Retention and effects of miniature transmitters in juvenile American eels


A R T I C L E   I N F O

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A B S T R A C T

The purpose of this study was to assess the effects of an acoustic micro transmitter (tag) on survival and swimming ability of juvenile American eels (Anguilla rostrata). The transmitter was designed for implantation through a < 3 mm opening into the body cavity of anguilliform fishes without the need for sutures. Potential transmitter effects on swimming performance were examined by comparing critical swimming speeds (U_{crit}, an index of prolonged swimming performance) for six size groups (n = 120, 113–175 mm) of tagged and non-tagged eels. There was no significant difference in U_{crit} between tagged and non-tagged eels. Median U_{crit} for tagged eels ranged from 50.2 cm/s for the smallest group tested (113–119 mm) to 63.9 cm/s for eels 141–150 mm in length. Non-tagged group median U_{crit} ranged from 47.2 cm/s for the smallest group to 66.9 cm/s for the 141–150 mm group. An additional 26 eels (115–208 mm) were tagged and held for 38 days (without undergoing swimming performance tests) to assess survival and tag loss. No mortality occurred during the holding period and a tag loss of 3.8% (n = 1) was observed within the first 20 days post-tagging, which is the current projected battery life of the tag at a 5 s ping rate interval. Tag loss increased to 50% overall (n = 13) for eels held up to 38 days. Our results indicate that micro acoustic tags can be successfully implanted in juvenile American eels with no apparent effects on swimming ability or survival, and would be a viable option for examining eel movement patterns in river systems and near hydroelectric facilities.

1. Introduction

American eels (Anguilla rostrata) were once abundant throughout all tributaries of rivers flowing into the Atlantic Ocean and upstream through the St. Lawrence River to Lake Ontario. In recent decades American eels have experienced dramatic declines in stock abundance ranging from 50% in Chesapeake Bay to as much as 97% in Lake Ontario (Dixon, 2003; MacGregor et al., 2013; ASMFC, 2006; DFO, 2014). American eels are listed as Endangered under the Ontario (Canada) Endangered Species Act. This population decline has been attributed to several factors, including the construction of hydroelectric dams, fragmentation and loss of habitat, and commercial harvesting (MacGregor et al., 2013). The development of hydropower on the East Coast of the United States has had major adverse effects on eel populations because the species is catadromous and dams impede the riverine migrations of both juvenile and adult eels. Additionally, hydroelectric turbines may contribute to higher injury and mortality rates of juvenile eels (i.e., during the elver or yellow-phase) as they migrate upstream and then fall back (Normandeau, 2006). The ability to implant acoustic transmitters and track the movement of juvenile eels would help researchers better understand migration routes and survival rates to make better informed management decisions regarding new and existing hydroelectric facilities.

Previous tagging studies of American eels have focused primarily on the use of passive integrated transponder (PIT) tags to detect adults at hydro facilities during downstream migrations. The tags in these studies were implanted by both incision (Boubee and Williams, 2006) and injection ( McGrath et al., 2003; Verdon et al., 2003) in eels ranging from approximately 200–1200 mm in length; however, differences in the tag retention rates using these techniques are unknown. By contrast Normandeau (2006) implanted PIT tags in 291 American eel elvers (mean ± SD of 156.5 ± 26.1 mm) and reported a tag retention rate of 99% for fish held up to 4 days. Radio tags have also been used to assess downstream movements of silver eels on the Connecticut River (Haro et al., 2000) and acoustic tags have recently been used to assess downstream movements of silver-phase longfin eels (Anguilla dieffenbachia) in New Zealand (Jellyman and Unwin, 2017). To our knowledge, controlled laboratory studies to assess potential transmitter...
effects on anguilliform behavior and transmitter retention prior to use of the transmitter in field studies have not been conducted, and such studies are particularly important with the development of new transmitters.

The Pacific Northwest National Laboratory (PNNL) has developed a new, acoustic micro transmitter specifically for use in juvenile eels and lamprey, called the Eel/Lamprey Acoustic Tag (ELAT). The final version will have an operating frequency of 416.7 kHz. The tag can be monitored via autonomous receivers (hydrophones), at fixed structures or tracked by mobile systems. Prior studies have shown that fish outfitted with similar acoustic transmitters have been successfully tracked in the proximity to hydroelectric facilities (Skalski et al., 2014; Haro et al., 2000). The size of the prototype transmitter used in this study (11.4 mm length x 2 mm diameter, weighing 0.088 g in air, and having a specific density of 2.54 g, and a volume of 0.035 cm³) has been designed for implantation into anguilliform fishes without the need for sutures to close the incision, in part because the incision is < 3 mm long. A small incision without sutures can shorten surgery and healing time, and minimize potential negative effects of surgical implantation on the eels (Mesa et al., 2012). Surgical implantation effects can vary in response to species, life stage, body cavity length, incision location, study duration, and environmental conditions (Brown et al., 1999; Zale et al., 2005; Panther et al., 2011; Økland and Thorstad, 2013). Tagging methods, tag loss, and healing rates have been documented on silver-phase eels in the laboratory (Baras and Jeandrain, 1998; Wargo et al., 1996). Transmitter weight is also an important consideration because it provides a measure of the tag burden (i.e., the weight of the tag relative to the weight of the fish) that, when coupled with the surgical implantation process (e.g., anesthesia, handling, surgery), can affect tag retention, survival, growth, swimming performance, or the ability of fish to avoid predation (Adams et al., 1998; Jepson et al., 2008; Brown et al., 2013; Walker et al., 2016). Moreover, although implanted or externally attached transmitters have been shown to adversely affect swimming performance of Atlantic cod (Gadus morhua) and white sturgeon (Acipenser transmontanus; Counihan and Frost, 1999; Cote et al., 1999, respectively), no studies have examined the swimming performance of yellow-phase American eels implanted with small acoustic or PIT tags. Thus, the objectives of this study were to evaluate the implantation effects of an ELAT on the swimming performance, survival, and tag retention in a wide size range (113–175 mm) of yellow-phase American eels.

2. Methods

2.1. Fish acquisition

Glass stage American eels (< 30 mm) were obtained from the Delaware Valley Fish Company, South Shore Trading Co. LTD (Port Elgin, NB, Canada) in June 2014. The eels were reared indoors in 38 L aquaria at PNNL’s Aquatic Research Laboratory (Richland, WA). They were fed a mixture of live artemia and Otohime commercial feed (size A through C2) during the glass and yellow life stages. At the time of testing, the eels had reached the yellow-phase (1.5 years post glass-stage) and were 113–175 mm in total length and 1.7–7.5 g in weight (Table 1). All test eels were reared in flow-through Columbia River water that was sand-filtered and passed through ultraviolet light. The water temperature followed the ambient river cycle until approximately one month prior to tagging when it was increased to 16 ± 0.5 °C (median ± SD) and then maintained throughout the study period. Dissolved oxygen was recorded via an electronic monitoring system and ranged from 88 to 101% (median ± SD of 94.4 ± 1.9%). The eels experienced a natural photoperiod provided by clerestory windows.

2.2. Surgical procedures

There were two treatment groups: eels implanted with a non-functioning ELAT (tagged group), and eels that were not tagged (control group). The non-functioning ELAT housed a full duplex PIT tag (8.5 mm length x 1.4 mm diameter, 0.033 g; Biomark HPT-8, Boise, ID) for individual eel identification. The non-functioning ELAT had the same specifications of length, diameter, and weight as the prototype functioning ELAT. Food was withheld from all eels 24 h prior to surgery. The tags were implanted by one surgeon throughout the duration of the study. Prior to surgery, the eels were anesthetized in 240 mg/L of tricaine methanesulfonate (MS-222) buffered with equal parts of sodium bicarbonate. Time to stage four sedation (Summerfelt and Smith, 1990) was ~3.5 min. Eels were tagged by placing them ventral side up on a closed-cell foam pad saturated with 150 μL/L Fish Protector® (Kordon LLC, Hayward, CA; Harnish et al., 2011). A 2–3 mm incision was made ~25 mm posterior to the base of the pectoral fin on the left lateral side (i.e., approximately 1/3 of the total length of the eel) with a sterile 3.0 mm microsurgical scalpel (15° blade; Beaver Visitec, Wal.tham, MA). The disinfected (submersed in 70% ethanol for 20 min, then submersed in sterilized water for 10 min) ELAT was then inserted anteriorly into the body cavity by hand (Fig. 1). The tagging procedure took < 60 s, after which eels were placed into recovery buckets with fresh aerated river water at 16 °C, then transferred to segregated holding troughs (300 L) that had the same environmental conditions as the holding tanks. Control eels did not undergo surgery or receive an

![Fig. 1. Tagging procedure before incision (a), after incision (b), and after anterior insertion of an ELAT (c). All pictures were taken of the same eel (138 mm, 4.0 g).](image-url)
ELAT, but were anesthetized, measured, and handled similarly (i.e., held on the closed-cell foam pad for ~60 s) to the tagged eels to minimize handling bias.

2.3. Swimming performance trials

Swimming performance was evaluated from January 15 to March 7, 2016. Six size bins of 10 mm increments (111–120, 121–130, 131–140, 141–150, 151–160, and 161–175 mm) were used for each treatment, with transmitter burdens ranging from 1.1–5.2% (Table 1). Trials consisted of 10 tagged and 10 control eels from each size bin, for a total sample size of 120 eels. Tagged and control eels were randomized for each size bin and all eels from one size bin were either tagged or handled prior to starting the next size bin. The sequence of size bins was randomized mix of approximately 5 eels per day). A Blažka-type respirometer was used for swimming performance trials (2736 cm³ swimming area; 9 cm inside diameter; 43 cm chamber length; and 122 cm overall length). A 560-W electric motor was used to control water velocity and conduct tests of critical swimming speed (Ucrit), as described by Brett (1964):

\[ U_{crit} = u_1 + \left\{ \left( \frac{t_1}{t_0} \right) \times u_0 \right\} \]

where \( u_1 \) is the highest velocity (cm/s) maintained for the prescribed period, \( u_0 \) is the velocity increment (cm/s), \( t_1 \) = time (min) for which the eels swam at the “fatigue” velocity, and \( t_0 \) = prescribed period of swimming (min). The swim trial methods used by Janak et al. (2012) were applied to the current study, except that the swimming velocity for juvenile eels was increased by 12 cm/s every 6 min, compared to 10 cm/s every 15 min for juvenile salmonids. All swimming tests were conducted at approximately 16°C with dissolved oxygen between 88 and 101%.

A black shade cloth was placed over the swimming chamber to reduce visual disturbance of the eels and provide a darkened viewing environment for observation. Light levels were monitored using a portable light meter (Extech model 101036, Nashua, NH) to observe any variability in light during the test period that could affect swimming performance, and ranged from 0–10 Lux. All swim trials were monitored continuously with the aid of an infrared bullet security camera (CCTV model CSP-IPB-B) and remote monitor. A portion of the swimming performance trials were recorded using a CCTV 960H digital video recorder (Cherry Hill, NJ). The relationship between water velocity in the swimming chamber and motor speed was determined using a pitot tube (United Sensor, model S-065-10-250-8-125, Amherst, NH) and a linear regression model (\( y = 0.6034x + 6.965 \)). Plastic tubes (n = 126, 150 mm in length \times 6 mm diameter) were bundled together to form an 80 mm diameter cylinder that was then inserted at the upstream end of the respirometer to maintain a uniform water velocity within the swimming chamber. An electric grid was placed at the downstream end of the respirometer to move idle eels into the swimming area.

Eels were allowed to acclimate for 10 min, during which the water velocity in the swimming chamber was set to 2.2 cm/s. Following the acclimation period, the velocity was increased by 12 cm/s every 6 min. If the eel stopped swimming and fell back to the downstream end of the swimming chamber during the test period, they were given a mild shock from the electric grid (6–12 V, 0.75–1.5 amp for 1 s). Eels that did not swim away from the grid were shocked consecutively for 1 s at 3 s intervals for 10 s. If the eel remained on the grid at the end of the 10 s period, it was considered fatigued and the test was terminated. All eels were removed from the swimming chamber and held for 20 days to determine delayed mortality. We did not conduct a long term tag loss assessment for the swimming groups because repeated impingement and shocking events in the swimming chamber could have affected tag retention.

2.4. Long-term holding

A separate group of n = 26 eels were tagged on January 7, 2016 using the same protocols as described in the surgical procedures. This group (115–208 mm and 2.4–14.1 g) had a median transmitter burden of 2.0% (Table 2), and were held in a separate trough at 16°C for 38 days to assess transmitter loss and survival. While the current tag design has a 20 day battery life at a 5 s ping rate interval (PRI), we held fish for a longer period as the PRI can be increased to extend battery life (e.g., 10–11 s PRI provides 35–40 day life). Visual observations were made once per day. If a tag was dropped in the trough, it was removed from the tank and the tag code was recorded. During the holding period, three randomly selected tagged eels were examined on the day after tagging to assess wound healing (i.e., hemorrhaging, infection at the incision site). These additional examinations were performed while the eels were anesthetized (as described in the surgical procedures). Fish that lost tags were also examined to determine if the cause could be determined.

2.5. Statistical analysis

The Ucrit data of the tagged group of American eels were not normally distributed; therefore, differences in Ucrit among tagged and untagged groups were tested using the Wilcoxon Rank Sum Test (\( \alpha = 0.05 \)). Because swimming trials were conducted during two distinct time periods (January 15–February 4 and February 24–March 7), Ucrit was also compared between time periods for fish within tagged and untagged groups to determine whether extended holding affected the swimming performance of the fish tested later in the study. Due to non-normality of the data, the Wilcoxon Rank Sum Test (\( \alpha = 0.05 \)) was used to test for a difference in length between eels that retained their tag for the duration of the long-term holding study and those that expelled their tag. The relationship between tag retention duration and fish length was explored using simple linear regression.

3. Results

3.1. Swimming performance

The median Ucrit for all test groups combined was 59.7 cm/s for the tagged eels and 55.5 cm/s for the control eels (Fig. 2). The Ucrit values did not differ significantly between tagged and untagged eels (\( p = 0.745 \)). No differences in Ucrit were detected between early and late testing periods within groups of tagged and untagged eels (\( p ≥ 0.343 \)). Boxplots illustrating the variability in Ucrit for each size group in cm/s is shown in Fig. 3. Although the initial trend suggests that Ucrit increased with body lengths of up to approximately

<table>
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<th>Size Bin</th>
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<th>Tag Loss (%)</th>
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<td>111–120</td>
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served (Table 2). Only one of the 26 eels (3.8%) lost its tag within the transmitters during the 38 day holding period, no mortality was observed. The duration of tag retention was significantly correlated with fish size (Fig. 4). The median length of eels that retained their tag was 150 mm and the median length of eels that lost tags was 130 mm. However, differences in lengths between groups that retained or lost tags was not significant (p = 0.124).

Necropsies were performed on five eels that lost their tags. There were no observed abnormalities, although it appeared the tags were all expelled through the incision because there was redness or discoloration around the incision site, and the incision was either still open or had not completely healed. Two eels were imaged post-mortem with a commercial x-ray imaging system using a tungsten anode tube source (MXR-160 HP/11; Comet, Stamford, CT) to illustrate the exact tag position within the body cavity (Fig. 5). The tag location was just below the swim bladder and near the duodenum. For eels that did not lose tags, visual observation indicated that healing began at 1 day post-tagging. To determine whether eels could bend their heads and disturb the incision region with their mouths, we physically tested the eel’s flexibility when anesthetized and determined that it was not possible for them to reach this area.

4. Discussion

4.1. Swimming performance

The swimming performance of juvenile American eels implanted with an ELAT was similar to those that were not tagged for the size range of eels tested in this study. Variability in $U_{crit}$ was noted in all control and tagged groups but was most apparent in the 121–131 and 131–141 mm control groups. The duration over which these trials were conducted (~7 weeks) may have contributed to these differences; however, the $U_{crit}$ of the last two groups tested did not differ significantly from the groups tested at the beginning of the study period. Additionally, we do not believe that the extended duration for testing had a significant impact on $U_{crit}$ observed because all eels were held in a similar holding tanks that had consistent water temperatures and feeding schedules. All test eels were at the beginning stages of the yellow-phase and no skin color variability was observed. The majority of changes in eel hormones do not start to take effect until the intermediate phase (between yellow and silver-phase; van den Thillart et al., 2009).

This study was the first examination of swimming performance in tagged American eels at this developmental stage, so direct comparisons with similar studies are difficult. Evidence suggests, however, that both water velocity and temperature have a high likelihood of affecting swimming performance in eels. Wuenschel and Able (2008) found that water temperature was the main factor controlling $U_{crit}$ in untagged Conger eel (Conger oceanicus) at life stage ER-M1 and glass stage American eel. The $U_{crit}$ increased linearly with increasing temperature in both species, whereas $U_{crit}$ was unaffected by fish length for Conger eels (mean ± 95% confidence interval of 18.64 ± 1.365 cm/s for

Fig. 2. Box plots of critical swimming speed in cm/s for ELAT-tagged and untagged control eels. 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.

Fig. 3. Box plots of critical swimming speed for the six size groups of eels tested in cm/s for ELAT tagged and control eels. 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.
80–118 mm fish at 14–24.5 °C) and glass stage American eels (mean ± SD of 13.27 ± 0.675 cm/s for 48–60 mm fish at 10–15 °C). These U\text{crit}s are considerably lower than those reported in our study for American eels ≥111 mm, although we did not attempt to determine the influence of water temperature on swimming ability.

In another controlled swimming ability study, Barbin and Krueger (1994) observed that American eel elvers (56 mm, median) were able to maintain swimming speeds at 40 cm/s for very brief intervals. They also noted that 50% of the elvers tested were not able to maintain their position in the swimming chamber at velocities > 30 cm/s and suggested that they could not make forward progress against currents > 40 cm/s. Our smallest control group tested (113–119 mm) had a median U\text{crit} of 47.2 cm/s, which indicated that the swimming ability increased by ~17 cm/s for eels that were ~50 mm longer than those observed by Barbin and Krueger (1994). Moreover, the lack of significant differences in swimming performance between tagged and untagged eels is similar to observations made by Mueller et al. (2006) for 12 mm PIT-tagged and untagged juvenile Pacific lamprey (Entosphenus tridentatus). In that study, the median U\text{crit} for the tagged group was 76 cm/s and 82 cm/s at 16 °C water temperature for the untagged group with lamprey ranging in length from 128 to 171 mm. Interestingly, the median U\text{crit} for lamprey was slightly higher than the median U\text{crit} observed for implanted eels in this study (59.7 cm/s, median), although the overall size ranges were comparable.

Tag burden, or the weight of the transmitter relative to the weight of the eels used for the swimming trials, ranged from 1.2 to 5.2%. These values are comparable to those reported by Anglea et al. (2004) for juvenile Pacific salmonids of similar size ranges (122–198 mm) surgically implanted with acoustic transmitters (tag burden range 1.4–6.7%). However, these values were less than the tag burdens for acoustic transmitters and PIT tags (0.74 g) implanted into smaller juvenile salmon (93–116 mm) by Brown et al. (2010); tag burden range was 4.5–8.6%. Winter (1996) suggested that tag to body weight ratio should be at 2% or less than the fish’s weight but other studies suggest that some species do not experience negative impacts (mortality, tag loss or sublethal impacts) when ratios approach 8–12% (Brown et al., 1999). Jepsen et al. (2008) found that adult brown trout (Salmo trutta) 150–300 mm in length with high tag/body weight ratios were more likely to shed tags than those with lower ratios (mean: 2.7 vs 2.4). Jepsen et al. (2003) describes other important factors should be
considered such as tag shape, wound healing rates, feeding ability, social rank and water temperature among others may contribute to how well fish are able to cope and function with an implanted tag. Based on the swimming performance results for eels in the smallest test bin (which had the highest tag burdens), we conclude that the added weight of the ELAT would not be a detriment to swimming ability.

4.2. Extended holding

The current ELAT has a projected battery life of 20 days at a 5 s PRI. Based on our initial findings, we would not expect tag loss to be a concerning factor because all but one eel from the long-term holding groups retained their tags past the 20 day period. Additional studies may be needed to determine the extent of tag loss over a longer duration and the mechanism of tag loss if a longer PRI is used or if future battery technology improves performance, as there was an increase in tag loss between days 21 and 38 (n = 12 additional eels). This study did not determine why some of the eels retained their tags and the incision area healed in a short period of time while others experienced tag loss. However, we observed a positive correlation between the duration of tag retention and fish length, indicating fish size may contribute to tag expulsion. To prevent tag loss, a single suture could be added at the incision site. This was shown to be effective at eliminating tag loss for PIT-tagged juvenile Pacific lamprey (120–171 mm) during a prolonged holding study (40 days) at PNWL (Mueller et al., 2006). However, the increased handling/surgical time and possible attachment sites for fungal infections would need to be considered.

For the few tag loss studies that have been conducted on eels, PIT tags as opposed to acoustic tags were used. However, the ELAT used in the current study is similar in size and weight to a 12 mm PIT tag (12 × 2 mm). Short-term PIT tag loss has been reported with fish of similar morphologies (i.e., lampreys), but tag loss rates were relatively low. Normandieu (2006) observed 1.4% tag loss in 291 American eel elvers implanted with 12 mm PIT tags over a 4.4 day period. Mueller et al. (2006) observed a tag loss rate of 4% over 40 days in juvenile Pacific lamprey (n = 75) implanted with 12 mm PIT tags with no suturing.

Past field studies have commonly used PIT tags to assess movement patterns of adult eels around hydroelectric dams (Verdon et al., 2003; McGrath et al., 2003). Implantation of PIT-tags can be useful to track fish movements if fish are directed into detection structures; however, low detection ranges prohibit fish enumeration at most large passage structures at dams (e.g., spillways and overflow weirs). With the development of the new ELAT, it will be possible for researchers to tag juvenile eels and use acoustic telemetry to provide a better assessment of short-term movement patterns including the ability to track in-river movements in 3D (Li et al., 2014).

5. Conclusions

This research was the first to evaluate the swimming performance of yellow-phase American eels implanted with a small acoustic transmitter (11.4 × 2 mm, 0.088 g) in a controlled laboratory study. The results of this research found no negative effects from the ELAT associated with swimming performance when comparing tagged and control groups for the size range tested (113–175 mm). Tag loss occurred in the extended holding group. However, the majority of the tag loss occurred after the swimming performance results for eels in the smallest test bin (which had the highest tag burdens), we conclude that the added weight of the ELAT would not be a detriment to swimming ability.

such as impacts on growth, wound healing, and other physiological responses to the transmitter and/or tagging process, were also not the primary objectives of this study and should be further examined. Further assessments on tag effects, swimming performance, and tag retention in juvenile eels will aid researchers in better understanding eel behavior related to use of the ELAT in field studies. The ELAT allows researchers to track eel movements within river systems and near hydroelectric dams, and potentially make better informed management decisions near these facilities. Other possible benefits of using acoustic tags to study juvenile eel movements include the ability to estimate survival, fallback rates at spillways or via turbines, recension rates, passage delays and behavior as fish approach dams, and travel time within reservoirs or free-flowing river systems.

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References


