From: Heppell, Scott [mailto:scott.heppell@oregonstate.edu]
Sent: Thursday, August 16, 2007 3:17 PM
To: Bao Le
Subject: RE: radio and balloon tag technology for Pacific lamprey macrophthalmia passage and survival

Hello Bao-

I'm not sure I'm going to be able to help you here, but I'll try. I'm not sure what a Hi-Z balloon tag is. Can you tell me in what document the Pers. Comm. appeared? I did do some work a few years ago investigating whether we could use harmonic resonance tags to detect movement of lamprey through dams, but they didn't quite have the range we wanted. We also tried externally affixed radio tags to lamprey outmigrants, but they kept tying themselves in knots and pulling the tags off. I have attached a draft of the report that we wrote, as I can't find the final copy. I'm sorry I can't be more encouraging than that right now, but please let me know if I can answer any other questions.

Cheers-
Scott

From: Bao Le [mailto:baol@dcpud.org]
Sent: Wednesday, August 15, 2007 4:58 PM
To: Heppell, Scott
Subject: radio and balloon tag technology for Pacific lamprey macrophthalmia passage and survival

Hi Scott, I am currently engaged in the development of assessments for Pacific lamprey here at Wells Dam and had seen a pers. comm.. by you that said that Hi-Z balloon tagging coupled with radio-tag technology could be used for juvenile lamprey for route specific dam passage studies. I wanted to try to get more information regarding the available technology, it's feasibility towards implementation in the near future, and whether anyone has utilized it to assess route passage and survival for juvenile migrating Pacific lamprey. I was unaware that we have come this far with such radio-tag technology that would provide us with a tag that did not affect behavior or swimming capability. If this is the case, and the technology is available, this is very exciting. If you could please provide me with more information regarding my request, I would really appreciate it. Please feel free to give me a call if you have any questions.

Regards, Bao

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DETERMINATION OF PASSAGE OF JUVENILE LAMPREY: DEVELOPMENT OF A TAGGING PROTOCOL

BPS-P-0015-b

Draft Completion Report

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EXECUTIVE SUMMARY

- The smallest available radio tag is too large for implantation in the body cavity. External application is feasible, but the animals remove the tags within the first week and fish performance is affected to some degree A reduction in the size of current radio-tag technology is limited in part by current battery size technology. Exploration of methods for reduction in battery size were unsuccessful, and the smallest transmitters constructed are no longer being manufactured. This renders this technology unusable for implantation in juvenile lamprey at this time.

- Harmonic radar, a promising technology for tracking small terrestrial organisms, becomes non-functional in water due to rapid attenuation of signal strength. While it has been used to track small invertebrates at ranges of up to 800 meters in air, range is limited to 20-25 cm in water.

- At this time, there appears to be few viable options to internally implanted PIT tags for the tracking of juvenile lamprey around USACE projects. While there are severe limitations to this technology, including limited range and the ability to detect lamprey only where receivers are set up and not throughout the movement range of the lamprey, it is the only technology that is suitable to the combined small size and morphology of juvenile lamprey.

- Suitable fungicides have been identified for the long-term care and maintenance of juvenile lamprey in the laboratory. Protocols for their use are critical to future research on juvenile lamprey.

- Continued exploration into the physiology and behavior of juvenile outmigrant lamprey is critical to understanding the processes and events that may affect mortality during their passage from freshwater to saltwater. In addition, telemetry technologies should continue to be monitored in order to identify those which may be applicable to tracking juvenile lamprey through USACE hydropower projects.

OBJECTIVES

The goal of this study was to investigate a means whereby the passage of post-metamorphic Pacific lamprey (*Lampetra tridentata*) through Columbia River dams could be evaluated. Investigations were initiated because of concerns that hydroelectric power plants along the Columbia river are having an adverse impact on Pacific lamprey populations. Of particular concern were potentially undesirable levels of mortality among juveniles as they leave their natal streams and head to sea. Our efforts concentrated on development of a methodology for tagging fish such that tag recoveries or recaptures (through remote and/or physical sampling) would yield useful information regarding lamprey passage, behavior, and routes through dams and under various dam operation procedures. This information could then be utilized to assess potential problem areas (*e.g.*, physical attributes) of Columbia River hydroelectric projects that may reduce successful migration of juvenile lamprey. The ultimate application would be to facilitate successful migration of these juveniles by avoiding mortality associated with dam passage, such as that due to impingement on screens.

Specific objectives were as follows:

1. Evaluate extant tagging technologies for application on outmigrant lamprey.
    a. Determine characteristics of all tags currently available. This includes conducting a thorough literature review to identify tags successfully employed with other species having a similar morphology as the lamprey and gleaning

information from vendors regarding dimensions and applications of their products.

   b. Determine the characteristics of a tag that specifically relate to effective use at Columbia River dams. This includes assessment of data “recapture” feasibility.
   c. Investigate the feasibility of custom manufacture of batteries suitable for implantation.

2. Investigate new technologies (harmonic radar) that may be employed to track lamprey around Columbia River dams, including evaluation of the maximum detectable range of this technology used in water conditions similar to that found in the Columbia in the vicinity of the John Day dam.

3. Determine the characteristics of a tag and tagging procedures that can be applied without affecting migratory behavior of outmigrant lamprey.
   a. Conduct laboratory tests of swimming performance and behavior to assess candidate tag(s). This includes evaluation of effects of tags on the overall health and well-being of the animals.
   b. Evaluate the relative effectiveness (affects on behavior, tag retention time) of different tag implantation sites.
   c. Conduct field trials at John Day Dam (or other recommended dam) to assess effects of fish-diverting screens and other potential impediments. This includes:

1. Tagging procedures
2. Study design (e.g., how and where to release the fish)
3. “Recapture” methodology and gear placement
4. Robustness of the data to allow for statistical interpretation

4. Identify fungicides that can be used to treat infected juvenile lamprey while they are being held under laboratory conditions, and develop protocols for their use.

INTRODUCTION

Lamprey populations are experiencing a precipitous decline in abundance throughout the Columbia basin. While very little is known about the migratory biology of post-metamorphic lamprey, successful outmigration is dependent on safe passage through dams. To date, the only engineering or management measures aimed at making downstream passage better for fish has been targeted specifically at salmonids. It is not known where major bottlenecks for lamprey passage are in hydropower systems, but diversion screens designed explicitly for salmonids are one potential source of direct or indirect mortality in hydropower systems.

Because of this there is pressing need to develop a means whereby outmigrant lamprey can be studied at Columbia River projects. Two issues appear paramount. One is developing a method of "visualizing" fish in the vicinity of and within projects; hence the need for an appropriate tagging system. Second, a study design for answering critical questions needs to be developed that considers the constraints imposed by physical and water management aspects of Columbia dams.

Recovery of declining pacific lamprey populations is dependent on identification of sources of mortality. While the decline of lamprey is widespread throughout the Pacific Northwest, it is of great concern in the Columbia River system (Close *et al*., 1995). The sharply downward trend in counts of lamprey passing Columbia River dams is shown in the *Status Report* for this species written by our group (Close et al., 1995); recent numbers are alarmingly low. There is a paucity of information on dam-related mortality for this species. For example, it is not known if, or which, elements of the hydropower system are particularly lethal or debilitating to outmigrants or returning adults.

Lamprey have a life history cycle somewhat akin to salmonids (see Close et al., 1995, for a complete literature review), and hence one can postulate that these fish can be affected to a certain extent where salmonids are affected. While little is known about the migratory biology of lamprey, there is anecdotal information that bar screens could have a major impact on downstream passage.

Migration to the ocean appears to primarily be in the spring (Beamish and Levings, 1991); March to June is when outmigrants arrive in collection facilities at John Day and Bonneville Dams (Hawkes et al., 1991, 1992). This is a time when dams must also be managed for salmonids, primarily spring chinook smolts but others as well. Flow can apparently affect migration (Applegate, 1950; Potter, 1980; Beamish and Levings, 1991), which could relate lamprey passage to dam operation. It is possible that downstream migration is passive (i.e., the fish may not actively swim downstream) (Applegate, 1950), and thus water management or movement at dams can potentially have a major effect on where and how fish pass through a project. For example, perhaps only 10% of the outmigrant lamprey use the smolt bypass facilities of Columbia dams (Bill Muir, cited in Close, 1995). Further, it has been suggested that most migrating lamprey may enter turbine intakes at their lower end (Long, 1968) and are hence not diverted into bypass systems. Since it is possible for some post-metamorphic lamprey to remain in fresh water for up to 10 months (Beamish, 1980), they could be vulnerable to mortality factors for time frames well beyond what we are used to thinking about with salmonids.

We must obtain information on the general nature of lamprey and their migratory behavior at dams if this species is going to be managed towards recovery. This necessitates development of innovative approaches to learn about the species.

METHODS AND RESULTS

Animal collection and husbandry

All animals used in this study were collected from the smolt monitoring and bypass system at John Day dam. Animals were collected during normal bypass operations by Pacific States Marine Fisheries Commission personnel, and held in a communal tank with flow-through river water until a sufficient number (>200) had accumulated. This process usually took 7-10 days. Animals were then transported in river water supplemented with oxygen to the Fish Performance and Genetics Laboratory (Smith Farm) at Oregon State University, where all experimental procedures were conducted. A total of approximately 1015 animals were collected and transported over the two-year period. Collections for 1999 occurred on three dates: May 5 (218 fish), June 8 (250 fish), and 6 Aug (32 fish). Collections for 2000 occurred on two dates: April 19, (200 fish) and May 3 (315 fish). Fish at FPGL were held in 1 meter circular tanks with flow-through water at a temperature of 12^{o} C +/- 1^{o} C and maintained under a natural photoperiod. All animals were anesthetized (250 mg MS 222 • L $^{-1}$, pH 7.0) prior to experimental procedures.

Objective 1: Evaluate extant tagging technologies for application on outmigrant lamprey.

Several tag properties have been identified as desirable for use in tracking the movements of outmigrant juvenile lamprey. The tags must be small, as juvenile lamprey are only 3-5 grams and 100-160 mm total length during their migratory phase. In addition to a small overall size, juvenile lamprey have a relatively small body cavity, which severely restricts internal tag implantation. A detection range on the order of at least several meters is desirable, so that animals can be monitored as they move from a release point above the dam, through the pool, and into the various passage points around the dam. Finally, a tag life of at least 4-6 days was deemed necessary in order to allow tracking of juveniles from a release point 2-3 km above the dam through the dam itself.

We determined characteristics of tags currently available or those contemplated by vendors to be available in the near future that have potential for application in juvenile lamprey. A thorough review of the literature was conducted to determine if there are any products or concepts available with which we are not already familiar. It is remotely possible that someone had a system that could work in lamprey but that was developed for some other animal such as small eels, snakes or perhaps even annelids. For example, there has been work with telemetry of eels (Baras and Jeandran, 1998; Baras et al. 1998). We contacted all vendors of tags (including those having tags requiring direct sampling or those allowing for remote sensing) used in aquatic or terrestrial organisms to determine if tag characteristics and the sensing characteristics could be used for our application. Physical characteristics of the tags and their life expectancy and durability were assessed relative to their functionality in lamprey and characteristics of tag recovery or sensing technology were assessed relative to constraints of Columbia dams. How the tags must be applied to the fish and numbers of fish necessary was also assessed relative to potential study designs for the Columbia.

We constructed a matrix of tag types including functional and mechanical variables in order to provide a readily accessible means of comparing potential advantages and disadvantages of various tag types. Tag variables of interest include size, weight, shape, broadcast distance, durability, performance life, tagging procedure, and sources that could limit or interfere with tag data “recovery”. Few manufacturers specify the broadcast distance of their tags. Those companies that do declare a specific broadcast distance have, in many instances, either tested tag detection in air or under ideal laboratory conditions. Realistically, the broadcast distance of a tag varies with depth under water, temperature, weather conditions, and mode of detection (*e.g.*, from land, from water, or remote sensing). Therefore, any practical determination of broadcast distance must be tested under the conditions in which the work will be conducted. Hence we have not listed broadcast distance within the variables considered in the matrix (appendix A-1).

Concurrently, we assessed the Columbia dam and reservoir system, using John Day Dam, to identify potentially relevant fish tagging and fish release requirements with respect to the use of radio-tags and PIT-tags. Dam operation and physical aspects needing assessment were established by working with USACE and others familiar with the system.

Radio Telemetry

We have spoken with, or investigated the products of, what we believe to be every company manufacturing radio-, acoustic-, or PIT-tags in the world (see appendices A-1 and A-2 for specific company information). The smallest tag produced by the majority of these companies was many times too large for this application. We inquired of all companies whether they foresaw or had an interest in developing smaller tags. These companies concur that small tag size is limited by reductions in battery size.

The smallest radio tag currently available is produced by Titley Electronics PTY LTD from Ballina, Australia. This tag weighs approximately 400 mg in air and its dimensions are 12.5mm X 6mm X 2mm, and has a maximum lifetime of 12 days. Although we made several attempts to surgically implant this radio-tag, combined with the morphology of outmigrant pacific lamprey, the size of this tag prevents an internal application even in the largest of the animals we received this year.

We conducted several experiments examining the method and location for an external attachment. A frequently used method of attachment is by non-absorbable suture. We conducted a series of experiments attaching the tag by suture (Ethicon 5.0 non-absorbable), at two anchor points, from the attachment loop at the battery end of the tag in addition to a suture loop around the base of the tag where the antenna exits the transmitter. In an effort to avoid potential lateral bias (left or right) we attached the tag just below the last gill pore on the animals dorsum. This position however allowed the fish to remove the tag within several hours post-tagging. Several more attempts were made to attach the tag dorasally, but at various locations toward the caudal fins. Each of these experiments resulted in tag removal within several hours post-tagging. The best success, as measured by the amount of time it takes the animals to remove the tag, has been obtained by mounting the tag approximately mid body and laterally on the fish. In an experiment using 45 animals nearly 75% of the tags remained attached by the third day post-tagging, however, 100% were detached by day 15 (Fig. 1). It was thought that a more elastic suture might prevent or reduce the frequency of tag removal, although no significant differences were found among three types of suture (as measured by time to removal).

Three other, non-dermal sites were also considered for tag attachment: the first dorsal fin, the second dorsal fin, and the ventral surface of the caudal fin. For each attachment site, four lamprey were fitted with dummy transmitters designed to simulate the Titley tag in size and weight. Lamprey were anesthetized using MS-222 and each tag was attached using a single suture point. Animals were allowed to recover in 10 liters of fresh water, then transferred to 15 liter circular tanks with flow through water (12^{o} C), and observed for 1 hour post-treatment, and twice daily for 10 days following tag attachment. Two animals, (ventral dermal attachment, first dorsal attachment) demonstrated the characteristic twisting motion common to animals responding to external irritants within 15 minutes of recovery from anesthesia. This behavior continued throughout the initial one hour observation period. Neither of the other two animals (second dorsal attachment, caudal attachment) demonstrated this behavior at any observed time. By six hours post attachment, the animal with the tag attached to its ventral surface had succeeded in removing its tag. By 24 hours post-attachment, the lamprey with the caudal fin tag had also removed its tag. Neither of the two animals with tags attached to the dorsal fins succeeded in removing their tags by the end of 10 days.

Based on these initial results, an expanded experiment was conducted investigating the attachment of external transmitters to the fins of juvenile lamprey. During this round of experiments, the effects of water current were incorporated to determine if behavior would be altered under a simulated stream environment. Nine pairs of lamprey were anesthetized, and one animal from each pair was fitted with a transmitter attached to the second dorsal fin. Animals were then transferred to a raceway in which a submersible pump had been placed in order to create a water current. Behavior was observed for one hour following placement of lamprey in the tank, and was scheduled to be observed twice daily for the proceeding 10 days.

Control animals displayed behavior typical to that observed in animals held in communal tanks, where they remained attached (held fast) to the tank walls just below the waterline or at the at the tank edge/tank bottom interface. Tagged animals, on the other hand, remained on the bottom of the tank and exhibited the twisting behavior characteristic of lamprey trying to remove an external irritant. This behavior was evident immediately upon recovery from the anesthetic, and continued unabated until each animal had removed its tag. By 24 hours post-attachment, all tagged animals had removed their tags, and were exhibiting behavior similar to that of the control lamprey. The experiment was discontinued at this time.

Because battery size and shape is a major limiting component to the reduction in overall transmitter size, effort was put forth to locate a commercial, academic, or private interest capable of manufacturing a battery smaller than the currently smallest available 1.5V hearing aid battery, which weighs approximately 250 mg. In addition, it is also desirable to have the shape of the battery altered, from a disc or "button" type battery commonly used in hearing aids, watches, etc., to a more cylindrical type (like an "AA" battery), which would fit better in an elongated body cavity. Inquiries to multiple "custom" battery manufacturers met with no success. There were no manufacturers interested in or capable making a battery of our required specifications, and without a market larger than that needed to support lamprey research, it is unlikely that any manufacturer will put forth an effort to reduce battery size and alter battery shape in the near future. This does not preclude this event, but demand must be great enough to make the process economically viable to the manufacturer. Minimum estimates on order size to entice a major manufacturer to begin production of a custom sized battery were on the order of 10^6 pieces. Inquiries to electrical engineering faculty at Oregon State University met with a similar fate. At best estimate, according to one contact, the battery technology we desire is at least several years out.

PIT-tags

Another approach to monitoring migratory behavior is the use of PIT tags. PIT tags are small enough for internal implantation (8 mm X 2 mm X 2 mm) in juvenile lamprey. In an initial experiment, 45 juvenile lamprey (each group) were either implanted with PIT tags using a single scalpel-incision, given an incision only (sham) or left intact (control). Approximately 25% of both the PIT-tagged and sham animals died by day 6 post-tagging; there was no mortality over this period in the control group (Fig. 2). This evidence suggests that this lethality was not due to the mere presence of the tag but rather the surgery methodology itself. In a second experiment 36 animals were either PIT-tagged using the single incision method described above, but with the use of a dissecting microscope to avoid damaging the digestive tract and given oxygen enriched recovery, or left intact. The results of this experiment reduced mortality by 10% over the same period (Fig. 2). Regression analysis of the length and weight data from the previous two experiments indicates that 99% of the mortality in both trials was those animals that fell below 150 mm in length (Fig 3). In a third experiment 35 animals were selected for large size (150 mm total length) and either PIT-tagged by the method in the second experiment described above or left intact. These results suggest that nearly 100% survival can be obtained by PIT-tagging animals with total lengths greater than 150 mm. (Fig 2). Forty-five days post-implantation 90% of the juvenile lamprey selected for size greater than 150 mm in length survived (Fig 4). We believe that the 10% mortality found might have been confounded by problems associated with aquatic fungus, which will be discussed below.

Other potential tracking methods

We are exploring several other methods that may prove to be applicable to reaching the goals (or some aspects) of this project. Tagging juvenile lamprey with color or luminescent tags may provide a method for visualizing fish passage with the use of video cameras, some of which could be installed on the bar screens at John Day Dam. The smallest florescent light stick available (Cyalume®, Omniglow Corporation, West Springfield, MA.) is 38 mm X 5 mm. X 5 mm (length, width, height) and lasts approximately 4 hours. Longer lasting sticks exist (12 hours) and may have potential for use in smaller capsules. While existing light stick sizes are far too large for implantation, reductions in size and the relationship between size and longevity needs to be investigated. Another light marking method we are recently exploring is Photonic Marking™ (NEWWEST Technologies, Santa Rosa, CA). Photonic Marking™ is a minimally invasive, externally visible marking technology that uses fluorescent microspheres for individual identification. Current drawbacks to utilizing this technology include the relatively short life of the tag and the need for a specific scanning laser or UV lamp for detection. Obviously any method using videography or manual counting methods to record data can often be extremely demanding of time and labor.

<u>Objective 2:</u> Determine the characteristics of a tag and tagging procedures that can be applied without affecting migratory behavior of outmigrant lamprey.

We determined the potential effects of tagging lamprey with candidate tags at our Fish Performance and Genetics Laboratory. Functional radio-tags and dummy radio-tags that simulate candidate tags in physical characteristics were used. In addition, we tested the potential effects of implantation of PIT-tags. We made these assessments at various times after tagging to ensure that there is no drop-off in performance of the fish with time. Additionally we field tested the effects of radio tags on lamprey migration in the Columbia River at John Day Dam

Experimental Swim Tube

We designed an experimental chamber that allowed us to subject juvenile lamprey to flows ranging from 0.5 ft.• sec^{-1} to 5.5 ft.• sec^{-1}. The swim tube consists of three-3' sections of 2" inside diameter, clear, schedule 40 PVC pipe; two feet of 2" diameter schedule 40 white PVC pipe; three-2" diameter aluminum cam-locks; six-2" diameter slip-by-thread PVC bushings; three-2" diameter perforated 30 gage stainless steel screens; five-45° slip-by-slip couplers; a Leader Ecosub 420A 68 gpm submersible pump; and a variable speed motor control. Water velocity was determined for different voltage settings on the variable speed control. Output over time at the discharge end of the swim tube was measured, and flow was calculated based on the cross-sectional area of the pipe. Each section of the swim tube consists of a single 3' length of clear PVC with a slip-by-thread PVC bushing on either end. Attached to either bushing is a male or female component of the cam-lock. At the downstream end of each section a stainless steel screen is attached with silicone caulking. Sections can be coupled to create either a single or multiple chamber tube. The 45° slip-by-slip couplers and white PVC is used at the pump and at the end to maintain head pressure. The 2" inside diameter of the clear pipe was large enough to allow complete side-to-side undulations of the juvenile lamprey, and therefore should not have interfered with their swimming ability.

Swimming Behavior

We experimented with several types of screening within our swim tube to prevent lamprey from holding fast to the tube. Researchers with Pacific States Marine Fisheries Comission, employees of the USACE at John Day Dam and researchers with the Pacific Northwest National Laboratory provided us with various types of mesh screening typically used on the bar screens in the gatewells at both John Day and McNary dams. Moderate success was obtained using a 1/8” square plastic mesh; however, the swimming performance of juvenile lamprey was extremely variable and thus prevented any useful interpretation or analysis.

We dedicated many nighttime hours to video taping lamprey in the swim tube using standard video camera equipment and miniature closed circuit infrared cameras on loan from **e**cological **p**lanning and **t**oxicology inc., Corvallis, Oregon. Additionally we designed and conducted several experiments at night examining the effects of disturbance (light, noise, and water flow changes) on triggering movement or swimming behavior of lamprey. Juvenile lamprey did not exhibit any regular patterns of movement in either the provoked or unprovoked condition. Hence we were not able to develop a standard or regular behavioral assay based on swimming performance in which to compare tagged and untagged fish.

Finally we developed methodology to assess the effects of external attachment of radio-tags and implantation of PIT-tags in juvenile lamprey by allowing the animals to attach to the experimental chamber, rather than trying to force them to swim. In separate experiments, radio-tagged and PIT-tagged juvenile lamprey were subjected to flows similar to what they may encounter in the Columbia River and around Columbia River hydroelectric projects. For the radio-tag experiment 6 animals were tagged as described previously and along with 6 untagged fish were placed into one of three chambers (2 tagged and 2 control fish per chamber) of the swim tube previously described. Animals were allowed to attach or "hold fast" to the side of the tube at which time flows were gradually (within 1 minute) increased to 2.5 ft./sec. Animals were then monitored by video for a twenty-four hour period and time of detachment was recorded. By 12 hours 100% of the radio-tagged animals had been detached, forced to the end of each experimental tube, and impinged on the screening. Untagged fish remained attached by 24 hours post-treatment (Table 1).

PIT-tagged fish exhibited similar behavior to controls in the swim tube attachment experiment. Eight PIT-tagged and 8 untagged fish were subjected to the same experiment described above. By 24 hours post-treatment all animals (tagged and untagged) remained attached (Table 1)

Field Work

A pilot field test was conducted at John Day Dam in early August 1999, during which time radio tag receiver aerials maintained by USGS-BRD Columbia River Research Laboratory, Cook, WA were operational. Unfortunately underwater antenna arrays were not available at that time. First we validated the range and range at depth of the Titley tag and compared this to the range of a commonly used salmonid smolt tag (Advanced Telemetry Systems Inc.) in the Willamette and Columbia Rivers. Slightly greater distance was achieved during the Willamette River trial (data not shown) at the zero-depth level (just below the water surface). The ATS tag was nearly twice the size of the Titley tag by weight, hence we would expect the slightly reduced range exhibited by the Titley tag (Fig 5). In separate trials on the same day for each river, each tag was attached to the end of a weighted rope, which was marked in meters and half meters. One researcher, stationed on the bank of the river, incrementally lowered the tag as a second researcher stationed on a prop-boat listened for the tag and recorded "hits" by depth and distance from the source. Distance was measured using the differential Global Positioning System mounted on the boat (Fig. 5).

The field trial was conducted utilizing 5 radio-tagged lamprey and all of the antenna/receivers positioned at the powerhouse, spillway, and navigation lock on the forebay side of the dam. Juvenile lamprey were "heard" for a maximum of 30 seconds after releases approximately 1 km up river from the dam. We downloaded data from the remote receivers every two days for 12 days (Maximum expected life of the tags was 10-12 days). Unfortunately these fish were not "heard" by the aerial antennas as they presumably passed through the dam. When considered with tag range data, these results suggest that juvenile lamprey migrate at depths below 7 meters in the water column, if the fish did indeed migrate to the dam (Fig 5).

Aquatic Fungus

Approximately 5% of the animals received on 5 May, 1999 had visible signs of fungal infection. The number of individuals infected and mortalities (confirmed to be associated with fungal infection by Richard Holt, senior fish pathologist, ODFW) rose steadily over the following few weeks. Greater than 25% of the animals received on 8 June exhibited visible signs of fungal infection. These animals were held separate from the first collection and their health declined rapidly during the ensuing weeks. Handling animals appeared to adversely effected them and made them therefore more susceptible to fungal infection. All animals were placed on a treatment regime of 167 mg• L^{-1} Formalin, 3 times weekly for 15 minutes in static water. Initially formalin treatment appeared to slow the progression of the aquatic fungus, however there were periodic bouts of mortalities throughout the season, especially among animals collected on 8 June. It is clear that this issue needed to be dealt with in a systematic manner if long-term experimentation on juvenile lamprey were to be conducted in the future.

Objective 3: Investigate new technologies (harmonic radar)

Harmonic Radar

Another potential candidate for tracking juvenile lamprey is harmonic radar. Harmonic radar has been utilized to track bees, butterflies, moths, caddis flies, and snakes (Engelstoft *et al.*, 1999; Mascanzoni and Wallin, 1986; Riley *et al.*, 1996; Roland, *et al.*, 1996). We have been investigating the possible use of this microwave technology for tracking juvenile lamprey. The “tag” is comprised of a diode and wire antenna weighing approximately 0.04g. A signal is transmitted from a transmitter/receiver unit. Upon reaching the target, the diode is activated, thus doubling the frequency, which is received back at the transmitter/receiver unit. Current transmitter/receiver technology operates at a frequency of 917 MHz and receives at a frequency of 1834 MHz. At present, this technology does not allow for individual identification. Three-dimensional position of animals is determined by triangulation of the signal.

Under these conditions signals can be detected at a maximum depth under water of 25 cm (personal communication, J.H.R. Gee, Institute of Biological Sciences, The University of Wales). Several researchers have investigated the effects of attenuation and absorption of discrete microwave frequencies in the atmosphere due to water vapor, rain, and other forms of precipitation (Kerr, D. E., 1951; Yasmin, K. and Armstrong, R. L., 1990), in liquid media in the laboratory (Yiannis *et al.*, 1984), and the propagation of low level frequencies in sea water (King and Wu, 1993). Currently it is unknown what ranges of detection at depth in fresh or seawater may be possible with alternative frequencies within the microwave range. If current technology permits, we hope to explore the use of various frequencies in bodies of water at depth. It may be that this information is known, but is sensitive and protected by the U.S or other military organizations as it has application with regard to underwater navigational radar. We have contacted RECCO AB in Lindig ¯Sweden, the manufacturer of the transmitter/receiver unit currently used by other researchers. They have forwarded our inquiry regarding attenuation of microwaves in water to an engineer in Sweden who we are currently working with and exploring possible methods to obtain our objectives. Current options include assembling an inexpensive method of frequency delivery if we can network with an appropriate engineer, or purchasing/renting equipment.

The final technology we investigated was harmonic radar (for which preliminary investigations began in FY 1999), which has proven to be useful in tracking small terrestrial organisms over large distances. Harmonic radar is a passive/active system like PIT tags. A passive diode is affixed to the animal, and a radar transmitter-receiver unit probes for the tag. Unlike normal radar, which would detect any reflective object in the path of the transmission beam, harmonic radar works on the principle that the wave reflected by the diode reports back at exactly twice the frequency of the transmission beam. A version of the harmonic radar system is what is used for merchandise security in shops, where a small magnetic strip attached to an item will activate a transmitter/receiver unit at the entrance to the store if the diode has not been previously deactivated.

The primary advantage to harmonic radar is the small size of the tag itself, approximately 40 mg. In terrestrial systems, harmonic radar has been used to track free-ranging bees, moths, beetles, and butterflies over a range of hundreds of meters. This system appeared ideally suited for the tracking small aquatic organisms, including juvenile lamprey. Unfortunately, the physical properties of water reduce the transmission range of the microwave generator from hundreds of meters to several centimeters. Roy Llewellan (a private consultant in Portland, OR) was hired to perform computer simulations of harmonic radar transmission frequencies through water, given the conductance of Columbia river water at John Day dam, while manipulating the potential ouptut of the device, antenna length, and signal frequency. The theoretical range determined through Mr. Llewallan's simulations was 25 cm in water, well below the 800 meter detection range that has been reported for terrestrial objects. This is on order with the figure quoted (20 cm) by a customer representative at Recco AB (Switzerland), the manufacturer of the transmitter/receiver unit. Therefore, harmonic radar represented little improvement over that of PIT tags or visual marks.

Objective 4: Identify fungicides that can be used to treat infected juvenile lamprey while they are being held under laboratory conditions, and develop protocols for their use.

During FY 1999 studies it was observed that juvenile lamprey are extremely prone to fungal infection when held in the laboratory, and initial treatments with formalin were done in order to mitigate this problem. Because future studies into tagging, physiology, and behavior depend on the ability to house animals long-term under laboratory conditions, an investigation into suitable fungicides was conducted.

Experimental setup

In consultation with Dr. Rich Holt, Oregon Department of Fish and Wildlife/Oregon State University Fish Pathology Laboratory, the following compounds were selected for evaluation: formalin (37% stock), hydrogen peroxide (30% stock), Aquamedix PX-700 (an experimental algicide that had demonstrated antifungal properties, Aquamedix Co., Ft. Lauderdale, FL), and salt (Instant ocean). Formalin was used at a dose of 1:6000, a standard dose used for the treatment of other fishes. Similarly, H_2O_2 was administered at a standard concentration of 1:3500. Aquamedix was used at two doses, 1:500 and 1:2000, based on recommendations from the manufacturer. Instant Ocean was mixed up to concentrations of 0.35%, 1%, and 3.5%, the latter value simulating full strength seawater.

Two sets of 15 liter tanks were plumbed with flow through water. Both sets were maintained at an ambient well-water temperature of 12^{o} C (+/- 1^{o} C), until after addition of experimental animals, when one set was warmed over a two hour period and maintained at an elevated temperature of 22^{o} C (+/- 1^{o} C) throughout the experiment. Ten lamprey, five each from the first collection and the second collection dates, were randomly distributed to each of the 15 tanks in the cold-water bank and the 15 tanks in the warm water bank (300 animals total). Animals were allowed to acclimate to the tanks for two days prior to the onset of treatment.

Experiments

In experiment 1 three tanks in each set were randomly assigned to each of the following treatments: formalin, hydrogen peroxide, Aquamedix high dose, Aquamedix low dose, and control. Following acclimation of animals to the tanks, treatments were administered in static water for one hour every other day for 21 days. Mortalities were removed each day and tallied.

Hydrogen peroxide proved to be acutely toxic within 24 hours at the dose administered to animals in the warm water tanks . These animals were replaced, and each of the three replicate tanks was assigned to one of the following reduced dose treatments: 1:7000 (1/2 dose), 1:14,000 (1/4 dose), and 1:28,000 (1/8 dose). Mortalities did not differ among the three different warm-water hydrogen peroxide treatments throughout the remainder of the experiment, and data were therefore pooled from these three tanks for analysis.

Cumulative mortality for this experiment is shown in figure 6. Mortality was low overall in the cold-water treatment, although it was highest for Aquamedix treated animals. The majority of mortalities occurred within the first nine days of the experiment. Mortality was substantially higher in the warm-water treatment, with an associated increase in the amount of fungus observed. Mortalities were highest for control animals and animals treated with the low dose of Aquamedix, followed by the high dose of Aquamedix, formalin, and finally hydrogen peroxide. Similar to that observed for the cold-water treatment, the majority of mortalities occurred within the first nine days of the experiment.

The second experiment used the same experimental set-up, but was run only at the warmer temperature and focused on three different salt treatment doses, formalin, and control tanks. As before, all tanks were assigned randomly, but due to limitations in the number of animals, two animals from the first collection and eight animals from the second collection were added to each tank. In addition, control tanks and formalin tanks were limited to two replicates each.

Mortality was low overall for the second experiment, and therefore our ability to judge salt as an effective antifungal agent was impaired. This may have been due to one of several factors. One possibility is that the animals obtained from the stock tank which had been maintained in the laboratory for several weeks and had been treated on a regular basis with formalin. Therefore, those animals prone to fungal infection had either already been eliminated from the population or cured of their infections. Another possibility for low overall mortality may be due to the difference in time taken to warm the tanks between the first and second experiment. In the first experiment, water temperature was elevated over a 2 hour time period, while in this experiment water temperature was elevated over a longer (24 hour) time period.

DISCUSSION

Telemetry

We have determined that the maximal size for an implantable telemetry tag for juvenile pacific lamprey is the size of a miniature PIT tag (see appendix A-1 for specifications). Mortality associated with implantation can be significantly reduced by improved surgical techniques and the use of animals greater than 150 mm in total length. External application of the smallest radio-tag currently available may be able to provide some information about migratory behavior through hydroelectric projects on the Columbia River if combined with detection technology at depth. Reductions in the size of radio-tags would be of great benefit, especially if the size could be reduced to that of a PIT-tag or smaller.

Important information about migratory patterns of fish has been gained through the use of PIT tags in salmonids. The findings of our laboratory and researchers at USGS-BRD Columbia River Research Laboratory, Cook, WA indicate that the potential exists for these tags to be used to obtain similar information for migratory lamprey in the Columbia River and other river systems. However, the range of readability for PIT tag detectors is limited. Advancements in the range at which PIT tags can be detected would greatly enhance the value of this information. Availability of sufficient numbers of animals to gain an accurate estimate of survival may also be a limiting factor.

At this time, there appears to be few viable options to internally implanted PIT tags for the tracking of juvenile lamprey around USACE projects. While there are limitations to this technology, including a short detection range and the fact that lamprey can only be detected where receivers are set up and not throughout their free-movement range, it is the only technology at this time that is suitable to the combined small size and morphology of juvenile lamprey. It is deemed preferable to visual marks because it does not require constant human monitoring for passage of lamprey, as the PIT tag system is already automated. PIT tags are preferable to radio tags because they can be implanted and therefore they do not affect the behavior of the animal to the same extent that an external tag will affect behavior, and because they cannot be removed by the animal. It is our intent to continue to monitor technology advances and explore other options for the tagging of these animals, with the intent that when such technology is identified or developed, we will be poised to initiate research to characterize the behavior and movement patterns of juvenile outmigrant lamprey.

Analysis of fish performance is desirable to insure that the implantation or external attachment of telemetry devices does not alter the natural migratory behavior of outmigrant lamprey. To accurately assess the effects of tagging on animal performance and behavior it is necessary to design behavioral assays such that treated animals may be compared to untreated animals. Developing methods for assessing swimming performance of juvenile lamprey has proved to be a difficult task.

Respirometry is typically used to assess changes in environmental conditions or physiological well being. Measuring respiration in fishes can reveal important information about recent activity, acclimation to changing environments, and stress (Cech, 1990). Very low levels of oxygen uptake and wide ranges of variability among untreated animals (Close, unpublished data and this study) have confounded the use of this technique for measuring activity in lamprey.

We explored differences in lamprey performance and behavior both during the day and at night. The literature suggests that juvenile lamprey exhibit most of their downstream movement during the night (Long, 1968), while we detected no consistent diel pattern of movement. These results led us to conduct the “attachment” experiments described previously, where we determined that juvenile lamprey (tagged or untagged) prefer to hold fast rather than continually move around their environment.

A comparison of the effects of tagging on salmonids and juvenile lamprey yields contrasting results. We have found that lamprey withstand longer periods of exposure out of water and require anesthesia doses five times that of salmonids. However, we have also found that juvenile lamprey are highly compromised by handling necessary for tagging; leaving them vulnerable to disease problems associated with aquatic fungi. The results of this tagging-stress may indicate that stresses associated with downstream migration through hydroelectric facilities on the Columbia River may compromise the immune systems of post-metamorphic outmigrant lamprey. In fact, it is possible that studies of any sort that necessitate handling of these animals could be compromised if they subsequently become diseased. This could be a “generic” difficulty inherent in work with this species.

Fungus

Previously, we have found that malachite green, formalin, glutaraldehyde, and hydrogen peroxide effectively controls mortality due to fungus in salmonids (Fitzpatrick *et al.*, 1995). Salt has also been suggested as a potential suppressor of fungal growth. The U.S. Food and Drug Administration (FDA) revoked its limited permit for use of malachite green in 1991. Testing for glutaraldehyde ceased due to extensive testing requirements by the FDA because of potential toxicity problems for mammals (Marking *et al.*, 1994). Presently formalin is the only registered aquatic fungicide (for salmonids and esocids only) and during the FY99 phase of this project we have found that it effectively reduces fungi-induced mortality in juvenile lamprey in the laboratory, at least over short periods. However, there are concerns regarding user safety and effluent effects on the environment (Fitzpatrick *et al.*, 1995). Prevention or control of fungal infection is possible. Suitable, FDA acceptable, disease prophylaxis and treatment must be developed. Any effective approach to tagging and tracking juvenile lamprey, or studies involving capture and/or handling of these fishes, will need to consider the use of an anti-fungal agent prior to and post-handling.

Based on our experiments, hydrogen peroxide appears to be the best fungicide for use on juvenile lamprey, provided the dose is regulated. Formalin was also effective, although not quite to the degree of hydrogen peroxide, and there is potentially less operator and environmental risk in the use of hydrogen peroxide. Aquamedix was nominally viable as a treatment, but would not be an ideal choice due to the volume and cost of material used. At the high dose, which demonstrated some anti-fungal effects, 675 ml were used to treat three 15-liter tanks each week. At a cost of $10/liter, this results in a weekly cost for anti-fungal treatment of $6.75 to treat 45 liters of water containing 30 animals. Because of the low overall mortality, it was difficult to discern a treatment effect of salt as a fungicide. However, anecdotal observations were that juvenile lamprey react vigorously to the intrusion of salt into their tank, and that the degree of their reaction coincided with the concentration of salt added. This may preclude salt from being a viable fungicide for these animals. All animals appeared to return to typical behavior within the one hour treatment time, although there may have still been some osmoregulatory stresses that were not observed.

We now have the ability to hold animals long-term in a laboratory environment, which will greatly facilitate future work on the physiology and behavior of outmigrant juvenile lamprey in the Columbia River basin.

There are numerous questions that remain regarding juvenile lamprey behavior, migration, and survival that ultimately are important. We do not know the success rate of lamprey migration to the estuary in natural or altered systems. And, ultimately, we do not know the return rates (*i.e.*, juvenile to adult escapement) of lampreys in natural or altered systems. Explicit design of the field aspects of this study is dependent on the nature of the tag(s) deemed appropriate. Different tags (*e.g.*, radio-tags, harmonic radar, PIT-tags) could be used to address different questions.

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FIGURE and TABLE LEGENDS

Figures

1. Cumulative percentage of radio-tags removed by juvenile lamprey. Lamprey were externally tagged with radio-tag dummies that were the same weight, shape and density as real tags. Tags were attached by suture mid-body and lateral on the fish. All of the tags were removed by day 15 post-tagging.

2. Cumulative percent mortality for three PIT tagging procedures. Animals were PIT-tagged using a scalpel incision only, using a scalpel incision under a dissecting microscope and provided and oxygen enriched recovery, or selected for size greater than 150 mm and tagged using the scope + O_2 method. Fourteen days post-tagging, the size biased group had fewer mortalities.

3. Regression analysis of body size for PIT-tagged lamprey. Plotted is mass and total length of lamprey subjected to the scalpel only tag implant or implant under dissecting scope and held for two weeks. Closed circles represent survivors; open squares represent mortalities. There was no mortality in animals greater than 150 mm in total length.

4. Long term cumulative percent mortality of PIT-tagged juvenile lamprey. Animals were PIT-tagged using a scalpel incision only, using a scalpel incision under a dissecting microscope and provided and oxygen enriched recovery, or selected for size greater than 150 mm and tagged using the scope + O_2 method and then held for 45 days. Animals selected for greater size exhibited 90% survival over this period.

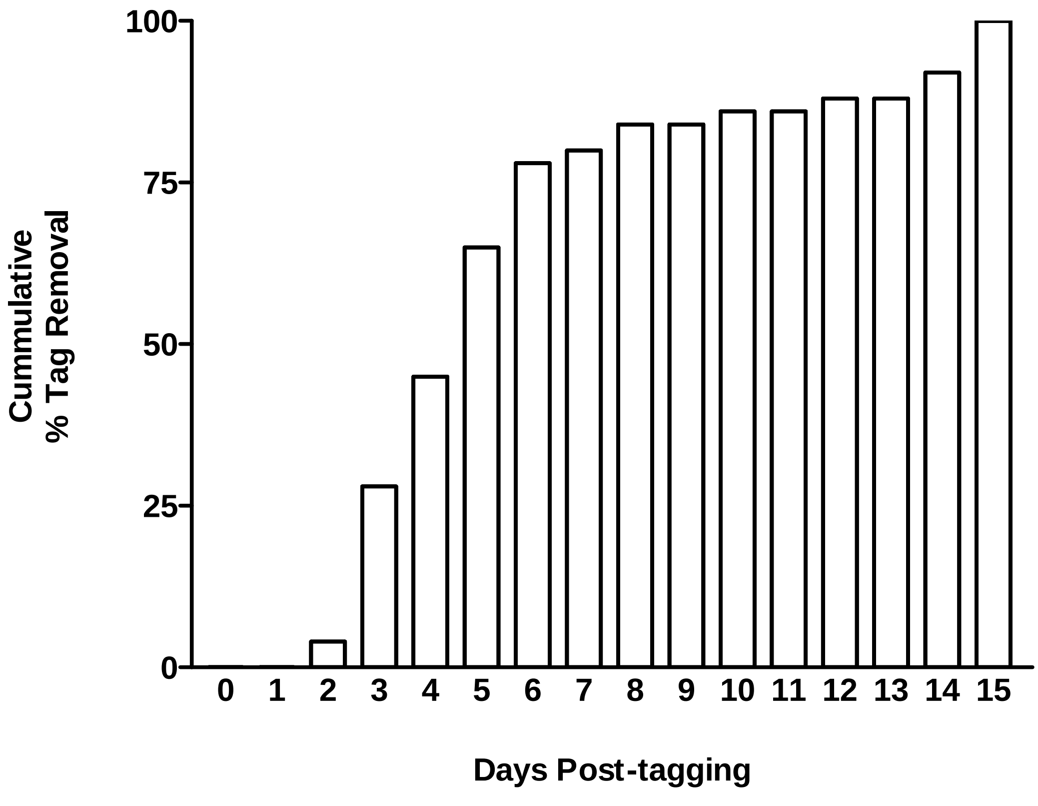
5. Range parameters for two radio tags tested in the Columbia River above John Day Dam on 6 August 1999. A miniature tag (0.4g in air) produced by Titley electronics was compared to a typical smolt tag (1.0 g, ATS). Smaller tag size did result in slighter shorter range parameters and battery life.

6. Figure 1. Cumulative mortality over the 21 day experimental period for juvenile lamprey held at 12^o C (upper panel) and 22^o C (lower panel) and treated with either formalin (1:6000), H_2O_2 (1:3500 in 12^o C treatment, 1:7,000, 14,000, or 28,000 in the 22^o C treatment), Aquamedix (1:500 or 1:2000), or control.

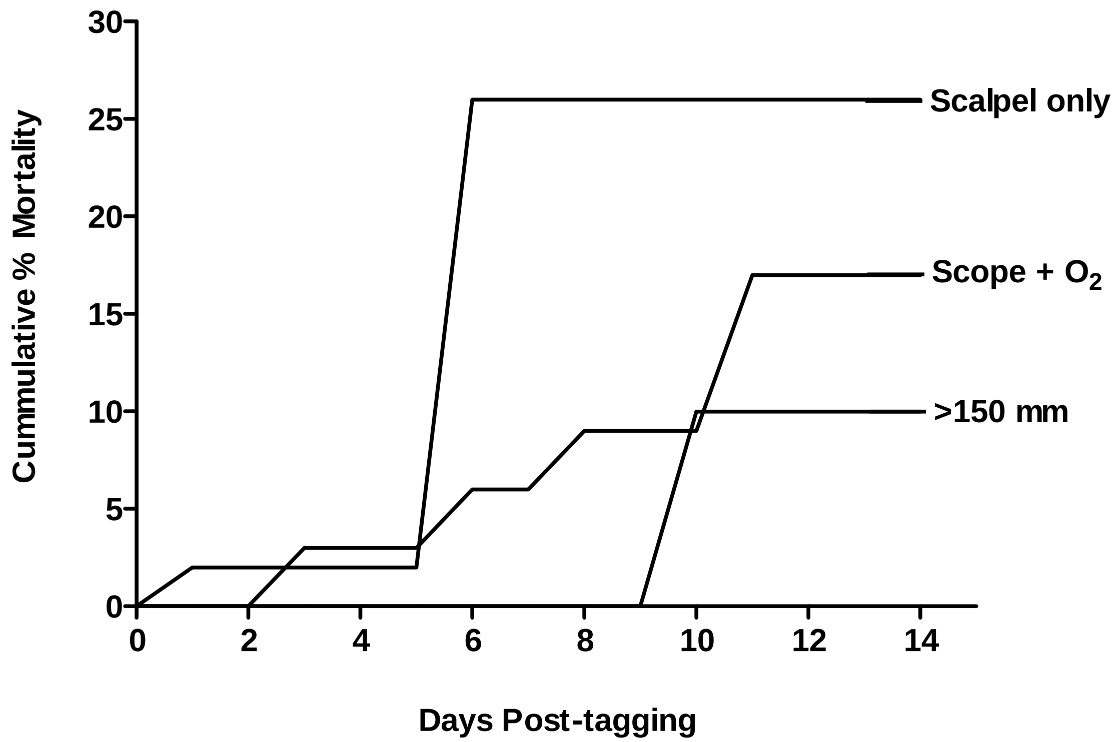
Tables

1. Results from an attachment behavior experiment showing number of juvenile lamprey remaining attached to a swim tube after being subjected to flow of 2.5 ft./sec. for 24 hours. Animals were either radio-tagged, PIT-tagged or remained untagged. Radio-tagged animals exhibited a reduced ability to remain attached by 12 hours post-treatment. PIT-tagged animals were not different from controls.

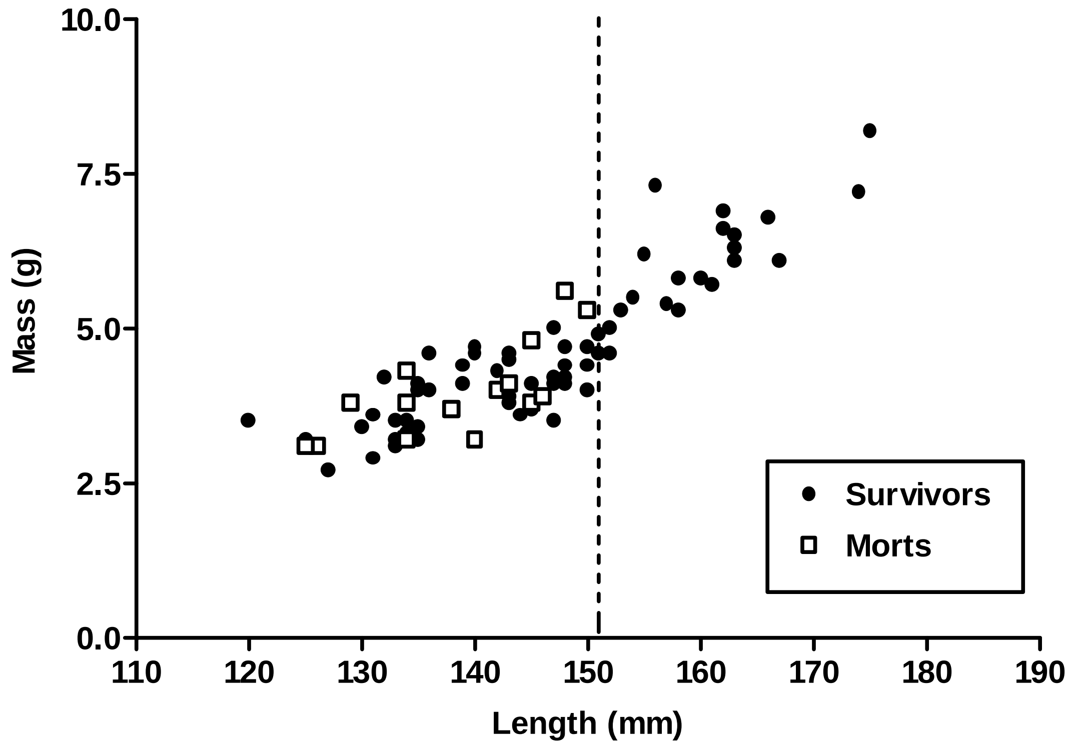
2. Summary table of telemetry technologies investigated for the tagging and tracking of juvenile Pacific lamprey (*Lampetra tridentata*), and the advantages and disadvantages of each. See appendix A-1 for a complete listing.

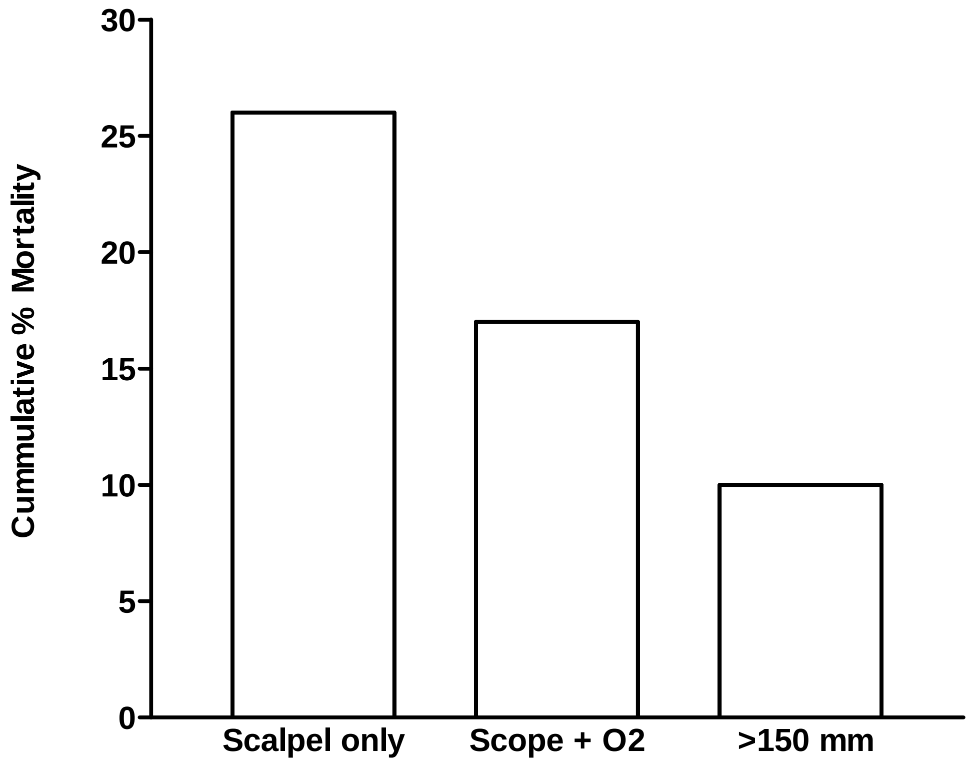


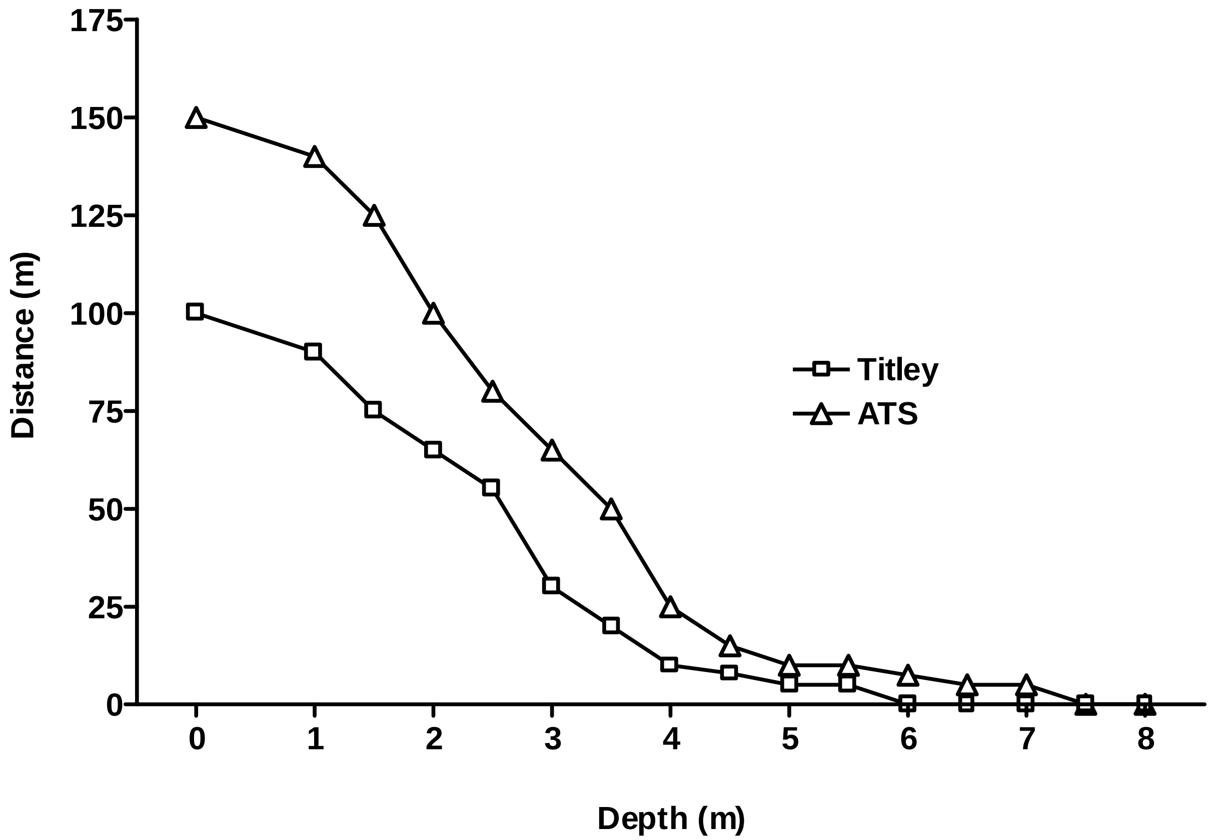
Cummulative
% Tag Removal
100
75
50
25
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Days Post-tagging

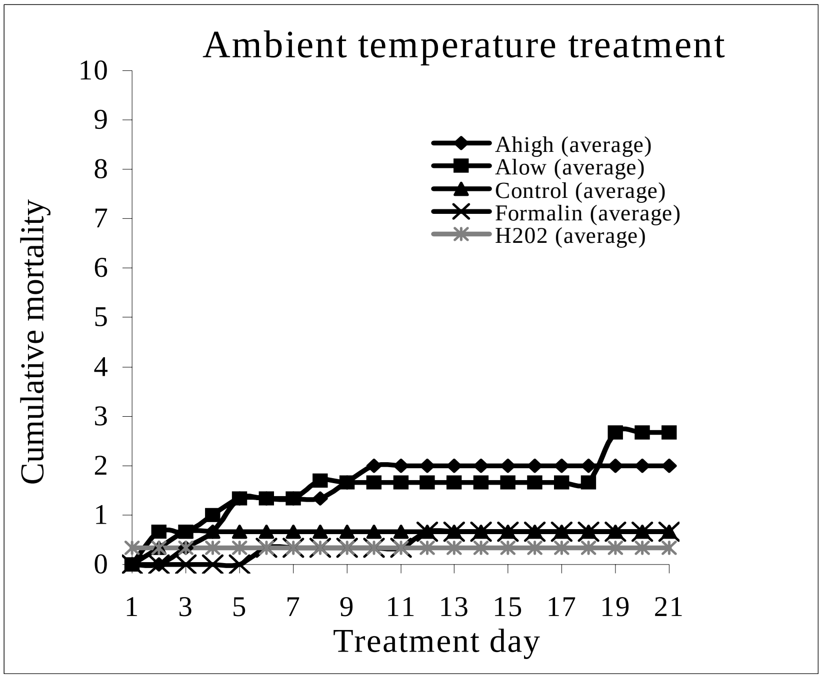


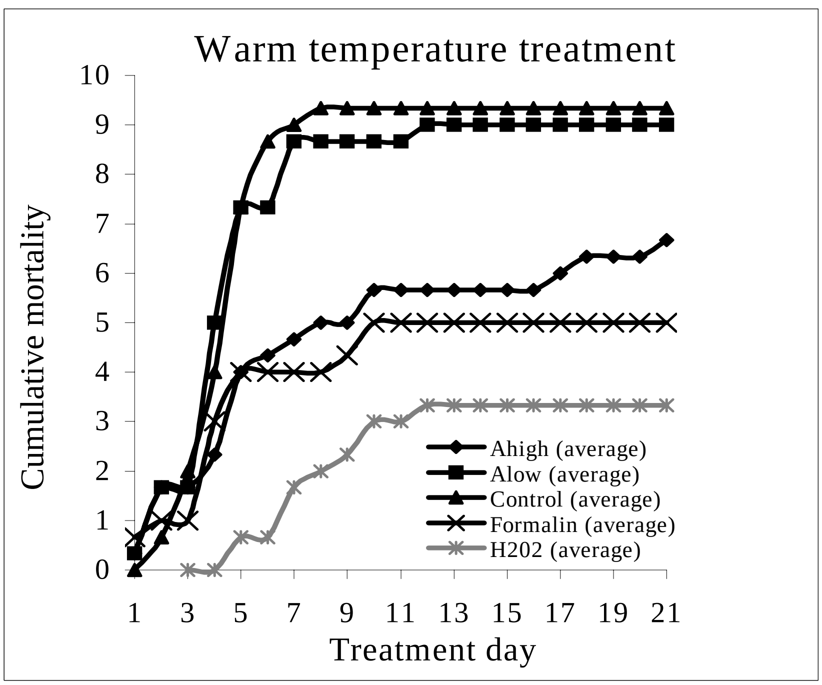
Cummulative % Mortality
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
Days Post-tagging
Scalpel only
Scope + O2
>150 mm


10.0
7.5
5.0
2.5
0.0
Mass (g)
110
120
130
140
150
160
170
180
190
Length (mm)
Survivors
Morts


30
25
20
15
10
5
0
Cummulative % Mortality
Scalpel only
Scope + O2
>150 mm


175
150
125
100
75
50
25
0
Distance (m)
0
1
2
3
4
5
6
7
8
Depth (m)
Titley
ATS


Ambient temperature treatment
Cumulative mortality
Treatment day
Ahigh (average)
Alow (average)
Control (average)
Formalin (average)
H202 (average)
0
1
2
3
4
5
6
7
8
9
10
1
3
5
7
9
11
13
15
17
19
21


Warm temperature treatment
Cumulative mortality
Treatment day
Ahigh (average)
Alow (average)
Control (average)
Formalin (average)
H202 (average)
0
1
2
3
4
5
6
7
8
9
10
1
3
5
7
9
11
13
15
17
19
21

Treatment	Attached @ 0 hrs.	Attached @ 12 hrs.	Attached @ 24 hrs.
Radio-tagged	6	0	N/A
Control	6	6	6
PIT-tagged	8	8	8
Control	8	8	8

Technology	Advantages	Disadvantages
Radiotags	Long-range sensing Track animals anywhere Multiple suppliers Custom construction	Smallest transmitter is too large to implant Lamprey remove externally attached objects
PIT tags	Small Inexpensive Receivers already in place on many projects Can be implanted successfully	Short range (30-40 cm) Cannot be used to track lamprey, only to detect passage past receivers
Harmonic Radar	Extremely small transmitter (<50 mg) Passive transmitter (requires no power) Can track terrestrial animals >500 meters	Limited transmission range in water (<30 cm) Technology untested for use with fish
Visual Marks	Inexpensive Easy Quick	Similar monitoring limitations to PIT tags Require extreme commitment of manpower resources