PIT-tagging Italian spined loach (Cobitis bilineata): Methodology, survival and behavioural effects

PIT-tagging Italian spined loach (Cobitis bilineata) – methodology, survival, and behavioral effects

Daniel Nyqvist[1*], Alfredo Schiavon[2,3], Alessandro Candiotto[4], Gloria Mozzi[1], Florian Eggers[5], and Claudio Comoglio[1]

[1] Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Italy

[2] Department of Ecohydrology and Biogeochemistry, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

3 Department of Biology, Chemistry and Pharmacy, Free University of Berlin, Germany

[4] Ittiologo libero professionista, Predosa, Italy

[5] Department of Environmental and Life Sciences, Karlstad University, Sweden

*Daniel Nyqvist, Via Enrico d'Ovidio, 10, c/o Ufficio Posta Interna Politecnico di Torino, 10129, Torino

Emails and ORCID:

Daniel Nyqvist: daniel.nyqvist@polito.it; 0000-0002-3098-0594

Alfredo Schiavon: alfredo.schiavon@igb-berlin.de; 0000-0002-4386-4912

Alessandro Candiotto: ale.candiotto@libero.it

Gloria Mozzi: gloria.mozzi@polito.it; 0000-0002-5173-769X

Florian Eggers: florian.eggers@kau.se

Claudio Comoglio : claudio.comoglio@polito.it; 0000-0002-7962-0653

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Abstract

The Italian spined loach (*Cobitis bilineata*) is an elongated, small sized (<12 cm) spined loach native to Northern Italy, Slovenia and Croatia. As for loaches in general, little is known about their individual movements in nature. Passive integrated transponders (PIT-tags) are small (typically 7–32 mm), relatively cheap, and allow tracking of individual fish movements and behavior. A fundamental assumption in animal telemetry is that the performance of a tagged animal does not deviate substantially from its natural performance. Although PIT-tagged fish often display high survival and tag retention, the effect varies between species and contexts, and few studies have looked at behavioral effects of PIT-tagging. Here we demonstrate a PIT-tagging methodology for spined loaches, and compare survival, activity, and provoked escape response (maximum swimming speed) between tagged and control fish. We also track tag retention in the tagged fish. Italian spined loaches tagged with 12 mm PIT-tags displayed high tag retention and no extra mortality, and no effects on tagging on activity or maximum swimming speed were observed. The tag-to-fish weight and length ratios in our study ranged from 2 – 5 % and 10 - 16 % respectively, and we conclude that PIT-tagging, within these ratios, appears suitable for Italian spined loach.

Introduction

The Italian spined loach (*Cobitis bilineata*) is an elongated, small sized (<12 cm) spined loach native to Northern Italy, Slovenia and Croatia. It is a benthic fish that typically lives close to sand-, mud- or gravel banks in shallow and slow flowing waters in lakes and rivers. Although a relatively tolerant species, it is threatened by habitat alterations as well as competition from invasive species (Fortini, 2016; IUCN, 2010). While spined loach ecology has been studied using capture sampling techniques (e.g. Bohlen, 2003; Copp & Vilizzi, 2004; Robotham, 1978), as for loaches in general, little is known about their individual movements in nature.

In animal telemetry, electronic transmitters are commonly used to study the movement of individual animals (Cooke et al., 2004; Thorstad et al., 2013). Passive integrated transponders (PIT-tags) are small (typically 7–32 mm) and relatively cheap. They do not carry an internal battery but instead transmit a unique signal when within the electromagnetic field of a reader antenna. This limits detection range but nevertheless allows detection of recaptured animals and animals passing strategically placed antennas (Gibbons & Andrews, 2004). Animals with relatively restricted or constrained movements can also be tracked manually using a portable antenna (Nzau Matondo et al., 2019; Watz et al., 2019). PIT-telemetry has, for example, been used to study habitat use (Quintella et al., 2005; Watz et al., 2019), survival (Keeler et al., 2007), home range (Breen et al., 2009), migration patterns (Brönmark et al., 2008; Schwinn et al., 2017), activity (Závorka et al., 2016), and fish passage performance (Castro-Santos et al., 1996) in stream fish in nature. Telemetry has accelerated our understanding of fish behavior, and is an effective tool to bolster evidence-based river management and conservation (Crossin et al., 2017).

A fundamental assumption in animal telemetry is that the performance of a tagged animal does not deviate substantially from its natural performance (Brown et al., 2011; Crossin et al., 2017). Many researchers refer to a relatively arbitrary "2 % rule" - assuming normal behavior if the PIT tag weighs less than 2% of body weight - based on the capability of the swim bladder to compensate for the weight of the tag (Baras et al., 1999; Brown et al., 1999; Winter, 1983). For salmonids and PIT-tags, a fish-tag-length ratio of 17.5% has been suggested as a no effect threshold based on a meta-study taking survival and growth into account (Vollset et al., 2020). Tag effects can, however, differ between species, and even based on environmental conditions (Clark, 2016; Jepsen et al., 2015; Wargo Rub et al., 2020), making studies of tag effects an important component of fish telemetry.

Most studies on effects of PIT-tags on small stream fish have focused on survival, growth and tag retention, typically showing high survival and tag retention, as well as growth effects mainly in the short term (Clark, 2016; Vollset et al., 2020). Importantly, however, some species may display high tagging mortalities (Clark, 2016; Watson et al., 2019). While potentially of high importance, behavioral effects are much less explored. In a rare study, Newby et al., (2007) did not see any difference in feeding behavior between PIT-tagged and control rainbow trout (*Oncorhynchus mykiss)*. Fish swimming performance involves both behavior and physical performance (Tudorache et al., 2013) and has been tested without detectable effects of tagging on sustained swimming in cyprinids (Ficke et al., 2012), salmonids (Newby et al., 2007), and lampreys (Mueller et al., 2006) and on burst speeds in bullheads (Knaepkens et al., 2007) and lampreys (Mueller et al., 2006).

Whereas most swimming tests constitute forced or provoked swimming, an open field test consists of letting an animal freely explore an arena after having been given a time to acclimatize, and hence measures a type of voluntary swimming behavior. It is commonly used in behavioral ecology to test for individual animals inclination to explore an area, as a proxy for boldness, activity or exploratory behavior (Mittelbach et al., 2014; Perals et al., 2017). In fish, individual open field test scores have correlated with activity (Závorka et al., 2016) and movement (Fraser et al., 2001; Watz, 2019) in nature, as well as passage behavior at a hydropower dam (Haraldstad et al., 2021). Outside of ecology, it is also used to test for chemically induced alterations in animal behavior (Echevarria et al., 2008; Gould et al., 2009; Hong & Zha, 2019). In this study, we apply an open field test, in combination with provoked swimming, to test for behavioral differences between tagged and control fish.

Although a potentially important tool for studying their behavior and ecology in nature, spined loaches have not yet, to our knowledge, been tagged and tracked in nature, nor have potential effects of tags on their behavior been evaluated. Here we draw on typical incision based procedures (e.g. Bolland et al., 2009) and previous work on pond loaches (Kano et al., 2013) to demonstrate a PIT-tagging methodology for spined loaches. We compare survival, total distance moved during an open field test (activity), and provoked escape response (maximum swimming speed) between tagged and control fish. We also track tag retention in the tagged fish.

Material and methods

C. bilineata were collected in slow flowing water, over muddy sediment, in the Lemme River, province of Alessandria, Italy (UTM 485300E, 4947307N; UTM 484407E, 4948227N; zone 32T) using electrofishing on 15 November 2021. The fish were brought to the Predosa Hatchery (Predosa, Alessandria, Italy), and left to recover in a spring-fed flow-through tank for three days before tagging. Only healthy-looking fish were included in the study.

Fish were anesthetized in clove oil (Aromlabs, USA; approximately 0.05 ml clove oil / l water) for an average of 234 seconds (sd = 74 s). Treatment fish were placed lying on the side on a wetted dishcloth with the head covered with another wetted dishcloth. A 2 - 4 mm incision was made on the ventral side of the fish, slightly offset from the center, and anterior of the pelvic fins. The Passive Integrated Transponder (PIT-tag; Biomark, USA; 12 mm * 2.1 mm; 0.10 g) was inserted manually and pushed forward in the abdominal cavity to align with the fish body (see film in supplementary material online). After tagging, fish were measured for length and weight before being let to recover in an aerated fresh water tank. Controls were subject to the same anesthesia protocol but then only measured for length and weight.

After tagging, fish were kept in a spring fed flow through tank (length*width*depth = 1.1 m * 1.2 m * 0.4 m) with shelters available, dampened natural light regimes (windows in the hatchery) and a stable temperature of 13°C. The fish were fed commercial aquaria fish pellets for bottom feeding fish (Tetra TabiMin) and occasionally wild-caught aquatic invertebrates (Bohlen, 1999). The tank was inspected daily for mortalities.

Three weeks after tagging, a random subset of fish was subject to an open field test followed by a series of provoked escape responses to score the fish movement activity and maximal swimming speed. An individual fish was netted from the holding tank, placed in a small bucket and gently released into an arena (length*width*depth = 565*365*100 mm). The fish was left in the arena for approximately ten minutes: five minutes to habituate to the new environment and 4.5 minutes for an open field test (Miklósi et al., 1992; Watz, 2019). After approximately 10 minutes in the arena, an escape response was provoked in the fish to estimate maximum swimming speed; a circular weight was released from about one meter's height to land in the proximity of the fish. The fish typically displayed an immediate escape response followed by some time swimming around. When the fish stopped, another escape response was provoked by dropping another spherical weight close to the fish. This was repeated a third time, to provoke in total three escape responses (Knaepkens et al., 2007; Tudorache et al., 2008). After stopping for a third time, the fish was netted, anesthetized, checked for presence of a tag, measured for length and left to recover in an aerated tank. When the full subset of fish had been tested and recovered, they were returned to the larger holding tank. Two trials were run in parallel. Water in the test tanks was changed regularly to maintain a stable temperature across trials. Temperature was measured continuously in a separate tank, subject to identical conditions as the test tanks.

The arena was filmed with an overhead video camera (Sony 4K, FDR-AX43 , 50fps), and fish positions were manually tracked using a custom-made MATLAB (R2021b, The MathWorks Inc, Natick, Massachusetts, USA) script (https://github.com/SilverFox275/manual-point-tracking). The fish position (center of mass) was manually determined with a frequency of one frame per second during the open field test, and ten frames per second during the provoked escape response. Known dimensions of the arena were used to translate distances in pixels to distances in meters. During the open field test, total distance moved was quantified (eg. Haraldstad et al., 2021; Watz, 2019). The fastest 400 ms (that is, longest distance moved over 4 tracked frames) during the provoked escape response period were used as an estimate of maximum swimming speed (Knaepkens et al., 2007; Tudorache et al., 2008). Both the actual tests and the manual tracking were performed with the investigator being blind to the treatment.

On 20 January 2022, 63 days after tagging, the experiment was ended, and all remaining fish were checked for tag retention and measured for length and weight. The tank was searched for missing tags. If a fish lost the PIT tag, unless the incision could still be seen, it would be included in the control group in the later length and weight calculations.

Data management, plotting and statistical tests were performed in R (R Core Team, 2021) involving the following packages: dplyr (Wickham & Francois, 2015), ggplot2 (Wickham, 2016) and plyr (Wickham & Wickham, 2017). Difference in maximum swimming speed between tagged and control was tested using a two-sided t-test. Other data did not fulfil the criteria for parametric tests and therefore Wilcoxon-Mann-Whitney tests were used to compare tagged and control fish with respect to the length and weight at the start and end of the study and the total swimming distance for the subset of fish that did move during the open field test. As maximum swimming speed typically scales with body size, the swimming speed was normalized to the length of the fish (Domenici & Blake, 1997).

The study was performed in accordance with the Ufficio Tecnico Faunistico e Ittiofauna of the Provincia di Alessandria (n.65493 of 11 november 2021), under the provisions of art.2 of the national Decree n.26/2014 (implementation of Dir. 2010/63/EU).

Results

In total, 43 fish were tagged (median = 4.6 g, IQR = 3.6 - 5.3 g; median = 98 mm IQR = 90 - 103 mm) and 43 fish were kept as control (median = 3.6 g, IQR = 2.9 - 5.4 g; median = 91mm, IQR =85 – 101 mm). Fish were anesthetized for an average of 234 seconds (sd = 74 s). Handling times were on average 71 s (sd = 22s) for treatment fish, and 26 s (sd = 11 s) for control. There was no difference in length or weight between tagged and control fish (Wilcoxon, $p > 0.08$). The range of tag-to-fish weight ratio was 2 – 5%, while the tag-to-fish length ratio ranged between 10 - 16 %.

No extra mortality from tagging was detected. In total, two treatment fish (5%) and ten control fish (23%) died during the course of the study, all but one during the last week of the study (Fig. 2). Two fish (5%) lost their tags, noteworthy the first two fish tagged. Not all tagged fish displayed visible scars. Neither the length or weight differed between the tagged (n= 39; median = 4.4, IQR = 3.5 - 5.1 g; median = 99 mm, IQR 92 – 104 mm) and control fish (n = 35; median = 3.5 g, IQR = 2.6 – 5.4 g; median = 91 mm, IQR = 85 - 115 mm; included two fish with tag losses) alive by the end of the study (Wilcoxon; p > 0.12) but both groups had lost weight in comparison to the start of the study.

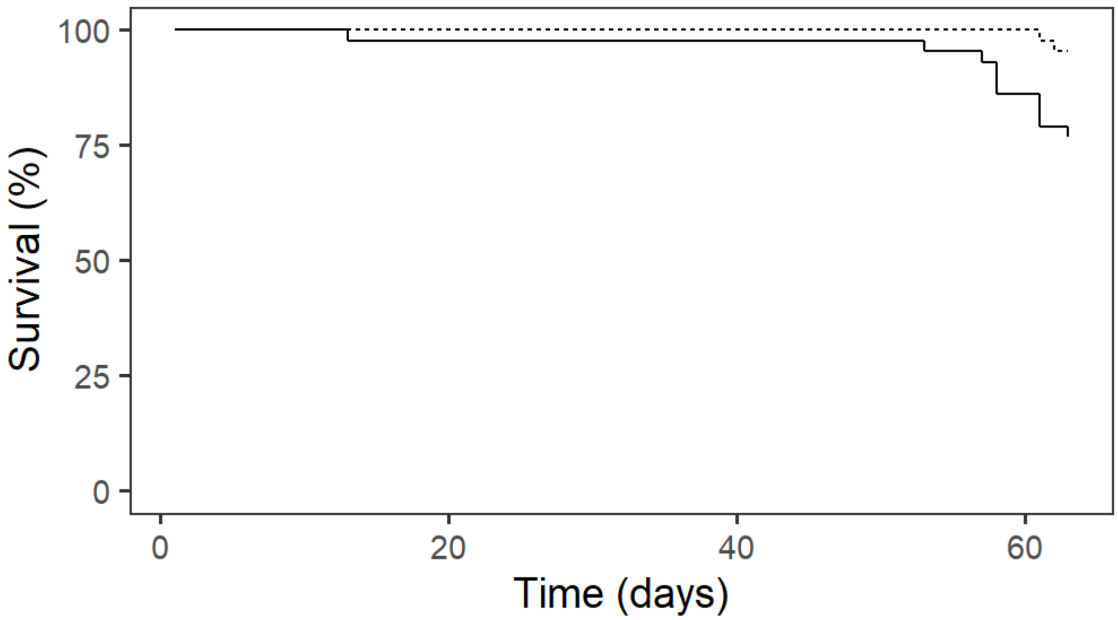

Figure 1. Survival plot showing the proportion of control (solid line) and tagged fish (dashed line) alive throughout the study period. Proportion alive on the y-axis and days after tagging on the x-axis. 100% corresponds to 43 fish for both groups.

For the open field and provoked escape tests, a subset of 23 tagged (median length = 94 mm, IQR = 89 – 99 mm) and 23 control fish (median length = 88 mm, IQR = 80 – 93 mm) was used. There was no significant difference in length between tagged and control fish (Wilcoxon, p = 0.06). Mean water temperature was 12.6°C (sd = 0.3°C) during the experiments. The median distances moved during the open field test were 4.3 m (IQR = 1.4 – 7.1 m) for control fish and 6.1 m for the tagged fish (IQR = 1.4 – 8.5 m; Fig 2). Six control fish and five tagged fish did not move during the entire time period. The total distance swam among moving fish was not different between tagged and control fish (Wilcoxon, p = 0.31).

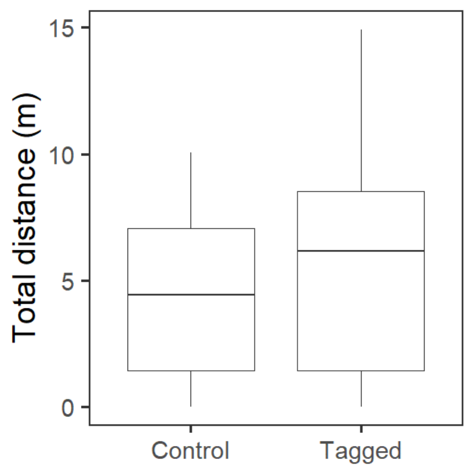

Figure 2. Total distance moved during the 270 s of the open field test. (n = 46). The horizontal line represents the median value, the box the interquartile range, and the vertical lines the total range of data.

During the provoked escape response test, all fish reacted to the dropped circular weights by escape responses of different intensities. Maximum swimming speeds recording over 400 ms were on average 1.03 ± 0.23 m/s (mean ± 1SD) for control fish and 0.95 m/s ± 0.27 m/s (mean ± 1SD) for tagged fish. This corresponds to normalized swimming speeds of 12.1 ± 3.0 BL/s and 10.2 ± 3.0 BL/s for control and tagged fish respectively (Fig. 3). There was no significant difference in maximum swimming speed between tagged and control fish. (t-test, $p = 0.08$).

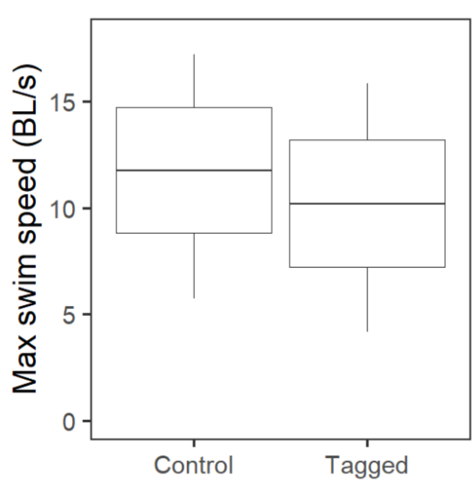

Figure 3. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment (n = 46). The horizontal line represents the mean value, the box the standard deviation, and the vertical lines the total range of data.

Discussion

The method of implanted PIT-tags was evaluated and found suitable to tag and track individual Italian spined loaches. The fish displayed high tag retention and no extra mortality when tagged with 12mm PIT-tags. In addition, no effect on distance moved nor maximum swimming speed was observed.

Only 5% (two fish) of the tagged fish compared to 23% (7 fish) of the control group died during the experiment resulting in no extra mortality related to tagging, a result not uncommon for PIT-tags (Clark, 2016). All but two control fish died during the last week of the study, indicating an emerging problem with the fish holding not related to the experimental treatment. Although the fish were regularly fed, both the tagged and control group tended to lose weight during the experimental period. Perhaps, their condition gradually deteriorated to cause increased mortality towards the end of the study, after about two months in captivity.

No difference in activity (total distance moved) was seen between the tagged and control fish in the open field test. This is, to our knowledge, the first time an open field test is used to evaluate the effects of tagging on fish. This metric measures the motivation and capability of the fish to move, often framed as boldness, activity and/or exploratory tendencies of the fish (Gould et al., 2009; Perals et al., 2017). It seems reasonable to think that any severe tagging effects on the fish's physiology would be detected by the test, as for effects of chemical compounds where it is more widely applied (Echevarria et al., 2008; Gould et al., 2009). Any tag effect detected in the arena might then correlate with movement and activity in nature (Fraser et al., 2001; Watz, 2019; Závorka et al., 2016), and hence constitute a warning signal concerning the suitability of the tagging method.

Escape responses and maximum swimming speeds have a high ecological relevance, being crucial for predator-prey interactions (Domenici, 2010). In the provoked escape response test, we estimated the maximum swimming speed by dropping a circular weight in the vicinity of the fish and measured its escape velocity (Knaepkens et al., 2007; Tudorache et al., 2008). We did not find any significant difference between tagged and control fish, reproducing what was found for bullhead (*Cottus gobio*) using a similar provoked escape response test (Knaepkens et al., 2007). As for lampreys (Mueller et al., 2006), however, the untagged control fish attained the highest velocities, and there was a tendency of higher mean speeds in the control group. This might be an artefact due to the swimming speed being scaled to the body length, tagged fish being on average slightly longer than the control group, and the correlation between speed and length lacking in the data. A minor persisting tagging effect can, however, not be excluded, especially given the rather limited sample size. Regarding the absolute swimming speeds, Tudorache (et al., 2008) used the same type of test to estimate maximum swimming speeds for untagged stone loach (*Barbatula barbatula),* a species relatively similar to spined loaches, to 10-15 BL/s depending on temperature. A result overlapping with our spined loaches.

Tag retention was relatively high, with only 5% (two fish) of tagged fish losing their tag during the two-month duration of the experiment. This confirms the high retention of PIT-tags in a range of studies on other groups of fish (Clark, 2016). This is encouraging given the small size and elongated body of the spined loach potentially making them susceptible to tag losses. Interestingly, the two fish that did shed their tags during the study were the two first fish tagged during the study, suggesting the possibility of improved handling with time, and an even higher tag retention among the bulk of fish in a larger study.

The tag-to-fish weight and length ratios in our study ranged from 2 – 5 % and 10 - 16 % respectively, placing it within 17.5% of the tag-to-fish length ratio recommended for salmonids based on survival and growth effects (Vollset et al., 2020), but higher than the often questioned 2 % weight rule often cited in fish telemetry (Brown et al., 1999; Jepsen et al., 2005; Winter, 1983). Following the 2 % rule would have excluded 13% of our fish, and, in the future, potentially interesting fish behavioral findings (Fullerton et al., 2010). A controlled field tagging effect experiment, where the fish is exposed to predation and has the possibility to disperse, might complement our study (Jepsen et al., 2015). As for now, we can conclude that PIT-tagging, within our tag-to-fish ratios, appears suitable for Italian spined loach.

Contributions

Conceived and designed the investigation: DN, CC, AS, AC
Data Generation: DN, AS, CC, AC, FE
Analyzed the data: DN, AS, GM, FE, CC
Manuscript preparation: DN, CC, AS, GM, FE, AC
Funding: CC, AC

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