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Assessing the effects of passive integrated transponders (PIT tags) on the survival of Po brook lamprey (*Lampetra zanandreai*) ammocoetes

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ABSTRACT
Small-sized freshwater species with little or no direct economic value, such as many endemics, are poorly known in terms of habitat requirements, foraging strategies, distribution and movement behaviour. Passive Integrated Transponder (PIT) telemetry has proven useful for studying the ecology of small-sized species in confined environments. To use this technology in the wild, it is necessary to verify that PIT tags do not affect the survival and natural performance of tagged individuals. Such studies have been carried out on some small fish, usually showing low mortality and no effect on behaviour, but few have been carried out on lampreys. We investigated the effects of PIT-tagging on ammocoetes of *Lampetra zanandreai*, a freshwater lamprey endemic to northern Italy for which very little ecological knowledge is available. In a 2-week experiment, the tagged lampreys showed a high mortality rate (74%), while untagged controls showed no mortality. This result demonstrates that PIT-tagging is not a safe technology for ammocoetes of this species, at least not with the standard 12 mm tags.

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Telemetry; PIT tags; endemic species; freshwater; lamprey

Introduction

Freshwater habitats support a large proportion of biodiversity (Dudgeon et al. 2006; Tedesco et al. 2017). In Europe, the Mediterranean region is recognised as a biodiversity hotspot for freshwater organisms, hosting a large number of endemics (Reyjol et al. 2007; Freyhof & Brooks 2011; Tierno de Figueroa et al. 2013). These include lampreys (Cyclostomata: Petromyzontiformes: Petromyzontidae), a group of archaic vertebrates for which Europe is a centre of diversity and evolution (Freyhof & Brooks 2011).

The Po brook lamprey (*Lampetra zanandreai* Vladykov, 1955) is a non-parasitic lamprey that lives its entire life cycle in freshwater (Kottelat & Freyhof 2007; Fortini 2016). It is endemic to the Padano-Veneto district (northern Italy) and has populations in central Italy, Switzerland and Slovenia (Bianco 1992; Fortini 2016). This species has a larval phase of 4–5 years, after which it undergoes metamorphosis and becomes sexually mature. The larvae (ammocoetes) are blind and live in lowland reaches with moderate currents, sunken in soft (sandy or silty) substrates (Negro et al. 2023), where they feed by filtration. After metamorphosis, which occurs in autumn or winter, the adults are believed to make a short upstream migration where they spawn on gravelly bottoms and then die (Kottelat & Freyhof 2007; Fortini 2016). Apart from this general information, very little is known about the ecology of this species (Zerunian 2004).

One technology that has proven effective in increasing knowledge of animal ecology, particularly movement, migration and habitat use, is telemetry (Cooke et al. 2013; Thorstad et al. 2013). Passive integrated transponder (PIT) tags are small (typically 7–32 mm) and have therefore proven useful for studying small-sized fish (Cooke et al. 2013; Thorstad et al. 2013; Musselman et al. 2017). PIT tags are glass-encapsulated microchips, each with a unique identification code (Musselman et al. 2017). The tags are activated by the electromagnetic field of a reader antenna, and the lack of an internal battery has allowed a significant reduction in size while ensuring long-term viability (Cooke et al. 2013; Thorstad et al. 2013; Musselman et al.

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2017). Tagged individuals can be detected by antennas installed at strategic locations or by portable antennas (Thorstad et al. 2013). PIT tags can be used to estimate population size, growth, survival, movement, habitat use, predation, behaviour, and sampling efficiency (Musselman et al. 2017; Schiavon et al. 2024), as well as to evaluate and refine conservation measures (Castro-Santos et al. 1996; Watz et al. 2019) and to map the spread of invasive species (Thorlacius et al. 2015).

A fundamental assumption for the use of this technology is that the performance of tagged animals does not deviate significantly from their natural performance (Brown et al. 2011; Crossin et al. 2017). To test this assumption, studies focusing on the effect of PIT tags on mortality, tag retention and growth, as well as behaviour of species, are needed. Such studies have been carried out on a variety of freshwater fish, usually demonstrating the suitability of PIT tags for the species studied with no or small effects on survival or behaviour (e.g. Ficke et al. 2012; Nyqvist et al. 2023), given that the tag is not too large compared to the tagged fish (e.g. 16% length ratio in salmonids; Vollset et al. 2020). For small-sized fish, PIT tags have been found to be suitable for example for Cypriniformes (Schiavon et al. 2023; Nyqvist et al. 2024), loaches (Nyqvist et al. 2023), salmonids (Newby et al. 2007; Vollset et al. 2020), bullheads (Knaepkens et al. 2007), and gobids (Nyqvist et al. 2024).

Some studies are also available for lampreys, in particular ammocoetes or juveniles of Pacific lamprey (*Entosphenus tridentatus* Gairdner in Richardson, 1836) and sea lamprey (*Petromyzon marinus* Linnaeus, 1758), two anadromous, semelparous and parasitic species. Some of these studies found no effect of PIT tags on mortality and reported good tag retention for both species (Quintella et al. 2005; Mueller et al. 2006; Mesa et al. 2012; Moser et al. 2017; Simard et al. 2017), while Dawson et al. (2015) reported high mortality rates for sea lamprey larvae and Hanson and Barron (2017) for Pacific lamprey larvae. In terms of behavioural effects, some parameters indicative of swimming performance were found to not differ between tagged and untagged lampreys (Mueller et al. 2006; Moser et al. 2017), while others, including substrate burrowing speed, were lower in tagged lampreys than in controls (Dawson et al. 2015; Mueller et al. 2006). To our knowledge, no studies on the effects of PIT-tagging, or PIT telemetry studies for that matter, on Po brook lamprey ammocoetes has been published.

While the effects of PIT-tagging have been studied in sea and Pacific lampreys, and PIT telemetry has been successfully used to study sea and river lampreys (Pereira et al. 2017; Tummers et al. 2018; Moser et al. 2019), its application on other species is still an open question. In this study, we evaluated the effects of PIT-tagging on *Lampetra zanandreai* ammocoetes, in terms of survival, to assess the feasibility of using telemetry to improve our understanding of this particular endemic.

Materials and methods

On 27 September 2024, 40 lampreys (ammocoetes) were caught by electrofishing (direct current; ELT60IIGI, Scubla, Italy) in the Roggia Marina, a semi-natural, spring-fed stream located in Vercelli Province, Italy (UTM 433891E, 5006821N, zone 32T). The lampreys were transferred to the Alessandria Province Fish Hatchery (Predosa, Italy) and placed in a 150 x 44 x 20 cm spring-fed tank with a constant water exchange and equipped with shelters (tiles and perforated bricks), where they were left to acclimatise. After 3 days, the lampreys were anaesthetised with clove oil (Aromalabs, France; approximately 0.2 mL clove oil/L water), and their length and weight were measured. Thirty lampreys were tagged with 12 mm (12 mm × 2.1 mm; 0.10 g) passive integrated transponders (PIT tags; HDX, 134.2 kHz, ISO 11,784/11785; Oregon RFID, USA).

Tagging was performed by making a 2–3 mm latero-ventral incision with a scalpel, approximately 12 mm posterior to the gill openings, and pushing the tag in and forward in the abdominal cavity to align with the lamprey's body, following the procedure described by Mesa et al. (2012) and Moser et al. (2017). Lampreys were tagged by three different operators (10 each), all with experience in fish tagging. Two lampreys died during tagging and were not included in the subsequent stages of the study. After tagging and before transfer to the experimental tanks, the lampreys were placed in tanks with clean spring water and an oxygenator to recover from anaesthesia.

Two 52 x 35 x 18 cm holding tanks were set up within a larger 150 x 44 x 20 cm spring-fed tank with constant water exchange (Figure 1). The tanks were perforated on the bottom and all four sides with a series of 8 mm holes to promote oxygenation of the substrate and lined internally with non-woven fabric to prevent escape of substrate and lampreys. One tank was filled with the original substrate (SUB) and the other

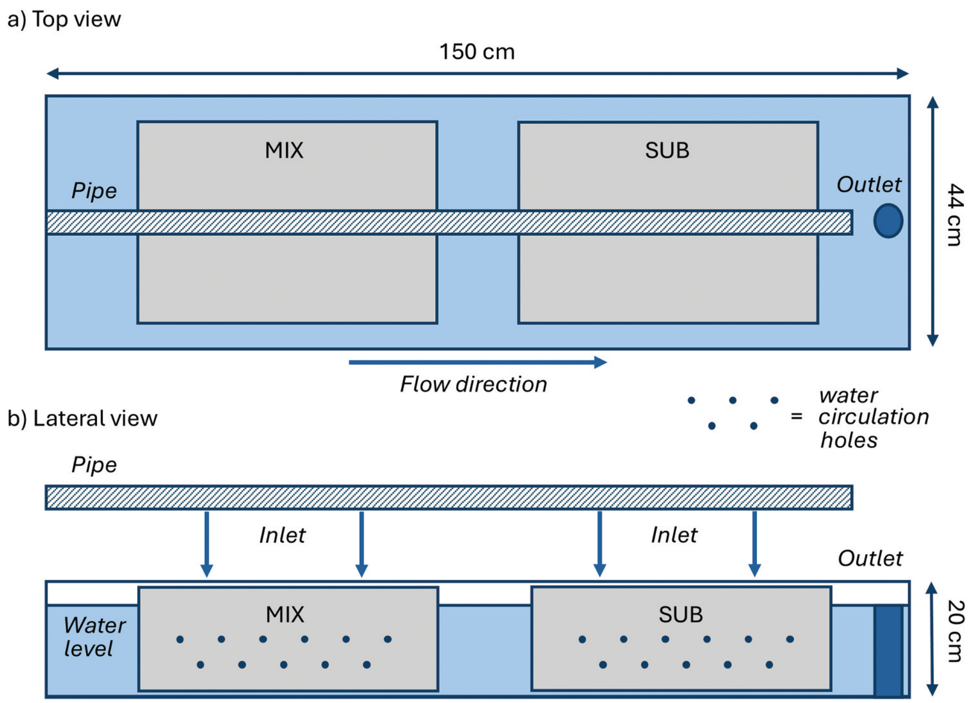

Figure 1. Experimental set-up. The set-up consisted of two 52 x 35 x 18 cm perforated and internally lined non-woven tanks, one filled with the original substrate (SUB) and the other with a 50:50 mixture of substrate and sand (MIX). These were placed in a 150 x 44 x 20 cm spring-fed tank with two water jets directed into each of the two smaller tanks. Panel (a) shows a top view of the set-up and panel (b) a lateral view.

with a mixture of the original substrate and aquarium sand with a grain size of 2–3 mm in a 50:50 ratio (MIX). Both were filled with a 14 cm layer of substrate. The substrate of the capture site consists of very fine sediment (silt) and Fine Particulate Organic Matter (FPOM) and is likely to provide a suitable environment for the lampreys, including food. We tested the original substrate mixed with sand to make it more permeable and facilitate oxygen exchange, which was identified as a potential issue in the hatchery environment. The bottoms of the smaller tanks were slightly raised to allow some water exchange also from below. A rigid pipe with four holes also supplied water directly into the two holding tanks. The water level was set at 16 cm, and the temperature was monitored using a data logger (MX2202, HOBO, USA) installed in the larger tank.

Fourteen tagged lampreys and five untagged lampreys (controls) were placed in each of the two tanks. After 2 weeks, the experiment was ended and the tanks checked for alive and dead lampreys. A handheld reader (TracKing-1 Portable Reader, Datamars, USA) was used to read the PIT tags and identify the tagged lampreys individually. One tagged lamprey was missing from the SUB tank (likely an escape) and was therefore not included in subsequent analyses. Surviving lampreys were released at the capture site.

Parametric tests (Student's test, Welch's test and analysis of variance (ANOVA)) were used to test for differences in length between lampreys tagged by different operators, between lampreys in the two tanks, between tagged lampreys and controls, and between surviving tagged lampreys and dead tagged lampreys. Shapiro's test and Levene's test were used to test the normality and homogeneity of variance of the lamprey length data. Fisher's exact tests were used to assess whether mortality differed between tagged lampreys and controls, among lampreys tagged by the three operators, and between lampreys in the two tanks. Data management, plotting and statistical tests were performed in R (R Core Team 2023; Posit Team 2024) using the packages car (Fox & Weisberg 2019), dplyr (Wickham et al. 2023), ggplot2 (Wickham 2016), and ggfortify (Horikoshi & Tang 2016; Tang et al. 2016).

Results

In total, 38 lampreys were included in the experiment, 28 tagged and 10 controls, equally divided between the SUB and MIX tanks. The controls (10.2 ± 0.8 cm) were significantly smaller than the tagged lampreys (12.7

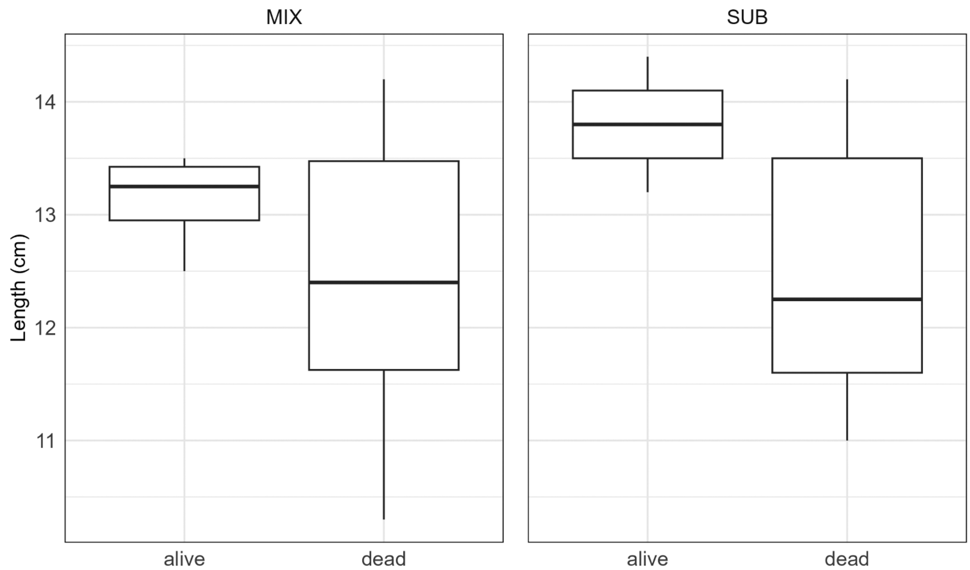

Figure 2. Box plot of lengths (cm) of alive ($n = 7$) and dead ($n = 20$) tagged lampreys in MIX and SUB tanks.

± 1.2 cm; t test: $t = -7.14$; df = 22.68; $p = 3.1e^{-07}$). No significant differences in size were found among lampreys tagged by the three operators (ANOVA: F = 2.78; df = 2; $p = 0.08$) or between lampreys placed in the two tanks (t test: $t = 0.21$; df = 35.21; $p = 0.83$).

At the end of the experiment, 20 tagged lampreys had died (10 in each tank), 7 survived (4 in the MIX tank and 3 in the SUB tank), and one was missing. All controls survived ($n = 10$). Mortality was significantly higher among the tagged lampreys than in controls (Fisher test: $p = 5.58e^{-05}$; test performed on a 2 × 2 contingency table). No difference in mortality was found among lampreys tagged by different operators (Fisher test: $p = 1$; test performed on a 3 × 2 contingency table) or between lampreys in the tanks with different substrates (Fisher test: $p = 1$; test performed on a 2 × 2 contingency table), suggesting that these two variables (operator and type of substrate) had no effect on mortality. Among the tagged lampreys, those that did not survive were significantly smaller than those that survived (alive: 13.4 ± 0.59 cm, dead: 12.5 ± 1.21 cm; Welch test: $t = 2.70$; df = 21.71; $p = 0.01$), suggesting an effect of size on mortality as a result of the tagging procedure (Figure 2).

After tagging, during recovery from anaesthesia, 15 tagged lampreys showed a change in colouration behind the tag location, a symptom which indicates that the tag was blocking blood flow through the dorsal aorta. Of the 15 lampreys that showed symptoms of blood flow obstruction, 12 did not survive, two survived and one was missing. By the end of the 2-week experiment, the surviving lampreys had incisions that were still open, partially closed or closed but not fully healed, but no wound infections were observed (Figure 3).

The mean water temperature was 14.3 ± 0.6°C during the experiment.

Discussion

This study assessed, for the first time, the effects of PIT tagging on the ammocoetes of the Po brook lamprey, endemic to the Padano-Veneto district. Most of the tagged lampreys (74%) did not survive the experiment, while all untagged control lampreys survived the experimental period. No effect of tagger or substrate type (MIX and SUB) on lamprey mortality was detected.

Among the tagged lampreys, smaller individuals seemed to fare worse than larger individuals, as the tagged lampreys that did not survive were significantly smaller than those that did. In particular, all lampreys shorter than 12.5 cm died. Several other studies have shown higher mortality of smaller compared to larger tagged lampreys of other species (Schreck et al. 1999; Mesa et al. 2012; Dawson et al. 2015; Hanson & Barron 2017; Simard et al. 2017). Different threshold lengths between 120 and 150 mm have been proposed for tagging (Schreck et al. 1999; Mueller et al. 2006; Mesa et al. 2012;

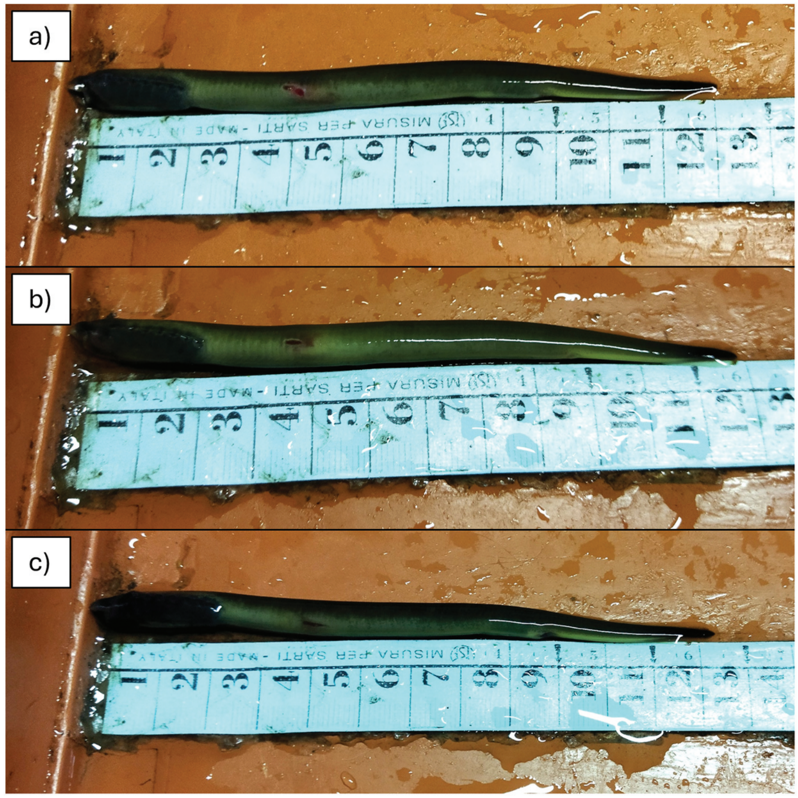

Figure 3. Different stages of healing at the end of the 2-week experiment: (a) incision still open; (b) incision partially closed; (c) incision closed but not fully healed.

Simard et al. 2017). In our study, however, even many large tagged lampreys died. Considering only lampreys larger than 12.5 cm, the mortality is still very high (53.8%), indicating that tagging resulted in high mortality of Po brook lampreys of all sizes.

The 100% survival of the control lampreys indicates that the tank conditions in the artificial environment of the experiment were adequate to maintain the lampreys, at least for the duration of the experiment (2 weeks). Although there was a large overlap in size between the tagged and control groups, the tagged lampreys were significantly longer than the controls. This is a bias likely introduced by the operators, who unintentionally selected the easiest lampreys to tag, i.e. the largest. Hanson and Barron (2017) and Mesa et al. (2012) also reported a difference in length between the PIT-tagged lampreys and the controls at the start of their experiments. Despite this bias, the fact that all of our controls survived excludes the possibility that factors other than tagging were the main cause of lamprey mortality.

After the 2-week experiment, none of the surviving lampreys had a fully healed incision. Studies of lampreys that have assessed wound healing over time have shown longer healing times, e.g. Moser et al. (2017) calculated 88 days for the complete healing of all individuals involved in their study, while after 15 days very few individuals showed signs of healing. It is therefore possible that our experiment was too short to allow the incisions to heal completely.

Our results clearly show that PIT-tagging causes high mortality in Po brook lamprey ammocoetes. Lampreys have a slim body profile and relatively small body cavity, and spatial constraints within the body cavity are likely to be a major issue in tagging mortality (Dawson et al. 2015; Hanson & Barron 2017). Indeed, 54% of tagged lampreys showed a colour change posterior to the tag within minutes of tagging, indicating that the tag had caused a disruption in blood flow through the dorsal aorta. Eighty percent of the lampreys with this symptom, which was also observed by Dawson et al. (2015), did not survive our experiment. Therefore, it appears that the tagging itself was the cause of mortality, rather than possible subsequent complications such as infection. Some studies have identified fungal infection as a cause of mortality in tagged lampreys of other species (Schreck et al. 1999; Mueller et al. 2006; Mesa et al. 2012; Simard et al. 2017), but we did not observe such issues.

Although smaller tags (down to 8 mm) than the one we used (12 mm) are available and could improve the results obtained (Cooke et al. 2013; Dawson et al. 2015), reducing the size of the tag usually also reduces the detection range (Zydlewski et al. 2006), making it more difficult to detect tagged individuals in the field. Given that *L. zanandreai* is a fossorial species, the use of smaller tags would likely prevent active tracking along watercourses with portable backpack antennas and limit the detection of the tagged lampreys only to times when they are actively moving through narrow sections equipped with stationary antennas or are recaptured during subsequent electrofishing. Nevertheless, future studies should test the use of smaller tags, which could open the door to tagging and tracking Po brook lamprey ammocoetes (Nyqvist et al. 2024). As for 12 mm tags, the high mortality of Po brook lamprey ammocoetes advises against using this method to study the movements and ecology of this species.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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