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Survival and swimming performance of a small-sized Cypriniformes (*Telestes muticellus*) tagged with passive integrated transponders

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ABSTRACT

Italian riffle dace (*Telestes muticellus,* Bonaparte 1837) is a small-bodied Leuciscidae native to the Italian Peninsula, of which little is known about the ecology and individual movements in nature. Passive Integrated Transponder (PIT) telemetry is used to track fish movements and behaviour. The basic assumption is that the PIT-tagged organism's performances do not differ considerably from their natural behaviour. Here we present the first evaluation of potential tagging effects in the genus Telestes. The survival rate and tag retention were compared between two different tag implantation methods – injector gun and scalpel incision - and pit-tagging effects on swimming performance were evaluated. Five weeks after tagging, Italian riffle dace demonstrated high survival rates in all treatments: 94.8% for fish tagged with injector gun (n=58), 100% for scalpel incision method (n=58), and 98.3% for controls (n=58). The tag retention was 96.6% for gun treatment and 100% for scalpel treatment. Prolonged swimming performance, tested 22-23 days after tagging, showed a reduction in endurance (time-to-fatigue) for scalpel treatment (n=22) compared to the control group (n=21), while no difference in maximum swimming velocity was observed. We conclude that PIT tagging is a suitable technique for Italian riffle dace, showing high survival and PIT retention and no effect on maximum swimming speed. Significantly lower prolonged swimming performance, although likely less ecologically important, shows that tagging is not without costs. Potential biases need to be evaluated on a study-by-study basis, and future studies should explore behavioural tagging effects in nature.

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Key words: PIT-telemetry; tagging effects; maximum swimming speed; endurance swimming; tagging methods; Leuciscidae.

Contributions: AS, DN, CC, AC, FH, study concept, investigation design; AS, DN, CC, AC, MUA, data generation; AS, DN, data analysis; AS, DN, CC, MUA, FH, manuscript drafting; CC, AC, FH, funding.

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INTRODUCTION

Freshwater ecosystems constitute hotspots for biodiversity and host about half of the world's fish fauna (Hughes, 2021; Maasri *et al.*, 2022). At the same time, many freshwater fish populations are under threat from a series of anthropogenic effects, including habitat degradation, flow modification, overexploitation, and invasive species (Dudgeon *et al.*, 2006; Reid *et al.*, 2019). Knowledge about fish ecology and movement is fundamental for the management and conservation of fish populations (Fullerton *et al.*, 2010; Smialek *et al.*, 2019). Unfortunately, there is a lack of information on the ecology and behaviour of freshwater fish in general and for small-sized fish species in particular, especially for species without economic and cultural importance (Smialek *et al.*, 2019; Negro *et al.*, 2021). Telemetry is a common tool for studying individual animals' ecology and movement (Cooke *et al.*, 2004; Thorstad *et al.*, 2014). For small-sized species, Passive Integrated Transponder (PIT) tags are widely used. These tags do not have an internal battery but transmit a unique signal when placed within the electromagnetic field of a reader antenna and are hence relatively inexpensive and small, usually 7-32 mm (Musselman *et al.*, 2017). The spatial range defined by the electromagnetic field limits the detection range of the tagged animals and is typically limited to within a few

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meters or less. (Gibbons and Andrews, 2004). Animals are then tracked with fixed (Castro-Santos *et al.*, 1996) or portable antennas (Nzau Matondo *et al.*, 2019; Watz *et al.*, 2019). PIT telemetry is widely used to study the ecology and behaviour of fish in marine and freshwater systems (Kessel *et al.*, 2014), for example, allowing the study of migratory patterns (Brönmark *et al.*, 2008), home ranges (Breen *et al.*, 2009), survival (Keeler *et al.*, 2007), activity (Závorka *et al.*, 2016) and fish passage performance (Castro-Santos *et al.*, 1996). The use of telemetry has accelerated our understanding of fish behaviour and has shown to be an effective tool in strengthening evidence-based river management and conservation efforts (Crossin *et al.*, 2017). A critical requirement for telemetry is that both the tags and the tagging procedure do not substantially affect the behaviour of the tagged animals (Brown *et al.*, 2011; Crossin *et al.*, 2017).

Most PIT-tag effects studies on small-sized fish focus on survival and tag retention, typically but not always, showing high survival and high retention rates (Clark, 2016; Vollset *et al.*, 2020). Potential behavioural and performance effects caused by PIT tags, on the other hand, are much less investigated despite their importance for fish behavioural studies in a natural environment (Nyqvist *et al.*, 2022). Here, swimming performance represents an important ecological trait involving both physical and behavioural components that can be quantified in the laboratory (Tudorache *et al.*, 2013). The effects of PIT tagging on the swimming performance of small-bodied fish have been studied in only a handful of species, where tagging has not been shown to affect either prolonged swimming performance (Newby *et al.*, 2007; Ficke *et al.*, 2012) or maximum swimming speeds (Nyqvist *et al.*, 2022; Swarr *et al.*, 2022). Not only the tag but also the tagging procedure (i.e., handling and tagging technique) can have potential effects on the tagged animal (Brown *et al.*, 2011). For example, the use of anaesthesia, antibiotics, suturing, and capture and holding conditions can potentially affect the fish welfare and consequently its behaviour (Mulcahy, 2003; Brown *et al.*, 2011; Carter *et al.*, 2011; Oldenburg *et al.*, 2011). For PIT tags, in particular, two principal tagging techniques are used – surgical incision and needle injection. While needle injection is considered a faster technique, it has also been associated with higher mortality among the tagged fish (Baras *et al.*, 1999; Archdeacon *et al.*, 2009), but available studies are very few. The MK25TM Implant Gun (Biomark, Boise, ID, USA) constitutes a special case of the injection technique, widely used (Bjørnevik *et al.*, 2017; Chen *et al.*, 2017) but, to our knowledge, not yet scientifically evaluated.

Italian riffle dace (*Telestes muticellus,* Bonaparte 1837) is a small-sized (typically <15 cm) Leuciscidae native to the Italian peninsula, spread both between the Adriatic and Tyrrhenian basins, as well as in neighbouring areas of France (Bevera stream) and southern Switzerland. It is a gregarious rheophilic fish that inhabits piedmont rivers and creeks with clear, cold water, but it can also be found in lowland springs. It is omnivorous and feeds mainly on aquatic invertebrates and epilithic algae. Spawning occurs in spring on gravel substrates with swift and shallow water (Fortini, 2016). Although genetics and biogeography have been studied both on *T. muticellus* and the genus *Telestes* in general (Stefani *et al.*, 2004; Marchetto *et al.*, 2010; Buj *et al.*, 2017), very little is known about the ecology and individual movements of the species.

As a prerequisite for studying natural behaviour and individual movement of Italian riffle dace in the wild, we evaluated tag retention and survival of Italian riffle dace tagged using surgical incisions or an injector gun. In addition, prolonged swimming performance, as well as the volitional maximum swimming speed of PIT-tagged fish, were compared to untagged control fish in an open channel flume.

METHODS

Italian riffle daces were collected on 15 November 2021 with electrofishing in Lemme River, province of Alessandria, North-Western Italy (UTM 484564E, 4947986N, zone 32T), and brought to Predosa Hatchery (Predosa, AL, Italy). Fish were held in a spring-fed flow-through tank for two days before being tagged. Despite excluding a few large individuals from the study, all healthy-looking fish were included in the study. Fish were anaesthetized in clove oil (Aroma Labs, Kalamazoo, MI, USA; approximately 0.2 ml clove oil / l water) and randomly assigned to the two tagging techniques: incision with scalpel or injection with the injector gun (MK25TM Implant Gun, Biomark), or to an untagged control group. Treatment fish were tagged with a Passive integrated transponder (PIT-tag; Biomark; 12 mm * 2.1 mm; 0.10 g). The scalpel technique involved an incision of 2-4 mm on the ventral side of the fish, offset slightly from the centre and anterior to the pelvic fins (Bolland *et al.*, 2009; Nyqvist *et al.*, 2022). The tag was pushed forward in the abdominal cavity to align with the fish's body. For the gun injectors, the needle was inserted at a 45° angle in the same position as the incision, followed by a full insertion of the tag, almost parallel to the fish body, into the abdominal cavity. Following the tagging procedure, fish were measured for fork length and weight and left to recover in aerated tanks. Controls received the same anaesthetic treatment but were only measured and weighed. Following the tagging procedure, fish were held in a spring-fed flow-through tank (length*width*depth = 1.1 m * 1.2 m * 0.4 m) under a natural light regime and a stable temperature of 13°C. The rearing tank was equipped with artificial shelters comprised of perforated bricks.

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Fish were fed with commercial pellets (Sera Koi Royal pellets®) and wild-caught macrozoobenthos. The tank was inspected for mortalities daily, and missing tags were checked at the end of the experiment on day 35. Due to time constraints and marginally lower survival observed in gun-tagged fish, effects on swimming performances were evaluated only on control fish (n=21) and fish tagged with the scalpel incision technique (n=22). On the 22nd-23rd days after tagging, fish were subject to a swimming performance test in a recirculating open channel flume. The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of 60 cm. A honeycomb diamond structured flow straightener installed at the upstream end of the flume made the flow uniform in the test section. A downstream grid placed 60 cm from the upstream limited the downstream end of the test arena. The flume side and bottom walls were made of transparent plexiglass material, allowing us to record the fish swimming in the flume during experiments. Water depth and temperature were monitored using a depth and a temperature sensor installed on the flume, and a flow meter sensor (AquaTrans AT600, Baker Hughes, Houston TX, USA) attached to one of the pipes in the system was used to monitor the flow rate. The temperature was maintained at 12.1°C (SD=0.5°C) - using a TECO TK-2000 chiller. All sensors (depth, temperature, and flow meter) connected to a data acquisition device were controlled and operated through a LabVIEW program. Experiments were recorded using a camera (Sony 4K, FDR-AX43, 50 fps) positioned underneath the flume. Fish position (centroid) was subsequently tracked using an animal tracking system Trex (https://trex.run, accessed on 10 November 2022) (Walter and Couzin, 2021). Individual fish were netted from the holding tank and gently released into the experimental flume section. The testing protocol included 5 min of habituation to the new environment with a weak current of 0.19 m* s^{-1} (SD=0.11 m* s^{-1}). After habituation, the swimming performance test started with an initial flow velocity of 0.45 m*s^{-1} (SD=0.02 m* s^{-1}). If the fish did not fatigue within 10 min, the flow velocity was increased to 0.53 m*s^{-1} (SD=0.05 cm* s^{-1}). When the fish rested on the downstream grid, it was gently poked from the downstream side of the grid; a fish was considered fatigued when resting on the downstream grid and not reacting to poking stimuli (Videler and Wardle, 1991). The time to fatigue defined the prolonged swimming performance. During the experiment, fish displayed steady swimming as well as burst and coast behaviour (Peake and Farrell, 2006; Tudorache *et al.*, 2007), typically including bursts across the full flume length. The fish position tracked with Trex was used to calculate the maximum swimming speed when crossing the full flume length; in order to determine the highest crossing velocity, only the fastest crossing recorded for each individual fish was used in the subsequent comparison. Data management, plotting, and statistical tests were performed in Excel (Microsoft Corporation, 2018; and SPSS, IBM Corp., Released 2017., IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA). Nonparametric tests, Kruskall-Wallis, and Mann-Whitney tests were used to compare tagged and control fish fork length, weight, time-to-fatigue, and maximum swimming speed. The statistical differences related to mortality and tag retention between treatments were tested using Fisher's exact test. The swimming speed of the fish was normalized to its length (body length/second) since maximum swimming speed varies with body size (Domenici and Blake, 1997). 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

Survival and tag retention

Fish were anaesthetized for an average of 184 seconds (SD=59 s). Average handling time was 56 s (SD=16 s) for scalpel treatment, 46 s (SD=13 s) for injection gun treatment, and 25 s (SD=9 s) for control fish, significantly lower for gun-tagged compared to scalpel-tagged fish (Mann-Whitney, $p<0.05$).

There was no difference in length or weight between treatments (Kruskal–Wallis, df = 2, $p>0.05$). The range of tag-to-fish weight ratio was 0.4-3.7%, while the tag-to-fish length ratio ranged between 8.6 and 20.0%. Survival was high in all groups. One control and three gun-tagged fish died, while all scalpel-tagged fish survived during the study period (Tab. 1). Tag retention was high for both tagging techniques; two PIT tags were lost among gun-tagged fish, while no tag was lost among the fish subjected to scalpel incision. No statistically significant differences were observed for mortality and PIT retention between treatments (Fisher exact test, $p>0.05$). By the end of the study, some but not all tagged fish displayed visible scars.

Swimming performance: time-to-fatigue

For the swimming performance test, a subset of 22 fish tagged with scalpel (median length = 73 mm, IQR = 71 - 79 mm; median weight = 5.5 g, IQR = 4.9-6.5 g) and 21 control fish (median length = 75 mm, IQR = 74 -78 mm; median weight = 5.7 g, IQR = 5.4-6.3 g) were used. There was no significant difference in length (Mann-Whitney, $p=0.15$) or weight (Mann-Whitney, $p=0.39$) between tagged and control fish. Two control fish refused to swim and were removed from the analysis. Time-to-fatigue was lower for scalpel-PIT tagged fish compared to control fish

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(Mann-Whitney, p=0.02). Median time-to-fatigue was 360 s (IQR = 167-672 s) for control fish and 176 s (IQR = 94-330 s; Fig. 1) for the scalpel-PIT tagged fish.

Swimming performance: maximum swimming speed

All but six fish traversed the flume at least once. The median maximum swimming speed during the traversal was 12.0 body length (BL)*s^{-1} (IQR = 10.2-13.0 BL* s^{-1}) for control fish (n=17) and 10.8 BL*s^{-1} (IQR = 9.7-13.1 BL* s^{-1}; Fig. 2) for the scalpel-PIT tagged fish (n=18). There was no difference in maximum swimming speed between tagged and control fish (Mann-Whitney, p=1.0; Fig. 2). The maximum speed reached by a scalpel-PIT tagged individual was 15.9 BL* s^{-1}, while the maximum swimming velocity recorded for untagged fish was 15.3 BL*s^{-1}.

DISCUSSION

Italian riffle dace tagged with 12 mm PIT tags displayed high survival and tag retention rates. There was no significant difference between implantation methods, but implantation by incision showed no mortality or tag loss; a few fish tagged with the injection gun lost their tag or died. PIT tagged fish showed a lower prolonged swimming performance compared to the control, while no difference was detected in maximum swimming speeds.

The tag-to-fish length ratio ranged from 8.6 to 20.0% (mean=15.3%) and was higher than 17.5%, a threshold recommended for salmonids based on survival and growth effects (Vollset *et al.*, 2020), in 6.9% of tagged fish. The tag-to-fish weight ratio ranged from 0.4 to 3.7 % (mean=1.8%), also, in this case, placing it slightly above the often-cited threshold of 2% (Winter, 1983) for 36.2% of tagged fish. In both instances, none of the fish that exceeded the thresholds died or lost their tag during the study period, supporting claims of certain flexibility regarding these thresholds (Brown *et al.*, 1999).

Although generally low and not statistically different, we did observe some mortality and tag loss among the gun-tagged fish but not among the fish tagged with the scalpel incision. The deaths were recorded in the days immediately following the tagging procedure and perhaps related to damage to internal organs compatible with the over-insertion of the gun's needle (Archdeacon *et al.*, 2009). This slightly lower survival rate obtained with the

Tab. 1. Biometric measures, survival and PIT tag retention, of Italian riffle dace (*Telestes muticellus*) in subsequent stages of rearing day.

	Control	Gun	Scalpel
Fork length, median, IQR (mm), n	79, 74-85 (58)	78, 73-82 (58)	77, 72-81 (58)
Body weight, median, IQR (g), n	5.8, 4.8-7.8 (58)	5.6, 4.7-6.7 (58)	5.6, 4.5-6.7 (58)
Survival, % (n)			
Day 0	100.0 (58)	100.0 (58)	100.0 (58)
Day 7	98.3 (57)	94.8 (55)	100.0 (58)
Day 35	98.3 (57)	94.8 (55)	100.0 (58)
Retention, % (n)			
Day 35	n.a.	96.6 (56)	100.0 (58)

n.a., not applicable.

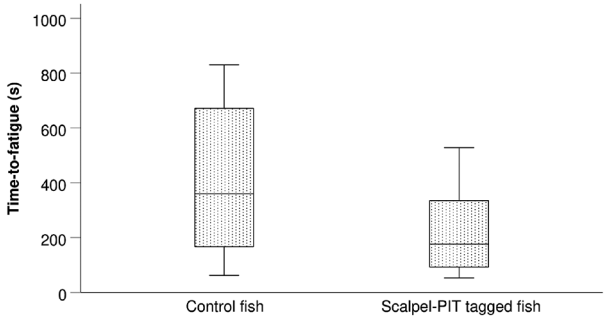

Fig. 1. Box plots of time-to-fatigue (s) during swimming performance tests. Control fish (n=19) and scalpel-PIT tagged fish (n=22).

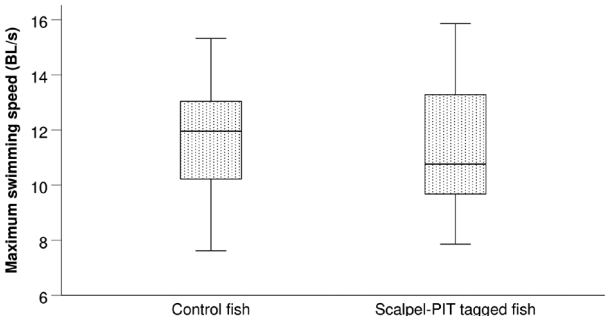

Fig. 2. Box plots of maximum swimming speed (BL/s) measured during a complete flume crossing. Control fish (n=17) and scalpel-PIT tagged fish (n=18).

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gun injector was likely attributable to the small size of the fish tested. A study on *Oreochromis niloticus* (Cichlidae) showed higher survival rates of fish tagged with injectors proportionally to fish size (Baras *et al.*, 1999). Moreover, the requirement of a larger hole entrance into the fish body wall for the insertion of the gun's needle may have led to a higher expulsion rate of the PIT tags, resulting in a lower PIT retention rate; two PIT tags were lost among fish tagged with the injector gun while no tag was lost with scalpel incision.

The PIT tagged fish showed a substantially lower prolonged swimming performance compared to the control fish, constituting a warning that tagging is not without cost for the tagged fish. Previous studies on salmonids (Newby *et al.*, 2007) and non-salmonid species (Ficke *et al.*, 2012; Clark, 2016; Swarr *et al.*, 2022) did not detect any tagging effects on prolonged swimming performance. No difference between tagged and control fish was also observed among species of the Leuciscidae family, although in fish with a relatively large size range and associated increased variance in swimming performance (Ficke *et al.*, 2012). The partially different results of our study might be due to interspecific differences (Brown *et al.*, 2006), perhaps in combination with study-specific effects (*e.g.*, sample size, variability, test design). It is possible that longer recuperation after tagging would reduce or even erase the difference between tagged and untagged fish (Adams *et al.*, 1998; Georgopoulou *et al.*, 2022). We studied the swimming performance 22-23 days after tagging, allowing a relatively short time for the fish to recover. Finally, the effect is likely smaller in larger fish or at lower tag-to-fish ratios, as found in radio-tagged Pacific salmon (Adams *et al.*, 1998). Future studies will need to study the potential attenuation of tagging effects on prolonged swimming capacity over time and under a wider range of tag-to-fish ratios.

It is generally acknowledged that swimming performance represents an important ecological trait, influencing a wide range of behaviours (Tudorache *et al.*, 2008; Downie *et al.*, 2020). In the laboratory, it is often tested using increasing or fixed velocity tests within prolonged swimming speeds, integrating aerobic and anaerobic swimming as well as physiology and behaviour (Hammer, 1995; Katopodis and Gervais, 2012; Tudorache *et al.*, 2013). Under *in situ* conditions, however, burst swimming speeds during shorter time intervals might be as, if not more important, directly influencing survival (Wardle, 1975), predator-prey interaction (Domenici, 2010), and passage of high-velocity barriers (Starrs *et al.*, 2011; Katopodis and Gervais, 2012). To evaluate tag effects on burst swimming performance, we tracked the highest swimming speed by which the fish traversed the flume. Although these values do not represent the maximum speeds achievable by each fish, they constitute a good basis to compare a semi-volitional performance between the treatments. We did not find any significant difference in maximum swimming velocities between tagged and untagged fish. The maximum swimming speeds found are in agreement with swimming capacities reported for Leuciscidae species in North America (Leavy and Bonner, 2009). No difference in maximum swimming speed between PIT tagged fish and control fish also corroborates the result from escape response-based swimming tests in spined loaches (Nyqvist *et al.*, 2022), bullheads (Knaepkens *et al.*, 2007) and lampreys (Mueller *et al.*, 2006).

Although not the main objective, this study also constitutes the first published estimate of maximum and prolonged swimming performance in *Telestes muticellus*. From an applied perspective, these traits are important for predicting the fish's capability to overcome high flow velocity barriers in fishways and hence for fish passage design (Katopodis and Gervais, 2012). Although the need for fish passage has been acknowledged for hundreds of years (Montgomery, 2004), the functionality of fishway is still highly variable and especially challenging for small-sized fish (Bunt *et al.*, 2012; Noonan *et al.*, 2012; Marsden and Stuart, 2019). Given the high number of river barriers to movement, improved fish passage efficiency is significant for future riverine fish conservation (Silva *et al.*, 2018; Belletti *et al.*, 2020). Our results invite further inquiry into the swimming performance of *Telestes muticellus* and other small-sized fish species.

CONCLUSIONS

Italian riffle dace tagged with 12 mm PIT tags displayed high survival and tag retention rates. The endurance under prolonged swimming was significantly lower for PIT tagged fish compared to the control; the maximum swimming speeds achieved by the PIT tagged fish equalled those of the control fish. This leads us to conclude that Italian riffle dace above 60 mm can be tagged with 12 mm PIT-tags but not without costs for tagged fish and with the potential to introduce biases in the studied system. Although it is likely that for relatively stationary small stream fish, the maximum swimming speed achieved is at least of the same, if not higher, importance as the capability of steady swimming for several minutes (Domenici, 2010; Starrs *et al.* 2011). It is also likely that the tagging effect on prolonged swimming performance will decline with time (Adams *et al.*, 1998; Georgopoulou *et al.*, 2022). The importance of potential reduced prolonged swimming capacity on the study results needs to be weighted on a study-by-study basis. Future work should explore potential tagging effects over longer periods of time, with a particular emphasis on the behaviour in the natural environment.

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