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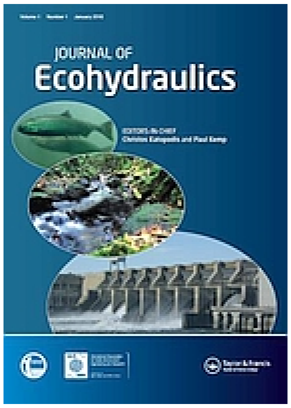

Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

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Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

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Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

A fundamental assumption in animal telemetry is that the behavior and performance of tagged animals does not substantially deviate from that of untagged animals. For fish, swimming behavior is fundamental for every part of a fish post-hatch life, influencing predator-prey interactions, movement ecology and habitat choice. Here, we study effects of PIT-tagging on survival and a range of swimming behaviors for South European nase (*Protochondrostoma genei*) and brook barbel (*Barbus caninus*), two small-sized, stream-dwelling cypriniforms native to the Italian peninsula. Effects on volitional swimming activity (sustained swimming) and maximum swimming speed (escape response; burst swimming) were tested in arena trials. Tagging effects on the prolonged swimming performance were tested in South European nase in an increasing velocity time-to-fatigue test, while a barrier passage test was designed to further investigate tagging effects in brook barbel. Both species displayed very high survival (95-100%), with no difference between tagged and control fish. No fish lost a tag during the 64 days of the study, and no tagging effect on swimming activity, prolonged swimming performance, barrier passage rate, or escape response was detected. Our results indicate that PIT-telemetry is a suitable tool to study the tested fish species.

Keywords: brook barbel; South European nase; swimming behaviour; telemetry; PIT-tags; tagging effects

Introduction

Electronic transmitters are commonly used to study the movement of individual animals but the methodology is limited by the size of the animal in relation to the size of the tags (Cooke *et al.*, 2004; Thorstad *et al.*, 2013). Passive integrated transponders (PIT-tags) are small (typically 7–32 mm) and relatively cheap electronic tags that transmit a unique ID code when within range of a reader antenna. As they don't carry their own battery, they allow tracking the same animal throughout its life. PIT tags are typically detected only within a relatively short range (< 1 m), but are used to identify recaptured animals, track passing animals with stationary antennas, or actively track animals using mobile antennas (Gibbons & Andrews, 2004; Nyqvist *et al.*, 2020). In fish biology, PIT-telemetry is widely applied and has, for example, been used to study survival (Keeler *et al.*, 2007), growth (Watz *et al.*, 2016), habitat use (Quintella *et al.*, 2005; Watz *et al.*, 2019b), home range (Breen *et al.*, 2009), migration patterns (Brönmark *et al.*, 2008; Schwinn *et al.*, 2017), and activity (Závorka *et al.*, 2016) in nature. From an applied perspective, PIT-telemetry is used to evaluate fish passage performance (Castro-Santos *et al.*, 1996; Moser *et al.*, 2019; Ovidio *et al.*, 2023) as well as effects of hydropeaking and habitat restoration (Bartoň *et al.*, 2022; Watz *et al.*, 2019b). In the laboratory, PIT-telemetry is used to keep track of individual identities over time or between experiments (Harbicht *et al.*, 2022; Haro *et al.*, 2004; Mulligan *et al.*, 2021). In short, PIT-telemetry has enhanced our understanding of fish behavior, and is a useful tool for evidence-based river management and conservation (Crossin *et al.*, 2017).

A fundamental assumption in animal telemetry is that the behavior and performance of a tagged fish does not substantially deviate from that of an untagged animal (Brown *et al.*, 2011; Crossin *et al.*, 2017). In fish telemetry, based on the capability of the swim bladder to compensate for the weight of the tag, a relatively arbitrary rule states that the tag should not exceed 2% of fish body weight (Baras *et al.*, 1999; Brown *et al.*, 1999; Winter, 1983). Alternatively, a meta-study on survival and growth of PIT-tagged salmonids concluded with a recommended fish-tag-length ratio of under 17.5% (Vollset et al., 2020). Tag effects, however, can differ between species (Clark, 2016; Jepsen *et al.*, 2015; Wargo Rub *et al.*, 2020), and effects may go beyond those affecting survival and growth in the laboratory (Connors *et al.*, 2002; Zakęś *et al.*, 2022). This makes studies of tag effects an important component of fish telemetry, and hence of ecohydraulic research.

Typically, PIT-tagged fish display high survival and tag retention, and negative effects on growth mainly in the short term (Clark, 2016; Vollset *et al.*, 2020). Notably, however, some species can experience high tagging mortalities, at least under some conditions (Clark, 2016; Watson *et al.*, 2019). While growth effects capture some sublethal tagging effects (Vollset *et al.*, 2020), and physiological sampling can reveal stress responses in fish (Zakęś *et al.*, 2022), behavioral effects on the tagged animals may be of very high importance to the animals performance in nature. Given this, behavioral effects of PIT-tags on the tagged fish are surprisingly little studied (Nyqvist *et al.*, 2022).

Swimming performance, involving both behavior and capability, is fundamental for the ecology of fish, influencing movement, reproduction and predator-prey interactions (Castro-Santos *et al.*, 2022; Tudorache *et al.*, 2013). Fish swimming can be categorized into three modes - sustained, prolonged, and burst swimming - differing in physiology and utilization (Videler, 1993). While bursting fish uses white muscles in anaerobic processes, for a short period of time, sustained swimming is powered with red muscles, aerobic processes and can, theoretically, be maintained indefinitely. Prolonged swimming is a mix of the two other modes where fish uses both red and white muscles, and fatigues after seconds or hundreds of minutes (Hammer, 1995; Videler, 1993). Burst swimming is fundamental for predator-prey interactions and for overcoming velocity barriers, whereas sustained swimming performance may be more important for continuous (e.g. patrolling, food seeking) and long-distance movements (Videler, 1993). Relating to applied fisheries science, estimated fish swimming performance is often used as a design criterion for the construction of fishways ((Baki *et al.*, 2020; Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012). Accordingly, testing performance under different swimming modes is highly relevant when evaluating tagging effects.

Conventional swimming tests constitute forced or provoked swimming in flumes or swim chambers where the fish swim to fatigue, and test prolonged or burst swimming capability while also deducing sustained swimming performance (Tudorache *et al.*, 2013). In addition, provoked escape response tests have been used to estimate maximum swimming speeds in the burst mode (Domenici, 2010; Tudorache *et al.*, 2008). Effects of PIT-tags on fish swimming performance have been tested without detectable effects 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), spiny loaches (Nyqvist *et al.*, 2022) and lampreys (Mueller *et al*., 2006). Schiavon *et al*. (2023), however, did detect an effect of PIT-tags on the prolonged swimming performance, but not on maximum burst speeds, in Italian riffle dace.

An open field test, on the other hand, consists of letting an animal freely explore an experimental arena, and consequently, when it comes to fish, measure a type of voluntary swimming behavior, typically within the sustained mode. Open field tests are commonly used in behavioral ecology to test for individual animal's willingness to explore an area, often as a proxy for activity, boldness or exploratory behavior (Mittelbach *et al.*, 2014; Perals *et al.*, 2017), and in the field of ecotoxicology to test for chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*, 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests, volitional swimming in open field tests has been seen to correlate with activity (Závorka *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to tag effects, open field tests have been used to study effects of PIT-tags on Italian spined loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

There is a general lack of information on the ecology and habitat preferences for many fish species, in particular for small-sized fish with little economical interest (Negro *et al*., 2021; Smialek *et al*., 2019). South European nase (*Protochondrostoma genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation concern while confronted with a high number of in-stream barriers as well as anthropogenically induced habitat change such as increased temperatures and water scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et al.*, 2017). The need to increase the knowledge of the ecology and behavior of these, and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-telemetry offers a valuable research tool, but the need to evaluate for potential tagging effects beyond mere survival remains.

Here, we study PIT-tagging effects on survival and a range of swimming behaviors – encompassing sustained, prolonged and burst swimming - in South European nase and brook barbel. Effects on volitional swimming activity (sustained swimming) and maximum swimming speed (escape response; burst swimming) were tested in arena trials. Tagging effects on the prolonged swimming performance was tested in South European nase in an increasing velocity test, while a barrier passage test was designed to further investigate tagging effects in brook barbel.

Material and methods

South European nase and brook barbel were caught in Lemme River, Italy using wading electrofishing (direct current; ELT60IIGI, Scubla, Italy) on 15 November (UTM 484564E, 4947986N, zone 32T) and 16 November 2021 (UTM 487799E 4941784N, zone 32T), respectively, and brought to Predosa Hatchery (Predosa, Alessandria, Italy). Fish were kept in spring-fed flow-through tanks for two to four days before tagging. All healthy-looking fish caught were included in the study.

In total, 120 South European nase (60 tagged and 60 control) and 112 brook barbel (56 tagged and 56 control) were included in the study. South European nase (60 tagged and 60 control) had a median length of 9.9 cm (range 6.2 – 13.7 cm, interquartile range = 8.2 – 11.9 cm) and weight of 8.6 g (range = 2.5 - 28.1 g; IQR = 3.9 – 13.4 g), while the corresponding metrics for the brook barbel (56 tagged and 56 control) were 9.3 cm (range = 6 – 13.7 cm; IQR = 8.6 – 10.2 cm) and 9.8 g (range = 2.5 - 28 g; IQR =7.9 - 12.5 g).

At the time of tagging, fish were anaesthetized in clove oil (Aromalabs, USA; approximately 0.2 ml clove oil / l water) and randomly assigned to either a tagging or control group. Fish were tagged with a passive integrated transponder (PIT-tag; Biomark, USA; 12 mm * 2.1 mm; 0.10 g) on 18-19 November, 4 and 2 days after capture for nase and barbel, respectively. An incision of 2-4 mm was made on the ventral side of the fish, offset slightly from the center and anterior to the pelvic fins, and the tag was pushed in and forward in the abdominal cavity to align with the fish's body (Bolland *et al.*, 2009; Schiavon *et al.*, 2023). Fish were then measured for fork length and weight before being left to recover in aerated tanks. Controls received the same anesthetic treatment but were only measured and weighed. After recuperating from anesthesia, fish were then held in spring-fed flow-through tanks. Tagged and control fish were kept together in one large tank for South European nase (length*width*depth = 110 cm * 120 cm * 40 cm) and two smaller tanks with higher water exchange rates for brook barbel (length*width*depth = 150 cm * 45 cm * 20 cm). Temperatures were kept stable around 13°C and the light regime followed natural seasonal rhythms. All holding tanks were equipped with artificial shelters comprised of perforated bricks. Fish were fed daily. Brook barbel were fed with

Sera Koi Royal pellets® while South European nase were fed Tetra TabiMin sinking pellets containing a higher proportion of vegetarian content. For both species, the commercial pellets were supplemented with wild-caught macrozoobenthos. The tanks were inspected for mortalities daily, and missing tags were checked at the end of the experiment, 64 days post tagging.

Flume experiments

A subset of fish was tested for swimming performance in an open channel flume made of plexiglass (Fig. 1). South European nase were tested in a traditional time to fatigue experiment. Brook barbel, on the other hand, did not perform (refused to swim) in traditional swimming trials, and were instead tested in a barrier passage experiment. The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of 60 cm for the time to fatigue trials, and 140 cm for the barrier passage experiments. A honeycomb flow straightener at the upstream end of the flume made the flow uniform in the test section and delimited the testing arena in the upstream direction. A downstream fine-meshed grid delimited the downstream end of the arena. Water depth, temperature, and flow were continuously monitored using dedicated sensors (Schiavon *et al*. 2023 for details). The temperature was maintained at 12.5°C (SD = 0.3°C) using a chiller (TECO TK-2000 chiller).

Time-to-fatigue experiment

The swimming performance of South European nase were tested in an increasing velocity swimming test (Schiavon *et al*., 2023) on 13-14 December, 24-25 days after tagging. Two days before the swimming trials the experimental fish were size sorted to acquire a subset (n = 41, 25 control and 16 tagged) of small and relatively uniformly sized fish (≤ 8.4 cm) for the swimming trials. These fish were kept in a separate holding tank until the swimming trials. At the start of a trial, an individual fish was netted and gently released in the swimming arena. In the arena, fish were given 5 min to habituate to the flume at a low flow velocity of 17-19 cm/s. At the start of the swimming trial, water velocity was increased to 45 cm/s. If the fish had not fatigued within 10 min, velocity was increased to 52 cm/s, corresponding to an approximate increase of one body length per second for the tested fish. Water depth during the swimming trial was 7-8 cm depending on velocity. When the fish rested on the downstream grid, it was gently poked from the downstream side of the grid, encouraging it to continue swimming. A fish was defined as fatigued after resting on the grid despite poking, and time to fatigue as the time from the start of testing velocity to the fish refusing to swim. After the swimming trial, the fish was scanned for PIT-ID and returned to the main holding tank.

Barrier passage experiment

The barrier experiment was conducted on 15-16 December, 27-28 days after tagging. A subset of brook barbel (n = 60, 27 control and 33 tagged) were randomly selected in groups of five (Amaral *et al.*, 2016). For the barrier passage experiment a wooden weir covered with black cloth was introduced in the flume. The barrier divided the flume in a downstream (90 cm) and upstream section (50 cm), creating a drop of 7 cm between them. Depth on the downstream section was 10.8 cm, while the depth on the upstream area was 17.8 cm. A PIT-reader antenna (HPR Plus PIT Tag handheld reader, Biomark, USA) was placed in the upstream part of the experimental arena to detect tagged fish. A solid brick was placed in the downstream part of the experimental arena to offer fish shelter from the flow, while a perforated brick offered shelter upstream the barrier to encourage fish not to return downstream (Fig. 1). At the start of the experiment, five brook barbel were netted from one of the holding tanks and gently released in the downstream section of the experimental arena. Fish were continuously observed by 1-2 researchers. Inter-individual differences in size and spot patterns allowed the observers to quickly distinguish the five fish in a trial (Watz *et al.*, 2019a). Time of passage for each fish was noted. After all had passed, or 40 minutes after the start of the trial, the trial was stopped and the fish were scanned for PIT-tags, measured for length and placed in a temporary holding tank. By the end of the passage experiment, all experimental fish were returned to the original holding tanks.

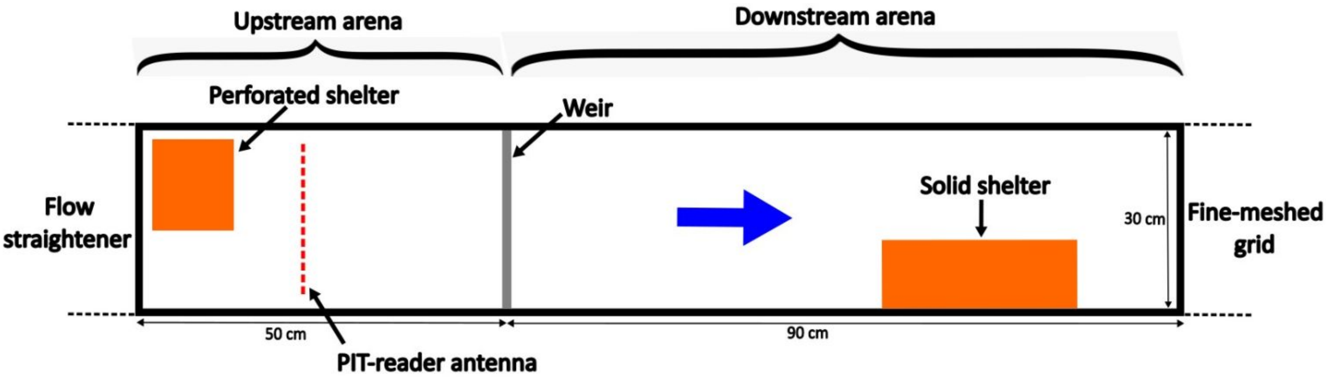

Figure 1. A scaled drawing of the experimental arena for the barrier passage experiment. For the Time-to-fatigue experiment the flow straightener delimited the experimental in an upstream direction while the fine-meshed grid did so in a downstream direction, with an arena length of 60 cm.

Open field and escape response test

A random subset of small sized South European nase (n = 34, 18 control and 16 tagged) and brook barbel (n = 54, 27 control and 27 tagged) were tested for activity score and maximum swimming speed in an open field test followed by a series of provoked escape response (Nyqvist *et al.*, 2022). South European nase were tested on 22 January (64 days after tagging) while brook barbel were tested on 20-21 January (63-64 days after tagging).

Fish were netted from the holding tank, and gently released in to the experimental arena. For the open field test, the fish were left in the arena for approximately ten minutes, five minutes to habituate and five minutes for the open field test (Miklósi *et al.*, 1992; Nyqvist *et al.*, 2022; Watz, 2019). After this time and to estimate maximum swimming speed, an escape response was provoked by dropping a spherical weight in the vicinity of the fish from a height of about 1 m. The fish typically showed an instant escape response followed by some time swimming around. When the fish stopped, another escape response was triggered by dropping another spherical weight near the fish. In total three escape responses were provoked (Knaepkens *et al.*, 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). After halting for the third time, the fish was netted, anaesthetized, checked for presence of a tag, and measured for length. The fish were left to recover in an aerated tank, as not to disturb fish in the main holding tank or risk using the same fish twice. Two trials were run in parallel. Water temperature was measured continuously in a separate tank that was identical to the test tank, and water was changed regularly to maintain a stable temperature across all trials (12.7 ± 0.3°C for nase; 13.1 ± 0.3°C for barbel).

The arena was video recorded with an overhead camera (Sony 4K, FDR-AX43, 50fps, Minato City, Tokyo, Japan). A custom-made MATLAB (R2021b; The Math-Works Inc., Natick, MA, USA) script (https://github.com/SilverFox275/manual-point-tracking) was used to track fish positions manually in one frame per second for the open field test and 10 frames per second during the provoked escape response. Distance in pixels was translated to distance in meters using the known dimensions of the arena. Total distance moved was quantified for the time from 5 min habituation to the time when the first spherical weight was dropped (Haraldstad *et al.*, 2021; Nyqvist *et al.*, 2022; Watz, 2019). Although the experiment was designed for this time to be 5 min, due to mis-timing during the execution of the experiment, and to achieve identical durations for all fish of the same species it was reduced to 204 s for nase and 230 s for barbel. For the escape response, the fastest 400 ms (i.e., the longest distance moved over four tracked frames) was used as an estimate of the maximum swimming speed (Knaepkens *et al.*, 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). As maximum swimming speed typically depends on the length of the fish, the swimming speed was normalized to the length of the fish (Domenici & Blake, 1997).

Statistics

As assumption of normality were not met for part of the data (Shapiro-Wilk test of normality), nonparametric Mann-Whitney tests were used to compare fork length, weight, time-to-fatigue, distance moved, and maximum swimming speed. Difference in survival between tagged and control fish was tested using chi2-tests. Effects of treatment (tagged or control) on passage rates in the barrier experiment were tested using Cox-regression, a type of time-to-event analysis (Castro-Santos & Perry, 2012; Hosmer *et al.*, 2008). Fish length was included in the model to control for any effect of fish size on passage rates. Fish not passing were included as censored observations. Fish were clustered on trial to control for non-independence between fish in the same trial. The assumption of proportionality of hazard was explicitly tested (Fox, 2002). Significance level of $p < 0.05$ was applied to all tests. Data management, plotting and statistical tests were performed in R 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria, URL https://www.R-project.org).

Ethical permission

The study was performed in agreement with the Ufficio Tecnico Faunistico e Ittiofauna (Wildlife and Ichthyofauna Office) of the Province of Alessandria (n. 65493 of 11 November 2021), pursuant to art. 2 of the National Decree n.26/2014 (implementation of Dir. 2010/63/EU).

Results

There was no difference in length or weight between tagged and control fish within any of species (Mann-Whitney, $p > 0.1$).

Survival and tag retention

Survival over the study period was high for both species, with no difference between tagged and control (Chi2, $p > 0.3$). In South European nase, only 3 of 60 tagged fish died, corresponding to a survival ratio of 95%. One control nase died, resulting in a 98% survival. For brook barbel, all tagged fish survived the study period (100% survival) while one control fish died (98% survival). No tag was lost, and correspondingly both species displayed 100% tag retention.

Time-to-fatigue experiment

All fish fatigued during the experiment. Median time-to-fatigue was 601 seconds (IQR 251 – 782 seconds, $n = 41$) with no difference between tagged and control South European nase (Mann-Whitney, $p = 0.3$; fig. 2). The median length of the tested fish was 7.0 cm (IQR = 6.6 – 7.2 cm) and not different between tagged and control fish (Mann-Whitney, $p = 0.49$).

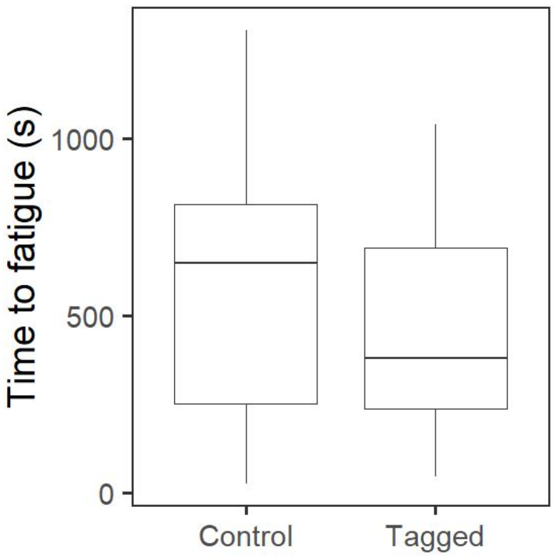

Figure 2. Time-to-fatigue for control (n =25) and tagged (n =16) South European nase. First 600 s in 45 cm/s, followed by 52 cm/s until fatigue. The horizontal line represents the median value, the box the interquartile range, the whiskers the range of data.

Barrier passage experiment

In total, 60 brook barbel, 27 control fish and 33 tagged, participated in the barrier experiment divided over 12 trials with five fish in each trial. Median length of the fish was 9.3 cm (IQR = 8.8 - 10.1 cm) with no difference between tagged and control fish (Mann-Whitney; p = 0.68). Passage success was 78% for control fish and 85% for tagged fish (Fig. 3). Longer fish passed at higher rates than shorter fish (Coef = 0.39, se = 0.17, p = 0.02), but no effect of tagging treatment (coef = 2.6, se = 1.93, p = 0.15) or any interaction between tagging treatment and length (coef = -0.29, se = 0.20, p = 0.13) was detected. Although jumping fish were observed, all fish successfully passing the obstacle did so by swimming against the overtopping flow.

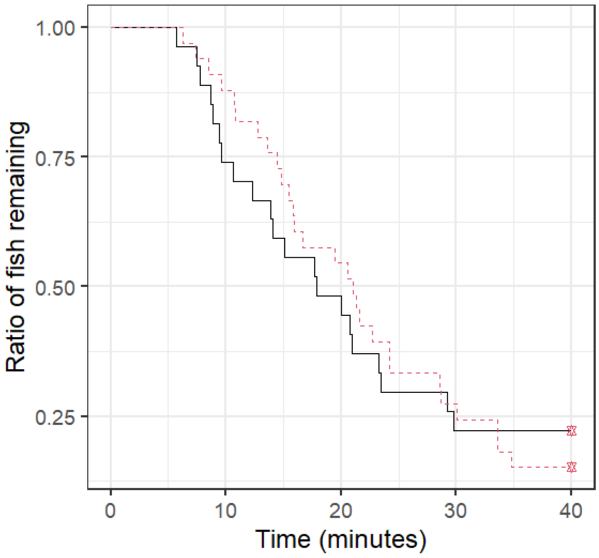

Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line; n = 27) and tagged fish (dashed line; n = 33) remaining downstream the barrier over time.

Open field test

A random subset of small sized South European nase and brook barbel were tested for activity score and maximum swimming speed in an open field test followed by a series of provoked escape responses. In total, 34 small-sized South European nase (18 control and 16 tagged) with an average length of 7.1 cm (median, IQR = 6.3 - 7.4) and 54 brook barbel (27 control and 27 tagged) with an average length of 9.7 cm (median, IQR = 9 - 10.4) were tested in the open field test. There was no difference in size between tagged and control fish for any of the species (Mann-Whitney, $p > 0.6$), and mean temperatures during the tests were 12.7°C (range 12.1 – 13.5 °C) for nase and 13.1°C (range 12.4 – 13.6 °C) for barbel.

Average distance moved during the 204 s open field test was 10.5 m (median, IQR = 8.5 – 13.7 m; Fig. 4a) with no difference between tagged and control (Mann-Whitney, $p = 0.6$) for South European nase. For brook barbel, the average distance moved during the 230 s open field test was 21 m (IQR = 19-24 m, Fig. 4b) with no difference between tagged and control fish (Mann-Whitney, $p = 1$).

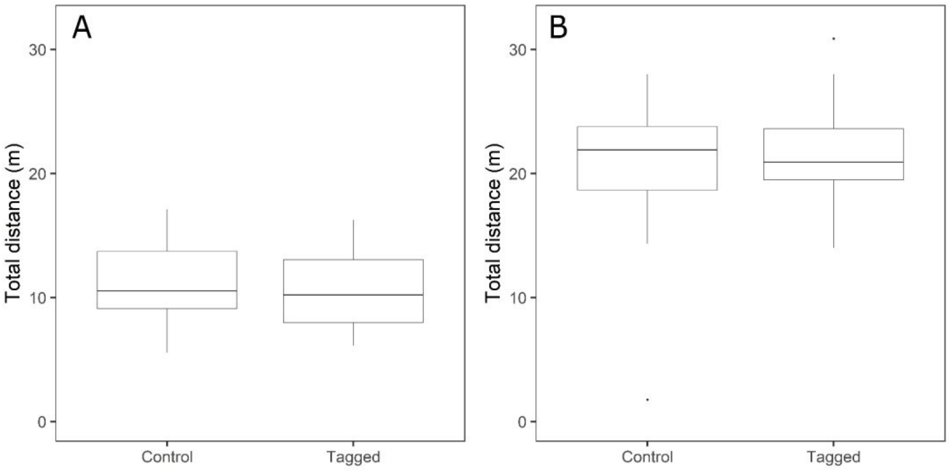

Figure 4. Total distance moved for control and tagged fish during the open field test for A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

Maximum swimming speed

In the provoked escape response test, all tested fish reacted to the dropped spherical weight by an escape response. Maximum swimming speed reached was 1.2 m/s (median, 1.0 – 1.4 m/s), corresponding to 17.1 BL/s (median, IQR = 14.1 – 19.1 BL/s) for South European nase. For brook barbel the maximum swimming speed was substantially lower and on average 1.0 m/s (median, IQR = 0.8 -1.1 m/s) or 10.1 BL/s (median, IQR = 7.8 – 11.2 BL/s). There was no difference in maximum swimming speed between tagged and control fish for any of the species (Mann-Whitney, $p > 0.8$; Fig. 5ab)

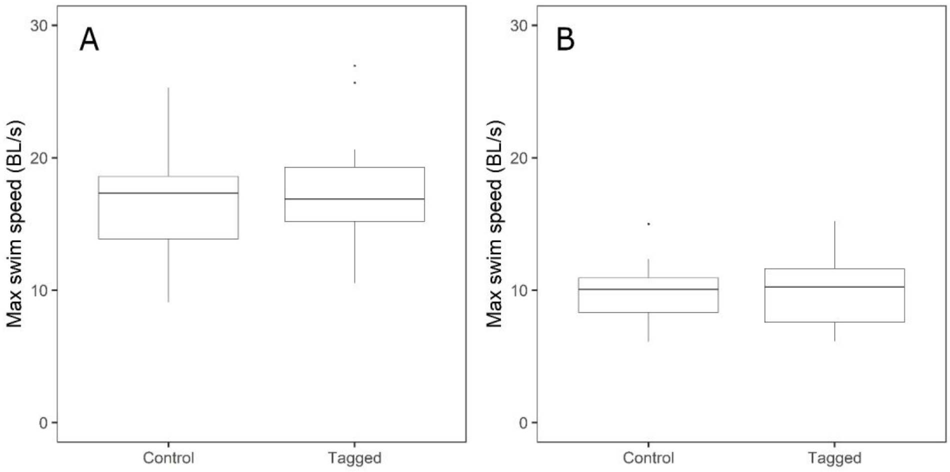

Figure 5. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment for A) South European nase (n = 34) and B) brook barbel (n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

Discussion

PIT-tagged South European nase and brook barbel displayed very high survival, not different from control fish, and no fish lost a tag during the 64 days of the study. In addition, no effect on swimming activity, prolonged swimming performance or escape response was detected for any of the species.

High survival and tag retention are in line with many PIT-tag effects studies on a range of species (e.g. Bolland *et al.*, 2009; Gries & Letcher, 2002; Hühn *et al.*, 2014; Nyqvist *et al.*, 2020; Ombredane *et al.*, 1998; Schiavon *et al.*, 2023). In particular, the results support a recent study on larger common nase (*Chondrostoma nasus*) and European barbel (*Barbus barbus)*, congeners to our tested species, also finding high survival and high retention rates after PIT-tagging (Nagel *et al.*, 2023). In relation to the often cited 2%-rule, recommending tag-to-fish-weight not to exceed 2%, our tag-to-fish ratios were higher than this in 30% of the nase and 9% of the barbel, in keeping with studies relativizing this rule (Brown *et al.*, 1999; Jepsen *et al.*, 2005). Corresponding proportions of fish exceeding the 17.5% tag-to-fish length ratio derived from a meta-analysis on salmonids (Vollset *et al.*, 2020), were 9% and 5% for nase and barbel, respectively. Potential tagging effects, however, may go beyond mere survival.

Swimming is a central part of a fish biology and of particular importance for migration, habitat selection, and predator-prey interaction, as well as for fish passage design (Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012; Tudorache *et al.*, 2008). Previous studies have found no effects of PIT-tags on sustained and prolonged swimming (Ficke *et al.*, 2012; Mueller *et al.*, 2006; Newby *et al.*, 2007) or burst swimming performance (Knaepkens *et al.*, 2007; Mueller *et al.*, 2006; Nyqvist *et al.*, 2022). Here we strengthen these results studying a large range of swimming behaviors. No effects of PIT-tagging were found on sustained swimming activity in the open field test, burst swimming in the provoked escape response, or prolonged swimming in the increased velocity test (nase) and barrier passage test (barbel). Sample sizes were relatively modest, so the result should be taken with some caution. The distribution of data, although displaying an expected large spread of performances (Katopodis & Gervais, 2012) , does not show any tendency of potential tagging effects, except perhaps for nase in the increasing velocity test (similar to Italian riffle dace; Schiavon 2023). Overall this constitutes encouraging results for PIT-tagging small sized Cypriniformes fish.

The range of swimming behaviors investigated covers a wide range of behaviors relevant to survival and movement of fish in their natural environment (Castro-Santos *et al.*, 2022; Videler, 1993), but future studies may go a step further to investigate potential tagging effects on the behavior in the wild. Studies on salmonids, pikes, and cyprinids show high survival and tag retention rates and no effect on growth also in nature (Hühn *et al.*, 2014; Ombredane *et al.*, 1998; Skov *et al.*, 2020), but not always (Dieterman & Hoxmeier, 2009; Šmejkal *et al.*, 2019). While growth and survival studies, to some extent, summarize the consequences of behavior for wild fish, specific studies on tagging effects on behavior in nature are scarce in the literature. For acoustic tags, the behavior of fish tagged in previous years has been compared to recently tagged fish, assuming diminishing tagging effect over time, to check for behavioral tagging effects (Wilson *et al.*, 2017). Recently this approach was extended to PIT-telemetry, revealing effects of tagging and handling on fish passage performance of PIT-tagged alewife (Sullivan *et al.*, 2023). A similar approach should be applicable also in a more natural context for PIT-telemetry.

The estimated maximum swimming speeds – as all swimming tests relying on both behavior and capability - were 17 BL/s for nase and 10 BL/s for barbel. This is within the range of what has been reported for other Cypriniformes fish with similar methodology (Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008), and over longer time windows using tracking technology within a flume (Schiavon *et al.*, 2023). The lower swimming performance in barbel, might be due to them relying more on camouflage than escape in their natural environment (Eilam, 2005), but perhaps also on having been more habituated to the artificial hatchery environment. When a researcher approached their respective holding tanks, nase typically hid int their shelters while the barbel anticipated feeding. Perhaps a combination of natural behavior and partial habituation to a predator free environment among the barbel explains their lower performance.

In the barrier test, most barbel did pass the barrier with no tagging effects detected. Both tagged and control fish passed the barrier by swimming over the streaming flow. Interestingly, larger barbel – independent of tagging – passed the barrier at a higher rate than smaller fish. Selection against shorter fish are known from both natural and artificial barriers (Haugen *et al.*, 2008; Volpato *et al.*, 2009), and may be explained by differential swimming performance (Katopodis & Gervais, 2012). In our experiments, however, the length of the smallest fish to pass did not differ from the length of the smallest fish to fail, indicating a rather subtle selection process of this barrier type on brook barbel. Further studies on the barrier passage capabilities of brook barbel, and other small stream fish, can help inform fish passage and barrier design for fish conservation (Jones *et al.*, 2021).

Both South European nase and brook barbel are species under conservation concern, endemic to the Italian peninsula, and listed as endangered on the regional IUCN redlist (Rondinini *et al.*, 2022). As for many other freshwater fish species with little direct economic value (Smialek *et al.*, 2019; Vøllestad, 2023), there is a lack of knowledge about their ecology and behavior. This is particularly pressing given that they are subject to range of anthropogenic stressors requiring efficient management (Carosi *et al.*, 2019; Dudgeon *et al.*, 2006). In this light, the present study encourages the use of PIT-telemetry to study, for example, movement dynamics, survival, habitat use, restoration success, and fish passage performance (eg. Brönmark *et al.*, 2008; Castro-Santos *et al.*, 1996; Keeler *et al.*, 2007; Watz *et al.*, 2019b) in these and similar species.

Conclusions

In agreement with many other studies on PIT-tagged fish, our results demonstrate high survival and tag retention. In addition, we investigated potential tagging effects on a range of fish swimming behaviors, relevant to survival and movement of fish in their natural environment, not finding any effects. (Castro-Santos *et al.*, 2022; Videler, 1993). Overall, our results indicate that PIT-telemetry is a useful method for studying small-sized South European nase and brook barbel.

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Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

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Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

A fundamental assumption in animal telemetry is that the behavior and performance of ~~a~~ tagged ~~fish~~animals does not substantially deviate from that of ~~an~~ untagged ~~animal. Fish~~animals. For fish, swimming behavior~~, in particular,~~ is fundamental for every part of a fish post-hatch life, influencing predator-prey interactions, movement ecology and habitat choice. Here, we study effects of PIT-tagging on survival and a range of swimming behaviors for South European nase (*Protochondrostoma genei*) and brook barbel (*Barbus caninus*), two small~~-~~-sized, stream-dwelling cypriniforms native to the Italian peninsula. Effects on volitional swimming activity (sustained swimming) and maximum swimming speed (escape response; burst swimming) were tested in arena trials. Tagging effects on the prolonged swimming performance were tested in South European nase in an increasing velocity time-to-fatigue test, while a barrier passage test was designed to further investigate tagging effects in brook barbel. Both species displayed very high survival~~,~~ (95-100%), with no difference between tagged and control fish. No fish lost a tag during the 64 days of the study, and no tagging effect on swimming activity, prolonged swimming performance, barrier passage rate, or escape response was detected. Our results indicate that PIT-telemetry is a suitable tool to study the tested fish species.

Keywords: brook barbel; South European nase; swimming behaviour; telemetry; PIT-tags; tagging effects

Introduction

Electronic transmitters are commonly used to study the movement of individual animals but the methodology is limited by the size of the animal in relation to the size of the tags (Cooke *et al.*, 2004; Thorstad *et al.*, 2013). Passive integrated transponders (PIT-tags) are small (typically 7–32 mm) and relatively cheap electronic tags that transmit a unique ID code when within range of a reader antenna. As they don't carry their own battery, they allow ~~to track~~tracking the same animal throughout its life. PIT tags are typically detected only within a relatively short range (< 1 m), but are used to identify recaptured animals, track passing animals with stationary antennas, or actively track animals using mobile antennas (Gibbons & Andrews, 2004; Nyqvist *et al.*, 2020). In fish biology, PIT-telemetry is widely applied and has, for example, been used to study survival (Keeler *et al.*, 2007), growth (Watz *et al.*, 2016), habitat use (Quintella *et al.*, 2005; Watz *et al.*, 2019b), home range (Breen *et al.*, 2009), migration patterns (Brönmark *et al.*, 2008; Schwinn *et al.*, 2017), and activity (Závorka *et al.*, 2016) in nature. From an applied perspective~~,~~ PIT-telemetry is used to evaluate fish passage performance ~~(Castro-Santos *et al.*, 1996; Ovidio *et al.*, 2023)~~(Castro-Santos *et al.*, 1996; Moser *et al.*, 2019; Ovidio *et al.*, 2023) as well as effects of hydropeaking and habitat restoration (Bartoň *et al.*, 2022; Watz *et al.*, 2019b). ~~In the laboratory, PIT-telemetry is used to keep track of individual identities over time or between experiments (Harbicht *et al.*, 2022; Haro *et al.*, 2004). In short, PIT-telemetry has enhanced our understanding of fish behavior, and is an~~In the laboratory, PIT-telemetry is used to keep track of individual identities over time or between experiments (Harbicht *et al.*, 2022; Haro *et al.*, 2004; Mulligan *et al.*, 2021). In short, PIT-telemetry has enhanced our understanding of fish behavior, and is a useful tool for evidence-based river management and conservation (Crossin *et al.*, 2017).

A fundamental assumption in animal telemetry is that the behavior and performance of a tagged fish does not substantially deviate from that of an untagged animal (Brown *et al.*, 2011; Crossin *et al.*, 2017). In fish telemetry, based on the capability of the swim bladder to compensate for the weight of the tag, a relatively arbitrary rule states that the tag should not exceed 2% of fish body weight (Baras *et al.*, 1999; Brown *et al.*, 1999; Winter, 1983). Alternatively, a meta-study on survival and growth of PIT-tagged salmonids concluded with a recommended fish-tag-length ratio of under 17.5% (Vollset et al., 2020). Tag effects, however, can differ between species ~~or~~

~~environmental conditions~~ (Clark, 2016; Jepsen *et al.*, 2015; Wargo Rub *et al.*, 2020), and effects may go beyond those affecting survival and growth in the laboratory (Connors *et al.*, 2002; Zakęś *et al.*, 2022). This makes studies of tag effects an important component of fish telemetry, and hence of ecohydraulic research.

Typically, PIT-tagged fish display high survival and tag retention, and negative effects on growth mainly in the short term (Clark, 2016; Vollset *et al.*, 2020). Notably, however, some species can experience high tagging mortalities, at least under some conditions (Clark, 2016; Watson *et al.*, 2019). While growth effects capture some sublethal tagging effects (Vollset *et al.*, 2020), and physiological sampling can reveal stress responses in fish (Zakęś *et al.*, 2022), behavioral effects on the tagged animals may be of very high importance to the animals performance in nature. Given this, behavioral effects of PIT-tags on the tagged fish are surprisingly little studied (Nyqvist *et al.*, 2022).

Swimming performance, involving both behavior and capability, is fundamental for the ecology of fish, influencing movement, reproduction and predator-prey interactions (Castro-Santos *et al.*, 2022; Tudorache *et al.*, 2013). Fish swimming can be categorized ~~in to~~into three modes ~~–~~- sustained, prolonged, and burst swimming - differing in physiology and utilization (Videler, 1993). While bursting fish uses white muscles in anaerobic processes, for a short period of time, sustained swimming is powered with red muscles, aerobic processes and can, theoretically, be maintained indefinitely. Prolonged swimming is a mix of the two other modes where fish uses both red and white muscles, and fatigues after seconds or hundreds of minutes (Hammer, 1995; Videler, 1993). Burst swimming is fundamental for predator-prey interactions and for overcoming velocity barriers, whereas sustained swimming performance may be more important for continuous (e.g. patrolling, food seeking) and long-distance movements (Videler, 1993). Relating to applied fisheries science, estimated fish swimming performance is often used as a design criterion for the construction of fishways ~~(Castro-Santos et al.,~~((Baki *et al.*, 2020; Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012). Accordingly, testing performance under different swimming modes is highly relevant when evaluating tagging effects.

Conventional swimming tests constitute forced or provoked swimming in flumes or swim chambers where the fish swim to fatigue, and test prolonged or burst swimming capability while also deducing sustained swimming performance (Tudorache *et al.*, 2013). In addition, provoked escape response tests have been used to estimate maximum swimming speeds in the burst mode (Domenici, 2010; Tudorache *et al.*, 2008). Effects of PIT-tags on fish swimming performance have been tested without detectable effects 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), spiny loaches (Nyqvist *et al.*, 2022) and lampreys (Mueller *et al.*, 2006). Schiavon *et al.* (2023), however, did detect an effect of PIT-tags on the prolonged swimming performance, but not on maximum burst speeds, in Italian riffle dace.

An open field test, on the other hand, consists of letting an animal freely explore an experimental arena, and consequently, when it comes to fish, measure a type of voluntary swimming behavior, typically within the sustained mode. Open field tests are commonly used in behavioral ecology to test for individual animal's willingness to explore an area, often as a proxy for activity, boldness or exploratory behavior (Mittelbach *et al.*, 2014; Perals *et al.*, 2017), and in the field of ecotoxicology to test for chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*, 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests, volitional swimming in open field tests has been seen to correlate with activity (Závorka *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to tag effects, open field tests have been used to study effects of PIT-tags on Italian spined loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

There is a general lack of information on the ecology and habitat preferences for many fish species, in particular for small-sized fish with little economical interest (Negro *et al.*, 2021; Smialek *et al.*, 2019). South European nase (*Protochondrostoma genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation concern while confronted with a high number of in-stream barriers as well as anthropogenically induced habitat change such as increased temperatures and water scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et al.*, 2017). The need to increase the knowledge of the ecology and behavior of these, and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-telemetry offers a valuable research tool, but the need to evaluate for potential tagging effects beyond mere survival remains.

Here, we study PIT-tagging effects on survival and a range of swimming behaviors – encompassing sustained, prolonged and burst swimming ~~– in South European nase (*Protochondrostoma genei*) and brook barbel (*Barbus caninus*), two small-sized cyprinids, native to streams on the Italian peninsula (Fortini, 2016).~~ in South European nase and brook barbel. Effects on volitional swimming activity (sustained swimming) and maximum swimming speed (escape response; burst swimming) were tested in arena trials. Tagging effects on the prolonged swimming performance was tested in South European nase in an increasing velocity test, while a barrier passage test was designed to further investigate tagging effects in brook barbel.

Material and methods

South European nase and brook barbel were caught in Lemme River, Italy using wading electrofishing (direct current; ELT60IIGI, Scubla, Italy) on 15 November (UTM 484564E, 4947986N, zone 32T) and 16 November 2021 (UTM 487799E 4941784N, zone 32T), respectively, and brought to Predosa Hatchery (Predosa, Alessandria, Italy). Fish were kept in spring-fed flow-through tanks for two to ~~fours~~four days before tagging. All healthy~~-~~-looking fish caught were included in the study.

In total, 120 South European nase (60 tagged and 60 control) and 112 brook barbel (56 tagged and 56 control) were included in the study. South European nase (60 tagged and 60 control) had a median length of 9.9 cm (range 6.2 – 13.7 cm, interquartile range = 8.2 – 11.9 cm) and weight of 8.6 g (range = 2.5 - 28.1 g; IQR = 3.9 – 13.4 g), while the corresponding metrics for the brook barbel (56 tagged and 56 control) were 9.3 cm (range = 6 – 13.7 cm; IQR = 8.6 – 10.2 cm) and 9.8 g (range = 2.5 - 28 g; IQR =7.9 - 12.5 g).

At the time of tagging, fish were anaesthetized in clove oil (Aromalabs, USA; approximately 0.2 ml clove oil / l water) and randomly assigned to either a tagging or control group. Fish were tagged with a passive integrated transponder (PIT-tag; Biomark, USA; 12 mm * 2.1 mm; 0.10 g) on 18-19 November, 4 and 2 days after capture for nase and barbel, respectively. An incision of 2-4 mm was made on the ventral side of the fish, offset slightly from the center and anterior to the pelvic fins, and the tag was pushed in and forward in the abdominal cavity to align with the fish's body (Bolland *et al.*, 2009; Schiavon *et al.*, 2023). Fish were then measured for fork length and weight before being left to recover in aerated tanks. Controls received the same anesthetic treatment but were only measured and weighed. After recuperating from anesthesia, fish were then held in spring-fed flow-through tanks. Tagged and control fish were kept together in one large tank for South European nase (length*width*depth = 110 cm * 120 cm * 40 cm) and two smaller tanks with higher water exchange rates for brook barbel (length*width*depth = 150 cm * 45 cm * 20 cm). Temperatures were kept stable around 13°C and the light regime followed natural seasonal rhythms. All holding tanks were equipped with artificial shelters comprised of perforated bricks. Fish were fed daily. Brook barbel were fed with Sera Koi Royal pellets® while South European nase were fed Tetra TabiMin sinking pellets containing a higher proportion of vegetarian content. For both species, the commercial pellets were supplemented with wild-caught macrozoobenthos. The tanks were inspected for mortalities daily, and missing tags were checked at the end of the experiment, 64 days post tagging.

Flume experiments

A subset of fish was tested for swimming performance in an open channel flume made of plexiglass~~.~~ (Fig. 1). South European nase were tested in a traditional time to fatigue experiment. Brook barbel, on the other hand, did not perform (refused to swim) in traditional swimming trials, and were instead tested in a barrier passage experiment. The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of 60 cm for the time to fatigue trials, and 140 cm for the barrier passage experiments. A honeycomb flow straightener at the upstream end of the flume made the flow uniform in the test section and delimited the testing arena in the upstream direction. A downstream fine-meshed grid delimited the downstream end of the arena. Water depth, temperature, and flow were continuously monitored using dedicated sensors (Schiavon *et al*. 2023 for details). The temperature was maintained at 12.5°C (SD = 0.3°C) using a chiller (TECO TK-2000 chiller).

Time-to-fatigue experiment

The swimming performance of South European nase were tested in an increasing velocity swimming test (Schiavon *et al.*, 2023) on 13-14 December, 24-25 days after tagging. Two days before the swimming trials the experimental fish were size sorted to acquire a subset (n = 41, 25 control and 16 tagged) of small and relatively uniformly sized fish (≤ 8.4 cm) for the swimming trials. These fish were kept in a separate holding tank until the swimming trials. At the start of a trial, an individual fish was netted and gently released in the swimming arena. In the arena, fish were given 5 min to habituate to the flume at a low flow velocity of 17-19 cm/s. At the start of the swimming trial, water velocity was increased to 45 cm/s. If the fish had not fatigued within 10 min, velocity was increased to 52 cm/s~~.~~, corresponding to an approximate increase of one body length per second for the tested fish. Water depth during the swimming trial was 7-8 cm depending on velocity. When the fish rested on the downstream grid, it was gently poked from the downstream side of the grid, encouraging it to continue swimming. A fish was defined as fatigued after resting on the grid despite poking, and time to fatigue as the time from the start of testing velocity to the fish refusing to swim. After the swimming trial, the fish was scanned for PIT-ID and returned to the main holding tank.

Barrier passage experiment

The barrier experiment was conducted on 15-16 December, 27-28 days after~~, and a subset of brook barbel (n = 60) were randomly selected in groups of five.~~ tagging. A subset of brook barbel (n = 60, 27 control and 33 tagged) were randomly selected in groups of five (Amaral *et al.*, 2016). For the barrier passage experiment a wooden weir covered with black cloth was introduced in the flume. The barrier divided the flume in a downstream (90 cm) and upstream section (50 cm), creating a drop of 7 cm between them. Depth on the downstream section was 10.8 cm, while the depth on the upstream area was 17.8 cm. A PIT-reader antenna (HPR Plus PIT Tag handheld reader, Biomark, USA) was placed in the upstream part of the experimental arena to detect tagged fish. A solid brick was placed in the downstream part of the experimental arena to offer fish shelter from the flow, while a perforated brick offered shelter upstream the barrier to encourage fish not to return downstream~~.~~ (Fig. 1). At the start of the experiment, five brook barbel were netted from one of the holding tanks and gently released in the downstream section of the experimental arena. Fish were continuously observed by 1-2 researchers. Inter-individual differences in size and spot~~-~~ patterns allowed the observers to quickly distinguish the five fish in a trial ~~(Watz *et al.*, 2019a)~~(Watz *et al.*, 2019a). Time of passage for each fish was noted. After all had passed, or 40 minutes after the start of the trial, the trial was stopped and the fish were scanned for PIT-tags, measured for length and placed in a temporary holding tank. By the end of the passage experiment, all experimental fish were returned to the original holding tanks.

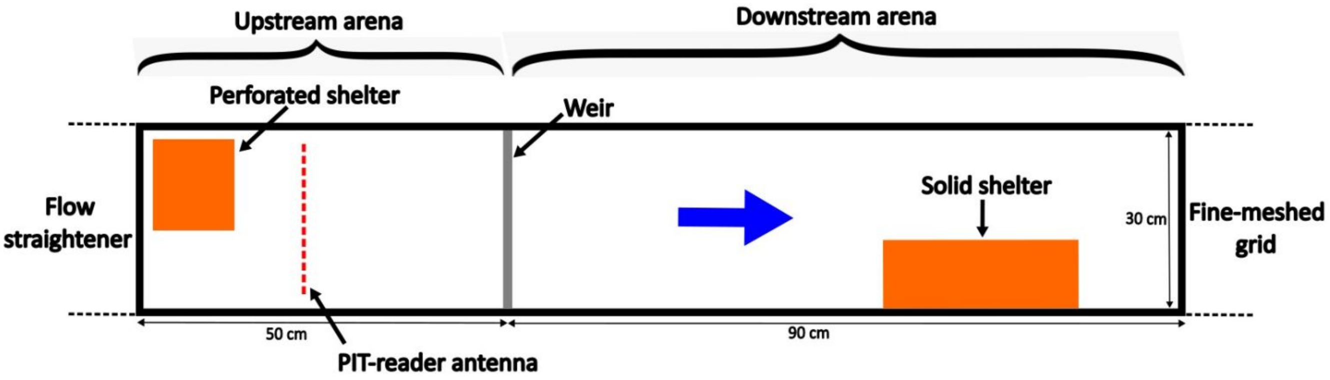

Figure 1. A scaled drawing of the experimental arena for the barrier passage experiment. For the Time-to-fatigue experiment the flow straightener delimited the experimental in an upstream direction while the fine-meshed grid did so in a downstream direction, with an arena length of 60 cm.

Open field and escape response test

A random subset of small sized South European nase (n = 34, 18 control and 16 tagged) and brook barbel (n = 54, 27 control and 27 tagged) were tested for activity score and maximum swimming speed in an open field test followed by a series of provoked escape response (Nyqvist *et al.*, 2022). South European nase were tested on 22 January (64 days after tagging) while brook barbel were tested on 20-21 January (63-64 days after tagging).

Fish were netted from the holding tank, and gently released in to the experimental arena. For the open field test, the fish were left in the arena for approximately ten minutes, five minutes to habituate and five minutes for the open field test (Miklósi *et al.*, 1992; Nyqvist *et al.*, 2022; Watz, 2019). After this time and to estimate maximum swimming speed, an escape response was provoked by dropping a spherical weight in the vicinity of the fish from a height of about 1 m. The fish typically showed an instant escape response followed by some time swimming around. When the fish stopped, another escape response was triggered by dropping another spherical weight near the fish. In total three escape responses were provoked (Knaepkens *et al.*, 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). After halting for the third time, the fish was netted, anaesthetized, checked for presence of a tag, and measured for length. The fish were left to recover in an aerated tank, as not to disturb fish in the main holding tank or risk using the same fish twice. Two trials were run in parallel. Water temperature was measured continuously in a separate tank that was identical to the test tank, and water was changed regularly to maintain a stable temperature across all trials (12.7 ± 0.3°C for nase; 13.1 ± 0.3°C for barbel).

The arena was video recorded with an overhead camera (Sony 4K, FDR-AX43, 50fps, Minato City, Tokyo, Japan). A custom-made MATLAB (R2021b; The Math-Works Inc., Natick, MA, USA) script (https://github.com/SilverFox275/manual-point-tracking) was used to track fish positions manually in one frame per second for the open field test and 10 frames per second during the provoked escape response. Distance in pixels was translated to distance in meters using the known dimensions of the arena. Total distance moved was quantified for the time from 5 min habituation to the time when the first spherical weight was dropped (Haraldstad *et al.*, 2021; Nyqvist *et al.*, 2022; Watz, 2019). Although the experiment was designed for this time to be 5 min, due to mis-timing during the execution of the experiment, and to achieve identical durations for all fish of the same species it was reduced to 204 s for nase and 230 s for barbel. For the escape response, the fastest 400 ms (i.e., the longest distance moved over four tracked frames) was used as an estimate of the maximum swimming speed (Knaepkens *et al.*, 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). As maximum swimming speed typically depends on the length of the fish, the swimming speed was normalized to the length of the fish (Domenici & Blake, 1997).

Statistics

~~Nonparametric~~As assumption of normality were not met for part of the data (Shapiro-Wilk test of normality), nonparametric Mann-Whitney tests were used to compare fork length, weight, time-to-fatigue, distance moved, and maximum swimming speed. Difference in survival between tagged and control fish was tested using chi2-tests. Effects of treatment (tagged or control) on passage rates in the barrier experiment were tested using Cox-regression, a type of time-to-event analysis (Castro-Santos & Perry, 2012; Hosmer *et al.*, 2008). Fish length was included in the model to control for any effect of fish size on passage rates. Fish not passing were included as censored observations. Fish were clustered on trial to control for non-independence between fish in the same trial. The assumption of proportionality of hazard was explicitly tested (Fox, 2002). Significance level of $p < 0.05$ was applied to all tests. Data management, plotting and statistical tests were performed in R 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria, URL https://www.R-project.org).

Ethical permission

The study was performed in agreement with the Ufficio Tecnico Faunistico e Ittiofauna (Wildlife and Ichthyofauna Office) of the Province of Alessandria (n. 65493 of 11 November 2021), pursuant to art. 2 of the National Decree n.26/2014 (implementation of Dir. 2010/63/EU).

Results

~~South European nase (60 tagged and 60 control) had a median length of 9.9 cm (range 6.2 – 13.7 cm, interquartile range = 8.2 – 11.9 cm) and weight of 8.6 g (range = 2.5 - 28.1 g; IQR = 3.9 – 13.4 g), while the corresponding metrics for the brook barbel (56 tagged and 56 control) were 9.3 cm (range = 6 – 13.7 cm; IQR = 8.6 – 10.2 cm) and 9.8 g (range = 2.5 - 28 g; IQR =7.9 - 12.5 g)~~ There was no difference in length or weight between tagged and control fish within any of species (Mann-Whitney, $p > 0.1$).

Survival and tag retention

Survival over the study period was high for both species, with no difference between tagged and control (Chi2, $p > 0.3$). In South European nase, only 3 of ~~56~~60 tagged fish died, corresponding to a survival ratio of 95%. One control nase died, resulting in a 98% survival. For brook barbel, all tagged fish survived the study period (100% survival) while one control fish died (98% survival). No tag was lost, and correspondingly both species displayed 100% tag retention.

Time-to-fatigue experiment

All fish fatigued during the experiment. Median time-to-fatigue was 601 seconds (IQR 251 – 782 seconds, n = 41) with no difference between tagged and control South European nase (Mann-Whitney, $p = 0.3$; fig. ~~1~~2). The median length of the tested fish was 7.0 cm (IQR = 6.6 – 7.2 cm) and not different between tagged and control fish (Mann-Whitney, $p = 0.49$).

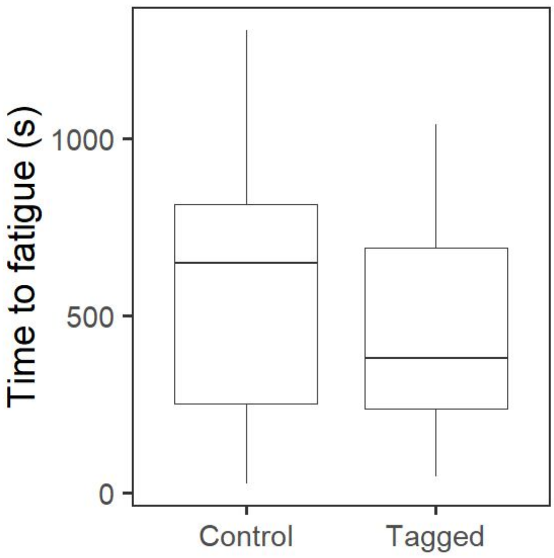

Figure 2. Time-to-fatigue for control (n =25) and tagged (n =16) South European nase. First ~~600s~~600 s in 45 cm/s, followed by 52 cm/s until fatigue. The horizontal line represents the median value, the box the interquartile range, the whiskers the range of data.

Barrier passage experiment

In total, 60 brook barbel, 27 control fish and 33 tagged, participated in the barrier experiment divided over 12 trials with five fish in each trial. Median length of the fish was 9.3 cm (IQR = 8.8 - 10.1 cm) with no difference between tagged and control fish (Mann-Whitney; p = 0.68). Passage success was 78% for control fish and 85% for tagged fish~~.~~ (Fig. 3). Longer fish passed at higher rates than shorter fish (Coef = 0.39, se = 0.17, p = 0.02), but no effect of tagging treatment (coef = 2.6, se = 1.93, p = 0.15) or any interaction between tagging treatment and length (coef = -0.29, se = 0.20, p = 0.13) was detected. ~~There was no difference in length between tagged and control fish in the barrier experiment (Mann-Whitney, p = 0.68).~~ Although jumping fish were observed, all fish successfully passing the obstacle did so by swimming against the overtopping flow.

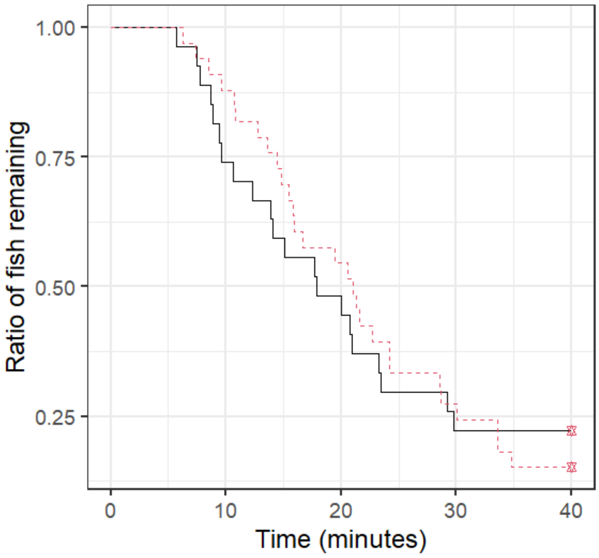

Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line; n = 27) and tagged fish (dashed line; n = 33) remaining downstream the barrier over time.

Open field test

A random subset of small sized South European nase and brook barbel were tested for activity score and maximum swimming speed in an open field test followed by a series of provoked escape responses. In total, 34 small-sized South European nase (18 control and 16 tagged) with an average length of 7.1 cm (median, IQR = 6.3 - 7.4) and 54 brook barbel (27 control and 27 tagged) with an average length of 9.7 cm (median, IQR = 9 - 10.4) were tested in the open field test. There was no difference in size between tagged and control fish for any of the species (Mann-Whitney, p > 0.6), and mean temperatures during the tests were 12.7°C (range 12.1 – 13.5 °C) for nase and 13.1°C (range 12.4 – 13.6 °C) for barbel.

Average distance moved during the 204 s open field test was 10.5 m (median, IQR = 8.5 – 13.7 m; Fig. 4a) with no difference between tagged and control (Mann-Whitney, p = 0.6) for South European nase. For brook barbel, the average distance moved during the 230 s open field test was 21 m (IQR = 19-24 m, Fig. 4b) with no difference between tagged and control fish (Mann-Whitney, p = 1).

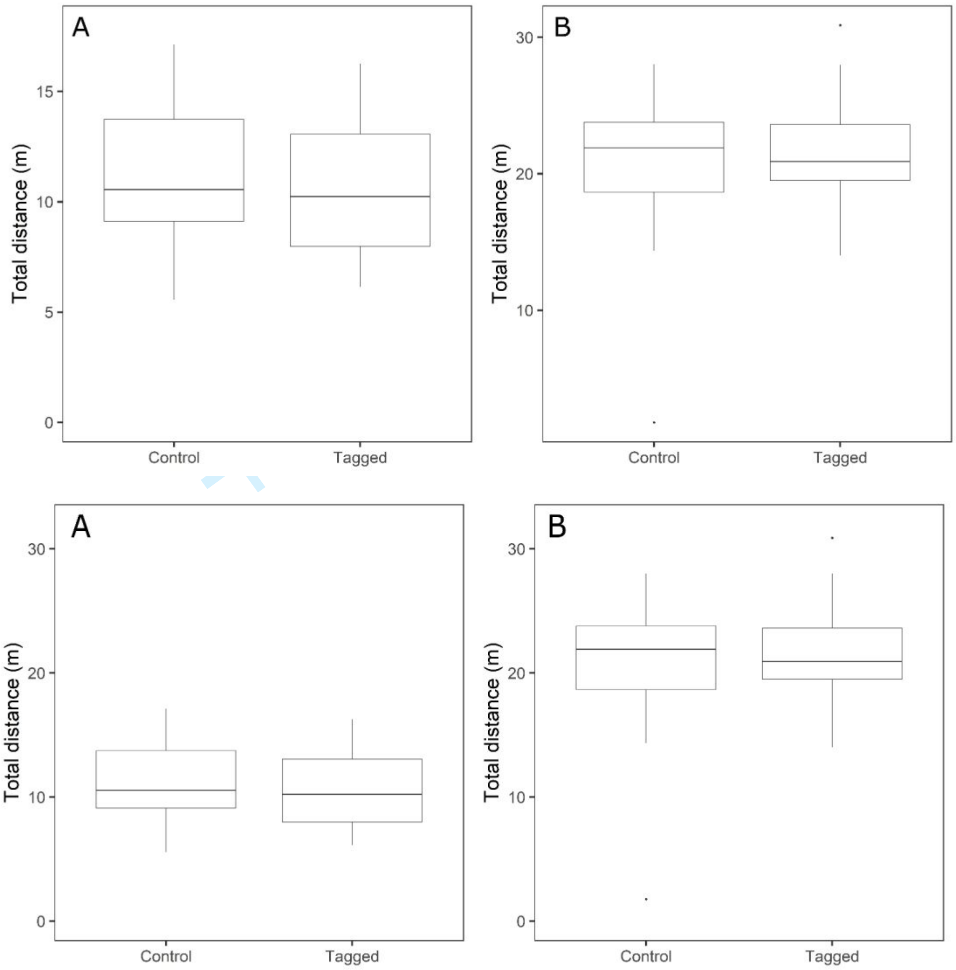

Figure ~~3~~4. Total distance moved for control and tagged fish during the open field test for A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

Maximum swimming speed

In the provoked escape response test, all tested fish reacted to the dropped spherical weight by an escape ~~responses~~response. Maximum swimming speed reached was 1.2 m/s (median, 1.0 – 1.4 m/s), corresponding to 17.1 BL/s (median, IQR = 14.1 – 19.1 BL/s) for South European nase. For brook barbel the maximum swimming speed was substantially lower and on average 1.0 m/s (median, IQR = 0.8 -1.1 m/s) or 10.1 BL/s (median, IQR = 7.8 – 11.2 BL/s). There was no difference in maximum swimming speed between tagged and control fish for any of the species (Mann-Whitney, p > 0.8; Fig. 5ab)

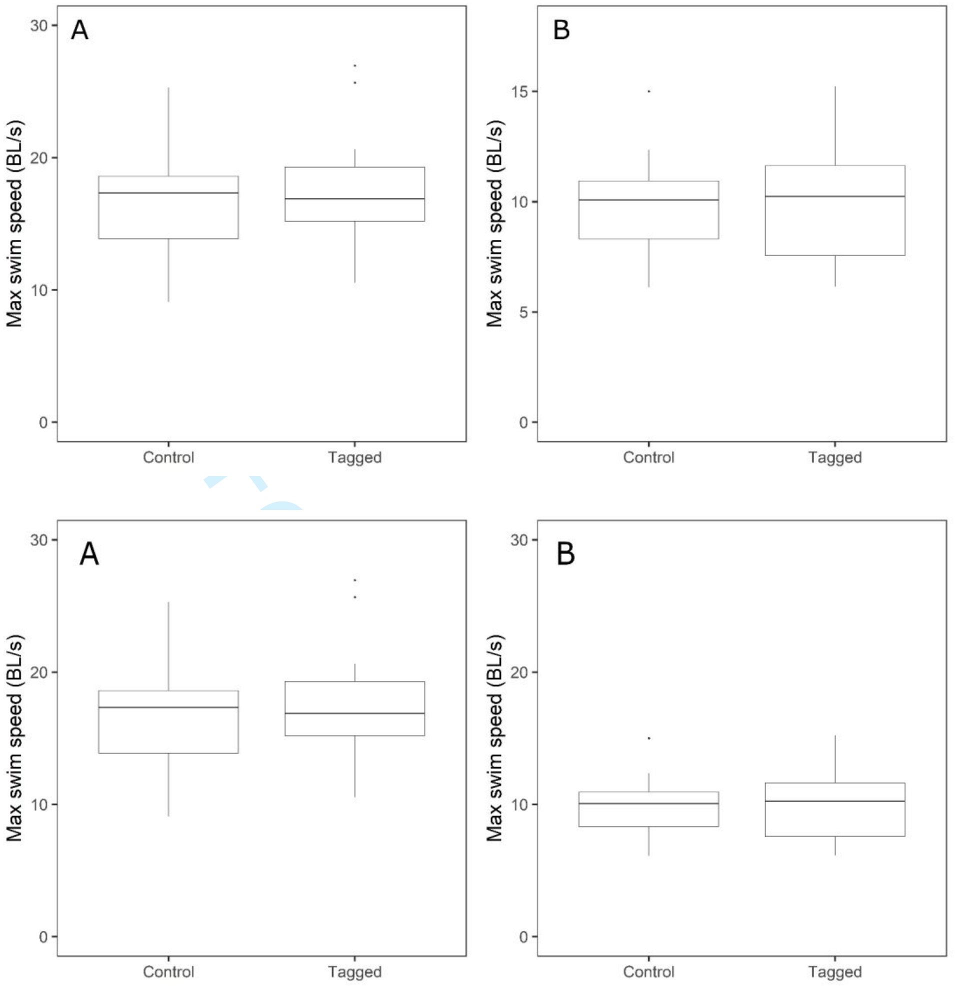

Figure 45. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment for A) South European nase (n = 34) and B) brook barbel (n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

Discussion

PIT-tagged South European nase and brook barbel displayed very high survival, not different from control fish, and no fish lost a tag during the 64 days of the study. In addition, no effect on swimming activity, prolonged swimming performance or escape response was detected for any of the species.

High survival and tag retention are in line with many PIT-tag effects studies on a range of species (e.g. Bolland *et al.*, 2009; Gries & Letcher, 2002; Hühn *et al.*, 2014; Nyqvist *et al.*, 2020; Ombredane *et al.*, 1998; Schiavon *et al.*, 2023). In particular, the results support a recent study on larger common nase (*Chondrostoma nasus*) and European barbel (*Barbus barbus)*, congeners to our tested species, also finding high survival and high retention rates after PIT-tagging (Nagel *et al.*, 2023). In relation to the often cited 2%-rule, recommending tag-to-fish-weight not to exceed 2%, our tag-to-fish ratios were higher than this in 30% of the nase and 9% of the barbel, in keeping with studies relativizing this rule (Brown *et al.*, 1999; Jepsen *et al.*, 2005). Corresponding proportions of fish exceeding the 17.5% tag-to-fish length ratio derived from a meta-analysis on salmonids (Vollset *et al.*, 2020), were 9% and 5% for nase and barbel, respectively. Potential tagging effects, however, may go beyond mere survival.

Swimming is a central part of a fish biology and of particular importance for migration, habitat selection, and predator-prey interaction, as well as for fish passage design (Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012; Tudorache *et al.*, 2008). Previous studies have found no effects of PIT-tags on sustained and prolonged swimming (Ficke *et al.*, 2012; Mueller *et al.*, 2006; Newby *et al.*, 2007) or burst swimming performance (Knaepkens *et al.*, 2007; Mueller *et al.*, 2006; Nyqvist *et al.*, 2022). Here we strengthen these results studying a large range of swimming behaviors. No effects of PIT-tagging were found on sustained swimming activity in the open field test, burst swimming in the provoked escape response, or prolonged swimming in the increased velocity test (nase) and barrier passage test (barbel). Sample sizes were relatively modest, so the result should be taken with some caution. The distribution of data, ~~however~~although displaying an expected large spread of performances (Katopodis & Gervais, 2012) , does not show any tendency of potential tagging effects, except perhaps for nase in the increasing velocity test (similar to Italian riffle dace; Schiavon 2023). Overall this constitutes encouraging results for PIT-tagging small sized Cypriniformes fish.

The range of swimming behaviors investigated covers a wide range of behaviors relevant to survival and movement of fish in their natural environment (Castro-Santos *et al.*, 2022; Videler, 1993), but future studies may go a step further to investigate potential tagging effects on the behavior in the wild. Studies on salmonids, pikes, and cyprinids show high survival and tag retention rates and no effect on growth also in nature (Hühn *et al.*, 2014; Ombredane *et al.*, 1998; Skov *et al.*, 2020), but not always (Dieterman & Hoxmeier, 2009; Šmejkal *et al.*, 2019). While growth and survival studies, to some extent, summarize the consequences of behavior for wild fish, specific studies on tagging effects on behavior in nature are scarce in the literature. For acoustic tags, the behavior of fish tagged in previous years has been compared to recently tagged fish, assuming diminishing tagging effect over time, to check for behavioral tagging effects (Wilson *et al.*, 2017). ~~A similar approach should be applicable also for PIT-telemetry.~~Recently this approach was extended to PIT-telemetry, revealing effects of tagging and handling on fish passage performance of PIT-tagged alewife (Sullivan *et al.*, 2023). A similar approach should be applicable also in a more natural context for PIT-telemetry.

The estimated maximum swimming speeds – as all swimming tests relying on both behavior and capability - were 17 BL/s for nase and 10 BL/s for barbel. This is within the range of what has been reported for other Cypriniformes fish with similar methodology (Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008), and over longer time windows using tracking technology within a flume (Schiavon *et al.*, 2023). The lower swimming performance in barbel, might be due to them relying more on camouflage than escape in their natural environment (Eilam, 2005), but perhaps also on having been more habituated to the artificial hatchery environment. When a researcher approached their respective holding tanks, nase typically hid int their shelters while the barbel anticipated feeding. Perhaps a combination of natural behavior and partial habituation to a predator free environment among the barbel explains their lower performance.

In the barrier test, most barbel did pass the barrier with no tagging effects detected. Both tagged and control fish passed the barrier by swimming over the streaming flow. Interestingly, larger barbel – independent of tagging – passed the barrier at a higher rate than smaller fish. Selection against shorter fish are known from both natural and artificial barriers (Haugen *et al.*, 2008; Volpato *et al.*, 2009), and may be explained by differential swimming performance (Katopodis & Gervais, 2012). In our experiments, however, the length of the smallest fish to pass did not differ from the length of the smallest fish to fail, indicating a rather subtle selection process of this barrier type on brook barbel. Further studies on the barrier passage capabilities of brook barbel, and other small stream fish, can help inform fish passage and barrier design for fish conservation (Jones *et al.*, 2021).

Both South European nase and brook barbel are species under conservation concern, endemic to the Italian peninsula, and listed as endangered on the regional IUCN redlist (Rondinini *et al.*, 2022). As for many other freshwater fish species with little direct economic value (Smialek *et al.*, 2019; Vøllestad, 2023), there is a lack of knowledge about their ecology and behavior. This is particularly pressing given that they are subject to range of anthropogenic stressors requiring efficient management (Carosi *et al.*, 2019; Dudgeon *et al.*, 2006). In this light, the present study encourages the use of PIT-telemetry to study, for example, movement dynamics, survival, habitat use, restoration success, and fish passage performance (eg. Brönmark *et al.*, 2008; Castro-Santos *et al.*, 1996; Keeler *et al.*, 2007; Watz *et al.*, 2019b) in these and similar species.

Conclusions

In agreement with many other studies on PIT-tagged fish, our results demonstrate high survival and tag retention. In addition, we investigated potential tagging effects on a range of fish swimming behaviors, relevant to survival and movement of fish in their natural environment, not finding any effects. (Castro-Santos *et al.*, 2022; Videler, 1993). Overall, our results indicate that PIT-telemetry is a useful method for studying small-sized South European nase and brook barbel.

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