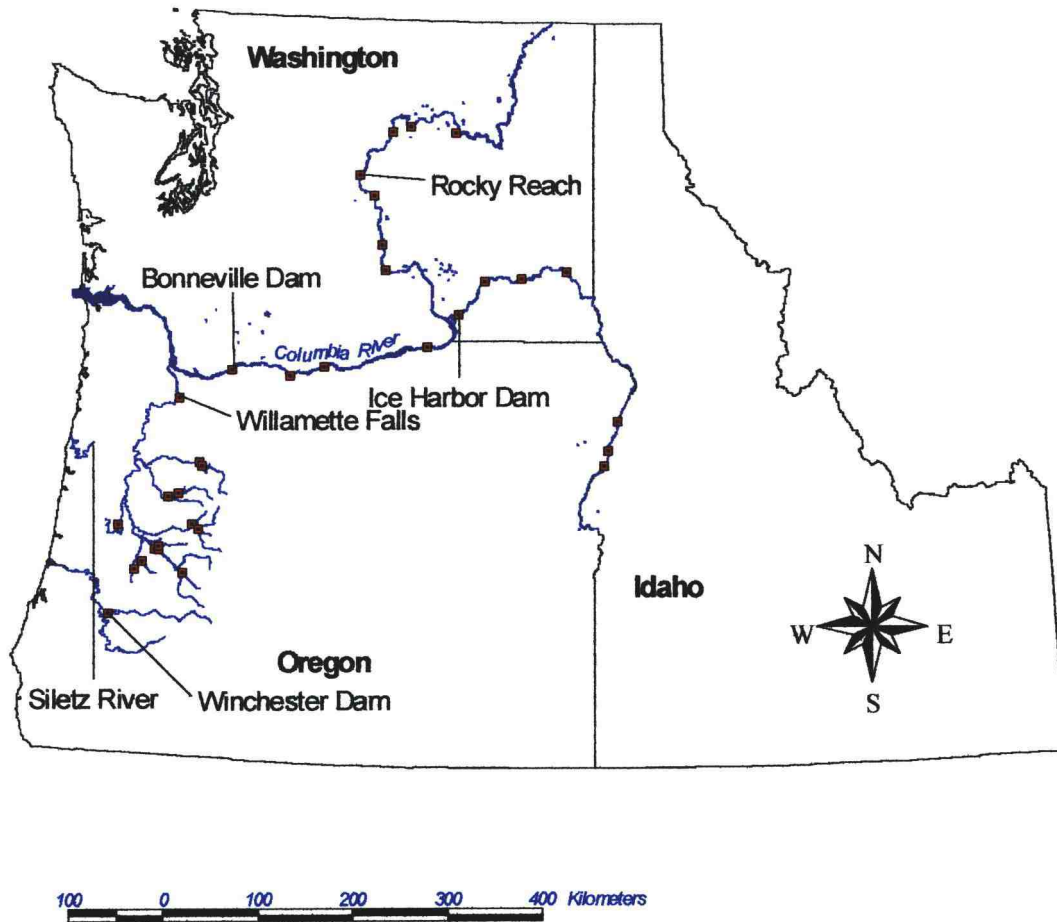


Figure 2.2 Map of locations where lamprey counts and harvest data were collected by Oregon Department of Fish and Wildlife, U.S. Army Corps of Engineers, and Chelan County Public Utility Department. Locations are labeled as place names except the Siletz River, which was anecdotal information from the Siletz tribe.

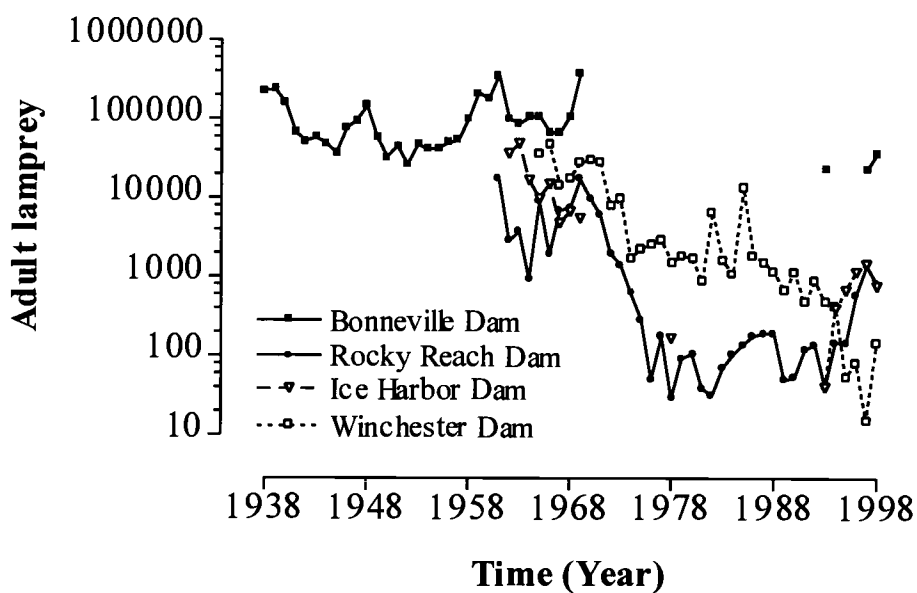


Figure 2.3 Lampreys counted at dams in the Columbia (Bonneville, Rocky Reach), Snake (Ice Harbor), and Umpqua (Winchester) Rivers.

The construction of mainstem dams as early as the 1930s may have also affected the trends in abundance. For example, the numbers of upstream migrants in the Willamette River seemed to be much greater than those past Bonneville Dam (Fig. 2.4) during the 1940s when data were available for both sites. During 1946, commercial harvest of lampreys at Willamette Falls peaked at approximately 500,000 calculated from reports on total weight of landings and from fish collected at Willamette Falls in 1996 and 1997 that had an average of 350 g lamprey<sup>-1</sup>. Mattson (1949) speculated that commercial catch in the 1940s at Willamette Falls was between 10 to 20% of the run, which suggests that the actual number of upstream migrants in 1946 was between 2.5 to 5 million in the Willamette River. Today, the number of adults harvested at Willamette Falls is roughly equal to the numbers counted at Bonneville Dam (Fig. 2.4). This may have at least two possible explanations. The Willamette River may always have been a bigger producer of lampreys than the rest of the Columbia River Basin. Alternatively, the construction of Bonneville Dam may have diverted large numbers of lampreys into the Willamette River. Without historical data, rejecting either one of these explanations is difficult. Return of more lampreys to the Willamette River than the rest of the Columbia River Basin under natural conditions seems unlikely because the Columbia River contains much higher flows. Stream flow is a major factor in the distribution of upstream migrant sea lampreys in the Great Lakes. Morman et al. (1980) reported if environmental factors were favorable, the largest flows would attract and contain the largest spawning runs of lampreys. In Quebec, upstream migrants preferred large volume rivers (Vladykov 1952).



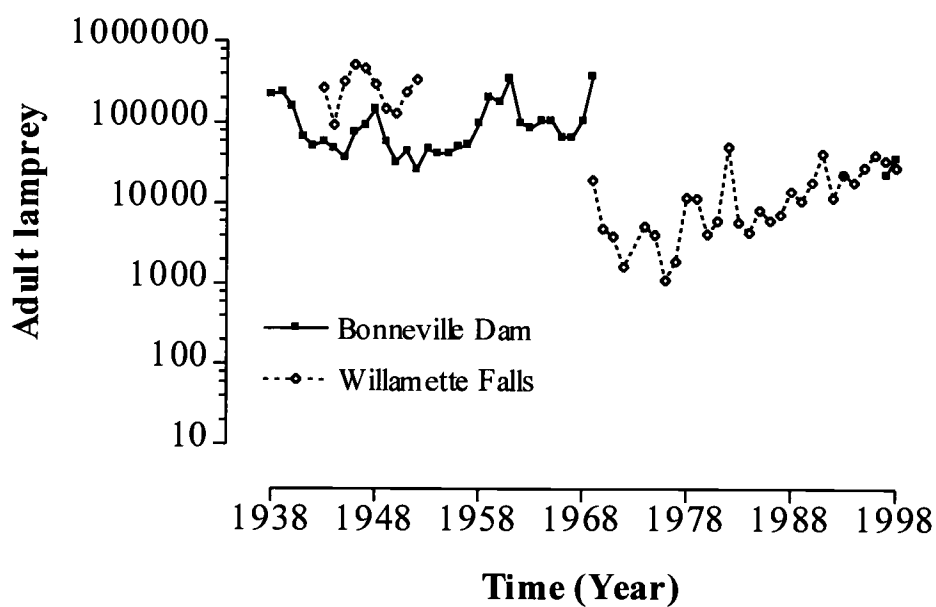


Figure 2.4 Lampreys counted at Bonneville Dam on the Columbia River and estimates of lampreys harvested at Willamette Falls on the Willamette River.

If Pacific lampreys have the same preferences for high flows, large returns to the Willamette River may partially reflect disturbances to natural migration patterns. The construction of Bonneville Dam in 1938 may have been one such disturbance. Physical barriers such as dams were very effective in limiting migration and reproductive success of sea lampreys (Hunn and Youngs 1980). In the 1800's, the decline of sea lampreys on the east coast of the U.S. was blamed on dams (Goode 1884). Hydroelectric dams were also responsible for the decline in European river lampreys in Finland (Tuunainen et al. 1980; Eklund et al. 1983; Ojutkangas et al. 1995). Likewise, the large abundance of lampreys in the Willamette River and the counts of lampreys at mainstem dams follow closely the construction of Bonneville Dam, which limited access to spawning habitat in the Columbia River. Lampreys blocked at Bonneville Dam may have dropped downstream and entered the Willamette River. However, the Willamette River Basin followed the same fate as the Columbia River Basin as much of the river was dammed. Unfortunately, without data in the Willamette River prior to Bonneville Dam construction, there's no way to determine if the number of Willamette River lampreys is a reflection of damming or natural production.

The development of dams may have drastically reduced numbers in the Columbia River Basin and may have contributed to the declines along the Oregon Coast. Mainstem hydroelectric dams have limited access to spawning areas in the interior Columbia River Basin. As the Columbia River and tributaries were blocked off, numbers of lampreys decreased, finally recruitment to coastal rivers ceased. The Siletz Tribe complained of decreased harvest of lampreys in the Siletz River (Downey et al. 1993). In addition, the

Umpqua River lampreys followed the same pattern of decrease during the same time period (Fig. 2.3).

## **Ecological Benefits**

Evidence suggests that Pacific lampreys were well integrated into the native freshwater fish community and as such had positive effects on the system. It was likely a big contributor to the nutrient supply in oligotrophic streams of the basin as the adults died after spawning (Beamish 1980).

Lampreys were an important part of the food web for many species of freshwater fishes, birds, and mammals (Table 2.1). Juvenile lampreys may have played an important role in the diets of many freshwater fishes. Larvae and spawned out carcasses of lampreys were important dietary items for white sturgeon (*Acipenser transmontanus*) in the Columbia and Fraser Rivers (Semakula and Larkin 1968; Galbreath 1979; pers.comm. Ken Witty ODFW 1997). Lampreys are found in the diets of northern pikeminnow (*Ptychocheilus oregonensis*) and channel catfish (*Ictalurus punctatus*) in the Snake River system (Poe et al. 1991). Further, lamprey larvae are commonly used for bait to catch the exotic small mouth bass (pers. comm. P. Bronson, Confederated Tribes of the Umatilla Indian Reservation Fisheries Program 1999) in the lower reaches of John Day River, Oregon. Pfeiffer and Pletcher (1964) found emergent ammocoetes and lamprey eggs were eaten by salmon fry.

Table 2.1 Predators of lampreys.

Name	Lamprey life stage	Source
channel catfish ( <i>Ictalurus punctatus</i> )	larvae	Poe et al. (1991)
northern pikeminnow ( <i>Ptychocheilus oregonensis</i> )	larvae	Poe et al. (1991); Pfeiffer and Pletcher (1964)
smallmouth bass ( <i>Micropterus dolomieu</i> )	larvae and metamorphosed lampreys	Perlmutter (1951)
northern pike ( <i>Esox lucius</i> )	larvae	Perlmutter (1951)
Eel ( <i>Anguilla rostrata</i> )	larvae	Perlmutter (1951)
coho salmon ( <i>Oncorhynchus kisutch</i> )	Egg and newly hatched Larvae	Pfeiffer and Pletcher (1964)
white sturgeon ( <i>Acipenser transmontanus</i> )	adults and larvae	Semakula and Larkin (1968); Galbreath (1979)
Sablefish ( <i>Anoplopoma fimbria</i> )	adults	Beamish (1980)
spiny dogfish ( <i>Squalus scanthias</i> )	adults	Beamish (1980)
california gull ( <i>Larus californicus</i> )	Larvae and metamorphosed lampreys	Merrell (1959)
ringbill gull ( <i>Larus delawarensis</i> )	Larvae and metamorphosed lampreys	Merrell (1959)
western gull ( <i>Larus occidentalis</i> )	Larvae and metamorphosed lampreys	Merrell (1959)
Forster's tern ( <i>Sterna forsteri</i> )	Larvae and metamorphosed lampreys	Merrell (1959)
great blue heron ( <i>Ardea herodias</i> )	adults	Wolf and Jones (1989)
california sea lion ( <i>Zalophus californianus</i> )	adults	Roffe and Mate (1984)
steller sea lion ( <i>Eumetopias jubatus</i> )	adults	Roffe and Mate (1984); Jameson and Kenyon (1977)
pacific harbor seal ( <i>Phoca vitulina richardsi</i> )	adults	Roffe and Mate (1984)
sperm whale ( <i>Physeter catodon</i> )	adults	Pike (1950)
Mink ( <i>Mustela vison</i> )	adults	Beamish (1980)
Human ( <i>Homo sapiens</i> )	adults	Mattson (1949)

Juvenile lampreys migrating downstream may have buffered salmonid juveniles from predation by fishes and sea gulls. Merrell (1959) found that lampreys comprised 71% by volume of the diet of gulls and terns below McNary Dam during early May.

Likewise, we suspect that adult lampreys may have been an important buffer for upstream migrating adult salmon from predation by marine mammals. From the perspective of a predatory sea mammal, lampreys have at least three virtues: (1) they are easier to capture than adult salmon; (2) they have higher in caloric value per unit weight than salmonids; and (3) their migration in schools means fertile feeding patches. Pacific lampreys are extraordinarily rich in fats much richer than salmon. Caloric values for lampreys' ranges from 5.92-6.34 kcal gm<sup>-1</sup> wet weight (Whyte et al. 1993); whereas; salmon average 1.26-2.87 kcal gm<sup>-1</sup> wet weight (Stewart et al. 1983). In fact, the work of Roffe and Mate (1984) revealed that the most abundant dietary item in seals and sea lions was Pacific lampreys. As a result, marine mammal predation on salmonids maybe much more severe because lamprey populations have declined.

It is unlikely that restoration of the Pacific lamprey will impede the recovery of Columbia River salmonids. There should be little fear that the Pacific lamprey will mimic the role of the sea lampreys, after its invasion into the Laurentian Great Lakes (e.g., Eschmeyer 1955, Moffett 1956, Coble *et al.* 1990). That was a case of an entire community of naive prey being exposed to an exotic predator; whereas, Pacific lampreys have co-adapted with its community, including Pacific salmon (Table 2). Beamish (1980) could find no evidence that increased lamprey production in the Skeena River

Table 2.2 Prey of lampreys (from Beamish 1980 and Pike 1951).

Name
sockeye salmon ( <i>Oncorhynchus nerka</i> )
coho salmon ( <i>Oncorhynchus kisutch</i> )
pink salmon ( <i>Oncorhynchus gorbuscha</i> )
chinook salmon ( <i>Oncorhynchus tshawytscha</i> )
steelhead salmon ( <i>Oncorhynchus mykiss</i> )
rockfish ( <i>Sebastes aleutianus</i> and <i>S. reedi</i> )
pacific cod ( <i>Gadus macrocephalus</i> )
lingcod ( <i>Ophiodon elongatus</i> )
sable fish ( <i>Anoplopoma fimbria</i> )
pacific halibut ( <i>Hippoglossus stenolepis</i> )
greenland turbot ( <i>Reinhardtius hippoglossoides</i> )
arrowtooth flounder ( <i>Atheresthes stomias</i> )
kamchatka flounder ( <i>Atheresthes evermanni</i> )
pacific ocean perch ( <i>Sebastes alutus</i> )
finback whale ( <i>Balaenoptera physalus</i> )
humpback whale ( <i>Megaptera nodosa</i> )
sei whale ( <i>Balaenoptera borealis</i> )
sperm whale ( <i>Physeter catodon</i> )

would lead to predation problems on its sockeye salmon. Although Pacific lampreys will prey on salmonids, lampreys also feed on a variety of midwater species such as Pacific hake (*Merluccius productus*) and walleye pollock (*Theragra chalcogramma*) in the open ocean (Beamish 1980). However, this raises the question of the role that the intense commercial harvest of Pacific hake and walleye pollock has on the food chain dynamics of the north Pacific and on Pacific lamprey stocks in particular.

### **Cultural Significance**

The utilization of lampreys is quite interesting due to the fact that both early EuroAmericans and Native Americans found them important in the Pacific Northwest. Fur trappers seeking coyote utilized lampreys as bait around the turn of the century (Mattson 1949; pers. comm. Milo Bell, University of Washington 1995). In the late 1800s, Oregon began developing artificial propagation of salmonids. Fish culturists soon discovered a need to hold fish longer and to increase growth while doing so. They needed to find a large and cheap food supply to accomplish the task. The fish culturists experimented with different foodstuffs and found ground raw Pacific lampreys were a premium feed for young salmon. Adult lampreys were collected at Willamette Falls then transferred to cold storage to be processed (Fig. 2.5). During the year 1913, 27 tons were harvested for this purpose (Clanton 1913).

In the following years, lampreys became commercially important. In 1941 a commercial fishery for Pacific lampreys at Willamette Falls had started. Between 1943 and 1949, a total of 740,419 kilograms of lamprey were harvested. The primary use of

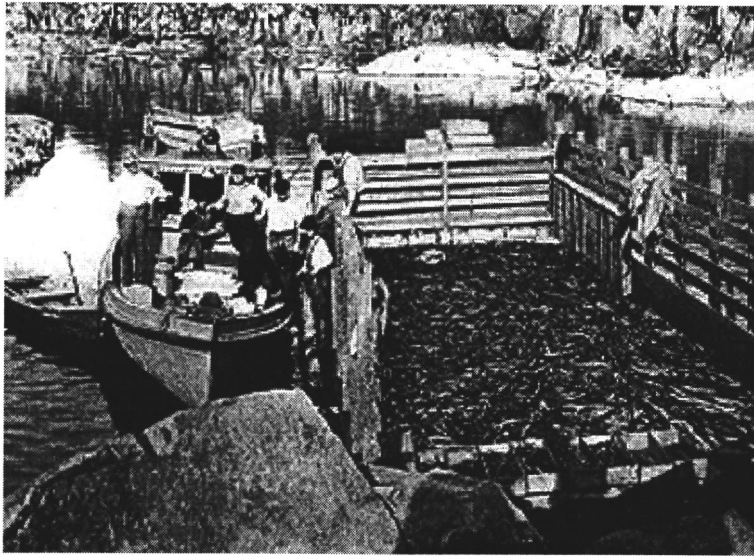


Figure 2.5 Fifteen tons of Pacific lampreys aboard a scow for delivery to a cold storage plant to be preserved as food for hatchery salmon fry (Clanton 1913).



the fish was for vitamin oil, protein food for livestock, poultry, and fishmeal (Mattson 1949). Presently, Pacific lampreys are important for scientific research in the area of medicinal anticoagulants, for teaching specimens (North Carolina Biological Supply House regularly collects at Willamette Falls), and for food (in 1994, approximately 4,000 lbs. were exported to Europe).

Tribal peoples of the Pacific Coast and interior Columbia Basin harvest these fish for subsistence, ceremonial, and medicinal purposes (Fig. 2.6). In the native tongue (Sahaptin) of the Columbia River Plateau tribes, lampreys are called *ksuyas* or *asum*. However, many Native Americans use the common name eel when in reference to Pacific lampreys. Lampreys are often harvested by hand, dip net, or jigging with a long pole and hook. Fishing for lampreys is usually done at night when the fish are most active. Generally fishing sites are located at falls or fast water areas that cause the lampreys to congregate. Before the dams turned the Columbia River into a series of lakes, many tribal peoples fished the rapids for lampreys. Three well known places where tribal members historically harvested Pacific lampreys on the mainstem Columbia River was near Umatilla Rapids (presently McNary Dam), near the mouth of the Snake River, and near the mouth of the Walla Walla River (Wallula). The fish were then prepared traditionally by drying or roasting. Lampreys continue to be part of the Columbia River tribal culture and are as important in ceremonies and celebrations as many other foods collected during seasonal harvests. Pacific lampreys also have medicinal value to tribal peoples in the Columbia Basin. Oil collected from drying lampreys is applied to skin or ailing parts of the body in conjunction with a purifying sweat bath. Some of the tribal

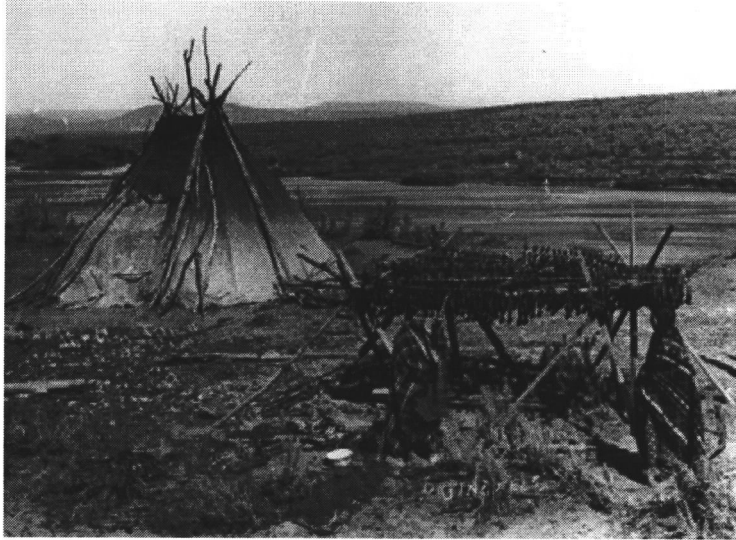


Figure 2.6 Umatilla tribal members drying Pacific lampreys on the Umatilla River, Oregon (Moorehouse collection 1903).

elders also talk about applying the oil to hair. Pacific lampreys were significant subsistence food, which is reflected in the myths and legends within the Columbia River tribes. Lampreys are an integral part of Columbia and Snake River tribal cultures and other tribes along the Pacific coast (Mattson 1949; Pletcher 1963; Anglin et al. 1979).

## **Summary**

Pacific lampreys have declined in numbers in the Columbia River Basin and in rivers along the Pacific coast. Dams may be the main cause; however, other factors such as ocean productivity, prey base depletion, and habitat changes cannot be ruled out.

Lampreys are also an important component to the ecosystem both as a predator and prey. As a predator, lampreys could play a role in culling the weak individuals in fish populations. As prey, lampreys are important to fish, birds, and mammals (including humans).

The cultural values of Pacific lampreys are recognized through a short history of utilization by early EuroAmericans, and a long history that continues today with Native Americans in the Columbia River Basin. However, the cultural values of the dominant society have affected management or lack thereof, to a greater degree than tribal or ecological values of Pacific lampreys in the Columbia River Basin. Regardless, conservation of this species is crucial to the integrity of the Columbia River ecosystem. Gaining knowledge about the problems that Pacific lampreys are experiencing and understanding the biology of this species are the first steps towards restoration of Pacific lampreys.

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## Chapter 3

Effects of Acute Stress on the Physiology of  
Pacific Lampreys (*Lampetra tridentata*)

by

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## **Abstract**

We identified clinical indicators of stress in adult Pacific lampreys, *Lampetra tridentata*. Plasma glucose became elevated soon after acute stress and remained elevated for one week. Plasma lactate also became elevated by 30 minutes; however, it decreased to resting levels by one hour after application of the stressor. Muscle lactate was shown to have an inverse relationship with glucose. Muscle lactate levels decreased by 4 hours and remained depressed for two days. Plasma chloride ions decreased by one hour, then returned to resting levels by 8 hours, decreased again at 24 hours, and then recovered by 48 hours. The steroid cortisol was not found in the plasma of Pacific lampreys. Our study suggests plasma glucose, lactate, chloride ions, and muscle lactate can be used as clinical indicators of stress in Pacific lampreys.

## Introduction

The reasons for the decline of Pacific lamprey (*Lampetra tridentata*) populations throughout the Columbia River Basin are unknown. One possible contributor to this precipitous fall may be migratory failure resulting from the inability of lampreys to negotiate passage around the dams within the Columbia River Basin. In order to determine if migrations of adult lampreys are impaired by hydroelectric projects, methods for examining the behavior of adult lampreys around dams must be developed. Currently, National Marine Fisheries Service (NMFS) biologists are using radio-telemetry to follow the movement of adult lampreys around Columbia River dams (Vella et. al 1999). One of the assumptions of biotelemetry studies is that radio-tagged animals behave like untagged animals; however, this assumption must be verified. Handling and tagging fish can cause stress in fish, which may lead to a decrease in performance and fish health (Pickering 1981). Acute stress in fish can be measured using both clinical indicators (Wedemeyer and Yasutake 1977) and behavioral tests (Sigismondi and Weber 1988). Our study objective was to identify clinical indicators of stress in adult Pacific lampreys. Experiments were designed to assess the utility of plasma cortisol, glucose, lactate, chloride, and muscle lactate as physiological indicators of stress for use in Pacific lampreys. Identification of clinical indicators of stress will be the first step toward evaluation of biotelemetry methods in lampreys.

## Materials and Methods

### *Experimental Animals*

Adult Pacific lampreys were collected from Willamette Falls, Oregon and transported to Oregon State University's Fish Performance and Genetics Laboratory, Corvallis, Oregon. Following their capture, the animals were treated with salt (50 g/37.8 L) during transport and subsequently with formalin (5.9 ml/37.8 L) to prevent fungal infection. After fish were anesthetized in tricaine methanesulfonate (MS-222; 200 mg/L), they were weighed and injected with oxytetracycline (0.5 ml/kg) to combat bacterial infection. Before the experiments, fish were maintained in flow-through 0.9 m diameter tanks (336 L) supplied by underground water at a temperature of 12-13 °C at least 1 week under natural photoperiod.

### *Experimental Design*

In experiment I, we examined the effects of acute stress on plasma glucose through time. Adult lampreys were distributed into 0.9 m diameter tanks (n=10/tank). Each tank was randomly assigned a sampling time (i.e. no individual fish was sampled more than once). Fish in all but one tank (control; time = 0) were exposed to a 5 minute dewatering stressor, then sampled 1, 2, 3, 4 and 24 hours after stress.

In experiment II, we examined the effects of acute stress on plasma glucose, lactate, chloride ion, and muscle lactate through time. Adult lampreys were distributed

into 0.9 m diameter tanks ( $n=5/\text{tank}$ ). Each tank was randomly assigned a sampling time. After two weeks of acclimation, the fish were sampled before the stress (controls; time = 0) or stressed by dewatering their tanks for 5 minutes and then returning water into tank. At each sampling time (0, 0.5, 1, 4, 24, or 48 hours after the stress), one tank of lampreys was sampled. This sampling design was conducted on one group of fish on 10/24/96 and another group of fish on 12/3/96.

In experiment III, we examined the time required for plasma glucose to recover after acute stress. Adult lampreys were distributed among tanks ( $n = 10/\text{tank}$ ; 2 tanks/sampling time), acclimated, and stressed as before; however, replicate tanks were sampled at 0 (controls), 1, 24, 72, and 168 hours (7 days) after the acute stress.

Experiment IV was designed to determine if plasma cortisol could be detected. Six adult lampreys were held in one tank filled with water. Three fish were immediately netted out and sampled for blood, while the other three fish were netted out and put into a dewatered 20 L bucket for 30 minutes, then returned to tank with water. After one hour the fish were sampled for blood.

Experiment V was designed to qualitatively assess corticosteroid production in Pacific lampreys. Two adult lampreys were transported to Oregon State University, held in garbage can filled with water. Fish were then anesthetized and injected with 5  $\mu\text{Ci}$  of radiolabeled pregnenolone. One fish was placed in bucket (20L) with water while the other was subjected to a 10 minute dewatering stress then transferred into a bucket (one fish per bucket) that contained water with air supply. Lampreys were then sampled for blood at 30, 60, and 180 minutes.

For experiments I and III, stress was imposed by netting the fish from the tank and placing them in a dewatered bucket for five minutes. After the stress, fish were placed back into their designated tanks. In experiment II, fish were dewatered in their tanks for five minutes. In experiment IV, 3 fish were placed in a dewatered bucket for 30 minutes. In experiment V, one fish was placed in a dewatered bucket for 10 minutes.

### *Sampling*

In all experiments, animals were netted from tanks and anesthetized in tricaine methansulfonate (MS-222; 800 mg/L for lethal sampling; 200 mg/L for non-lethal sampling) buffered with sodium bicarbonate. In experiment I, blood samples were collected by cardiac puncture using heparinized vacutainers. In the other experiments, blood was collected from the caudal vein (once the technique was perfected). Blood samples were kept on ice before plasma was separated by centrifugation at 1750 g for 5 min. Samples were kept frozen at -80°C until analysis. Muscle samples (~0.10 g) were collected with a scalpel after a lethal dose of MS-222. Muscle samples were taken just below the anterior dorsal fin, snap frozen in liquid nitrogen and stored at -80°C. Each muscle sample was homogenized in 1.0 ml of ice-cold 0.6N perchloric acid. Samples were centrifuged at 12,800 g for 10 minutes at 5°C. Lactate concentration of the supernatant was then measured by the spectrophotometric procedure of Passonneau (1974). Muscle lactate concentration was calculated by multiplying the homogenate lactate concentration by the total homogenate volume, then dividing the result by the sample weight.



### *Extractions*

Plasma samples (0.5 ml each) from experiment IV and V were extracted twice with 8 ml of diethyl ether. Tubes were vortexed vigorously for 30 seconds after the addition of ether. The organic phase was removed from the aqueous phase after snap freezing in liquid nitrogen. Combined extracts were dried in a Speed Vac centrifuge, resuspended in 1 ml of methanol, filtered through 0.45  $\mu$ m Acrodiscs and then redried. Dried extracts were resuspended in 0.1 ml of mobile phase and injected onto the HPLC.

### *HPLC System*

The HPLC consisted of a Waters system with a 600 controller, 717 autosampler, 996 photodiode array detector (steroids were monitored at 254 and 280 nm), a Digital Venturis computer, Millenium PDA software and a reverse phase C18 (Hewlett Packard) column. Extracts were examined on HPLC as described by Huang et al. (1983) and modified by Feist et al. (1990). Briefly, we used an isocratic mobile phase (flow rate 0.4 ml/min) of water : methanol : acetonitrile : isopropanol (62:28:5:5), followed by a linear gradient (3.3%/min) of water : methanol : butanol (35:45:20) for 30 minutes. This system allowed for the separation of 16 steroid standards (Table 3.1) when monitored at 254 nm with detection limits of 5 ng for each steroid. Fractions from the HPLC were collected at 1 minute intervals and counted on a Packard 1600CA scintillation spectrophotometer.

Table 3.1. Steroid standards for HPLC

name	abbreviation	Nomenclature
aldosterone	ALDO	4-pregnene-11b,21-diol-3,18,20-trione
cortisone	E	4-pregnene-17a,21-diol-3,11,20-trione
cortisol	F	4-pregnene-11b,17a,21-triol-3,20-dione
11-ketotestosterone	KT	4-androstene-17b-ol-3,11-dione
11b-hydroxyandrostenedion	OHA	4-androstene-11b-ol-3,17-dione
11b-hydroxytestosterone	OHT	4-androstene-11b,17b-diol-3-one
corticosterone	B	4-pregnene-11b,21-diol-3,20-dione
11-deoxycortisol	DOF	4-pregnene-17a,21-diol-3,20-dione
11-ketoprogesterone	KP	4-pregnene-3,11,20-trione
androstenedione	A	4-androstene-3,17-dione
17b-estradiol*	E2	1,3,5(10)-estratrien-3,17b-diol
testosterone	T	4-androstene-17b-ol-3-one
17a-hydroxyprogesterone	17-OHP	4-pregnene-17a-ol-3,20-dione
dihydroxyprogesterone	DHP	4-pregnene-17a,20b-diol-3-one
progesterone	P4	4-pregnene-3,20-dione
20b-hydroxyprogesterone	20-OHP	4-pregnene-20b-ol-3-one

\* detectable only at 280 nm.

### *Assay Methods*

Plasma glucose, lactate, and muscle lactate levels were determined by colorimetric assays (Wedemeyer and Yasutake 1977; Pasonneau 1974). Plasma chloride levels were determined by Bill LaVoie at the Idaho Cooperative Fishery Research Unit by the use of a Corning 920 M chloridometer. In experiments II, IV, and V, cortisol levels in plasma were determined by radioimmunoassay (RIA) techniques described by Foster and Dunn (1974) and modified by Redding et al. (1984).

### *Statistical Analysis*

In experiment I, individual fish were treated as the experimental unit. In the second experiment II, plasma glucose and plasma chloride data were pooled from each replicate treatment, and individual fish treated as the experimental unit because there was no evidence of tank effects within treatments (nested ANOVA) using SAS<sup>TM</sup>, release 6.10 (SAS Institute Inc., Cary NC, USA). Replicate tanks were not pooled for plasma lactate or muscle lactate data due to tank effects, and were analyzed as separate treatment groups. Glucose data from fish in replicate tanks were pooled in experiment III. Experiments IV and V were descriptive studies.

A one way analysis of variance (ANOVA) was performed to compare plasma glucose, lactate, chloride, and muscle lactate followed by multiple range testing using Duncan's LSD method for experiments I, II, and III. For all analysis, statistically significant differences were considered when the p-value (P) was less than 0.05.

## Results

Lampreys used in these experiments had a mean weight of  $387 \pm 9$  g (mean  $\pm$  SE). In experiment I, mean resting glucose level (time 0) rose significantly from  $38.8 \text{ mg dl}^{-1} \pm 1.6$  (SE), to  $53.0 \text{ mg dl}^{-1} \pm 1.3$  (SE) at one hour after stressor ( $P = 0.001$ ) and remained elevated for 24 hours (Fig 3.1). At 4 hours after the stressor, glucose levels were significantly lower than those at 1, 2, and 24 hours; however, these levels were still significantly higher than those at time 0 ( $P \leq 0.05$ ). In experiment II, plasma levels of cortisol were undetectable in all fish (data not shown; detection limit of assay 5 ng/ml plasma). Mean resting glucose level was  $47.8 \text{ mg dl}^{-1} \pm 2.4$  at time 0, and within 30 minutes after stress, circulating glucose had increased significantly ( $P < 0.05$ ) reaching maximum concentrations by 4 hours after stress (Figure 3.2). Glucose levels remained elevated for 48 hours. Mean resting plasma  $\text{Cl}^{-}$  level was  $97.7 \text{ meq l}^{-1} \pm 1.1$  at time 0; within 4 hours after stress, circulating  $\text{Cl}^{-}$  had decreased significantly ( $P = 0.002$ ) to  $93.0 \text{ meq l}^{-1} \pm 0.8$  (Fig. 3.3).  $\text{Cl}^{-}$  returned to resting levels by 8 hours; however at 24 hours, levels again were significantly decreased ( $P = 0.0001$ ) before returning to resting levels once again at 48 hours. The means for resting plasma lactate levels in the two control tanks were  $16.0 \text{ mg dl}^{-1} \pm 4.4$  ( $n = 5$ ); and  $19.0 \text{ mg dl}^{-1} \pm 3.7$  ( $n = 5$ ) respectively at time 0; within 30 minutes after stress, circulating lactate had increased significantly ( $P = 0.01$  and  $P = 0.0001$ ) reaching maximum concentrations (Fig. 3.4). Lactate levels then decreased to resting levels at one hour. Only one replicate treatment changed significantly through time for muscle lactate. In fish from this replicate, mean muscle lactate levels decreased significantly from  $53.6 \text{ mg g}^{-1} \pm 10.1$  at time 0 to  $13.6 \text{ mg g}^{-1} \pm 7.6$  by 4 hours after stress

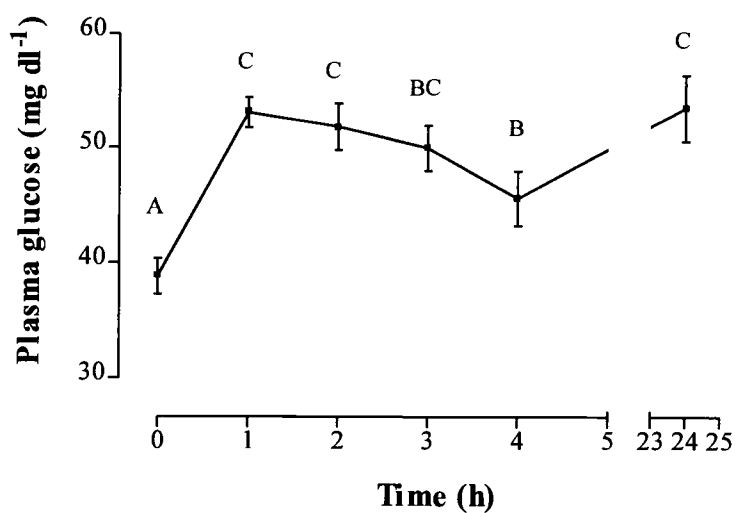


Figure 3.1. Mean plasma levels of glucose from Pacific lampreys at various times after acute stress in experiment I. Each point represents the mean ( $\pm$  SE) of 10 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ).

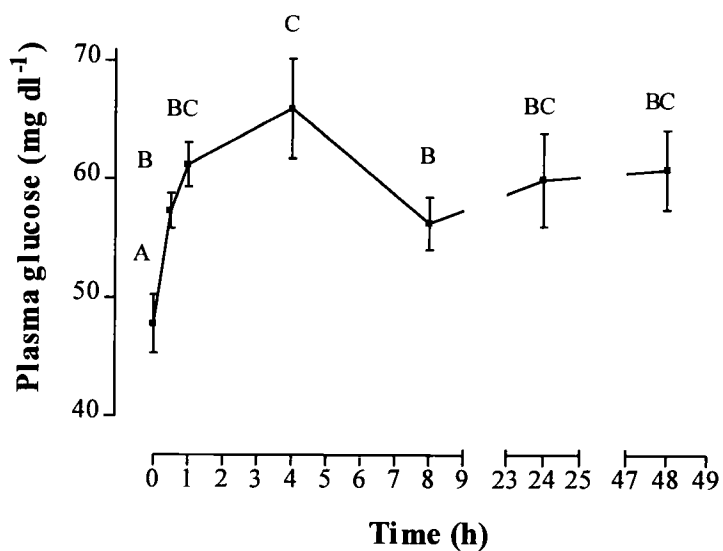


Figure 3.2. Mean plasma levels of glucose from Pacific lampreys at various times after acute stress in experiment II. Each point represents the mean ( $\pm$  SE) of 10 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ).

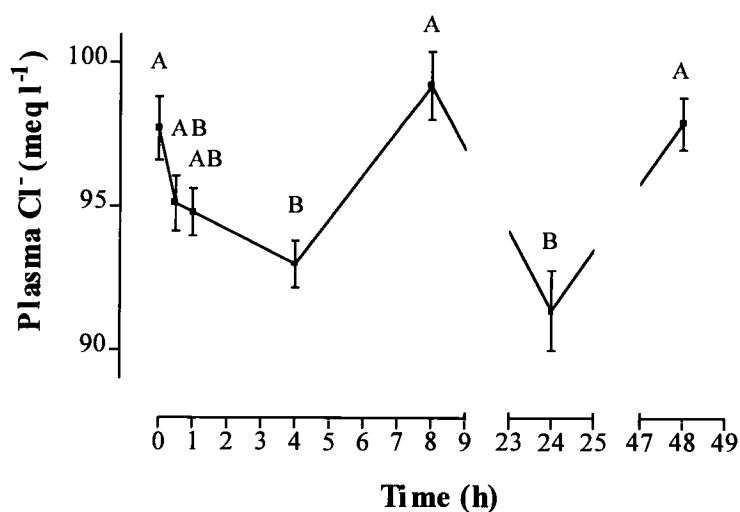


Figure 3.3 Mean plasma levels of chloride ion from Pacific lampreys at various times after acute stress in experiment II. Each point represents the mean ( $\pm$  SE) of 10 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ).

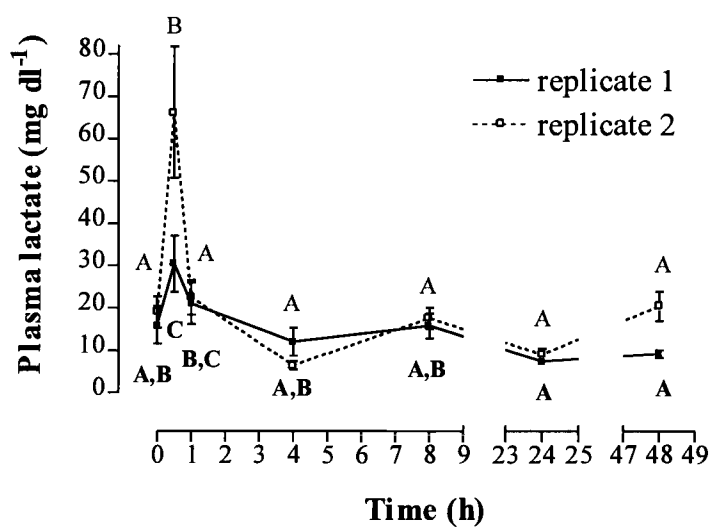


Figure 3.4 Mean plasma levels of lactate from Pacific lampreys at various times after acute stress in experiment II. Each point represents the mean ( $\pm$  SE) of 5 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ). Bold letters represent replicate 1, while regular letters represent replicate 2.



( $P = 0.0004$ ), and remained decreased by at 48 hours (Fig. 3.5). Muscle lactate levels in the second replicate were not significantly different ( $P=0.07$ ) through time; however, the mean followed a similar pattern as the other replicate. In experiment III, mean resting glucose levels increased significantly from  $45.4 \text{ mg dl}^{-1} \pm 1.7$  to  $59.0 \text{ mg dl}^{-1} \pm 1.7$  within 1 hour of applying the stressor (Fig. 3.6). Mean circulating level of glucose at 24 hours ( $52.1 \text{ mg dl}^{-1} \pm 3.1$ ) were not significantly different from the mean resting level ( $P = 0.07$ ); however, mean glucose at 72 hours was significantly higher than the mean at time 0. At 168 hours (7 days), glucose levels were not significantly different than at time 0. In experiment IV, no cortisol was found in fractions collected from HPLC (data not shown; detection limit of assay  $5 \text{ pg/ml}$  plasma). In experiment V, radiolabeled pregnenolone injections into two lampreys, produced compounds with similar retention times as some of the steroids in the standards. Corticosterone, 11-ketoprogesterone, testosterone, progesterone, pregnenolone and two unknown peaks were identified in plasma of lampreys at 30, 60, and 180 minutes after injections (Fig. 3.7). Validation of steroids was done by comparing retention times of known standards listed in Table 3.1.

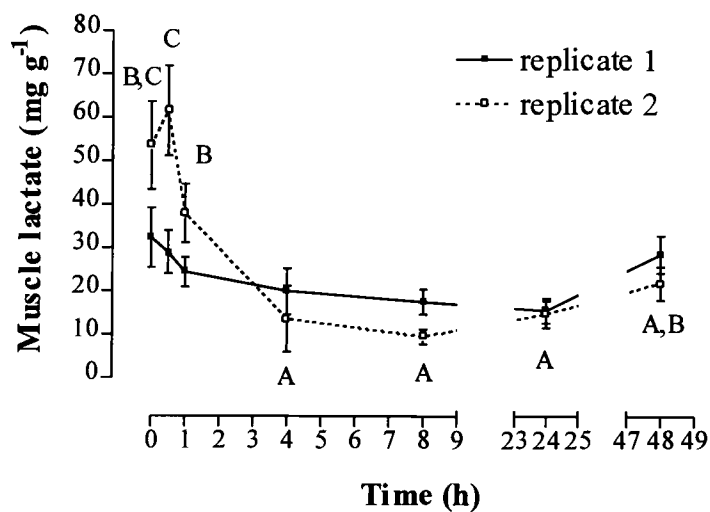


Figure 3.5 Mean muscle lactate levels from Pacific lampreys at various times after acute stress in experiment II. Each point represents the mean ( $\pm$  SE) of 5 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ). Letters represent replicate 2.

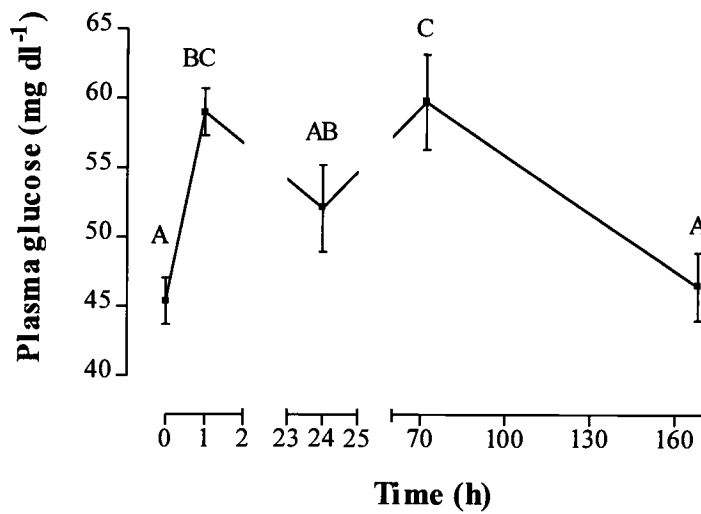


Figure 3.6 Mean plasma levels of glucose from Pacific lampreys at various times after acute stress in experiment III. Each point represents the mean ( $\pm$  SE) of 20 animals. Means without letters in common are significantly different by analysis of variance ( $P < 0.05$ ).

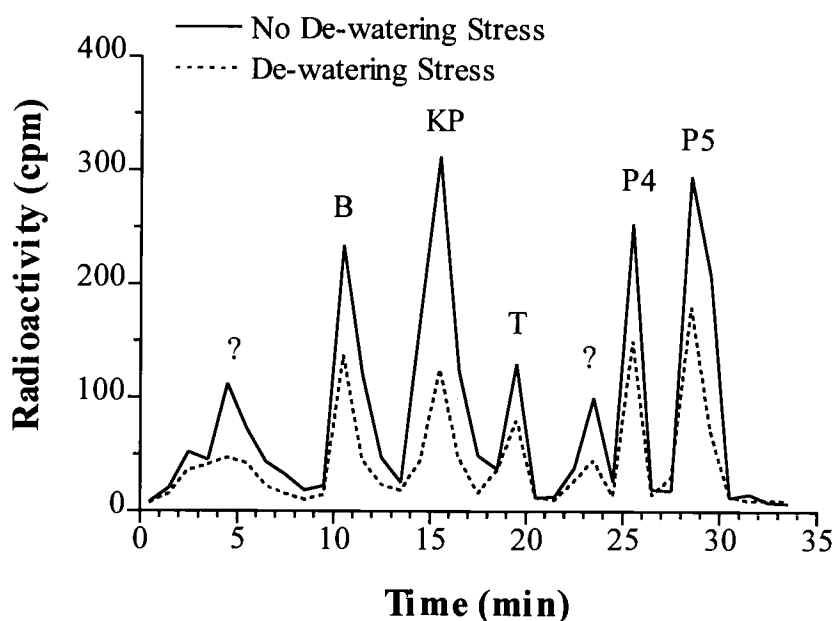


Figure 3.7 High Performance Liquid Chromatogram of radioactivity in plasma 180 minutes after  $^3\text{H}$ -pregnenolone injection in experiment V. Each line represents one lamprey. Each letter represents the following steroids: B=corticosterone, KP=11-ketoprogesterone, T=testosterone, P4=progesterone, P5=pregnenolone, ?=unknown.

## Discussion

The results obtained in this study show that the effects of acute stress on Pacific lampreys can be measured using clinical indicators. Pacific lampreys became hyperglycemic after stress in our studies. Resting levels of plasma glucose became elevated soon after the stressor was applied, then remained elevated for a week. Similarly, Larsen (1976) found hyperglycemia after stress in river lampreys (*Lampetra fluviatillis*) with glucose returning to resting levels after one week. Different mean resting levels of glucose were found in the current experiments conducted in the spring and the fall. The differences may be explained by a natural increase in glucose metabolism during maturation. Larsen (1976) found glucose levels increased as *Lampetra fluviatillis* approached sexual maturity in the spring. Different types of stressors such as dewatering and exercise (Stabrowsky 1967), anesthesia (Larsen 1976), handling (Morris and Islam 1969), and transportation (Leibson and Plisetskaya 1969) can induce hyperglycemia in lampreys. Stress can increase glucose levels for fish such as Atlantic salmon (Wendt and Saunders 1973) and has become a common indicator of stress in fish (Wedemeyer and Yasutake 1977; Wedemeyer et al. 1990). The elevation of glucose for such long periods raises the question of whether glucose is hormonally regulated in these fish. Insulin levels did not increase in *L. fluviatillis* after injecting a glucose load (Plisetskaya and Leibush 1972), nor did *L. fluviatillis* become hyperglycemic from increasing glucagon levels (Leibson and Plisetskaya 1969). It is thought that increased levels of circulating catecholamines stimulate glucogenolysis in fish (Mazeaud and Mazeaud 1981). Plasma levels of epinephrine and norepinephrine

were shown to increase in sea lampreys (Mazeaud 1969), and in rainbow trout (*Oncorhynchus mykiss*) (Gingerich and Drottar 1989; Barton and Iwama 1991) after various stressors. However, Dashow and Epple (1983) found that injections of epinephrine only had an effect on glycemia at superphysiological doses.

Plasma lactate and possibly muscle lactate were shown to have utility as clinical indicators of stress in Pacific lampreys. While plasma lactate levels increased rapidly (by 30 min) and decreased rapidly (by 1 hour), muscle lactate levels decreased slowly through time after acute stress. This may suggest plasma lactate is metabolized quickly, and muscle lactate remains depressed because gluconeogenesis is very efficient at metabolizing lactate from muscle tissue into circulating levels of glucose. However, Wood (1991) argues that lactate is metabolized in white mussel and not released into the blood stream. Lactate levels in fish generally increase rapidly after exercise or stress (Wedemeyer et al. 1984).

Circulating plasma  $\text{Cl}^-$  was shown to decrease through time after stress in lampreys. A similar response was shown by rainbow trout after exercise; however, recovery was stable after 12 hours (Postlethwaite and McDonald 1995). In our study,  $\text{Cl}^-$  levels were shown to recover by 8 hours; however at 24 hours, levels had decreased again before returning to resting by 48 hours. The reason for the second decrease at 24 hours is unknown. We are unsure how stress disrupts ion regulation in lampreys. Although, the loss of ions through the gills (chloride cells) or loss during filtration in the kidneys may be possible (Beamish 1980; Morris 1980). Postlethwaite and McDonald (1995) speculated that increased net influx of water at the gills above the level of urine production might increase extracellular fluid volume and dilute ions. Regardless, our

study has shown that stress alters ion regulation in Pacific lampreys. Disruption of osmoregulatory function by stress is common among other fish (Mazeaud et al. 1977; McDonald and Robinson 1993).

The steroid hormone cortisol becomes elevated after stress in many species of fish (Barton and Iwama 1991) such as salmon (Strange et al. 1978); however, experiments II and IV indicated that stressed Pacific lampreys do not have detectable levels of cortisol. Our results concur with Buus and Larsen (1975), in which they found no detectable levels of cortisol in river lampreys *L. fluviatilis*. However, lampreys injected with radiolabeled pregnenolone appeared to produce a compound with a similar retention time as corticosterone, a stress-related corticosteroid found in other animals. It may be possible that in response to stress, lampreys 1) produce a compound similar to cortisol with no cross-reactivity to the antibody in the assay; 2) have cortisol levels below detection of assay; or 3) corticosteroids are not stress-related steroids in lampreys. Katz et al. (1982) postulates that the role of sex steroids in agnathans may differ from other fish and that androstenedione may be a stress-related hormone in sea lampreys.

Our experiments have shown that Pacific lampreys exhibit a classical stress response similar to other fishes and that clinical indicators such as plasma glucose, lactate,  $\text{Cl}^-$ , and possibly muscle lactate can be measured to assess stress in Pacific lampreys. However, plasma cortisol was not detected in Pacific lampreys.

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## Chapter 4

### The Effects of Intraperitoneally Implanted Radio Transmitters on the Swimming Performance and Physiology of Pacific Lampreys (*Lampetra tridentata*)

by

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## Abstract

The swimming performance and physiological effects of surgical implantation of dummy radio transmitters into the peritoneal cavities of Pacific lampreys, *Lampetra tridentata*, were assessed. Intraperitoneal implantations of 3.4 g transmitters had no significant effect on circulating levels of glucose (an indicator of stress) 4 months after surgery, while 10 gram transmitters caused a significant increase in plasma glucose. Lampreys implanted with 7.4 g transmitters recovered from surgery by day 4 based on levels of plasma glucose. Lampreys implanted intraperitoneally with 7.4 g dummy transmitters showed no significant difference in circulating glucose 30, 60, 90, and 180 days after surgery in comparison to sham-implanted controls. Ventilation rate decreased significantly by 30 minutes after surgery and was stable by 60 minutes; suggesting initial recovery from surgery is rapid. Swimming performance was impaired immediately after surgery; however, swimming was not compromised at 1 and 7 days after surgery. Tagged fish showed a significant difference in oxygen consumption in fish tested immediately after surgery; however, oxygen consumption was at control levels at 1 and 7 days after surgery.

## Introduction

Pacific lampreys (*Lampetra tridentata*) have declined throughout the Columbia River Basin and along the Oregon Coast (see chapter 2). Many factors may have contributed to the decline of Pacific lampreys, including development of hydroelectric projects. Following construction of hydroelectric dams in Finland, European river lamprey (*L. fluviatilis*) populations declined (Tuunainen et al. 1980; Eklund et al. 1983; Ojutkangas et al. 1995). Further, in the 1800's, the decline of sea lampreys (*Petromyzon marinus*), on the east coast of the U.S. was blamed on dams (Goode 1884).

Columbia River hydroelectric projects may be causing migration delays and impeding passage of lampreys to spawning areas in the interior basin. In 1996, the National Marine Fisheries Service (NMFS) started using radio-telemetry to follow movement of lampreys around the Columbia River dams. These studies from 1996 to 1999 found that 59 to 79% of radio-tagged lampreys did not pass Bonneville Dam (pers. comm. M. Mosier, 2000, National Marine Fisheries Service).

One of the assumptions of a radio-telemetry study is that the tagged individuals are representative of the entire population. Specifically, the method should not affect the physiology, behavior, or survival of the fish.

While surgically-implanted transmitters have been evaluated and found to be successful in juvenile Atlantic salmon, *Salmo salar* L. (Moore et al. 1990) and juvenile chinook salmon *Oncorhynchus tshawytscha* (Martinelli et al. 1998), other studies have shown problems with transmitter expulsion in channel catfish *Ictalurus punctatus* (Marty and Summerfelt 1986) and rainbow trout *O. mykiss* (Lucas 1989). In addition, externally attached transmitters on juvenile white sturgeon *Acipenser transmontanus* decreased swimming performance (Counihan and Frost 1999). However, we are unaware of any published literature testing the effects of radio-transmitters on lampreys.

Our objective was to determine the effects of surgically-implanted radio transmitters on the physiology, swimming performance, and survival of Pacific lampreys.

## **Materials and Methods**

Adult Pacific lampreys were obtained from Willamette Falls, Willamette River, Oregon and transported to Oregon State University's Fish Performance and Genetics Laboratory, Corvallis, Oregon. Upon arrival, fish were anesthetized, weighed, measured and marked with passive integrated transponder (PIT) tags for identification. Before the experiments, fish were maintained in flow-through 0.9 m diameter tanks (336 L) supplied by underground water at a temperature of 12-13 °C at least 1 week under natural photoperiod. Dummy transmitters were made by dipping tags into rubber cast, then filling casts with resin (courtesy of Advanced Telemetry Systems, Isanti, Minnesota) used for actual transmitters. Steel split shot was attached to antennae and placed into wet resin within the molds. After drying, the transmitters weighed the same in air as functional tags (3.4 g and 7.4 g). Transmitters were then surgically-implanted into the body cavities of lampreys. The procedure was carried out on 131 adult lampreys.

### *Experimental Design*

In experiment I, adults lampreys ( $n = 5$  fish/treatment/time) were anesthetized and then implanted with a 3.4 g dummy transmitters. Control fish were treated the same as tagged fish, but no incision or sutures were used. At 1, 6, and 24 hours after completion

of surgery, the fish were anesthetized and sampled for blood. In experiment II, adult lampreys ( $n = 4$  fish/tank; 3 tanks/replicate treatment) were either implanted with a 3.4 g tag (tagged), put through implantation surgery but without tag implantation (sham), or anesthetized but otherwise left intact (control). In addition, other lampreys ( $n = 6$  fish/treatment) were implanted with either a 10 gram tag or left intact (control) by John Vella at the National Marine Fisheries Service in Pasco Washington, and then transported back to Oregon State University four weeks after surgery. Fish were sampled for blood at 4 months after completion of surgery.

In experiment III, fish were anesthetized in MS-222 buffered with sodium bicarbonate and implanted with pit tags (passive integrated transponder). Lampreys were distributed to holding tanks to acclimate for 2 weeks ( $n=10$ /tank; 2 tanks/sampling time). Each tank was randomly assigned a treatment: control or tagged, and no individual fish was sampled more than once. Each lamprey was anesthetized and implanted with a 7.4 g tag into the body cavity. Control fish were handled the same as tagged fish, except no surgery or tag implantation was performed. At 3, 24, and 96 hours after completion of surgery, the fish were anesthetized and sampled for blood. Lampreys were then maintained in tanks and sampled for blood at 30, 60, 90 and 180 days after surgery. In experiment IV, swimming performance of radio-tagged individuals ( $n=47$ , mean length 60.1 cm with a 95% confidence interval from 59.8 cm to 60.3 cm) were tested at 1, 24, and 168 hours after surgery. Eight control and eight tagged lampreys (except for seven tagged during 168 hour test) at each time were tested individually. Adult lampreys were anesthetized in MS-222 buffered with sodium bicarbonate and then surgically implanted

with the 7.4 g tag. Fish were acclimated in the flume one hour before starting the flow of water.

### *Surgical Procedure*

Fish were netted from tanks and anesthetized by immersion in tricaine methansulfonate (200 mg/L buffered with sodium bicarbonate). Fish were placed in a PVC pipe with a sealed T end. A portion of the pipe was cut away to allow room for surgery. A peristaltic pump added anesthetic solution to the pipe sufficient to submerge the head and gill pouches during surgery. Fish were laid ventral side up with towel underneath the fish to prevent slippage. An incision was made ventrally along the center of the body. The dummy transmitters, previously sterilized in ethanol, were inserted through the incision. The antennae was guided through a catheter starting at the incision through the muscle tissue and exiting through the skin 15 mm below the incision. The incision was treated with oxytetracycline and closed with four single stitches using cat gut sutures. The time of the surgical procedure was ~5 minutes (3.4 g tags) and ~10 minutes (7.4 g tags). The fish were transferred to recovery tanks until sampling or placed into flume for the swimming assessment. In addition to the tagged (3.4 g) group, twelve fish underwent surgery as previously described but did not receive implants (shams). All other fish in the experiments that had no operation were held as controls.



### *Swimming Performance*

Ventilation rate was counted (beats/min) at 5, 30, and 60 minutes after placement in the flume. The flume was lined with a high-density polyethylene aqua-net grid. The lining in the flume prevented the lamprey from attaching on the walls of the flume. After an hour, the flow was turned on and the lampreys were acclimated to swimming for 10 minutes (5 minutes each at both 20 and 30 cm/sec). After swimming acclimation, the flow was increased to 40 cm/sec and swimming time measured. An electrical current (12 volts) was applied to keep the lampreys off the back screen. Lampreys were considered exhausted when the animal could not avoid the back screen. After one hour of swimming or at exhaustion, the test was ended. The lamprey were then taken out of the flume and placed into a respirometer. The dissolved oxygen levels were recorded at 5 and 30 minutes after swimming exhaustion for all fish.

### *Sampling*

Each lamprey was anesthetized in tricane methansulfonate (MS-222) at 80 mg/L buffered with sodium bicarbonate and then a blood sample was collected from the caudal vein with a vacutainer needle. The plasma was separated by centrifugation and stored for analysis at -80°C.

Plasma samples were analyzed for glucose by colorimetric assay (Wedemeyer and Yasutake 1977). Observations of ventilation rate (beats/min) were recorded during the acclimation in flume. Time to swimming exhaustion was recorded by use of a stop

watch. Time spent on the back screen was subtracted from total swimming time.

Oxygen consumption after swimming performance was determined by containing fish in a respirometer and measuring dissolved oxygen with a YSI Dissolved Oxygen meter (Cech 1990).

### *Statistical Analysis*

In experiments I and II, plasma levels of glucose were compared by a one way analysis of variance (ANOVA) followed by multiple range testing using Newman-Keuls method. In experiment III, plasma levels of glucose were compared by two way ANOVA followed by one way ANOVA with multiple range testing using Newman-Keuls method. In experiment IV, plasma levels of glucose were compared by repeated measures ANOVA followed by multiple range testing using Newman-Keuls method. In experiment V.a, ventilation rates were compared by repeated measures ANOVA followed by multiple range testing using Newman-Keuls method. In experiment V.b, swimming time was compared by the nonparametric tool Mann-Whitney U test. In experiment V.c, oxygen consumption levels were compared using a two-tailed unpaired t-test. The significance levels were set at  $p \leq 0.05$  for all statistical tests.

### **Results**

In experiment I, plasma glucose levels at 1, 6, 24 hours after surgery did not differ significantly between control and 3.4 g tagged adult lampreys (Fig. 4.1).

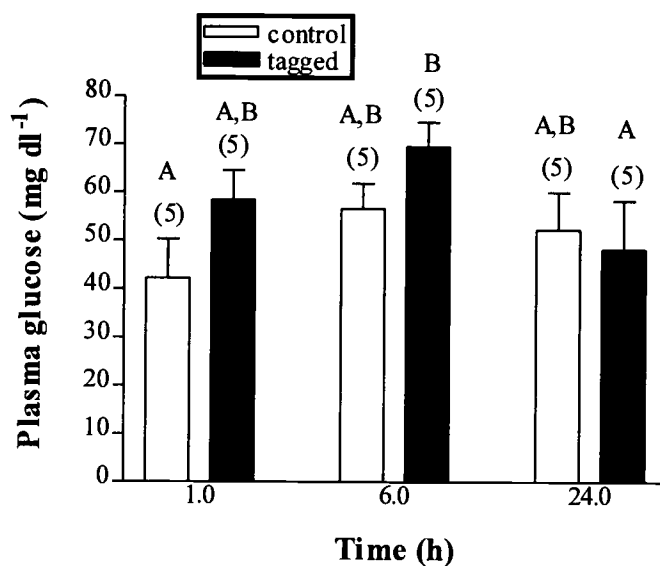


Figure 4.1. Mean plasma glucose levels for control and surgically implanted adult Pacific lampreys with 3.4 gram dummy transmitters. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by analysis of variance ( $p < 0.05$ ).

In experiment II, plasma glucose levels at 4 months post-surgery were not significantly different between control, 3.4 g tagged, and sham fish; however, lampreys implanted with 10 g tags had significantly higher glucose levels ( $p=0.01$ ) than controls (Fig 4.2).

In experiment III, mean plasma glucose levels between control and tagged fish were significantly different ( $p=0.0001$ ). In addition, a sex effect was shown to be significant ( $p=0.0001$ ). Therefore, we analyzed males and females separately. Mean plasma glucose levels in male control fish ( $55.7 \text{ mg dl}^{-1} \pm 3.7$ ) ( $n=11$ ) at 3 hours, were significantly lower ( $p=0.001$ ) than those in male tagged fish ( $79.6 \text{ mg dl}^{-1} \pm 5.4$ ) ( $n=9$ ). By 24 hours, mean glucose levels in control males ( $52.5 \text{ mg dl}^{-1} \pm 2.0$ ) ( $n=11$ ), were still significantly different ( $p=0.01$ ) than male tagged fish ( $70.4 \text{ mg dl}^{-1} \pm 3.9$ ) ( $n=10$ ). However, by 96 hours there was no difference in plasma glucose between control and tagged males.

Mean plasma glucose levels in female control fish ( $44.6 \text{ mg dl}^{-1} \pm 1.5$ ) ( $n=9$ ) at 3 hours were significantly lower ( $p=0.001$ ) than those in female tagged fish ( $72.1 \text{ mg dl}^{-1} \pm 5.6$ ) ( $n=10$ ). By 24 hours, mean glucose levels in control females ( $44.9 \text{ mg dl}^{-1} \pm 2.1$ ) ( $n=8$ ), were still significantly different ( $p=0.05$ ) from female tagged fish ( $61.2 \text{ mg dl}^{-1} \pm 4.9$ ) ( $n=10$ ). After 96 hours, there was no difference in glucose levels between control and tagged females (Fig. 4.3).

In experiment IV, plasma glucose levels at 30, 60, 90 and 180 days were not significantly different ( $p>0.05$ ) between control and tagged fish. However, there was a significant sex effect ( $p=0.005$ ) and overall time effect ( $p=0.0001$ ). Plasma glucose levels increased

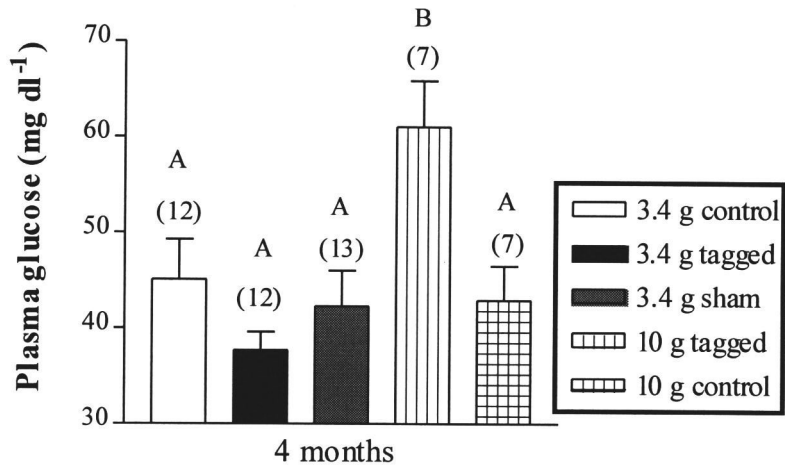


Figure 4.2. Mean plasma glucose levels for control, sham, and surgically implanted adult Pacific lampreys with 3.4 and 10.0 gram dummy transmitters 4 months after implantation. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by analysis of variance and unpaired t-test ( $p < 0.05$ ).

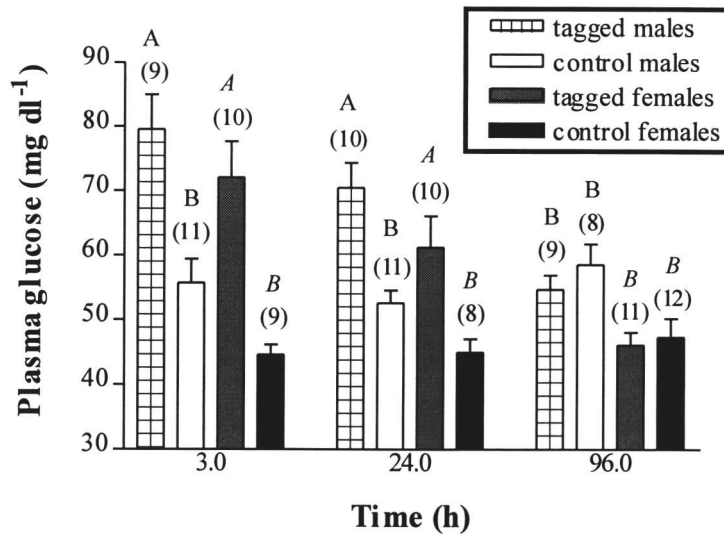


Figure 4.3. Mean plasma glucose levels for control and surgically implanted adult Pacific lampreys with 7.4 gram dummy transmitters 3, 24, and 96 hours after implantation. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by analysis of variance ( $p < 0.05$ ).

significantly ( $p=0.0001$ ) from 30 days to 60 days and from 90 days to 180 days ( $p=0.0058$ ) after surgeries. There was no significant increase or decrease from 60 to 90 days after surgeries ( $p=0.82$ ) (Fig. 4.4). Control and tagged lampreys appeared to sexually mature, developing secondary sexual characteristics (21.6 % of control ( $n=13$ ) and tagged ( $n=13$ ) lampreys became fully mature with loose eggs or flowing milt by 3/21/98).

In experiment V.a, ventilation rates decreased significantly ( $p<0.05$ ) from 5 to 30 minutes in both control and tagged fish at 1, 24, and 168 hours after tagging. In addition, ventilation rate did not differ significantly ( $p>0.05$ ) from 30 to 60 minutes between control and tagged fish at 1 and 24 hours; however, tagged fish at 168 hours did show a significant decrease ( $P=0.04$ ) from 30 to 60 minutes. Ventilation rates compared between control and tagged fish at 5, 30, and 60 minutes did not differ significantly in 1, 24, and 168 hours after surgeries (Fig. 4.5).

In experiment V.b, swimming performance measured in terms of duration was significantly lower ( $p=0.04$ ) in tagged lampreys at 1 hour after surgery in comparison to control fish. Swim time was not significantly different between control and tagged lampreys at 24 and 168 hours after surgeries (Fig. 4.6).

In experiment V.c, oxygen consumption was significantly lower ( $p=0.04$ ) in tagged lampreys 1 hour after surgery in comparison to control fish; however, there was no difference between control and tagged lampreys 24 and 168 hours after surgery (Fig. 4.7).

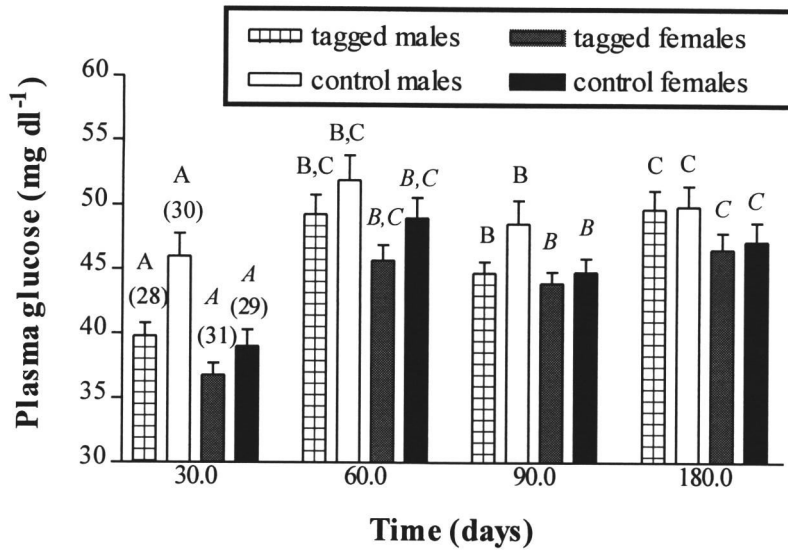


Figure 4.4. Mean plasma glucose levels for control and surgically implanted adult male and female Pacific lampreys with 7.4 gram dummy transmitters 30, 60, 90, and 180 days after implantation. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by repeated measures analysis of variance ( $p < 0.05$ ).



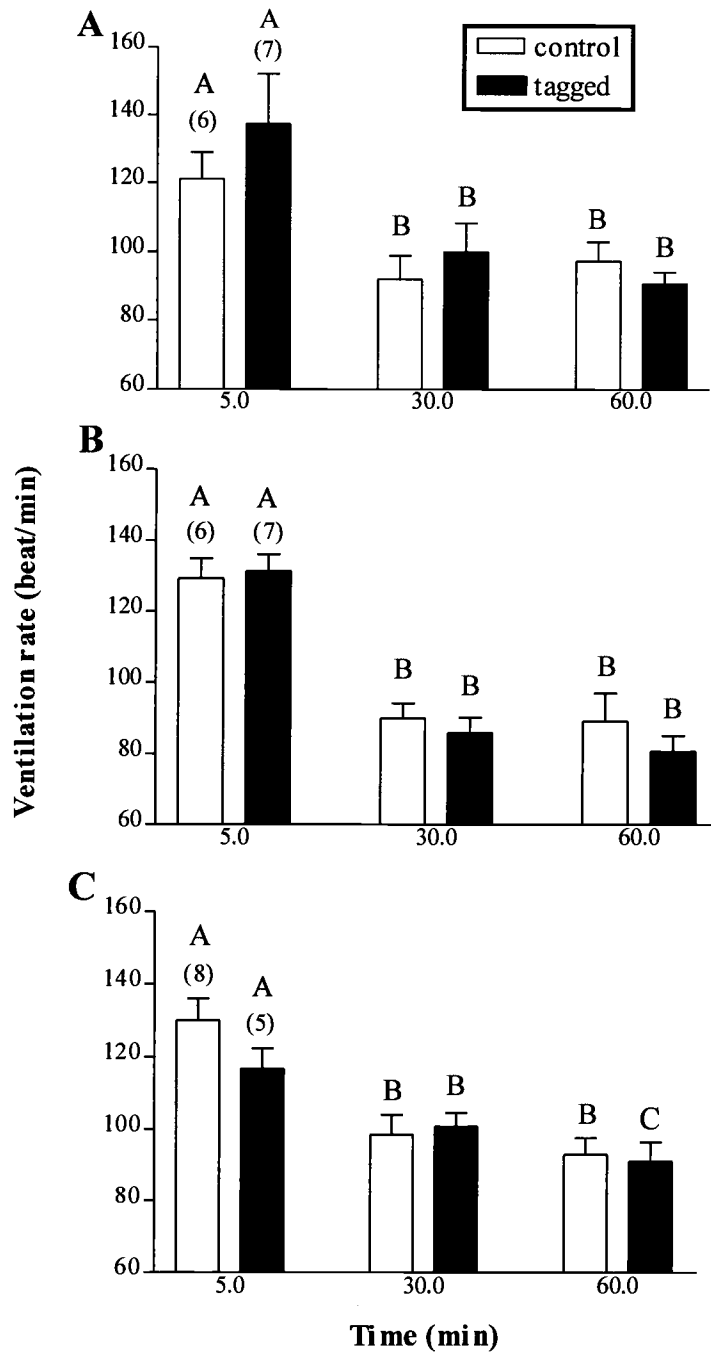


Figure 4.5. Mean ventilation rate of control and surgically implanted adult Pacific lampreys with 7.4 gram dummy transmitters after recovery from surgical anesthesia. **A**, **B**, and **C** represent groups of fish tested at 1, 24, and 168 hours after surgery. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by repeated measures analysis of variance ( $p < 0.05$ ).

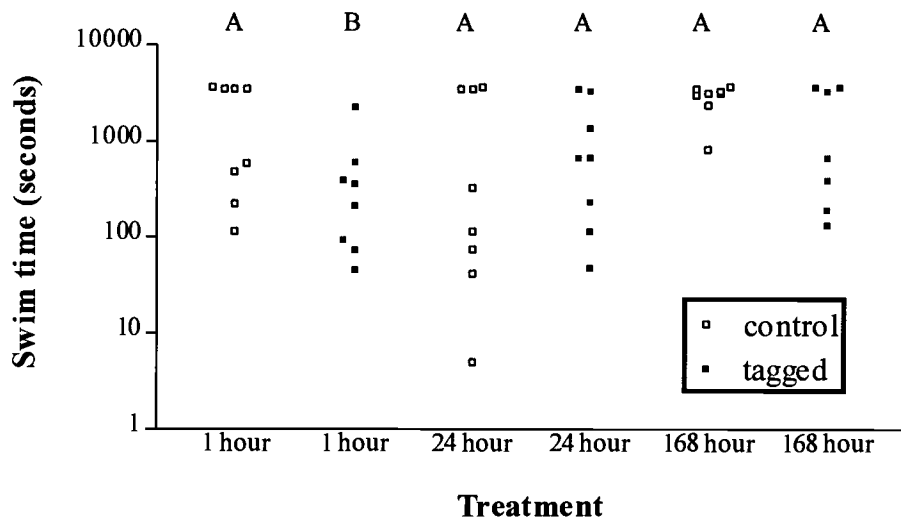


Figure 4.6. Individual swim times of control and surgically implanted adult Pacific lampreys with 7.4 gram dummy transmitters. Each point represents an individual fish swim time for 1, 24, and 168 hours after surgery. Treatments without letters in common are significantly different by Mann-Whitney U tests ( $p < 0.05$ ) within treatments.

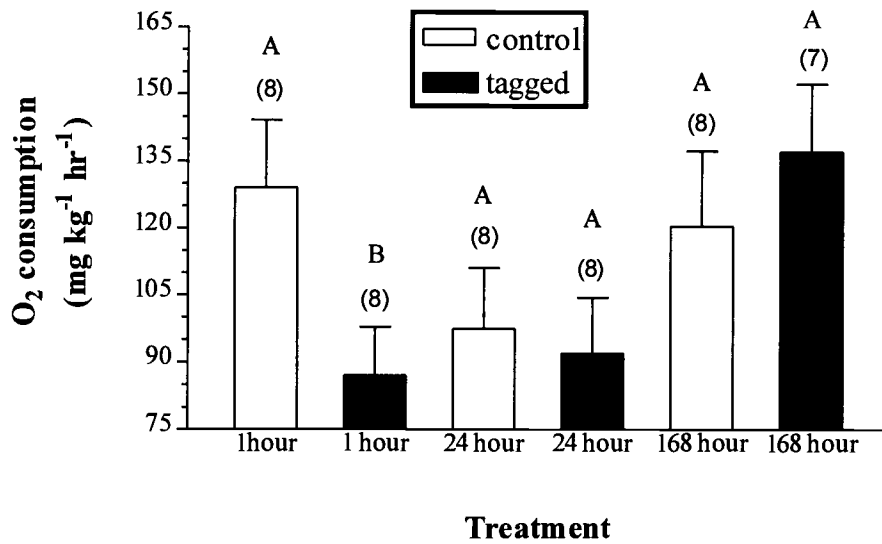


Figure 4.7. Mean O<sub>2</sub> consumption of control and surgically implanted adult Pacific lampreys with 7.4 gram dummy transmitters after recovery of swim performance test. Bars represent the mean, error bars are the standard error of the mean, and sample size is noted in parentheses above the bar. Bars without letters in common are significantly different by unpaired t-tests ( $p < 0.05$ ) within treatments.

## Discussion

Although there was 100% survival of surgically implanted adult lampreys during our study, 10.0 gram tags appeared to stress (as indicated by glucose levels) our study animals 4 months after surgeries. We found no difference between 3.4 g tagged and control lampreys in glucose levels during recovery and no chronic effect measured by plasma glucose at 4 months after surgeries. The 7.4 gram tagged fish did not recover from the stress of surgery and implantation until day 4. In addition, we found no evidence indicating chronic stress in lampreys implanted with 7.4 gram tags during the 6 months of monitoring plasma glucose levels. We found no tag expulsions in any of the study animals; however, necropsies did show clear tissue encapsulating the transmitters at 4 and 6 months. A similar response has been shown in surgically implanted Atlantic salmon (Moore et al. 1990).

Ventilation rate appeared stable by one hour, indicating some recovery before swimming performance assay. This one hour acclimation period is comparable to what NMFS uses on lampreys implanted with transmitters at Columbia River dams. Other research has shown ventilation rate decreases rapidly after handling and surgery. Moore et al. (1990) found tagged and sham juvenile Atlantic salmon increased opercular rate after surgeries; however, both groups returned to basal levels by 60 minutes.

The swimming performance tests of radio-tagged lampreys with 7.4 gram tags suggest that there is an immediate impact on Pacific lampreys, but the effects are reduced by 24 hours. Unfortunately due to time constraints, we did not conduct swimming performance tests on fish beyond one week.

Oxygen consumption after swimming performance suggested recovery was faster in control compared to tagged lampreys. There was no difference in oxygen consumption between control and tagged lampreys at 24 and 168 hours after surgery suggesting recovery in tagged fish.

Even though swimming performance was shown to recover by 24 hours, glucose did not recover to control levels until day 4; therefore, based on glucose levels, we suggest holding tagged lampreys a minimum of 4 days. It should be noted that in all tagged groups of fish during our experiments, glucose levels decreased slightly below control levels. However, the levels were not significantly different ( $p=0.054$ ) in 7.4 gram tagged fish at 30, 60, 90, and 180 days after surgery. We also found a sex- and time-effect in glucose levels. Glucose levels increased in tagged and control lampreys through time as fish matured. These finding also agree with previous studies with river lampreys in Europe (Larsen 1976). Our study suggests 3.4 and 7.4 gram tagged lampreys perform equally to controls. Therefore, inferences can be made to the larger free swimming lampreys in the Columbia River.

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## Chapter 5

### Summary

This thesis compiles what is known about the life history of Pacific lampreys. Where information was missing, we used information from other species of lampreys. Data collected in the form of window counts at dams suggests Pacific lampreys have declined over the last 30 years in the Columbia River Basin. Declines are thought to be primarily caused by migration barriers such as hydroelectric dams. However, there may be other factors impacting lampreys such as poor ocean conditions, prey base depletion, and freshwater habitat degradation. This review also suggests that Pacific lampreys are an important part of the food web in the Columbia River Basin. While it is recognized that Pacific lampreys are predators on fish during their parasitic phase, many animals feed on lampreys of various life stages. Native Americans have expressed concerns regarding the declines of lampreys in the Columbia River Basin and in the Siletz River on the Pacific coast. We suggest that fisheries agencies and the public have not been concerned about the declines due to the cultural biases toward the species. However, they are an important subsistence fish to Pacific Northwest tribes and ecologically important to the rivers.

This thesis also suggests that clinical indicators of stress typically used in other fish can be applied to Pacific lampreys with the exception of the steroid cortisol. These baseline data can be used to assess impacts of surgically implanted radio transmitters on Pacific lampreys. Furthermore, we demonstrated that 3.4 and 7.4 gram radio transmitters



are acceptable for use in Pacific lampreys, however, precautions should be taken to hold the fish until they have adequately recovered from implantation.

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