# Survival Assessment of Juvenile Pacific Lamprey Implanted with a Dummy Acoustic Tag for a Yakima Basin Acoustic Telemetry Study


(Cover Photo: Juvenile Pacific Lamprey and an accurately scaled image of an acoustic tag designed by Pacific Northwest National Laboratory for use in Pacific Lamprey.)

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## Highlights

- Thirty-one juvenile Pacific Lamprey were tagged with a dummy tag of a prototype acoustic transmitter developed by Pacific Northwest National Laboratory (PNNL, Pasco, WA) and were monitored for 30 days (to mimic the battery lifespan of the acoustic tag) for recovery and survival in 10-gallon aquaria in comparison with control (untagged) lamprey.
- Overall survival of dummy tagged lamprey was slightly lower than the control lamprey (83.9% and 96.2%, respectively). However, a small portion of dummy tagged lamprey were initially infected with fungus (prior to tagging) and all died after 19 days. Tagged lamprey that were not initially infected with fungus had a comparable survival rate to the control lamprey (96.3% and 96.2%, respectively).
- Our study demonstrated that the acoustic tag implantation process presumably had minimal impact on fungus infection occurrences and survival.

## Abstract

Juvenile Pacific Lamprey (macrophthalmia) were tagged with a prototype acoustic transmitter developed by Pacific Northwest National Laboratory (PNNL, Pasco, WA) to track their migration from the Lower Yakima River to the Lower Columbia River (Bonneville Dam). The small tag is 12 mm in length, weighs 0.08 gram, and has a battery life of 30 days with a pulse rate of 5 seconds. Reliable analysis of detection data during this study was dependent on a comprehensive understanding of lamprey recovery and overall survival from tag implantation. To address this uncertainty, a small group of juvenile lamprey were implanted with dummy acoustic tags and closely monitored for a 30-day period (to mimic acoustic tag battery lifespan) in a controlled laboratory environment (10-gallon aquaria; Prosser Hatchery). Dummy tagged lamprey were monitored for survival, surgery recovery, and overall changes in body condition (length and weight). In this study, dummy tagged lamprey showed high survival over the 30-day holding period. We observed that incisions healed well over the holding period, with the majority (84.6%) of incisions closed and healed with minimal scarring. Overall survival of dummy tagged lamprey was slightly lower than the control (untagged) lamprey held in the same aquaria (83.9% and 96.2%, respectively). However, a small portion of dummy tagged lamprey (n=3) were infected with fungus prior to tagging and all died after 19 days. Tagged lamprey that were not infected with fungus at the onset had a comparable survival rate to the control lamprey (96.3% and 96.3%, respectively). We also saw similar body condition changes over the holding period (length/weight) for both dummy tag and control fish, indicating the tag has minimal impact on physiological changes, at least for the 30-day battery life of the tag. Although only 35% of the dummy tagged lamprey met the minimum size threshold we established for the release study (140 mm), our study showed that the acoustic tag implantation process presumably had minimal impact on survival. As long as fish infected with fungus prior to tagging are excluded, fungus development was equal for control lamprey and dummy tagged lamprey, indicating that the tag implantation process appears to have no discernable impact on fungal infection occurrences.

# Introduction

In the spring of 2018, the United States Geological Survey (USGS), Yakama Nation Fisheries, Bureau of Reclamation, and several partnering agencies, initiated a multi-year acoustic telemetry study to track the migration patterns of ocean-bound juvenile (macrophthalmia) Pacific Lamprey in the Columbia Basin. The study area focuses on the lower reach of the Yakima River as well as the mainstem Columbia River downstream to Bonneville Dam. Juvenile Pacific Lamprey (hereinafter referred to as lamprey) are tagged with a prototype acoustic transmitter developed by the Pacific Northwest National Laboratory (PNNL; Pasco, WA). The small tag is 12 mm in length, weighs only 0.08 gram, and has a battery life of ~30 days. Reliable analysis of detection data collected during this study is dependent on a comprehensive understanding of lamprey recovery and overall survival from handling and tag implantation. In order to explore this uncertainty in project year one, a group of lamprey were implanted with dummy acoustic tags and closely monitored for a 30-day period in a controlled laboratory environment (10-gallon aquaria). This report summarizes the overall survival, recovery, and condition of dummy tagged lamprey, in an attempt to understand potential tag effects on acoustically tagged lamprey for the 30-day battery life of the acoustic tag. Methods and results of the field study monitoring the movements of acoustically tagged lamprey are reported in Liedtke et al. (in review).

# Methods

### Lamprey collection and initial holding

Lamprey used for this study were collected from the Yakima Subbasin (our preferred local source) and the Columbia River (McNary and John Day dams) during the months of April and May in 2018. In the Yakima Subbasin, lamprey were collected from the lower mainstem Yakima River (Chandler Diversion Juvenile Fish Monitoring Facility), and from screw traps in two tributary streams of the lower Yakima River (Ahtanum and Toppenish creeks). Lamprey collected from Columbia River dams were transported to Prosser Hatchery in 150 gallon totes (filled approximately half way with river water). Within the large transport tote, lamprey were held in green flow-through 5-gallon buckets, each with a tight-fitting lid, and held afloat by an inflated tire tube to minimize the stress on lamprey during handling and transfer. Based on a 10 g/L standard fish holding criteria (commonly used for juvenile salmonids), no more than 37 lamprey were held in a flow-through 5-gallon bucket during transport in order to limit stress from overcrowding [10 g/L x 15 L (water volume within bucket) = 150 g; 150 g / 4.0 g (typical weight of a juvenile Pacific Lamprey) = 37].

All collected lamprey were held at the Yakama Nation Prosser Hatchery (Prosser, WA). Lamprey were held for a maximum of 11 days between collection date and tag date (overall median hold time was 3 days); less holding time was always preferred and chosen whenever possible to limit holding stress and associated potential fungus growth. Prior to the first tagging date, lamprey were

held in green flow-through 5-gallon buckets (the same containers used for transport) suspended in a large flow-through tank in an approximately 50% mix of well and river water (water temperature were both at 14.5°C, +/- 1°C). However, even though these lamprey were initially fungus-free, on the first tagging date, we observed 70% incidence rate of fungus infection on lamprey that were held three days or longer in these conditions (n=23). After the first tagging date, due to the high rate of fungus infection, we modified our protocols and all collected lampreys were instead transferred to and held in flow-through aquaria with a flow rate of 4 L/min (turnover rate of 5.25 min) prior to tagging. A maximum of 52 lamprey were held per aquaria based on the standard holding capacity calculation. If any lamprey were observed to develop fungus during the holding period prior to tagging, they were separated from the overall group. After we began using the aquaria for holding, we had no occurrence of fungus infection prior to tagging even when lamprey were held longer than three days (n=8). See "Holding aquariua setup" section below for more details on the holding conditions.

**Tagging and handling**

Collected lamprey underwent one of three procedures: 1) acoustic tag implant, 2) dummy tag implant, or 3) no tag implant (control group). Acoustic, dummy and control groups were handled and measured on the same dates by the same tagger. All three groups underwent the same handling procedures. Length, weight and body condition comments (e.g. presence of fungus) were collected from each lamprey. All tagging and handling procedures followed a tagging protocol developed by USGS (Liedtke et al. 2012; Mesa et al. 2012) and PNNL (Deng et al. 2018). Tags were inserted into the body cavity anteriorly through a 2-3 mm incision located approximately 20 mm posterior to the last gill pore at a 45 degree angle on the lamprey body (whereby midline is 0 degrees and ventral side 90 degrees). Lamprey availability was dependent on the captures from the various source locations and varied widely from day to day (in general, higher discharge levels coincided with higher numbers of downstream migrating juvenile lamprey). The number of tagging events we scheduled was based on the number of lamprey that were available to tag; we aimed for a minimum of 20-25 acoustic tagged lamprey per tagging event. All lamprey (acoustic tag, dummy tag, and control) were held in flow-through 5-gallon buckets in a river water tank for up to 24 hours (overnight) before transfer to their destination. Dummy tag and control group lamprey were transferred to aquaria, and acoustic tagged lamprey were released into the Yakima River.

A total of 100 acoustic tags and 30 dummy tags were available starting in early May, 2018. Thirty lamprey were set aside to receive a dummy tag throughout the overall tagging duration to evaluate tag effects on acoustically tagged lamprey. A similar number of control lamprey and dummy tag lamprey were measured and held together in the same aquarium tanks in pairs of 4 to 8 fish at the start of the study. They were held together in same tanks to exclude effects from tank specific conditions or handling that are unrelated to the tag effects. A total length of 140 mm was the minimum size threshold used for the acoustic tagging of juvenile lamprey based on a recent study (Deng et al. 2018). The same size threshold was scheduled to be pursued for dummy tag and

control lamprey to help balance the lengths for the three treatment groups. However, lamprey collection numbers were limited and lamprey larger than the minimum size threshold were used first for the acoustic tagging group given its higher priority. As a result, the smallest fish were used as controls since they did not have to compensate for the weight of a tag. As a result, control group lamprey, and to a lesser extent dummy tag group lamprey, were considerably smaller compared to the acoustic tag lamprey. Tagger discretion also helped determine whether fish under the size threshold should receive a tag (acoustic or dummy) or not based on a combination of length and girth sizes. See results and discussion sections below. Dummy tag lamprey that were infected with fungus prior to tagging were held separately to prevent the spread of fungus to other lamprey.

**Holding aquaria setup**

Dummy tag and control lamprey were held in flow through 37.8 L (10-gallon) aquaria with approximately 21 L of water (Fig 1) for a minimum of 30 days. The 30-day hold time was designed to mimic the battery life of the acoustic tags. No more than 16 lamprey (maximum of eight dummy tag and eight control lamprey) were placed into an aquaria to minimize density related stress. Each aquarium was set to a flow of 4 L/min of well water (14.5°C, +/- 1°C). The aquaria were covered with a removable black mesh to reduce light-associated stress while providing easy access for our staff to make daily observations when needed. Coarse rounded pebbles were also added to each aquaria, covering approximately one-third of the bottom, to provide cover and further reduce holding stress. If too much cover was provided, it would have made it very challenging to make daily observations of all the study fish and would have precluded us from verifying the total number of fish in each tank or body condition, so a delicate and sensitive balance was sought between too much cover and too little cover.

**Figure 1. The overall aquaria set-up inside Prosser Fish Hatchery lamprey conex building where lamprey were monitored daily (left photo). The removable black mesh we used to cover the tanks and to keep them dark is also displayed (right photo).**

**Monitoring lamprey during the 30-day holding period**

Dummy tag and control groups were observed daily for mortality and signs of fungus growth. If a lamprey died, it was removed and placed into a freezer for later examination. Lamprey were monitored daily for signs of fungal infections. If it was thought that a lamprey was infected with fungus, all lamprey in the infected aquarium were briefly removed and examined for fungus. All infected dummy tag and control lamprey were tallied separately to understand if fungus infected either group differently. When an infected lamprey was confirmed in an aquarium, all fish in the aquarium were treated for fungus to limit the spread to other fish in the tank.

**Fungus and treatments during the 30-day holding period**

Two chemicals are known to be an effective treatment for fungal infections on fishes; formalin and hydrogen peroxide. Formalin (PARACIDE-F, Argent Laboratories, Redmond, WA) and hydrogen peroxide (35% PEROX-AID, Western Chemicals, Inc., Ferndale, WA), of varying concentrations, were first tested on untagged juvenile lamprey that were not part of the holding study to test their safety, before aquaria with holding study lamprey were treated with the prescribed treatments. Two approved and recommended dosages for salmonids are 167 µl/L of formalin and 50 mg/L of hydrogen peroxide in up to a 1-hour static bath treatment (Rach et al. 2000; Bowker et al. 2014). Our experiments on untagged lamprey explored higher concentrations, to ensure the study fish received the highest, yet safe, dosage to effectively treat fungus. Formalin concentrations of 222 – 1667 µl/L, hydrogen peroxide of 100 – 200 mg/L treatments in up to 1-hour static bath were tested. In addition, salt treatment of 3% (Bowker et al. 2014) was tested for one lamprey experimentally. Lamprey behavior was monitored closely during each treatment, and the observed behavior was used to determine if the experimental dosages were safe for use on study fish. Each experimental treatment was performed once for 30-60 min in an aerated static bath (within the holding aquaria). The treatment duration and use of an aerator was the same for both untagged test fish and study fish.

Study fish that became infected with fungus prior to completion of our behavior tests were treated with the recommended dosage of hydrogen peroxide (in one instance formalin was used). Once testing of higher concentrations was complete on May 25, 2018, the results from the behavior test were used to determine the new recommended dosage. Post treatment survival as well as the behavior were considered for the new recommended dosage. Each treatment was performed once a day for up to three consecutive days based on fish health specialist recommendation (David Thompson, U.S. Fish and Wildlife Service, personal communication), and all rocks were removed for easy observation and monitoring. Lamprey were removed and monitored for presence of fungus after the last treatment. If fungus was still present, we began a new 3-day treatment the following week after a 4-6 day break. Treatments continued until the end of the 30-day study, or until fungus was no longer visible on treated lamprey.

### Monitoring lamprey post-study

All lampreys were removed from their aquariums at least 30 days (31-33 days) after the initial placement date into the aquaria, except for those that died during the study. All dummy tagged lamprey were euthanized (with an approximate concentration of 600 mg/L MS-222), photographed, and measured for length and weight. The incision condition on each dummy tagged lamprey was categorized into the following condition ratings: "Good" if the incision is closed and healed, "Fair" if the incision was healed, but had a larger than normal scar, and "Bad" if the incision was not healed and open (for example, if the intestine was poking out). Each control lamprey was measured for length and weight, photographed, and tagged with an 8 mm PIT tag for later release.

### Analysis

The average length and weight data of lamprey (min, max, average) were summarized for each study group (acoustic, dummy, and control). Cumulative daily survival was calculated for both control and dummy tag groups, as well as for lamprey that had fungus at the time of tagging (and were left untreated). Biological changes (length and weight) over the 30-day holding period were summarized as an average for each aquarium, and only data for lamprey that survived the entire 30-day holding period were included. Treatments on untagged lamprey are summarized based on survival and behavior changes during the treatments. Treatment effectiveness on holding study lamprey is summarized as percentages of lamprey infected before and after the treatments.

## Results

### Lamprey collection

During the months of April and May, 2018, a total of 231 lamprey were collected from the Yakima Subbasin (50) and Columbia River (181) as shown in Table 1. Of the lamprey collected from the Yakima Subbasin, 70% were tagged with an acoustic tag, while 18% were either a dummy tagged or control group lamprey, and 12% were not used in this study (extra). Collected lamprey that were not used in this study were released (at the earliest possible time) into the Lower Yakima River (river km 6.3 and 189.0) after PIT and genetic tagging, or were released untagged due to fungus growth. In addition, two Western River Lamprey were collected from Chandler Juvenile Fish Monitoring Facility and one received an acoustic tag while the other received an 8-mm PIT tag.

**Table 1. Summary of trapping locations where lamprey were collected during the months of April-May, 2018. Also shown is the destination group of the lamprey in terms of numbers and percentage (i.e. acoustic tag, dummy tag, control, or extra).**

| Collection Source | | Total # (By Source) | # Acoustic Tag | # Dummy Tag | # Control (No Tag) | # Extra | % Acoustic Tag | % Dummy Tag | % Control (No Tag) | % Extra |
|---|---|---|---|---|---|---|---|---|---|---|
| Ahtanum Creek (Yakima) | | 7 | 5 | 2 | 0 | 0 | 71% | 29% | 0% | 0% |
| Toppenish Creek (Yakima) | | 8 | 5 | 2 | 1 | 0 | 63% | 25% | 13% | 0% |
| Chandler Diverion (Yakima) | | 35 | 25 | 1 | 3 | 6 | 71% | 3% | 9% | 17% |
| McNary Dam (Columbia) | | 110 | 49 | 20 | 19 | 22 | 45% | 18% | 17% | 20% |
| John Day Dam (Columbia) | | 71 | 14 | 6 | 3 | 48 | 20% | 8% | 4% | 68% |
| **Total** | **Yakima** | **50** | **35** | **5** | **4** | **6** | **70%** | **10%** | **8%** | **12%** |
| | **Columbia** | **181** | **63** | **26** | **22** | **70** | **35%** | **14%** | **12%** | **39%** |

## Biological data at time of tagging

Lamprey were tagged on three separate dates in May, 2018 (8th, 9th and 14th). Totals for each group of lamprey was 98, 31 and 26 for the acoustic tag, dummy tag, and control lamprey, respectively. Both lamprey groups that received an acoustic tag or a dummy tag were similar in size (Table 2 and Fig. 2). The average length was 149 mm, 138 mm and 130 mm for the acoustic tag, dummy tag, and control group lamprey, respectively. Control lamprey, on average were smaller that tagged lamprey due to the need to use the larger lamprey for tagging. Only 35% of the dummy tagged lamprey met the 140 mm threshold, compared to 93% of the acoustic tagged lamprey. The smallest lengths of lamprey tagged with acoustic and dummy tags were 138 and 130 mm, whereas the smallest weight were 3.2 and 2.9 g, respectively.

**Table 2. Biological summary of lamprey that received an acoustic tag, a dummy tag, or no tag (control). Biological data are presented for each tag date by tag type. "% Over 140 mm" shows the number of lamprey over 140 mm (our goal minimum tagging size threshold). The summary rows at the bottom of the table summarize biological data for each tag type from all tagging dates.**

| Tag Date | Tag Type | # Tagged / Handled | % Over 140 mm | Avg. Length (mm) | Min Length (mm) | Max Length (mm) | Avg. Weight (g) | Min Weight (g) | Max Weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| 5/8/2018 | | 20 | 85% | 147 | 138 | 173 | 4.3 | 3.2 | 6.4 |
| 5/9/2018 | Acoustic | 24 | 88% | 149 | 139 | 164 | 4.7 | 3.6 | 6.5 |
| 5/14/2018 | | 54 | 98% | 151 | 139 | 176 | 4.6 | 3.5 | 7.4 |
| 5/8/2018 | | 6 | 0% | 135 | 130 | 138 | 3.2 | 2.9 | 3.6 |
| 5/9/2018 | Dummy | 4 | 0% | 137 | 136 | 139 | 3.7 | 3.5 | 3.9 |
| 5/14/2018 | | 21 | 52% | 143 | 132 | 163 | 3.9 | 3.0 | 5.7 |
| 5/8/2018 | | 0 | - | | | - | | | |
| 5/9/2018 | Control | 5 | 0% | 125 | 114 | 136 | 2.8 | 2.0 | 3.7 |
| 5/14/2018 | | 21 | 19% | 137 | 125 | 154 | 3.4 | 2.2 | 4.8 |
| | **Acoustic** | **98** | **93%** | **149** | **138** | **176** | **4.5** | **3.2** | **7.4** |
| **Summary** | **Dummy** | **31** | **35%** | **138** | **130** | **163** | **3.6** | **2.9** | **5.7** |
| | **Control** | **26** | **15%** | **131** | **114** | **154** | **3.1** | **2.0** | **4.8** |

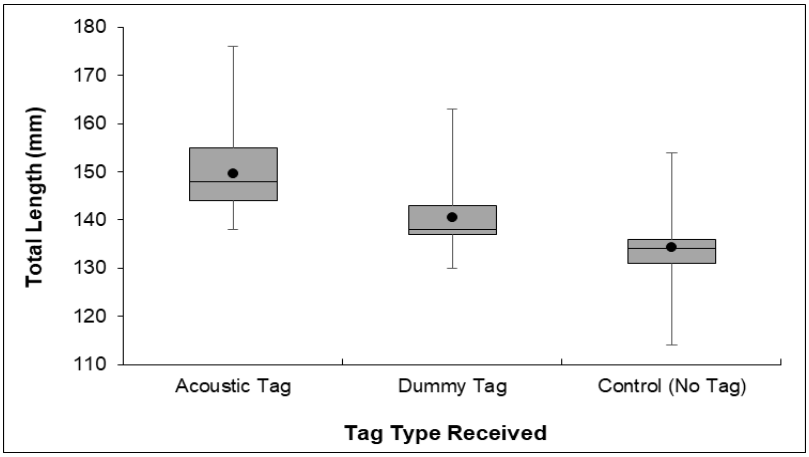


**Figure 2. Box plot of lamprey total lengths, shown for lamprey that received an acoustic tag, a dummy tag, or no tag (control). The upper and lower bounds of each box represent the 75$^{th}$ and 25$^{th}$ percentile, respectively. The line in the middle of each box represent the median length, and the black dot in each box represents the average length. Upper and lower whisker bounds indicate the maximum and minimum lengths, respectively. One Western River Lamprey received an acoustic tag, and is included in this figure.**

### Survival over the 30-day holding period

Lamprey used in this holding study were separated into six 10-gallon aquaria, separated by tagging date (Table 3). Each aquaria received a unique identification name based on their location within the group of aquaria. Overall, there was a similar number of dummy tag lamprey (n=31) and control lamprey (n=27). During the 30-day holding period, overall survival was higher for control lamprey compared to dummy tagged lamprey (83.9% and 96.2%, respectively). However, 4 of 31 dummy tagged lamprey (12.9%) were visibly infected by fungus at the time of tagging on May 8, 2018. These four infected lamprey were not treated for fungus, and all four lamprey died by day 19 (Fig. 3). Dummy tagged lamprey that were not initially infected by fungus (n=27) at the time of tagging had a much higher survival rate over the 30-day holding period (96.3%; Fig. 4) and an almost identical survival rate as the control lamprey (96.2%).

**Table 3. Summary of survival for lamprey held in aquaria over a minimum of 30 days. The "Holding Start Date" is the date that the lamprey were transferred to the aquaria after tagging. The "Holding End Date" is the date that lamprey were removed from the aquaria for final monitoring. The "Number of Hold Days" is the number of days the lamprey were held in each aquaria, or until all lamprey died. "Fungus at Time of Tagging?" shows aquaria that contained lamprey that had fungus on their body at the time of tagging.**

| Aquarium ID | Tag Type | Tag Date | Holding Start Date | Holding End Date | # of Hold Days | Fungus at Time of Tagging? | # Start | # End | # Died | % 30-Day Survival |
|---|---|---|---|---|---|---|---|---|---|---|
| RB2-4 | Dummy | 5/8/2018 | 5/9/2018 | 6/11/2018 | 33 | No | 2 | 2 | 0 | 100% |
| | Control | | | | | | 0 | 0 | - | - |
| RB2-5 | Dummy | 5/8/2018 | 5/9/2018 | 5/28/2018 | 19 | Yes | 4 | 0 | 4 | 0% |
| | Control | | | | | | 0 | | - | |
| RB3-5 | Dummy | 5/9/2018 | 5/10/2018 | 6/11/2018 | 32 | No | 4 | 4 | 0 | 100% |
| | Control | | | | | | 5 | 5 | 0 | 100% |
| RB2-1 | Dummy | 5/14/2018 | 5/15/2018 | 6/15/2018 | 31 | No | 5 | 5 | 0 | 100% |
| | Control | | | | | | 5 | 5 | 0 | 100% |
| RB2-2 | Dummy | 5/14/2018 | 5/15/2018 | 6/15/2018 | 31 | No | 8 | 7 | 1 | 88% |
| | Control | | | | | | 8 | 7 | 1 | 88% |
| RB2-3 | Dummy | 5/14/2018 | 5/15/2018 | 6/15/2018 | 31 | No | 8 | 8 | 0 | 100% |
| | Control | | | | | | 8 | 8 | 0 | 100% |
| **Summary** | **Dummy** | **-** | **-** | | **≤ 33** | **All** | **31** | **26** | **5** | **83.9%** |
| | **Control** | | | | | | **26** | **25** | **1** | **96.2%** |
| | **Dummy** | **-** | **-** | | **≤ 33** | **No** | **27** | **26** | **1** | **96.3%** |
| | **Control** | | | | | | **26** | **25*** | **1** | **96.2%** |
| | **Dummy** | **-** | **-** | | **19** | **Yes** | **4** | **0** | **4** | **0%** |
| | **Control** | | | | **-** | | | | **-** | |

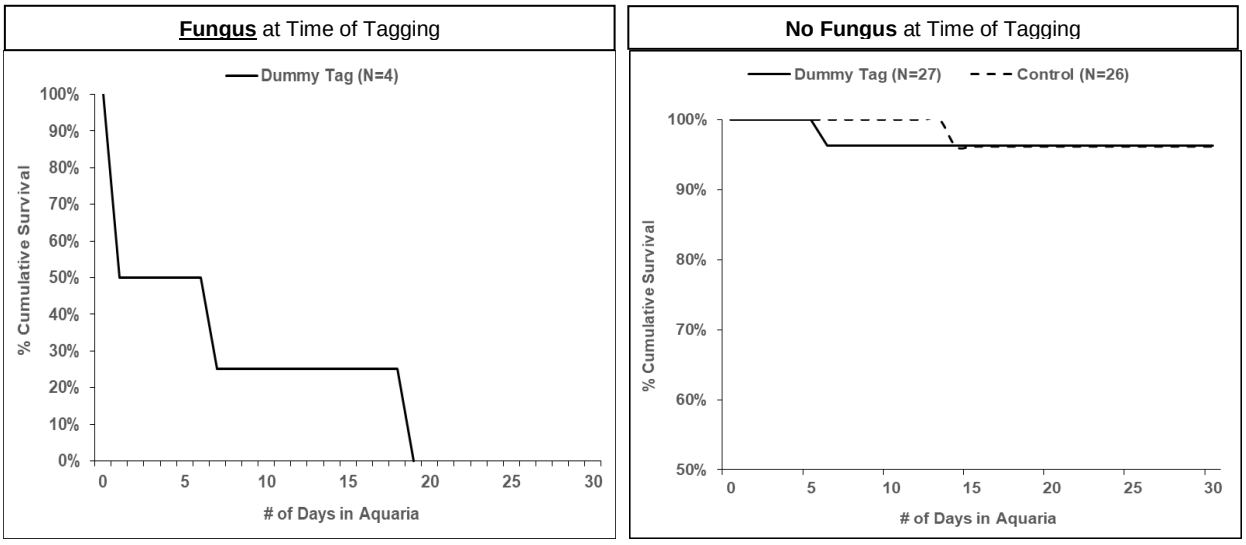


**Figure 3. Cumulative survival of control and dummy tagged lamprey over the 30 day holding period. The graph on the left shows lamprey that had fungus on their body at the time of tagging (no treatment was received). The graph on the right shows the cumulative survival for all lamprey with no fungus on their body at the time of tagging (some treatment received).**

**Dummy tag incision conditions after 30-day holding**

Overall, surgery wounds healed nicely over the 30-day holding period. A total of 22 out of 26 (85%) dummy tagged lamprey received an incision condition rating of "Good", where the wound was healed and there was minimal scarring (Fig. 4 and Fig. 5). Only 1 out of 26 (4%) lamprey received a "Bad" incision rating, where the intestine was poking through the incision.

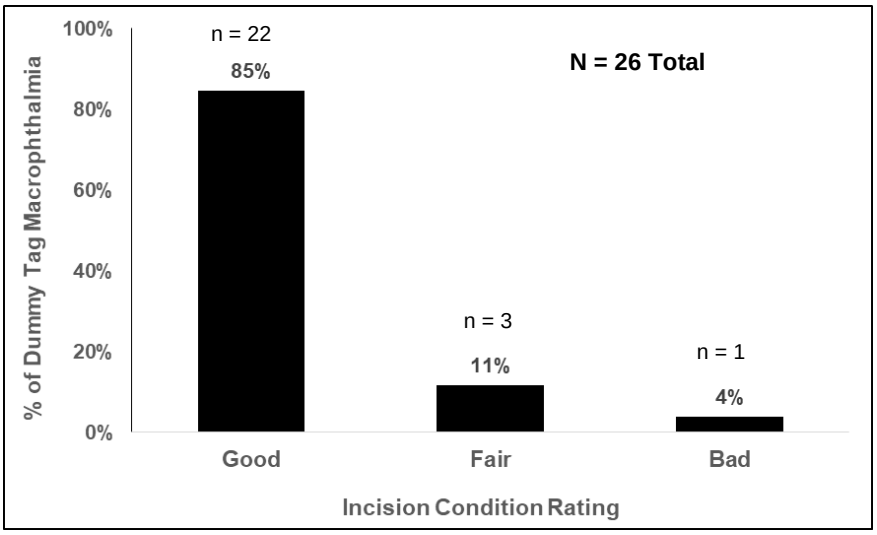


**Figure 4. Summary of incision condition ratings for dummy tagged lamprey that survived the 30 day holding period. Incisions were rated "Good" when the incision was healed with minimal scarring, "Fair" if the incision was healed, but the scarring was larger and more visible, and "Bad" if the incision was not fully healed.**

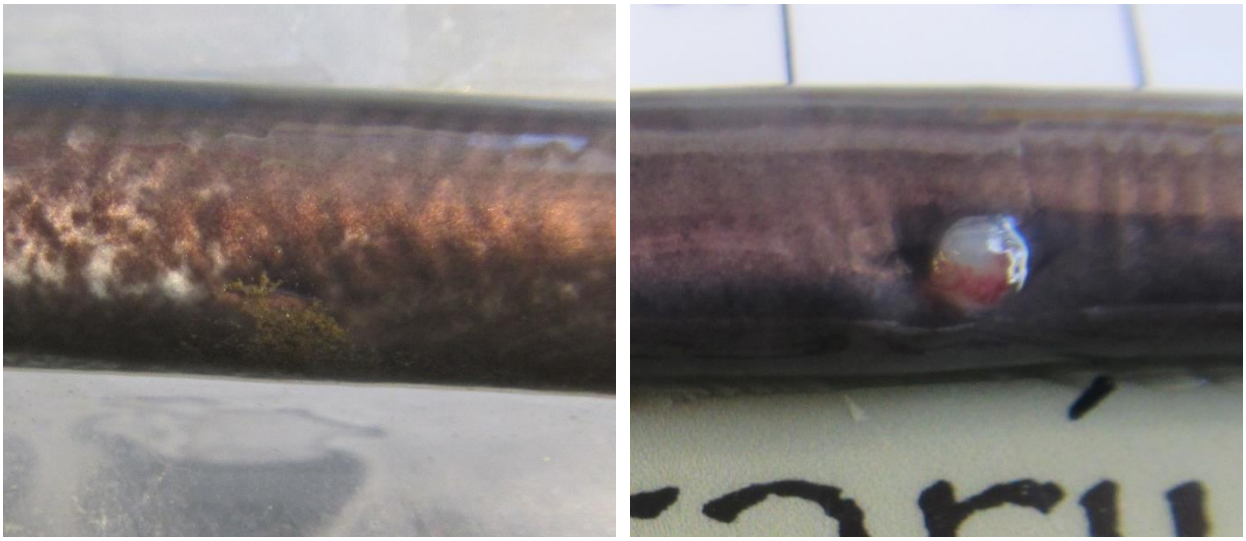

**Figure 5. Example of incision conditions ~30 days post tagging. The left photo is an example of a "Good" incision condition rating (closed, healed). The right photo is an example of a "Bad" incision condition rating (open, not healed; in this particular case the intestine is poking through the incision).**

## Biological changes after 30-day holding

On average, dummy tagged lamprey decreased in total length by 3 mm, and control lamprey decreased in length by 2 mm (2.3% and 1.6% decrease in total length, respectively; Table 4). Similarly, the overall weight loss by dummy tagged lamprey was close to control lamprey (0.20 grams and 0.28 grams, respectively). However, the percentage of weight change was greater for control lamprey compared to those with dummy tags (8.5% and 5.4%, respectively), resulting in a small increase in condition factor (0.98 to 1.00) while a modest decrease in condition factor was observed for the dummy tag group lamprey (0.96 to 0.95). A specific condition factor that minimizes length-based bias for lamprey was used for this calculation [weight (g) x 10^5 x (Length (mm)^(2.6))^(-1)].

**Table 4. Summary of biological changes of dummy tag and control lamprey held in aquaria for a minimum of 30 days. "Number of days held" is the length of time lamprey were held in each aquarium post tagging. Average lengths and weights (and their respective changes over the 30 day period) were calculated only from lamprey that survived the entire holding period. The average and percentage values in the summary rows are an average of all presented values.**

| Aquarium ID | Tag Type | # of Days Held | # at Start | # at End | # Died | Avg. Length Start | Avg. Weight Start | Avg. Length End | Avg. Weight End | Avg. Length Change | Avg. Weight Change | % Length Change | % Weight Change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RB2-1 | Dummy | 31 | 5 | 5 | 0 | 153 | 4.70 | 148 | 4.53 | -5 | -0.17 | -3% | -4% |
| RB2-2 | Dummy | 31 | 8 | 7 | 1 | 140 | 3.73 | 137 | 3.52 | -3 | -0.21 | -2% | -6% |
| RB2-3 | Dummy | 31 | 8 | 8 | 0 | 140 | 3.51 | 135 | 3.38 | -5 | -0.13 | -3% | -4% |
| RB3-5 | Dummy | 32 | 4 | 4 | 0 | 137 | 3.73 | 135 | 3.50 | -2 | -0.22 | -1% | -6% |
| RB2-4 | Dummy | 33 | 2 | 2 | 0 | 131 | 3.45 | 129 | 3.17 | -2 | -0.29 | -1% | -8% |
| RB2-1 | Control | 31 | 5 | 5 | 0 | 136 | 3.08 | 130 | 2.89 | -5 | -0.19 | -4% | -6% |
| RB2-2 | Control | 31 | 8 | 7 | 1 | 136 | 3.57 | 135 | 3.29 | -1 | -0.28 | -1% | -8% |
| RB2-3 | Control | 31 | 8 | 8 | 0 | 138 | 3.55 | 134 | 3.31 | -4 | -0.25 | -3% | -7% |
| RB3-5 | Control | 32 | 5 | 5 | 0 | 125 | 2.84 | 122 | 2.50 | -3 | -0.34 | -3% | -12% |
| **Summary** | **Dummy** | **≤ 33** | **27** | **26** | **1** | **140** | **3.82** | **137** | **3.62** | **-3** | **-0.20** | **-2.3%** | **-5.4%** |
| | **Control** | | **26** | **25** | **1** | **134** | **3.26** | **130** | **3.00** | **-3** | **-0.26** | **-2.6%** | **-8.3%** |

## Fungus and Treatments

To understand the effects of treatment (both positive and negative), some of the untagged extra juvenile lamprey from John Day Dam were treated experimentally with a range of formalin and hydrogen peroxide concentrations, which were higher (up to x10 dosage of formalin and x4 dosage of hydrogen peroxide) compared to the recommended dosage for salmonids (167 µl/L of formalin and 50 mg/L of hydrogen peroxide) (Table 5). Lamprey behavior was monitored during these treatments to assess their behavioral changes and responses in addition to survival. We observed no mortality during or after any of these high concentration treatments. Lamprey treated with 1667 µl/L of formalin had an adverse reaction during the treatment. Lamprey were removed from the treatment after 36 minutes, as lamprey were observed lying on their side, with abnormally fast breathing. No lamprey died immediately after this treatment (24 hours), but it was clear that their experience was traumatic and likely highly stressful. One lamprey which had heavy fungus at the start of this experimental testing died a little after 24 hours, but the cause of death was unclear

(may be related to the treatment or may have died from other causes, such as the fungus itself). Similarly, lamprey appeared restless in 200 mg/L hydrogen peroxide (although the overall degree of distress was noticeably less compared to the high formalin treatment). Lamprey behavior was normal and appeared calm in other formalin (222-667 µl/L), hydrogen peroxide (100 mg/L), and salt (3%) treatments. In the end, we made a decision to prescribe 167 µl/L formalin and 50-100 mg/L hydrogen peroxide treatment for our holding study fish based on these results (a level that appeared safe based on the experimental testing). However, more studies are needed to fully evaluate the effectiveness of the various treatments in addition to documentation of fish survival and behavior.

**Table 5. Results from fungus treatments on untagged juvenile lamprey. "Salmon Dosage Multiplication" is a multiplication factor in comparison to the recommended dosage of formalin and hydrogen peroxide for salmonids; 167 mg/L of formalin and 50 mg/L of hydrogen peroxide. Fish behavior observed in tank during each treatment concentration is summarized. "# Mort" (mortality) describes the number that died within 24 hours after the treatment. *Lamprey were removed from the high formalin dosage (1667 mg/L) after 36 minutes due to stressed behavior.**

| Treatment Type | Concentration | Salmonid Dosage Multiplication | Treatment Duration (Hours) | # In Tank | # Mort | Behavior Category | Summarized Behavior Observations |
|---|---|---|---|---|---|---|---|
| Formalin | 1667 µl/L | 10 x | 0.6* | 6 | 0 | Lethargic | **Lamprey appeared lethargic**, with heavy breathing, and swimming upside down or laying on their side. Took 1.5 hours for full recovery (although one fish showed minimal symptoms). |
| | 333 µl/L | 2 x | 1.0 | 4 | 0 | Calm | Lamprey hanging out near the inlet (where they normally hang out). No agitated swimming. Calm throughout treatment |
| | 222 µl/L | 1.5 x | 1.0 | 5 | 0 | Calm | Lamprey hanging out near the inlet (where they normally hang out). No agitated swimming. Calm throughout treatment |
| Hydrogen Peroxide | 100 mg/L | 2 x | 1.0 | 3 | 0 | Calm | Lamprey hanging out near the inlet (where they normally hang out). No agitated swimming. Calm throughout treatment |
| | 200 mg/L | 4 x | 1.0 | 3 | 0 | Restless | Lamprey restless, and swimming around tank. Although some were calm near the inlet, most appeared restless (can't stay in one place for more than several minutes). |
| Salt | 3% (Weight/Volume) | 1 x | 1.0 | 1 | 0 | Calm | Calm during the treatment. Normal behavior during the entire treatment; no movements. |

Fungus infected 28% of study fish (Table 6). Fungus did not appear to infect dummy tag lamprey differently than control lamprey (25% and 31% of total lamprey, respectively). There was no adverse behavior from the formalin treatment (167 mg/L) nor the hydrogen peroxide treatments (up to 100 mg/L). Fungus infections appeared in two of the five holding aquariums that did not originally have infected lamprey (Aquaria RB2-2 and RB2-3; Table 6). Fungus first appeared in

RB2-2 after 6 days of holding and in RB2-3 after 14 days of holding. Overall, our treatment using hydrogen peroxide 100 mg/L proved to be the most effective; in the two aquaria with 14 and 27% fungus infection rates, the treatment reduced the infection rate down to 0% after five daily treatments, spread out over a nine day period (Table 7).

**Table 6. Summary of fungus infected lamprey in the holding study. "# of Control (or Dummy Tag) in Tank" display the number of control (and dummy tagged) lamprey in the tank at the start of the study. "Day Fungus First Appeared" is the number of days after the holding start date when fungus was first observed in the aquarium. "# of Control (and Dummy Tag) with Fungus" is the number of lamprey that were observed to have fungus during the course of the holding period.**

| Aquarium ID | Day Fungus Frist Appeared | # Control in Tank | # Dummy Tag in Tank | # Control with Fungus | # Dummy Tag with Fungus | % Control with Fungus | % Dummy Tag with Fungus | % of Total with Fungus |
|---|---|---|---|---|---|---|---|---|
| RB2-2 | 6 | 8 | 8 | 3 | 2 | 38% | 29% | 31% |
| RB2-3 | 14 | 8 | 8 | 2 | 2 | 25% | 25% | 25% |
| **Summary** | **-** | **16** | **16** | **5** | **4** | **31%** | **25%** | **28%** |

**Table 7. Summary of fungus treatments used to treat holding study lamprey. "Day Fungus First Appeared" is the number of days after the holding start date when fungus was first observed in the aquarium. "# of Treatments" is the number of once a day 1-hour treatments that were performed for each treatment type. Lamprey were monitored 1-2 days after the treatment to confirm whether fungus was still present. * One lamprey infected with fungus died in RB2-2 prior to the 5/29/2018 treatment.**

| Aquarium ID | Day Fungus Frist Appeared | Treatment | Type | First Treatment Date | Last Treatment Date | # of Treat-ments | # in Tank at Start | # with Fungus at Start | # with Fungus at End | % with Fungus at Start | % with Fungus at End | # Died During Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RB2-2** | 6 | Formalin 167 mg/L | | 5/21/2018 | 5/21/2018 | 1 | 15 | 4 | 3 | 27% | 20% | 0 |
| | | Hydrogen Peroxide 50 mg/L | | 5/24/2018 | 5/25/2018 | 2 | 15 | 4 | 3 | 27% | 20% | 0 |
| | | Hydrogen Peroxide 100 mg/L | | 5/29/2018 | 6/7/2018 | 5 | 14* | 2 | 0 | 14% | 0% | 0 |
| **RB2-3** | 14 | Hydrogen Peroxide 100 mg/L | | 5/29/2018 | 6/7/2018 | 5 | 16 | 4 | 0 | 25% | 0% | 0 |

# Discussion

In conclusion, dummy tagged lamprey showed high survival over the 30-day holding period. We saw that incisions healed well over the holding period, with the majority (85%) of incisions closed and healed with minimal scarring. We also saw similar body changes in terms of length and weight over the holding period for both dummy tag and control lamprey, indicating the tag has had minimal impacts on physiological changes (at least for the 30-day battery life of the tag). Overall survival of dummy tagged lamprey was slightly lower than the control lamprey. However, lamprey that were not infected with fungus at the time of tagging had a comparable survival rate to the control group lamprey (96.3% and 96.2%, respectively). As a result, the negative impact from the tagging procedure appeared to be negligible so long as fungus infection was not present at the time of tagging. Furthermore, our study demonstrated that fungus infection can negatively impact survival, if not properly treated in a timely manner. It is strongly recommended that all juvenile

lamprey to be tagged in future studies be free of all fungal infection at the time of tagging to ensure the highest probability of survival. Although our testing was conducted strictly in a laboratory environment, which may be different from the natural conditions experienced in rivers, our study demonstrated that the acoustic tag implantation process presumably had minimal impact on survival.

The average length of dummy tagged and acoustic tagged lamprey were similar; however, due to the limited number of available study fish, we were not able to meet the goal of 140 mm minimum size threshold for most of the dummy tagged lamprey (only 32% met the threshold). Given that 68% of the dummy tagged group lamprey were under the 140 mm threshold, but still experienced similar rates of survival compared to the control group lamprey, it appears that these slightly smaller lamprey are able to overcome the tag burden (at least in relation to survival). The smallest dummy tag lamprey was 130 mm (3.3 g weight), and this lamprey survived the 30-day holding study. Another lamprey that was 132 mm and 3.0 g survived the 30 day holding period as well. However, there was another lamprey that had a weight of 2.9 g (138 mm in length), but this fish succumbed to fungus infection and died during the study period. Using lamprey body width (girth) or weight might provide a better measure for minimum tagging size thresholds given that the key factor is the amount of space in the body cavity (rather than length). Future analysis of tag survival in relation to girth and weight (in addition to length) is recommended to make this determination.

Control lamprey and dummy tagged lamprey developed fungus equally, so our results show that the tag implementation process appears to have no discernable impact on fungal infection occurrences. All lamprey were handled in the same fashion except for the tag implementation procedure, so it is possible that other aspects of their living conditions, such as the handling of lamprey or added stress from being confined to a holding tank, contributed to the growth of fungus. However, it is uncertain which lamprey were first infected with the fungus, which may be a critical factor; it may have been dummy tagged group lamprey (or control group lamprey) that were first infected in the two aquariums with fungus infection. More studies are needed to fully understand how rapidly the fungus can spread among the study fish within the tanks. It is also unknown whether juvenile lamprey with fungus in the wild are able to naturally overcome their fungus infection (due to higher flow rates, natural substrates, etc.).

As for treatment dosage, we discovered that a 1-hour formalin treatment at 333 µl/L was safe for them but a 1,667 µl/L dosage was too high, and a 1-hour hydrogen peroxide treatment at 100 mg/L was safe for them but a 200 mg/L dosage may be stressful for them. Schreck et al. (1999) experimented with a few treatment dosages of hydrogen peroxide in 1-hour warm water static bath (22˚C) and found that 286 mg/L dosage resulted in 0% survival, but a dosage of 143 mg/L dosage or less resulted in 100% survival. It would be worth testing dosages in between 150 and 200 mg/L to narrow down the most effective yet safe dosage for juvenile lamprey. Similarly, a safe formalin dosage between 333 and 1,667 µl/L should be investigated. Finally, more testing is needed with salt treatment to understand both its effectiveness in treating fungus on juvenile lamprey and its safety thresholds for them.

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