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Implantation of a New Micro Acoustic Tag in Juvenile Pacific Lamprey and American Eel

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TITLE:

Implantation of a New Micro Acoustic Tag in Juvenile Pacific Lamprey and American Eel

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KEYWORDS:

Pacific Lamprey, American Eel, tagging, acoustic transmitters, swimming ability, tag shedding

SUMMARY:

This article illustrates the procedure for implanting a micro acoustic tag in juvenile Pacific Lamprey and American Eel. Our application is based on laboratory trials, which indicate that acoustic tags can be successfully implanted with no apparent effects on swimming ability or survival and minimal tag loss.

ABSTRACT:

Juvenile Pacific Lamprey and American eels were used for laboratory evaluations to determine potential effects from tag implantation. Telemetry technology has been identified as a way to obtain more detailed information on movement and behavior across a broader spatial scale than is possible with other known technology. The purpose of this method is to provide a detailed step by step instruction on tag implantation for both lampreys and eel. For laboratory studies using actively migrating juvenile Pacific Lamprey (120-160 mm), we determined that the presence of the tag did not alter the swimming ability between tagged and untagged Individuals or have any significant tag loss (<3%). Similar results were determined during laboratory testing of Yellow phase American Eels (113–175 mm). No mortality occurred during a 38-day holding period and there was minimal tag loss (3.8%). The presence of the tag did not have any significant effect on the swimming ability or survival of tagged eels compared to untagged controls and there was minimal tag loss.

INTRODUCTION:

Understanding fish behavior and movements near river structures, such as hydropower dams, that may impede upstream or downstream migration routes is an ongoing research need. Although numerous studies have been conducted using existing technology, there are still many research questions about juvenile eels and lamprey survival and behavior. To help address these

questions, Pacific Northwest National Laboratory (PNNL) has developed a new acoustic micro transmitter specifically designed for use in juvenile eels and lampreys, called the lamprey/eel tag[1]. Prior to this development, existing acoustic tags were too large to be effectively implanted into the body cavities of juvenile eels and lampreys, and would result in a tag burden that exceeds accepted standards (a conservative value of tag to body weight ratio is 2% or less and other literature suggests 4-5%)[2-4]. The lamprey/eel tags emit unique coded signals at a frequency of 416.7 kHz, which are monitored via autonomous receivers (hydrophones) at fixed structures or in-river receivers[5-6].

In the Columbia River Basin in the United States, awareness and concern for understanding the entire life cycle of the Pacific Lamprey (*Entosphenus tridentatus*) has escalated because populations have significantly declined in the past 40 years[7]. Construction and operation of hydroelectric facilities may negatively affect juvenile lamprey, as their declines occurred after the period of major hydroelectric development[8]. One potential source of mortality is dam passage when juvenile Pacific Lamprey out-migrate to the ocean[9]. Passive Integrated Transponders (PIT) are commonly used for migrating fish species that may pass diversion structures (e.g., juvenile bypass systems), which allows tagged fish to be enumerated[10-11]. However, juvenile lamprey are thought to migrate deeper in the water column than juvenile salmon and are less likely to pass through the juvenile bypass systems[12]. Because of the low detection probabilities and a lack of hatcheries or other concentrated sources of juvenile lamprey for PIT tagging, information on juvenile lamprey passage, survival, or behavior is very limited. Knowledge of juvenile Pacific Lamprey behavior and survival are critical for developing mitigation strategies for downstream passage, including design of bypass systems for use at hydroelectric facilities and irrigation diversion structures[13].

The American eel (*Anguilla rostrata*) is catadromous with the adult phase migrating from freshwater toward the ocean to spawn. Their population levels have seen dramatic declines over the past several decades. Previously, they were very abundant in all major rivers flowing into the Atlantic Ocean and upstream through the St. Lawrence River to Lake Ontario; but since 1980, American eels have experienced significant declines in stock abundance ranging from 50% in Chesapeake Bay to as much as 97% in Lake Ontario[14-16]. The factors contributing to their decline include; the construction of hydroelectric dams, fragmentation and loss of habitat, and commercial harvesting[17]. They are currently listed as Endangered under the Ontario (Canada) Endangered Species Act. The past development of hydropower facilities along major rivers of Eastern U.S. states create obstacles that impede riverine migrations of both juveniles and adults.

The newly developed lamprey/eel acoustic tag was used in this study. The tags were manufactured at PNNL's accredited Bio-Acoustics & Flow Laboratory[18]. The tag measures 12 mm length x 2 mm diameter, and has a weight of 0.08 g in air. Because of the tag's small overall size, it can be effectively used for implantation into anguilliform or juvenile fish using a sutureless incision, due to the small incision required (<3 mm long). Additional benefits of sutureless incision include: a reduced amount of time required for surgical process, faster healing rates, and decreased possibility of infections at the implantation site[19]. Surgical implantation effects can vary in response to species, life stage, body cavity length, incision location, study duration, and

environmental conditions[20-23]. In addition to size, weight is an important variable because it provides a measure of the tag burden (i.e., the weight of the tag relative to the weight of the fish). The tag burden, in association with all other aspects of the surgical process (e.g., anesthesia, handling, surgery), can have a direct effect on tag retention, survival, growth, swimming performance, or the ability of fish to avoid predation[24-27].

PROTOCOL:

PNNL is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Eels and lamprey were handled in accordance with federal guidelines for the care and use of laboratory animals, and the protocols for our study were conducted in compliance with and approved by PNNL's Institutional Animal Care and Use Committee.

1. Tag preparation (timing 22 minutes)

1.1. Place tags in 70% ethanol solution for 20 min.

1.2. Remove tags using plastic tweezers and place in small glass dish containing sterile water.

1.3. Remove tags and place on a sterile pill cup.

2. Prepare anesthetic solution (timing ~ 5 minutes)

2.1. In a small plastic cup weigh 40 g of Tricaine.

2.2. Dissolve powder and tap water into a 500 mL beaker on a stirrer plate with mixer bar and then transfer to a plastic bottle to achieve an 80 g/L stock solution.

2.3. Repeat the same procedure with sodium bicarbonate and add to a separate 500 mL bottle.

2.4. Using a 10 L plastic tub container, fill with 5 L water (same water source as fish to be tagged).

2.5. Add 6.25 mL of Tricaine anesthetic solution and 6.25 mL of sodium bicarbonate using a measuring pouring gage attached to the top of the 500 mL bottles to obtain a 100 mg/L dose to the tub.

2.6. Stir to mix solution in water.

NOTE: Have a lid available to cover the tub to ensure lamprey do not escape while in the anesthetic solution.

3. Lamprey tagging (timing ~6 minutes)

3.1. Place juvenile lamprey (>140 mm) into anesthetic bath using a small dip net.

3.2. Wait 4–5 min for lamprey to become fully demobilized (no longer swimming in solution, while maintaining a slow by steady gill movement).

3.3. Use a dip net to remove lamprey, take length and weight measurements and record the unique identification code of the acoustic tag.

3.4. Prepare a 1.3 cm thick closed-cell foam pad saturated with water first and then 150 µL/L protective coating (see **Table of Materials**). The protective coating helps to counteract the disruption of mucus membranes during surgical procedure.

3.5. Place the lamprey ventral side up on the prepared foam. Position a small section of tubing with regulated water supply (from an elevated water tank) to flow through the mouth region during the surgical process. This allows for respiration while fish is undergoing tag implantation.

3.6. Locate the site where incision is to be made, ~20 mm posterior to the gill pores on the left or right lateral side. Use a ruler with makings or use markers placed on the foam pad for reference.

3.7. Using a sterile 3.0-mm microsurgical scalpel with a 15° blade (**see Table of Materials**) carefully make a 2.5–3 mm opening in the lateral direction ensuring that the scalpel is cutting just through the inner skin wall. See **Figure 1.**

3.8. Place the disinfected tag anteriorly into the body cavity by hand. Apply slight pressure to the tagging site to ensure that the tag has moved into the body cavity.

3.9. Place tagged lamprey into a recovery bucket containing fresh river water supplied with a fish tank oxygen pump, tubing, and air stone.

3.10. Ensure that lamprey have recovered from the anesthetic and transfer to holding tank for future study.

4. Eel tagging (timing ~6 minutes)

4.1. Replicate tag preparation and prepare anesthetic with a concentration dose of 240 mg/L for both Tricaine and sodium bicarbonate.

4.2. Add 15 mL of each to the 5 L water bath.

4.3. Place juvenile eels (>130 mm) into anesthetic bath using a small dip net. Wait 4–5 min for eel to become fully demobilized (no longer swimming in solution while maintaining a slow but steady gill movement).

4.4. Use a dip net to remove eel and place ventral side up on a closed-cell foam coated with Fish Protector.

4.5. Locate the site where incision is to be made, ~25 mm to the base of the pectoral fin on the left or right lateral side

4.6. Using a sterile 3.0-mm microsurgical scalpel with a 15° blade carefully make a 2.5–3 mm opening in the lateral direction ensuring that the scalpel is cutting just through the inner skin wall. See **Figure 2**.

4.7. Place the disinfected tag anteriorly into the body cavity by hand. Apply slight pressure to tagging site to ensure tag has moved into the body cavity.

4.8. Place tagged eels into a recovery bucket containing fresh river water supplied with a fish tank oxygen pump, tubing, and air stone.

4.9. Ensure that eel has recovered from the anesthetic and transfer to holding tank for future study.

REPRESENTATIVE RESULTS:

Juvenile Lamprey Laboratory Studies

A study was conducted at PNNL in 2015 to determine the feasibility of implanting a dummy lamprey/eel tag in actively migrating juvenile Pacific Lamprey. A total of 195 Pacific Lamprey (macrophthalmia life stage) were transported from a field collection site to PNNL. Two separate tasks were conducted. The first was to determine how fish respond to tag implantation. The variables determined included tag loss, delayed mortality, and healing rates for tagged fish as compared to an untagged (control) group. For the second task swim trials were conducted with tagged and untagged fish from 10 mm size categories (120–160 mm) to determine any adverse effects associated with swimming ability. Fish weights ranged from 1.8 to 7.0 g and tag burdens ranged from 4.8 to 1.25%. An x-ray illustrating the tag location inside the body cavity is shown in **Figure 3**[1]. A total of two fish dropped their tags during the first 2 days of holding and no additional dropped tags were found during the remainder of the 28-day holding period. The cumulate mortality during this period was 14.3% (7 fish) for the tagged lamprey and 9.6% (5 fish) for the control group. Individual tagged and control fish were placed in a swimming chamber and swam at a constant water velocity of 11 cm/s. The flow rate was selected based on previous laboratory testing which indicated a constant swimming motion at the lower end of the sustained swimming ability[9]. The mean swim time of the control group was 3.15 min (SE = 42.5 s) and the mean swim time of the implanted group was 2.3 min (SE = 30.2 s), which was not a significant difference (t = 0.958; p = 0.172). The results from the swimming performance tests showed no significant correlation between fish length and swimming duration (i.e., time to impingement) for either the tagged or control groups (**Figure 4**). Impingement is described as the fish no longer able to swim at the constant water velocity and rested at the downstream screen.

American Eel Laboratory Studies

To determine any possible l tag effects on swimming performance, we conducted g critical swimming speed tests using U_{crit}, (an index of prolonged swimming performance) for six size groups (n = 120; 113–175 mm) of untagged and tagged eels using dummy tags which measured 11.4 mm x 1.8 mm (length x diameter)[1]. There was no significant difference in U_{crits} between tagged and untagged eels as measured in cm/s (**Figure 5**) or median values measured in body length/s for all tagged and untagged groups combined (**Figure 6**). We also conducted a prolonged holding of tagged eels (38 days) and found no mortality from the tagged groups and a low tag loss of 3.8% (1 out of 26 fish)[1]. Based on our laboratory results, we conclude that micro acoustic tags can be effectively implanted in juvenile American eels with no significant effects on swimming ability, long term survival and had minimal tag loss during the tag life (30 days).

FIGURE LEGENDS:

Figure 1. Photographs illustrating tagging sequence for tag insertion in juvenile Pacific Lamprey. **(A)** Lamprey positioned on foam pad. **(B)** Incision made with microsurgical scalpel. **(C)** Tag being implanted. **(D)** Lamprey post-tagging. All pictures were taken of the same lamprey (148 mm).

Figure 2. Photographs illustrating tagging procedure for juvenile American Eels. (A) Before incision. **(B)** After incision. **(C)** After anterior insertion of the tag. All pictures were taken of the same eel (138 mm).

Figure 3. X-ray images of dummy lamprey/eel tag inside the body cavity of a juvenile Pacific Lamprey.

Figure 4. Impingement rates from swimming performance tests for control (untagged) and tagged lamprey, separated into 10 mm size categories with positive standard error bars. Sample sizes are listed in the individual bars.

Figure 5. Box plots of critical swimming speed for size groups of eels tested in cm/s for tagged and untagged eels (10 per treatment). The lines within each box represent the median; the top and bottom lines represent the 75th and 25th percentiles, respectively; the whiskers are the top 90th and bottom 10th percentiles; and the outliers are depicted by enclosed circles.

Figure 6. Box plots of critical swimming speed in body length/s for tagged and untagged eels (10 per treatment). The lines within each box represent the median; the top and bottom lines represent the 75th and 25th percentiles, respectively; the whiskers are the top 90th and bottom 10th percentiles; and the outliers are depicted by enclosed circles.

DISCUSSION:

The results of the laboratory studies demonstrate that the tagging procedure and tagging effects do not have adverse effects on fish survivability or swimming ability. Extended holding and monitoring showed that minimal tag loss occurred and was not apparent during the tag life (30 days). The tag implantation procedure was effective at placing tags in the body cavity without causing significant hemorrhaging or fungal infections at the tagging site. The duration of the entire tagging process (<6 min) is beneficial in that it reduces stress associated with the fish being

anesthetized. These findings characterize a new tool for juvenile lamprey and eel passage research and can be utilized in future studies. This technology will allow researchers to track lamprey and eel movements within river systems and as they approach hydroelectric dams or other structures that impede fish passage. In turn, the results can better inform management decisions at these facilities to help conserve these species throughout their juvenile life stages. The most critical steps of the tag implantation protocol include using the proper dose of anesthetic, depth and length of the incision, placement of the tags, and having the proper recovery holding tanks available. This technique has minimal limitations. Most notably, surgeon training is required to effectively make the <3 mm incision and place the tags at the desired location. Additionally, the tagging process would exclude smaller sized eels and lamprey (<140 mm) due to tag burden limitations. We do not recommend any modifications to the described protocols.

Alternate methods for tagging eels and lamprey include the use of PIT tags. However, PIT-tags do not actively transmit signals, therefore PIT-tagged fish can only be enumerated as they are entrained and diverted into bypass facilities, or pass close to a fixed detector[10-11]. Future applications of the lamprey/eel tag include the ability to tag and track populations of migrating lamprey and eels in any environment they inhabit. Additional benefits of using the lamprey/eel tag would include the ability to estimate survival, fallback rates at spillways or via turbines, travel time within reservoirs, passage delays and related behavior as fish approach dams.

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DISCLOSURES:

We have nothing to disclose.

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Figure 1

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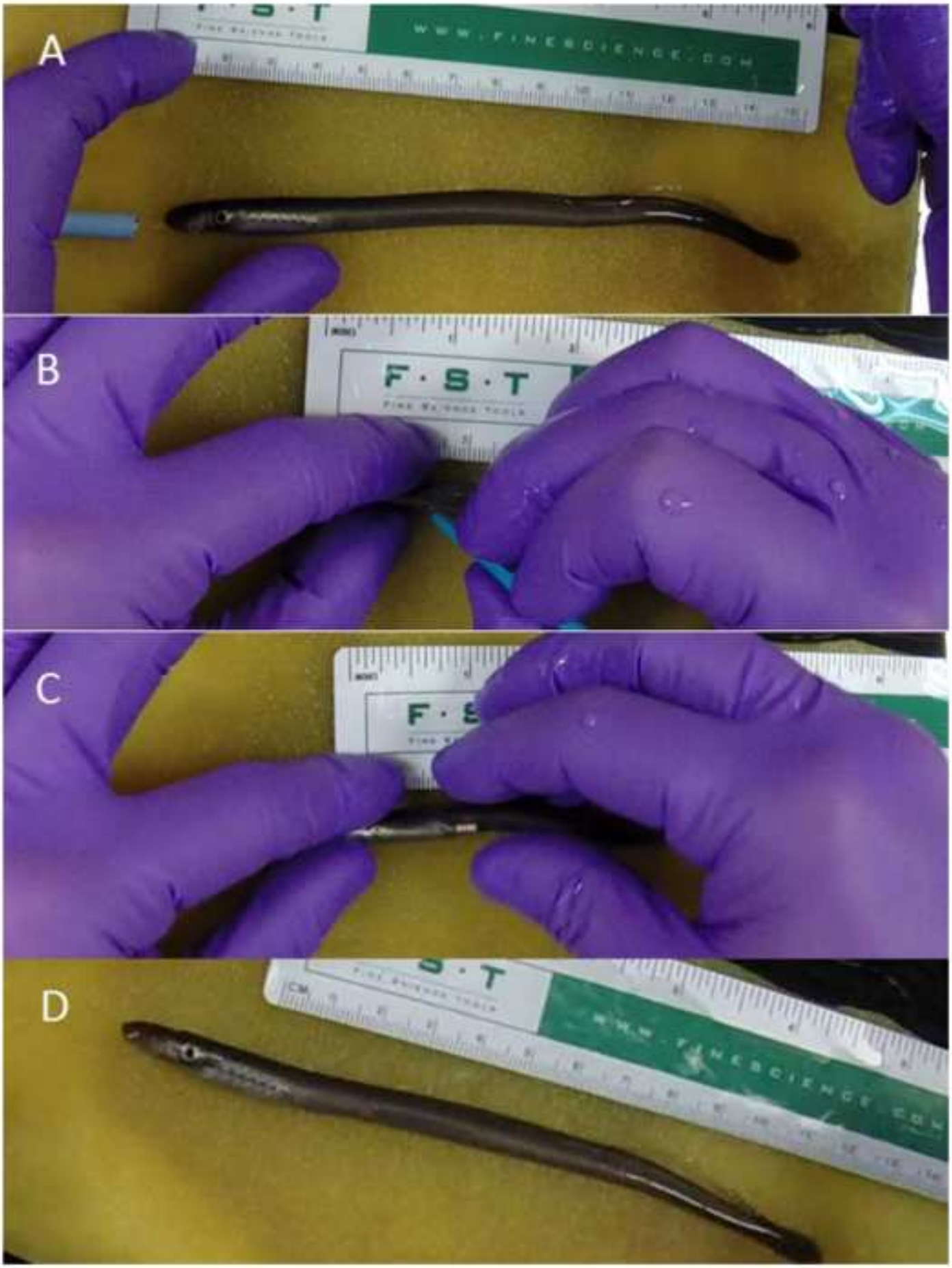

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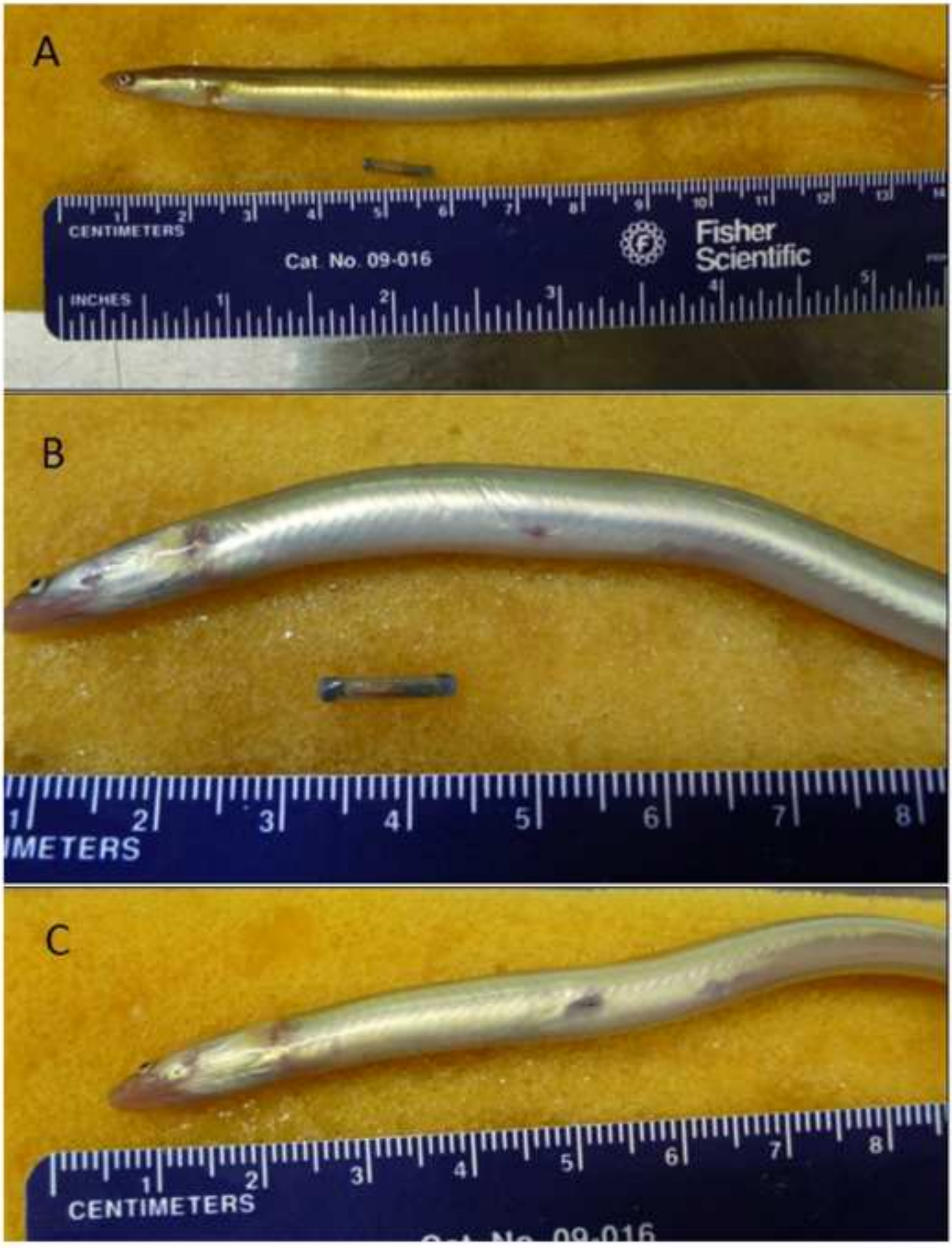

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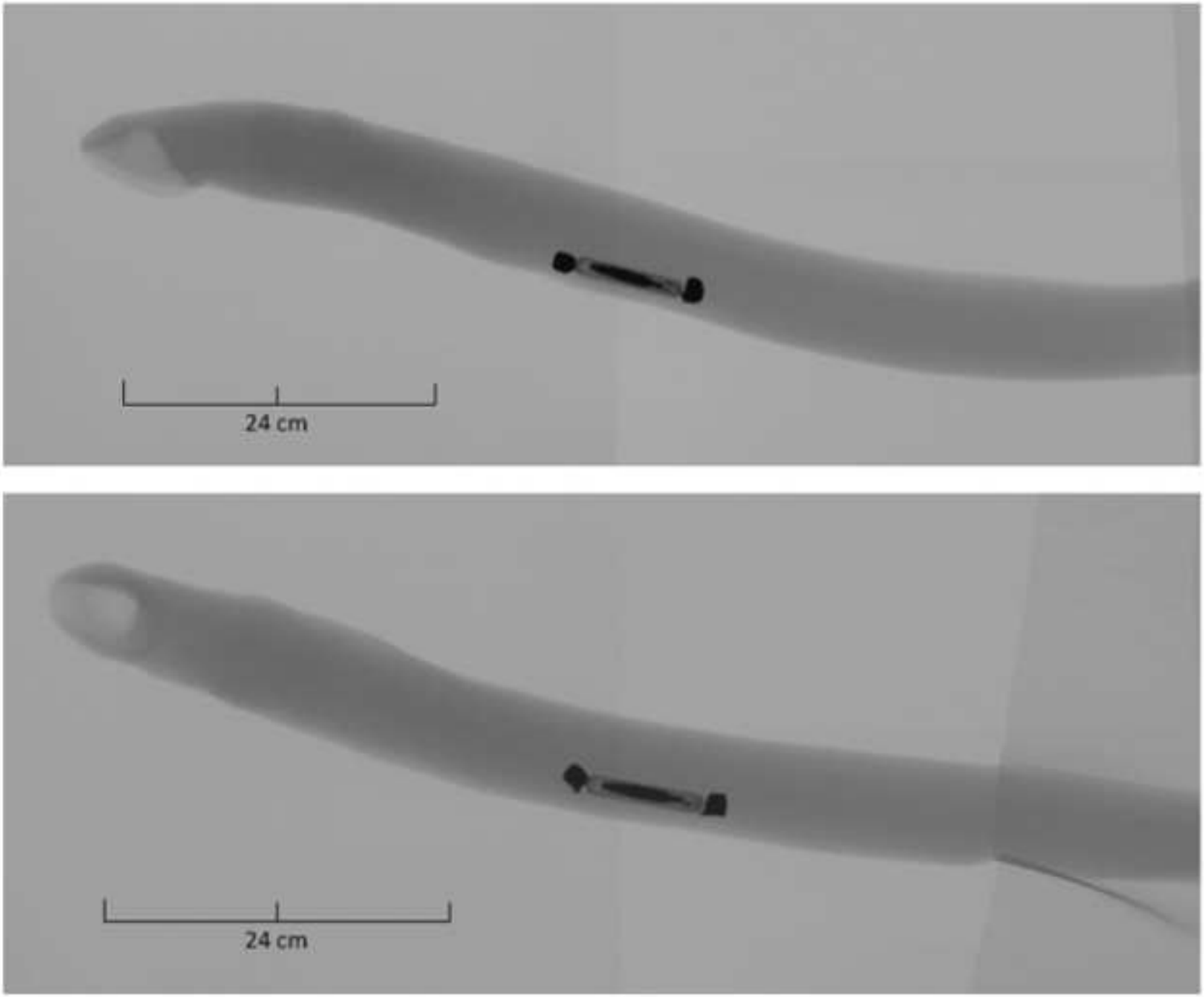

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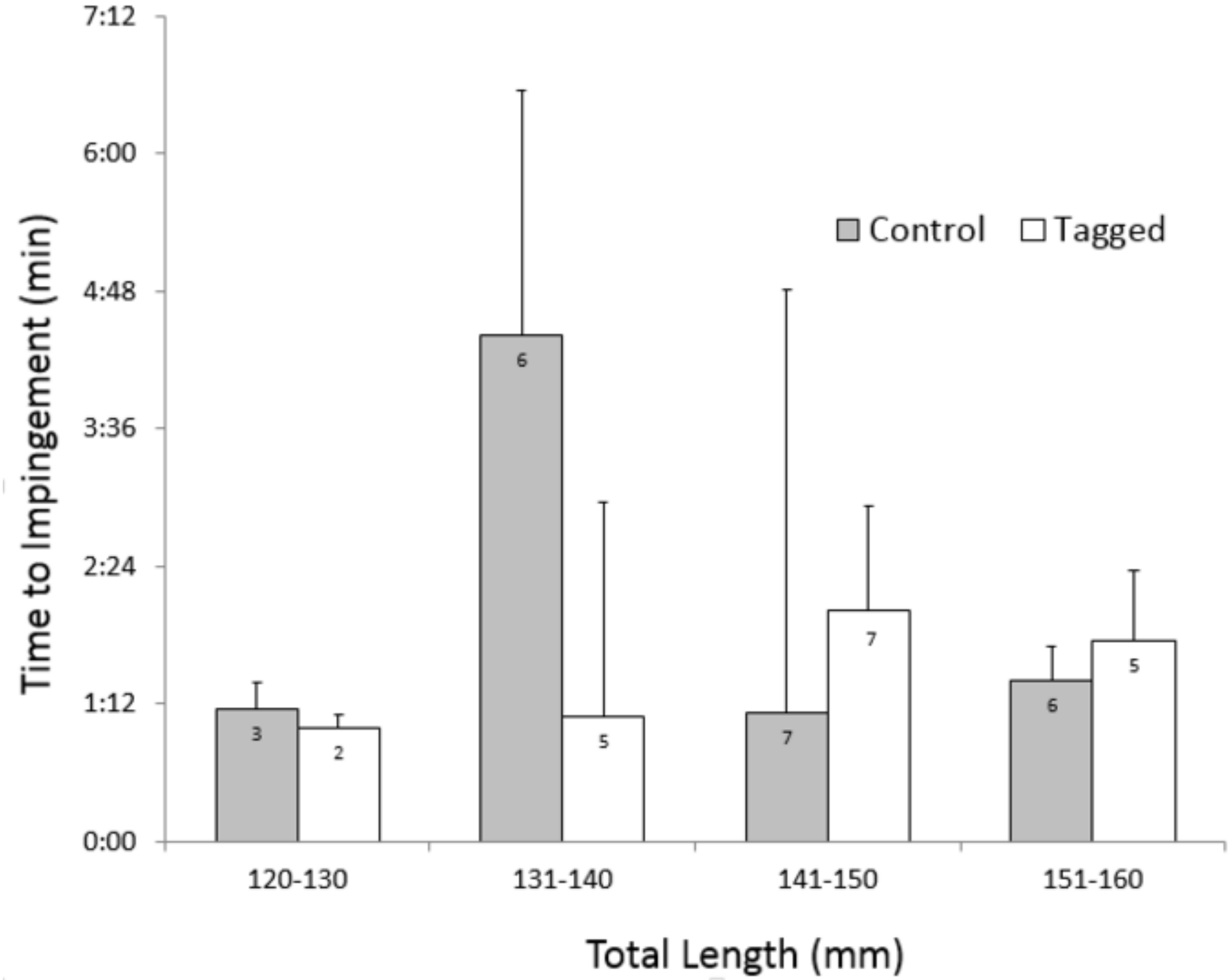

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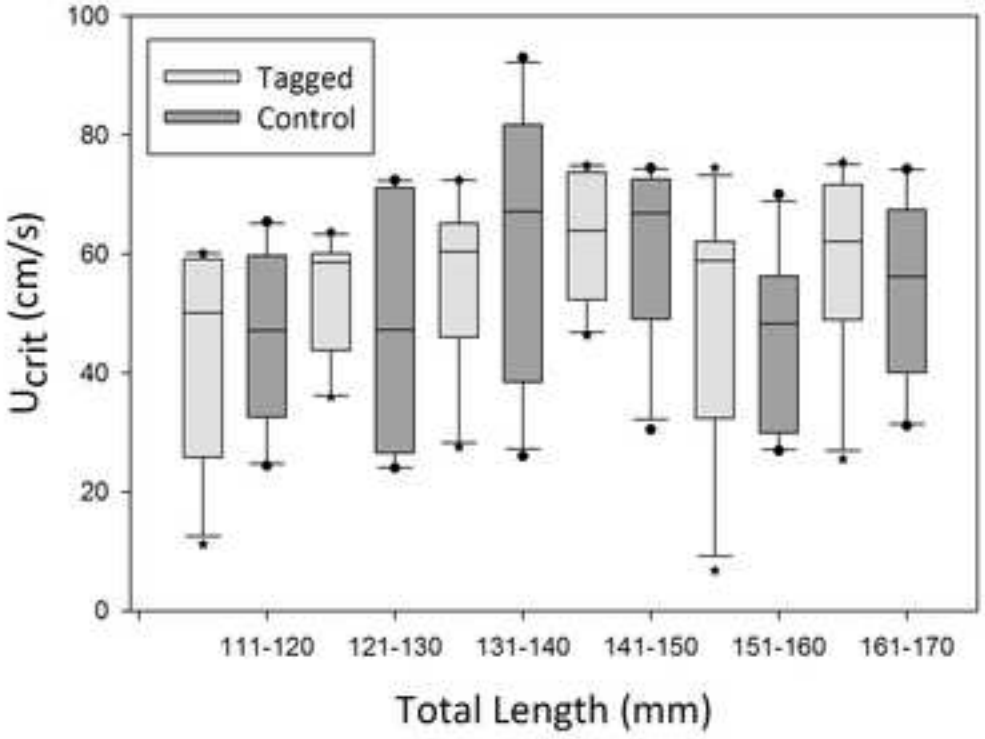

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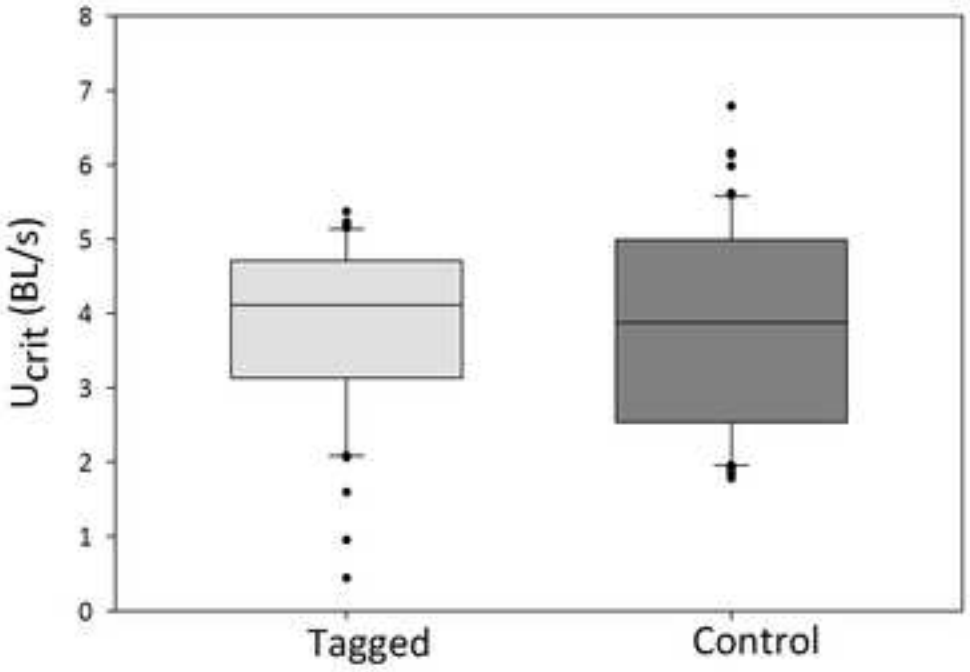

Table of Materials

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Name of Material/Equipment	Company	Catalog Number	Comments/Description
10 L plastic tub	N/A	N/A	
19 L pail	N/A	N/A	
3.00 mm scalpel	Beaver-Vistec	377513	
500 ml bottle	Nalgene	2089-0016	
Adam scale	Certified Material Testing Products	BCL-LBK12A	
Air pump	Amazon	N/A	
Air stone	Pentair	ALS3	
Dip net, 3/32 in mesh	N/A	N/A	
Ethanol (70%)	Sigma-Aldrich	24102	
Fish protector	Kordon LLC	31456	
Foam pad	N/A	N/A	
Gloves	Kimberly Clark	N/A	
Mixer bar	Sigma-Aldrich	F37110-1128	
Plastic tweezer Pouring dispenser gage	N/A Fischer Scientific	N/A 13-683-60C	
Scalpel handle	Beaver-Vistec	371360	
Sodium bicarbonate	Sigma-Aldrich	S5761	
Stirrer plate	Corning, PC-351	N/A	
Tricaine methanesulfonate, MS-222	Western Chemical Inc.	515388	Treated fish destined for food must be held in fresh water above 10°C (50°F) for 21 days before use
Tubing, 6 mm	N/A	N/A	

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Signature:	
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Changes to be made by the author(s) regarding the manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Completed.

2. Please revise lines 71-72, 74-79, 84-93, 180-181, 187-188, 238-240 to avoid previously published text.

These sections were reworded to eliminate duplicate text from previous journal report.

3. Are Figures 5 and 6 reprinted from a previous publication? If so, these figures must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]." If these figures are original, please rephrase their figure legends to avoid previously published text.

Yes. I submitted the copyright form which has the permissions granted. The captions were slightly altered.

4. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .pdf, .svg, .eps, .psd, or .ai file.

Completed and are now all in .tiff format.

5. Keywords: Please provide at least 6 keywords or phrases.

Completed.

6. Please revise the Abstract to focus on the method being presented rather than the results of a specific experiment. Include a statement about the purpose of the method. A more detailed overview of the method and a summary of its advantages, limitations, and applications is appropriate. Please focus on the general types of results acquired.

We revised the abstract to address this comment.

7. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

Completed.

8. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

The suggested change was made in the revised manuscript.

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The suggested changes were made in the revised manuscript.

10. Please describe in the protocol how the X-ray images are collected and how the swimming performance tests are done.

As per email from Alisha Dsouza on 1/26 these methods were referenced to our previous published journal article.

11. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

Added addition language in the discussion to address these concerns.

12. References: Please do not abbreviate journal titles.

Completed.

13. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

Completed.

Changes to be made by the Author(s) regarding the video:

1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

Changed the protocol text to better match the video narration.

2. 1:50: The video states "sterile water" while in line 98 of the written protocol it is "deionized water". Please revise to be consistent.

Changed in revised protocol text.

3. 1:54: A cup is used in the video while in line 100 of the written protocol a sterile foam pad is used.

Changed in protocol text.

4. 2:05, 2:19, 4:13, etc.: Please avoid commercial language (Finquel Tricaine, Nalgene, Fish Protector).

The suggested change was made in the revised video narration.

5. 2:10: What shows in the video and states in the written protocol is not the same. In the video the powder is dissolved with 500 mL of water and then transferred to a bottle.

Changed in protocol text.

6. 3:50-3:55: Such details in the video are not mentioned in the written protocol.

This section was added to the protocol (sec. 3.3).

7. 3:58: Please used the same length unit in the video (0.75 inch) and in the written protocol (1.3 cm).

Changed to 1.3 cm in the revised video narration.

8. 05:49-05:54: Such details in the video are not mentioned in the written protocol.

This section was added to the protocol (sec. 4.2).

9. Please upload a revised high-resolution video
here: https://www.dropbox.com/request/6pn0zGhWllkeNL6Ci7WP

10.The IACUC approval disclaimer for this procedure should be moved to before we see any animals in the video.

This disclaimer was moved to the beginning of video.

11. 0:00-1:36 - The background music is competing too much with the narration and interview audio. It should be lowered by at least 6 dB.

The background sound was lowered by 6 db.

12. 0:45 - The logos should be removed here. They can be included in a title card at the end of the video.

The suggested change was made in the revised video.

13. 6:26 - A chapter title card that reads "Representative Results" or something similar should be added here.

The suggested change was made in the revised video.

14. The protocol ID number (59274) should be included in the video file name for future submissions.

The suggested change was made in the revised video.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
It is well written with just enough details, but not too much detail.

Major Concerns:
No major concerns.

Minor Concerns:
Minor comments added to the pdf - please see those.

All of the reviewer comments were addressed in the revised manuscript using track changes.

Reviewer #2:
Manuscript Summary:
Overall, a very thorough description of a tagging process that will be very helpful to future researchers. Some minor concerns with the presentation of the laboratory trials.

Minor Concerns:
Sample size on figures 4, 5, and 6 above box-plots and bars, or provide these sample sizes in the text.

For figure 4, the sample sizes were added to the figure and for Figures 5 and 6 the samples sizes were added to the caption.

The discussion of fig. 4 in the text does not address the main concern (is there a tagging effect on swim duration and does this effect vary by size group), but rather states there is not a correlation between fish length and swimming duration. This should be reanalyzed/presented to provide the reader with information on tagging effects and if this varies by size class.

The text discussing this figure was changed to better explain the rational and the findings from this specific test. We also added a sentence providing with the statistical test used to explain the difference.

Comments that were called out in the separate PDF Document

All comments that were called out in the separate PDF document were addressed in the revised manuscript.

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