

Survival and swimming performance in small-sized South European Cypriniformes tagged with passive integrated transponders

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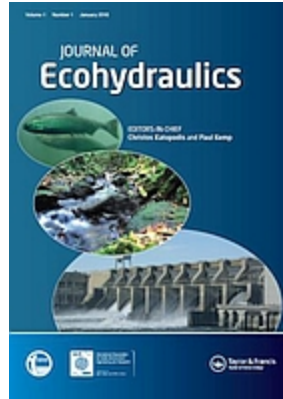
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## Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

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Keywords:	brook barbel, South European nase, swimming behaviour, telemetry, PIT-tags, tagging effects
Abstract:	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 ( <i>Protochondrostoma genei</i> ) and brook barbel ( <i>Barbus caninus</i> ), 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

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	study the tested fish species.

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3 **1 Survival and Swimming Performance in Small-sized South European**  
4 **2 Cypriniformes tagged with Passive Integrated Transponders**  
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3 15 **Survival and Swimming Performance in Small-sized South European**  
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5 16 **Cypriniformes tagged with Passive Integrated Transponders**  
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8 17 A fundamental assumption in animal telemetry is that the behavior and  
9 18 performance of tagged animals does not substantially deviate from that of untagged  
10 19 animals. For fish, swimming behavior is fundamental for every part of a fish post-  
11 20 hatch life, influencing predator-prey interactions, movement ecology and habitat  
12 21 choice. Here, we study effects of PIT-tagging on survival and a range of swimming  
13 22 behaviors for South European nase (*Protochondrostoma genei*) and brook barbel  
14 23 (*Barbus caninus*), two small-sized, stream-dwelling cypriniforms native to the  
15 24 Italian peninsula. Effects on volitional swimming activity (sustained swimming)  
16 25 and maximum swimming speed (escape response; burst swimming) were tested in  
17 26 arena trials. Tagging effects on the prolonged swimming performance were tested  
18 27 in South European nase in an increasing velocity time-to-fatigue test, while a  
19 28 barrier passage test was designed to further investigate tagging effects in brook  
20 29 barbel. Both species displayed very high survival (95-100%), with no difference  
21 30 between tagged and control fish. No fish lost a tag during the 64 days of the study,  
22 31 and no tagging effect on swimming activity, prolonged swimming performance,  
23 32 barrier passage rate, or escape response was detected. Our results indicate that PIT-  
24 33 telemetry is a suitable tool to study the tested fish species.

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34 34 Keywords: brook barbel; South European nase; swimming behaviour; telemetry;  
35 35 PIT-tags; tagging effects  
36

## 37 **Introduction**

38 Electronic transmitters are commonly used to study the movement of individual animals  
39 but the methodology is limited by the size of the animal in relation to the size of the tags  
40 (Cooke *et al.*, 2004; Thorstad *et al.*, 2013). Passive integrated transponders (PIT-tags)  
41 are small (typically 7–32 mm) and relatively cheap electronic tags that transmit a unique  
42 ID code when within range of a reader antenna. As they don't carry their own battery,  
43 they allow tracking the same animal throughout its life. PIT tags are typically detected  
44 only within a relatively short range (< 1 m), but are used to identify recaptured animals,  
45 track passing animals with stationary antennas, or actively track animals using mobile  
46 antennas (Gibbons & Andrews, 2004; Nyqvist *et al.*, 2020). In fish biology, PIT-  
47 telemetry is widely applied and has, for example, been used to study survival (Keeler *et al.*,  
48 2007), growth (Watz *et al.*, 2016), habitat use (Quintella *et al.*, 2005; Watz *et al.*,  
49 2019b), home range (Breen *et al.*, 2009), migration patterns (Brönmark *et al.*, 2008;  
50 Schwinn *et al.*, 2017), and activity (Závorka *et al.*, 2016) in nature. From an applied  
51 perspective, PIT-telemetry is used to evaluate fish passage performance (Castro-Santos  
52 *et al.*, 1996; Moser *et al.*, 2019; Ovidio *et al.*, 2023) as well as effects of hydropeaking  
53 and habitat restoration (Bartoň *et al.*, 2022; Watz *et al.*, 2019b). In the laboratory, PIT-  
54 telemetry is used to keep track of individual identities over time or between experiments  
55 (Harbicht *et al.*, 2022; Haro *et al.*, 2004; Mulligan *et al.*, 2021). In short, PIT-telemetry  
56 has enhanced our understanding of fish behavior, and is a useful tool for evidence-based  
57 river management and conservation (Crossin *et al.*, 2017).

58 A fundamental assumption in animal telemetry is that the behavior and  
59 performance of a tagged fish does not substantially deviate from that of an untagged  
60 animal (Brown *et al.*, 2011; Crossin *et al.*, 2017). In fish telemetry, based on the  
61 capability of the swim bladder to compensate for the weight of the tag, a relatively  
62 arbitrary rule states that the tag should not exceed 2% of fish body weight (Baras *et al.*,  
63 1999; Brown *et al.*, 1999; Winter, 1983). Alternatively, a meta-study on survival and  
64 growth of PIT-tagged salmonids concluded with a recommended fish-tag-length ratio of  
65 under 17.5% (Vollset *et al.*, 2020). Tag effects, however, can differ between species  
66 (Clark, 2016; Jepsen *et al.*, 2015; Wargo Rub *et al.*, 2020), and effects may go beyond  
67 those affecting survival and growth in the laboratory (Connors *et al.*, 2002; Zakeš *et al.*,  
68 2022). This makes studies of tag effects an important component of fish telemetry, and  
69 hence of ecohydraulic research.

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3 70 Typically, PIT-tagged fish display high survival and tag retention, and negative  
4 71 effects on growth mainly in the short term (Clark, 2016; Vollset *et al.*, 2020). Notably,  
5 72 however, some species can experience high tagging mortalities, at least under some  
6 73 conditions (Clark, 2016; Watson *et al.*, 2019). While growth effects capture some  
7 74 sublethal tagging effects (Vollset *et al.*, 2020), and physiological sampling can reveal  
8 75 stress responses in fish (Zakeš *et al.*, 2022), behavioral effects on the tagged animals  
9 76 may be of very high importance to the animals performance in nature. Given this,  
10 77 behavioral effects of PIT-tags on the tagged fish are surprisingly little studied (Nyqvist  
11 78 *et al.*, 2022).

12 79 Swimming performance, involving both behavior and capability, is fundamental  
13 80 for the ecology of fish, influencing movement, reproduction and predator-prey  
14 81 interactions (Castro-Santos *et al.*, 2022; Tudorache *et al.*, 2013). Fish swimming can be  
15 82 categorized into three modes - sustained, prolonged, and burst swimming - differing in  
16 83 physiology and utilization (Videler, 1993). While bursting fish uses white muscles in  
17 84 anaerobic processes, for a short period of time, sustained swimming is powered with red  
18 85 muscles, aerobic processes and can, theoretically, be maintained indefinitely. Prolonged  
19 86 swimming is a mix of the two other modes where fish uses both red and white muscles,  
20 87 and fatigues after seconds or hundreds of minutes (Hammer, 1995; Videler, 1993).  
21 88 Burst swimming is fundamental for predator-prey interactions and for overcoming  
22 89 velocity barriers, whereas sustained swimming performance may be more important for  
23 90 continuous (e.g. patrolling, food seeking) and long-distance movements (Videler, 1993).  
24 91 Relating to applied fisheries science, estimated fish swimming performance is often  
25 92 used as a design criterion for the construction of fishways ((Baki *et al.*, 2020; Castro-  
26 93 Santos *et al.*, 2022; Katopodis & Gervais, 2012). Accordingly, testing performance  
27 94 under different swimming modes is highly relevant when evaluating tagging effects.

28 95 Conventional swimming tests constitute forced or provoked swimming in  
29 96 flumes or swim chambers where the fish swim to fatigue, and test prolonged or burst  
30 97 swimming capability while also deducing sustained swimming performance (Tudorache  
31 98 *et al.*, 2013). In addition, provoked escape response tests have been used to estimate  
32 99 maximum swimming speeds in the burst mode (Domenici, 2010; Tudorache *et al.*,  
33 100 2008). Effects of PIT-tags on fish swimming performance have been tested without  
34 101 detectable effects on sustained swimming in cyprinids (Ficke *et al.*, 2012), salmonids  
35 102 (Newby *et al.*, 2007), and lampreys (Mueller *et al.*, 2006) and on burst speeds in

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3 103 bullheads (Knaepkens *et al.*, 2007), spiny loaches (Nyqvist *et al.*, 2022) and lampreys  
4 (Mueller *et al.*, 2006). Schiavon *et al.* (2023), however, did detect an effect of PIT-tags  
5 104  
6 105 on the prolonged swimming performance, but not on maximum burst speeds, in Italian  
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8 106 riffle dace.

9  
10 107 An open field test, on the other hand, consists of letting an animal freely explore  
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12 108 an experimental arena, and consequently, when it comes to fish, measure a type of  
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14 109 voluntary swimming behavior, typically within the sustained mode. Open field tests are  
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16 110 commonly used in behavioral ecology to test for individual animal's willingness to  
17  
18 111 explore an area, often as a proxy for activity, boldness or exploratory behavior  
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20 112 (Mittelbach *et al.*, 2014; Perals *et al.*, 2017), and in the field of ecotoxicology to test for  
21  
22 113 chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*,  
23  
24 114 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests,  
25  
26 115 volitional swimming in open field tests has been seen to correlate with activity (Závorka  
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28 116 *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as  
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30 117 downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to  
31  
32 118 tag effects, open field tests have been used to study effects of PIT-tags on Italian spined  
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34 119 loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

35  
36 120 There is a general lack of information on the ecology and habitat preferences for  
37  
38 121 many fish species, in particular for small-sized fish with little economical interest  
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40 122 (Negro *et al.*, 2021; Smialek *et al.*, 2019). South European nase (*Protochondrostoma*  
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42 123 *genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to  
43  
44 124 streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation  
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46 125 concern while confronted with a high number of in-stream barriers as well as  
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48 126 anthropogenically induced habitat change such as increased temperatures and water  
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50 127 scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et*  
51  
52 128 *al.*, 2017). The need to increase the knowledge of the ecology and behavior of these,  
53  
54 129 and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-  
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56 130 telemetry offers a valuable research tool, but the need to evaluate for potential tagging  
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58 131 effects beyond mere survival remains.

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60 132 Here, we study PIT-tagging effects on survival and a range of swimming  
133 behaviors – encompassing sustained, prolonged and burst swimming - in South  
134 European nase and brook barbel. Effects on volitional swimming activity (sustained  
135 swimming) and maximum swimming speed (escape response; burst swimming) were

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3 136 tested in arena trials. Tagging effects on the prolonged swimming performance was  
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5 137 tested in South European nase in an increasing velocity test, while a barrier passage test  
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7 138 was designed to further investigate tagging effects in brook barbel.  
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### 10 139 **Material and methods**

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12 140 South European nase and brook barbel were caught in Lemme River, Italy using wading  
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14 141 electrofishing (direct current; ELT60IIGI, Scubla, Italy) on 15 November (UTM  
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16 142 484564E, 4947986N, zone 32T) and 16 November 2021 (UTM 487799E 4941784N,  
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18 143 zone 32T), respectively, and brought to Predosa Hatchery (Predosa, Alessandria, Italy).  
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20 144 Fish were kept in spring-fed flow-through tanks for two to four days before tagging. All  
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22 145 healthy-looking fish caught were included in the study.

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24 146 In total, 120 South European nase (60 tagged and 60 control) and 112 brook barbel  
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26 147 (56 tagged and 56 control) were included in the study. South European nase (60 tagged  
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28 148 and 60 control) had a median length of 9.9 cm (range 6.2 – 13.7 cm, interquartile range =  
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30 149 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  
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32 150 corresponding metrics for the brook barbel (56 tagged and 56 control) were 9.3 cm (range  
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34 151 = 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).

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36 152 At the time of tagging, fish were anaesthetized in clove oil (Aromalabs, USA;  
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38 153 approximately 0.2 ml clove oil / 1 water) and randomly assigned to either a tagging or  
39  
40 154 control group. Fish were tagged with a passive integrated transponder (PIT-tag; Biomark,  
41  
42 155 USA; 12 mm \* 2.1 mm; 0.10 g) on 18-19 November, 4 and 2 days after capture for nase  
43  
44 156 and barbel, respectively. An incision of 2-4 mm was made on the ventral side of the fish,  
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46 157 offset slightly from the center and anterior to the pelvic fins, and the tag was pushed in  
47  
48 158 and forward in the abdominal cavity to align with the fish's body (Bolland *et al.*, 2009;  
49  
50 159 Schiavon *et al.*, 2023). Fish were then measured for fork length and weight before being  
51  
52 160 left to recover in aerated tanks. Controls received the same anesthetic treatment but were  
53  
54 161 only measured and weighed. After recuperating from anesthesia, fish were then held in  
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56 162 spring-fed flow-through tanks. Tagged and control fish were kept together in one large  
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58 163 tank for South European nase (length\*width\*depth = 110 cm \* 120 cm \* 40 cm) and two  
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60 164 smaller tanks with higher water exchange rates for brook barbel (length\*width\*depth =  
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166 150 cm \* 45 cm \* 20 cm). Temperatures were kept stable around 13°C and the light  
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shelters comprised of perforated bricks. Fish were fed daily. Brook barbel were fed with

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3 168 Sera Koi Royal pellets® while South European nase were fed Tetra TabiMin sinking  
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5 169 pellets containing a higher proportion of vegetarian content. For both species, the  
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7 170 commercial pellets were supplemented with wild-caught macrozoobenthos. The tanks  
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9 171 were inspected for mortalities daily, and missing tags were checked at the end of the  
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11 172 experiment, 64 days post tagging.

### 13 173 *Flume experiments*

14  
15 174 A subset of fish was tested for swimming performance in an open channel flume made  
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17 175 of plexiglass (Fig. 1). South European nase were tested in a traditional time to fatigue  
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19 176 experiment. Brook barbel, on the other hand, did not perform (refused to swim) in  
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21 177 traditional swimming trials, and were instead tested in a barrier passage experiment.  
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23 178 The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of  
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25 179 60 cm for the time to fatigue trials, and 140 cm for the barrier passage experiments. A  
26  
27 180 honeycomb flow straightener at the upstream end of the flume made the flow uniform in  
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29 181 the test section and delimited the testing arena in the upstream direction. A downstream  
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31 182 fine-meshed grid delimited the downstream end of the arena. Water depth, temperature,  
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33 183 and flow were continuously monitored using dedicated sensors (Schiavon *et al.* 2023 for  
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35 184 details). The temperature was maintained at 12.5°C (SD = 0.3°C) using a chiller (TECO  
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37 185 TK-2000 chiller).

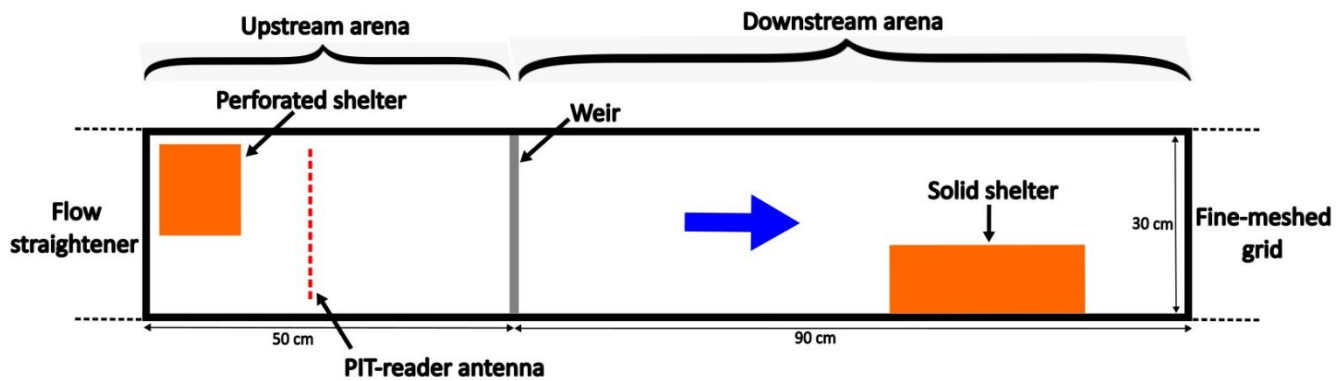
### 37 186 *Time-to-fatigue experiment*

38  
39 187 The swimming performance of South European nase were tested in an increasing  
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41 188 velocity swimming test (Schiavon *et al.*, 2023) on 13-14 December, 24-25 days after  
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43 189 tagging. Two days before the swimming trials the experimental fish were size sorted to  
44  
45 190 acquire a subset (n = 41, 25 control and 16 tagged) of small and relatively uniformly  
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47 191 sized fish ( $\leq 8.4$  cm) for the swimming trials. These fish were kept in a separate holding  
48  
49 192 tank until the swimming trials. At the start of a trial, an individual fish was netted and  
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51 193 gently released in the swimming arena. In the arena, fish were given 5 min to habituate  
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53 194 to the flume at a low flow velocity of 17-19 cm/s. At the start of the swimming trial,  
54  
55 195 water velocity was increased to 45 cm/s. If the fish had not fatigued within 10 min,  
56  
57 196 velocity was increased to 52 cm/s, corresponding to an approximate increase of one  
58  
59 197 body length per second for the tested fish. Water depth during the swimming trial was  
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198 7-8 cm depending on velocity. When the fish rested on the downstream grid, it was  
199 gently poked from the downstream side of the grid, encouraging it to continue

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3 200 swimming. A fish was defined as fatigued after resting on the grid despite poking, and  
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5 201 time to fatigue as the time from the start of testing velocity to the fish refusing to swim.  
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7 202 After the swimming trial, the fish was scanned for PIT-ID and returned to the main  
8  
9 203 holding tank.

10  
11 204 *Barrier passage experiment*

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13 205 The barrier experiment was conducted on 15-16 December, 27-28 days after tagging. A  
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15 206 subset of brook barbel (n = 60, 27 control and 33 tagged) were randomly selected in  
16  
17 207 groups of five (Amaral *et al.*, 2016). For the barrier passage experiment a wooden weir  
18  
19 208 covered with black cloth was introduced in the flume. The barrier divided the flume in a  
20  
21 209 downstream (90 cm) and upstream section (50 cm), creating a drop of 7 cm between  
22  
23 210 them. Depth on the downstream section was 10.8 cm, while the depth on the upstream  
24  
25 211 area was 17.8 cm. A PIT-reader antenna (HPR Plus PIT Tag handheld reader, Biomark,  
26  
27 212 USA) was placed in the upstream part of the experimental arena to detect tagged fish. A  
28  
29 213 solid brick was placed in the downstream part of the experimental arena to offer fish  
30  
31 214 shelter from the flow, while a perforated brick offered shelter upstream the barrier to  
32  
33 215 encourage fish not to return downstream (Fig. 1). At the start of the experiment, five  
34  
35 216 brook barbel were netted from one of the holding tanks and gently released in the  
36  
37 217 downstream section of the experimental arena. Fish were continuously observed by 1-2  
38  
39 218 researchers. Inter-individual differences in size and spot patterns allowed the observers  
40  
41 219 to quickly distinguish the five fish in a trial (Watz *et al.*, 2019a). Time of passage for  
42  
43 220 each fish was noted. After all had passed, or 40 minutes after the start of the trial, the  
44  
45 221 trial was stopped and the fish were scanned for PIT-tags, measured for length and  
46  
47 222 placed in a temporary holding tank. By the end of the passage experiment, all  
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49 223 experimental fish were returned to the original holding tanks.  
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225 *Figure 1. A scaled drawing of the experimental arena for the barrier passage*  
 226 *experiment. For the Time-to-fatigue experiment the flow straightener delimited the*  
 227 *experimental in an upstream direction while the fine-meshed grid did so in a*  
 228 *downstream direction, with an arena length of 60 cm.*

### 229 **Open field and escape response test**

230 A random subset of small sized South European nase ( $n = 34$ , 18 control and 16 tagged)  
 231 and brook barbel ( $n = 54$ , 27 control and 27 tagged) were tested for activity score and  
 232 maximum swimming speed in an open field test followed by a series of provoked  
 233 escape response (Nyqvist *et al.*, 2022). South European nase were tested on 22 January  
 234 (64 days after tagging) while brook barbel were tested on 20-21 January (63-64 days  
 235 after tagging).

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

1  
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3 250 changed regularly to maintain a stable temperature across all trials ( $12.7 \pm 0.3^\circ\text{C}$  for  
4  
5 251 nase;  $13.1 \pm 0.3^\circ\text{C}$  for barbel).  
6  
7 252 The arena was video recorded with an overhead camera (Sony 4K, FDR-AX43, 50fps,  
8  
9 253 Minato City, Tokyo, Japan). A custom-made MATLAB (R2021b; The Math-Works  
10  
11 254 Inc., Natick, MA, USA) script (<https://github.com/SilverFox275/manual-point-tracking>)  
12  
13 255 was used to track fish positions manually in one frame per second for the open field test  
14  
15 256 and 10 frames per second during the provoked escape response. Distance in pixels was  
16  
17 257 translated to distance in meters using the known dimensions of the arena. Total distance  
18  
19 258 moved was quantified for the time from 5 min habituation to the time when the first  
20  
21 259 spherical weight was dropped (Haraldstad *et al.*, 2021; Nyqvist *et al.*, 2022; Watz,  
22  
23 260 2019). Although the experiment was designed for this time to be 5 min, due to mis-  
24  
25 261 timing during the execution of the experiment, and to achieve identical durations for all  
26  
27 262 fish of the same species it was reduced to 204 s for nase and 230 s for barbel. For the  
28  
29 263 escape response, the fastest 400 ms (i.e., the longest distance moved over four tracked  
30  
31 264 frames) was used as an estimate of the maximum swimming speed (Knaepkens *et al.*,  
32  
33 265 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). As maximum swimming speed  
34  
35 266 typically depends on the length of the fish, the swimming speed was normalized to the  
36  
37 267 length of the fish (Domenici & Blake, 1997).

### 36 268 **Statistics**

38 269 As assumption of normality were not met for part of the data (Shapiro-Wilk test of  
39  
40 270 normality), nonparametric Mann-Whitney tests were used to compare fork length,  
41  
42 271 weight, time-to-fatigue, distance moved, and maximum swimming speed. Difference in  
43  
44 272 survival between tagged and control fish was tested using chi2-tests. Effects of  
45  
46 273 treatment (tagged or control) on passage rates in the barrier experiment were tested  
47  
48 274 using Cox-regression, a type of time-to-event analysis (Castro-Santos & Perry, 2012;  
49  
50 275 Hosmer *et al.*, 2008). Fish length was included in the model to control for any effect of  
51  
52 276 fish size on passage rates. Fish not passing were included as censored observations. Fish  
53  
54 277 were clustered on trial to control for non-independence between fish in the same trial.  
55  
56 278 The assumption of proportionality of hazard was explicitly tested (Fox, 2002).  
57  
58 279 Significance level of  $p < 0.05$  was applied to all tests. Data management, plotting and  
59  
60 280 statistical tests were performed in R 4.0.3 (R Foundation for Statistical Computing,  
281  
Vienna, Austria, URL <https://www.R-project.org>).

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2  
3 282 ***Ethical permission***  
4

5 283 The study was performed in agreement with the Ufficio Tecnico Faunistico e Ittiofauna  
6 284 (Wildlife and Ichthyofauna Office) of the Province of Alessandria (n. 65493 of 11  
7  
8 285 November 2021), pursuant to art. 2 of the National Decree n.26/2014 (implementation  
9 286 of Dir. 2010/63/EU).  
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13 287 **Results**  
14

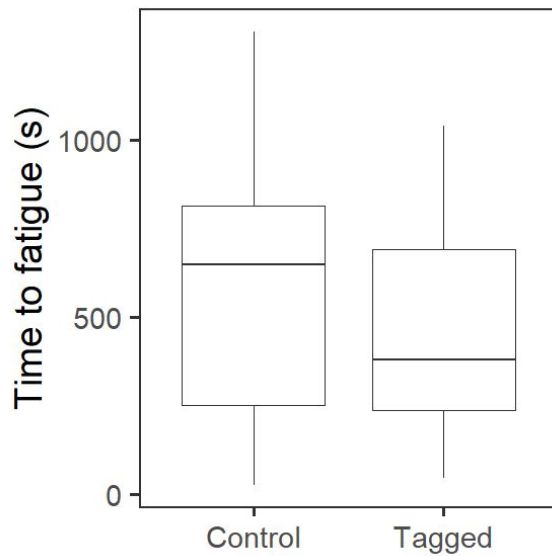
15 288 There was no difference in length or weight between tagged and control fish within any  
16 289 of species (Mann-Whitney,  $p > 0.1$ ).  
17  
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19

20 290 ***Survival and tag retention***  
21

22 291 Survival over the study period was high for both species, with no difference between  
23 292 tagged and control (Chi2,  $p > 0.3$ ). In South European nase, only 3 of 60 tagged fish  
24 293 died, corresponding to a survival ratio of 95%. One control nase died, resulting in a  
25 294 98% survival. For brook barbel, all tagged fish survived the study period (100%  
26 295 survival) while one control fish died (98% survival). No tag was lost, and  
27 296 correspondingly both species displayed 100% tag retention.  
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34 297 ***Time-to-fatigue experiment***  
35

36 298 All fish fatigued during the experiment. Median time-to-fatigue was 601 seconds (IQR  
37 299 251 – 782 seconds,  $n = 41$ ) with no difference between tagged and control South  
38 300 European nase (Mann-Whitney,  $p = 0.3$ ; fig. 2). The median length of the tested fish  
39 301 was 7.0 cm (IQR = 6.6 – 7.2 cm) and not different between tagged and control fish  
40 302 (Mann-Whitney,  $p = 0.49$ ).  
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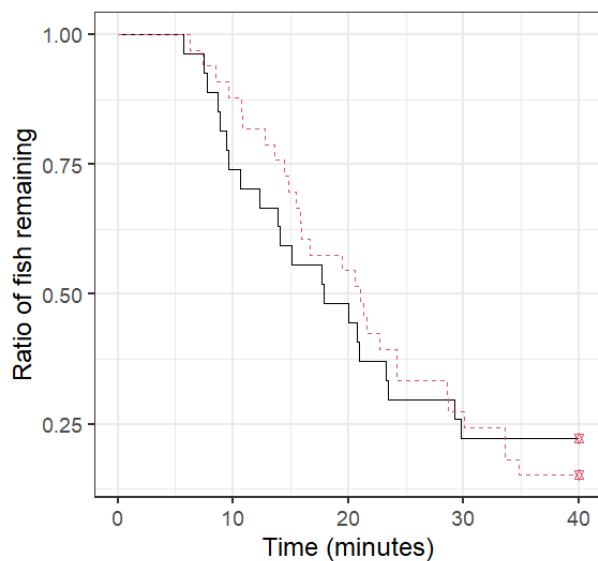


303

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

### 307 *Barrier passage experiment*

308 In total, 60 brook barbel, 27 control fish and 33 tagged, participated in the barrier  
 309 experiment divided over 12 trials with five fish in each trial. Median length of the fish  
 310 was 9.3 cm (IQR = 8.8 - 10.1 cm) with no difference between tagged and control fish  
 311 (Mann-Whitney;  $p = 0.68$ ). Passage success was 78% for control fish and 85% for  
 312 tagged fish (Fig. 3). Longer fish passed at higher rates than shorter fish (Coef = 0.39, se  
 313 = 0.17,  $p = 0.02$ ), but no effect of tagging treatment (coef = 2.6, se = 1.93,  $p = 0.15$ ) or  
 314 any interaction between tagging treatment and length (coef = -0.29, se = 0.20,  $p = 0.13$ )  
 315 was detected. Although jumping fish were observed, all fish successfully passing the  
 316 obstacle did so by swimming against the overtopping flow.



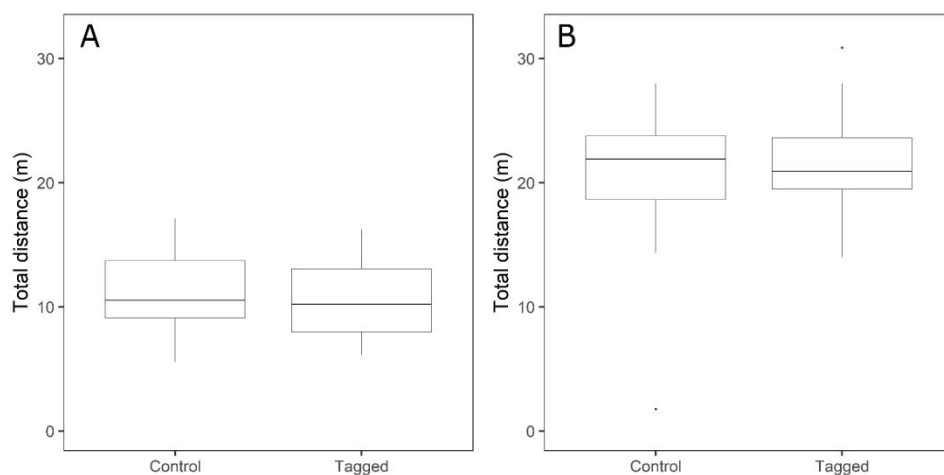
317

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

### 320 *Open field test*

321 A random subset of small sized South European nase and brook barbel were tested for  
 322 activity score and maximum swimming speed in an open field test followed by a series  
 323 of provoked escape responses. In total, 34 small-sized South European nase (18 control  
 324 and 16 tagged) with an average length of 7.1 cm (median, IQR = 6.3 - 7.4) and 54 brook  
 325 barbel (27 control and 27 tagged) with an average length of 9.7 cm (median, IQR = 9 -  
 326 10.4) were tested in the open field test. There was no difference in size between tagged  
 327 and control fish for any of the species (Mann-Whitney,  $p > 0.6$ ), and mean temperatures  
 328 during the tests were 12.7°C (range 12.1 – 13.5 °C) for nase and 13.1°C (range 12.4 –  
 329 13.6 °C) for barbel.

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

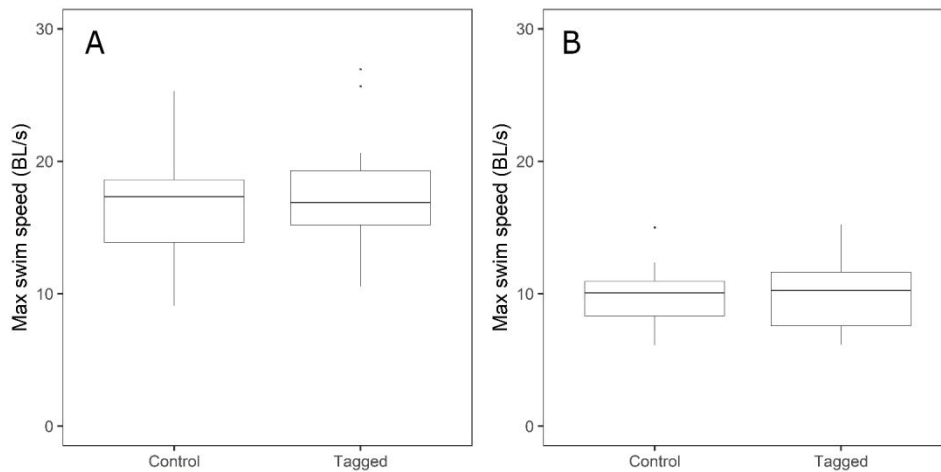


335

336 Figure 4. Total distance moved for control and tagged fish during the open field test for  
 337 A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The  
 338 horizontal line represents the median value, the box the interquartile range, the whiskers  
 339 1.5 IQR, and the point are outliers.

#### 340 *Maximum swimming speed*

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



349

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

### 354 Discussion

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

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

371 Swimming is a central part of a fish biology and of particular importance for  
 372 migration, habitat selection, and predator-prey interaction, as well as for fish passage

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3 373 design (Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012; Tudorache *et al.*, 2008).  
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5 374 Previous studies have found no effects of PIT-tags on sustained and prolonged  
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7 375 swimming (Ficke *et al.*, 2012; Mueller *et al.*, 2006; Newby *et al.*, 2007) or burst  
8  
9 376 swimming performance (Knaepkens *et al.*, 2007; Mueller *et al.*, 2006; Nyqvist *et al.*,  
10  
11 377 2022). Here we strengthen these results studying a large range of swimming behaviors.  
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13 378 No effects of PIT-tagging were found on sustained swimming activity in the open field  
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15 379 test, burst swimming in the provoked escape response, or prolonged swimming in the  
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17 380 increased velocity test (nase) and barrier passage test (barbel). Sample sizes were  
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19 381 relatively modest, so the result should be taken with some caution. The distribution of  
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21 382 data, although displaying an expected large spread of performances (Katopodis &  
22  
23 383 Gervais, 2012) , does not show any tendency of potential tagging effects, except  
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25 384 perhaps for nase in the increasing velocity test (similar to Italian ruffe; Schiavon  
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27 385 2023). Overall this constitutes encouraging results for PIT-tagging small sized  
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29 386 Cypriniformes fish.

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33 387 The range of swimming behaviors investigated covers a wide range of behaviors  
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35 388 relevant to survival and movement of fish in their natural environment (Castro-Santos *et*  
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37 389 *al.*, 2022; Videler, 1993), but future studies may go a step further to investigate  
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39 390 potential tagging effects on the behavior in the wild. Studies on salmonids, pikes, and  
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41 391 cyprinids show high survival and tag retention rates and no effect on growth also in  
42  
43 392 nature (Hühn *et al.*, 2014; Ombredane *et al.*, 1998; Skov *et al.*, 2020), but not always  
44  
45 393 (Dieterman & Hoxmeier, 2009; Šmejkal *et al.*, 2019). While growth and survival  
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47 394 studies, to some extent, summarize the consequences of behavior for wild fish, specific  
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49 395 studies on tagging effects on behavior in nature are scarce in the literature. For acoustic  
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51 396 tags, the behavior of fish tagged in previous years has been compared to recently tagged  
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53 397 fish, assuming diminishing tagging effect over time, to check for behavioral tagging  
54  
55 398 effects (Wilson *et al.*, 2017). Recently this approach was extended to PIT-telemetry,  
56  
57 399 revealing effects of tagging and handling on fish passage performance of PIT-tagged  
58  
59 400 alewife (Sullivan *et al.*, 2023). A similar approach should be applicable also in a more  
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401 natural context for PIT-telemetry.

402  
403 The estimated maximum swimming speeds – as all swimming tests relying on  
404  
405 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

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3 406 windows using tracking technology within a flume (Schiavon *et al.*, 2023). The lower  
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5 407 swimming performance in barbel, might be due to them relying more on camouflage  
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7 408 than escape in their natural environment (Eilam, 2005), but perhaps also on having been  
8  
9 409 more habituated to the artificial hatchery environment. When a researcher approached  
10  
11 410 their respective holding tanks, nase typically hid into their shelters while the barbel  
12  
13 411 anticipated feeding. Perhaps a combination of natural behavior and partial habituation to  
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15 412 a predator free environment among the barbel explains their lower performance.

16 413 In the barrier test, most barbel did pass the barrier with no tagging effects  
17  
18 414 detected. Both tagged and control fish passed the barrier by swimming over the  
19  
20 415 streaming flow. Interestingly, larger barbel – independent of tagging – passed the  
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22 416 barrier at a higher rate than smaller fish. Selection against shorter fish are known from  
23  
24 417 both natural and artificial barriers (Haugen *et al.*, 2008; Volpato *et al.*, 2009), and may  
25  
26 418 be explained by differential swimming performance (Katopodis & Gervais, 2012). In  
27  
28 419 our experiments, however, the length of the smallest fish to pass did not differ from the  
29  
30 420 length of the smallest fish to fail, indicating a rather subtle selection process of this  
31  
32 421 barrier type on brook barbel. Further studies on the barrier passage capabilities of brook  
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34 422 barbel, and other small stream fish, can help inform fish passage and barrier design for  
35  
36 423 fish conservation (Jones *et al.*, 2021).

37 424 Both South European nase and brook barbel are species under conservation concern,  
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39 425 endemic to the Italian peninsula, and listed as endangered on the regional IUCN redlist  
40  
41 426 (Rondinini *et al.*, 2022). As for many other freshwater fish species with little direct  
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43 427 economic value (Smialek *et al.*, 2019; Vøllestad, 2023), there is a lack of knowledge  
44  
45 428 about their ecology and behavior. This is particularly pressing given that they are  
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47 429 subject to range of anthropogenic stressors requiring efficient management (Carosi *et*  
48  
49 430 *al.*, 2019; Dudgeon *et al.*, 2006). In this light, the present study encourages the use of  
50  
51 431 PIT-telemetry to study, for example, movement dynamics, survival, habitat use,  
52  
53 432 restoration success, and fish passage performance (eg. Brönmark *et al.*, 2008; Castro-  
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55 433 Santos *et al.*, 1996; Keeler *et al.*, 2007; Watz *et al.*, 2019b) in these and similar species.

56 434

## 56 435 **Conclusions**

57  
58 436 In agreement with many other studies on PIT-tagged fish, our results demonstrate high  
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60 437 survival and tag retention. In addition, we investigated potential tagging effects on a

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3 438 range of fish swimming behaviors, relevant to survival and movement of fish in their  
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5 439 natural environment, not finding any effects. (Castro-Santos *et al.*, 2022; Videler, 1993).  
6  
7 440 Overall, our results indicate that PIT-telemetry is a useful method for studying small-  
8  
9 441 sized South European nase and brook barbel.

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17  
18 446 101074559 “Small fish, small streams, big challenges: conservation of endangered  
19  
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23  
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3 **Survival and Swimming Performance in Small-sized South European**  
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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 ~~of~~

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3 ~~environmental conditions~~ (Clark, 2016; Jepsen *et al.*, 2015; Wargo Rub *et al.*, 2020),  
4 and effects may go beyond those affecting survival and growth in the laboratory  
5 (Connors *et al.*, 2002; Zakęś *et al.*, 2022). This makes studies of tag effects an important  
6 component of fish telemetry, and hence of ecohydraulic research.  
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10 Typically, PIT-tagged fish display high survival and tag retention, and negative  
11 effects on growth mainly in the short term (Clark, 2016; Vollset *et al.*, 2020). Notably,  
12 however, some species can experience high tagging mortalities, at least under some  
13 conditions (Clark, 2016; Watson *et al.*, 2019). While growth effects capture some  
14 sublethal tagging effects (Vollset *et al.*, 2020), and physiological sampling can reveal  
15 stress responses in fish (Zakęś *et al.*, 2022), behavioral effects on the tagged animals  
16 may be of very high importance to the animals performance in nature. Given this,  
17 behavioral effects of PIT-tags on the tagged fish are surprisingly little studied (Nyqvist  
18 *et al.*, 2022).  
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26 Swimming performance, involving both behavior and capability, is fundamental  
27 for the ecology of fish, influencing movement, reproduction and predator-prey  
28 interactions (Castro-Santos *et al.*, 2022; Tudorache *et al.*, 2013). Fish swimming can be  
29 categorized ~~in to~~into three modes ~~--~~ sustained, prolonged, and burst swimming -  
30 differing in physiology and utilization (Videler, 1993). While bursting fish uses white  
31 muscles in anaerobic processes, for a short period of time, sustained swimming is  
32 powered with red muscles, aerobic processes and can, theoretically, be maintained  
33 indefinitely. Prolonged swimming is a mix of the two other modes where fish uses both  
34 red and white muscles, and fatigues after seconds or hundreds of minutes (Hammer,  
35 1995; Videler, 1993). Burst swimming is fundamental for predator-prey interactions and  
36 for overcoming velocity barriers, whereas sustained swimming performance may be  
37 more important for continuous (e.g. patrolling, food seeking) and long-distance  
38 movements (Videler, 1993). Relating to applied fisheries science, estimated fish  
39 swimming performance is often used as a design criterion for the construction of  
40 fishways (~~Castro-Santos et al.~~, (Baki *et al.*, 2020; Castro-Santos *et al.*, 2022; Katopodis  
41 & Gervais, 2012). Accordingly, testing performance under different swimming modes is  
42 highly relevant when evaluating tagging effects.  
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56 Conventional swimming tests constitute forced or provoked swimming in  
57 flumes or swim chambers where the fish swim to fatigue, and test prolonged or burst  
58 swimming capability while also deducing sustained swimming performance (Tudorache  
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3 *et al.*, 2013). In addition, provoked escape response tests have been used to estimate  
4 maximum swimming speeds in the burst mode (Domenici, 2010; Tudorache *et al.*,  
5 2008). Effects of PIT-tags on fish swimming performance have been tested without  
6 detectable effects on sustained swimming in cyprinids (Ficke *et al.*, 2012), salmonids  
7 (Newby *et al.*, 2007), and lampreys (Mueller *et al.*, 2006) and on burst speeds in  
8 bullheads (Knaepkens *et al.*, 2007), spiny loaches (Nyqvist *et al.*, 2022) and lampreys  
9 (Mueller *et al.*, 2006). Schiavon *et al.* (2023), however, did detect an effect of PIT-tags  
10 on the prolonged swimming performance, but not on maximum burst speeds, in Italian  
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19 An open field test, on the other hand, consists of letting an animal freely explore  
20 an experimental arena, and consequently, when it comes to fish, measure a type of  
21 voluntary swimming behavior, typically within the sustained mode. Open field tests are  
22 commonly used in behavioral ecology to test for individual animal's willingness to  
23 explore an area, often as a proxy for activity, boldness or exploratory behavior  
24 (Mittelbach *et al.*, 2014; Peralas *et al.*, 2017), and in the field of ecotoxicology to test for  
25 chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*,  
26 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests,  
27 volitional swimming in open field tests has been seen to correlate with activity (Závorka  
28 *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as  
29 downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to  
30 tag effects, open field tests have been used to study effects of PIT-tags on Italian spined  
31 loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

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42 There is a general lack of information on the ecology and habitat preferences for  
43 many fish species, in particular for small-sized fish with little economical interest  
44 (Negro *et al.*, 2021; Smialek *et al.*, 2019). South European nase (*Protochondrostoma*  
45 *genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to  
46 streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation  
47 concern while confronted with a high number of in-stream barriers as well as  
48 anthropogenically induced habitat change such as increased temperatures and water  
49 scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et*  
50 *al.*, 2017). The need to increase the knowledge of the ecology and behavior of these,  
51 and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-  
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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; ELT60HGI, 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~~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 / 1 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

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3 left to recover in aerated tanks. Controls received the same anesthetic treatment but were  
4 only measured and weighed. After recuperating from anesthesia, fish were then held in  
5 spring-fed flow-through tanks. Tagged and control fish were kept together in one large  
6 tank for South European nase (length\*width\*depth = 110 cm \* 120 cm \* 40 cm) and two  
7 smaller tanks with higher water exchange rates for brook barbel (length\*width\*depth =  
8 150 cm \* 45 cm \* 20 cm). Temperatures were kept stable around 13°C and the light  
9 regime followed natural seasonal rhythms. All holding tanks were equipped with artificial  
10 shelters comprised of perforated bricks. Fish were fed daily. Brook barbel were fed with  
11 Sera Koi Royal pellets® while South European nase were fed Tetra TabiMin sinking  
12 pellets containing a higher proportion of vegetarian content. For both species, the  
13 commercial pellets were supplemented with wild-caught macrozoobenthos. The tanks  
14 were inspected for mortalities daily, and missing tags were checked at the end of the  
15 experiment, 64 days post tagging.

### 26 *Flume experiments*

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28 A subset of fish was tested for swimming performance in an open channel flume made  
29 of plexiglass: (Fig. 1). South European nase were tested in a traditional time to fatigue  
30 experiment. Brook barbel, on the other hand, did not perform (refused to swim) in  
31 traditional swimming trials, and were instead tested in a barrier passage experiment.  
32 The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of  
33 60 cm for the time to fatigue trials, and 140 cm for the barrier passage experiments. A  
34 honeycomb flow straightener at the upstream end of the flume made the flow uniform in  
35 the test section and delimited the testing arena in the upstream direction. A downstream  
36 fine-meshed grid delimited the downstream end of the arena. Water depth, temperature,  
37 and flow were continuously monitored using dedicated sensors (Schiavon *et al.* 2023 for  
38 details). The temperature was maintained at 12.5°C (SD = 0.3°C) using a chiller (TECO  
39 TK-2000 chiller).

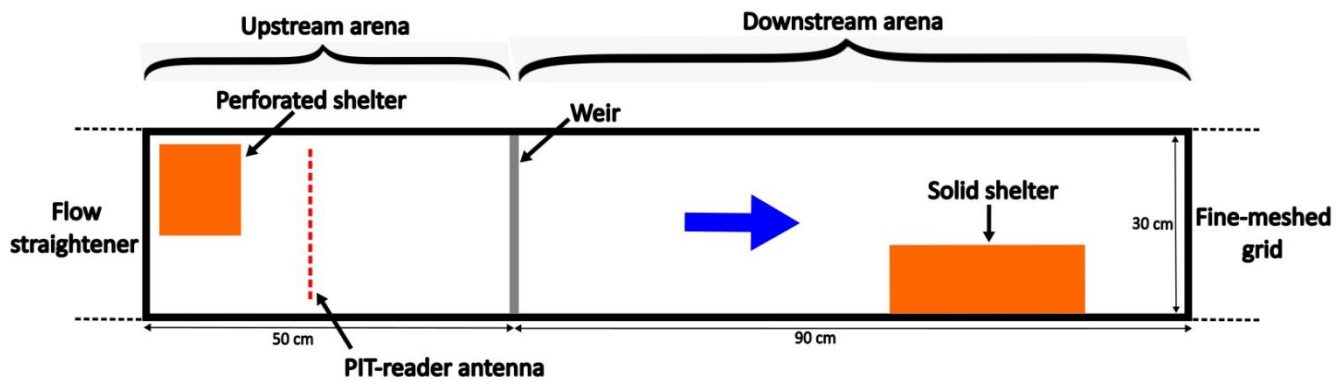
### 49 *Time-to-fatigue experiment*

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51 The swimming performance of South European nase were tested in an increasing  
52 velocity swimming test (Schiavon *et al.*, 2023) on 13-14 December, 24-25 days after  
53 tagging. Two days before the swimming trials the experimental fish were size sorted to  
54 acquire a subset (n = 41, 25 control and 16 tagged) of small and relatively uniformly  
55 sized fish ( $\leq 8.4$  cm) for the swimming trials. These fish were kept in a separate holding  
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3 tank until the swimming trials. At the start of a trial, an individual fish was netted and  
4 gently released in the swimming arena. In the arena, fish were given 5 min to habituate  
5 to the flume at a low flow velocity of 17-19 cm/s. At the start of the swimming trial,  
6 water velocity was increased to 45 cm/s. If the fish had not fatigued within 10 min,  
7 velocity was increased to 52 cm/s, corresponding to an approximate increase of one  
8 body length per second for the tested fish. Water depth during the swimming trial was  
9 7-8 cm depending on velocity. When the fish rested on the downstream grid, it was  
10 gently poked from the downstream side of the grid, encouraging it to continue  
11 swimming. A fish was defined as fatigued after resting on the grid despite poking, and  
12 time to fatigue as the time from the start of testing velocity to the fish refusing to swim.  
13 After the swimming trial, the fish was scanned for PIT-ID and returned to the main  
14 holding tank.  
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#### 25 *Barrier passage experiment*

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

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3 changed regularly to maintain a stable temperature across all trials ( $12.7 \pm 0.3^\circ\text{C}$  for  
4 nase;  $13.1 \pm 0.3^\circ\text{C}$  for barbel).  
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7 The arena was video recorded with an overhead camera (Sony 4K, FDR-AX43, 50fps,  
8 Minato City, Tokyo, Japan). A custom-made MATLAB (R2021b; The Math-Works  
9 Inc., Natick, MA, USA) script (<https://github.com/SilverFox275/manual-point-tracking>)  
10 was used to track fish positions manually in one frame per second for the open field test  
11 and 10 frames per second during the provoked escape response. Distance in pixels was  
12 translated to distance in meters using the known dimensions of the arena. Total distance  
13 moved was quantified for the time from 5 min habituation to the time when the first  
14 spherical weight was dropped (Haraldstad *et al.*, 2021; Nyqvist *et al.*, 2022; Watz,  
15 2019). Although the experiment was designed for this time to be 5 min, due to mis-  
16 timing during the execution of the experiment, and to achieve identical durations for all  
17 fish of the same species it was reduced to 204 s for nase and 230 s for barbel. For the  
18 escape response, the fastest 400 ms (i.e., the longest distance moved over four tracked  
19 frames) was used as an estimate of the maximum swimming speed (Knaepkens *et al.*,  
20 2007; Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008). As maximum swimming speed  
21 typically depends on the length of the fish, the swimming speed was normalized to the  
22 length of the fish (Domenici & Blake, 1997).  
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### 36 *Statistics*

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38 Nonparametric As assumption of normality were not met for part of the data (Shapiro-  
39 Wilk test of normality), nonparametric Mann-Whitney tests were used to compare fork  
40 length, weight, time-to-fatigue, distance moved, and maximum swimming speed.  
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42 Difference in survival between tagged and control fish was tested using chi2-tests.  
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44 Effects of treatment (tagged or control) on passage rates in the barrier experiment were  
45 tested using Cox-regression, a type of time-to-event analysis (Castro-Santos & Perry,  
46 2012; Hosmer *et al.*, 2008). Fish length was included in the model to control for any  
47 effect of fish size on passage rates. Fish not passing were included as censored  
48 observations. Fish were clustered on trial to control for non-independence between fish  
49 in the same trial. The assumption of proportionality of hazard was explicitly tested (Fox,  
50 2002). Significance level of  $p < 0.05$  was applied to all tests. Data management, plotting  
51 and statistical tests were performed in R 4.0.3 (R Foundation for Statistical Computing,  
52 Vienna, Austria, URL <https://www.R-project.org>).  
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### ***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 ( $\chi^2$ ,  $p > 0.3$ ). In South European nase, only 3 of ~~5660~~ 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. ~~12~~). 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,  $p = 0.49$ ).

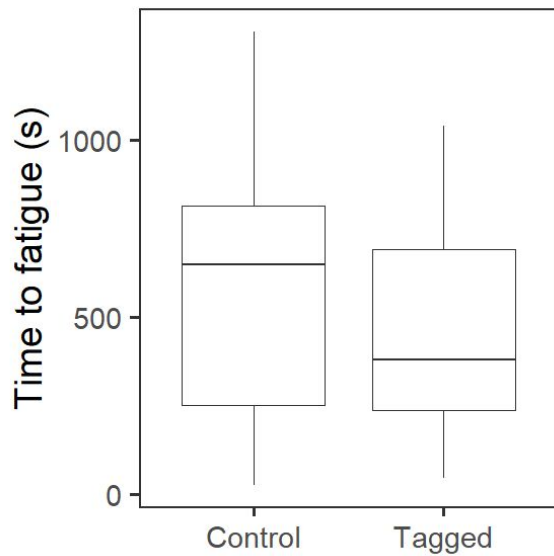


Figure 2. Time-to-fatigue for control (n =25) and tagged (n =16) South European nase. First ~~600s~~600 s in 45 cm/s, followed by 52 cm/s until fatigue. The horizontal line represents the median value, the box the interquartile range, the whiskers the range of data.

### ***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.

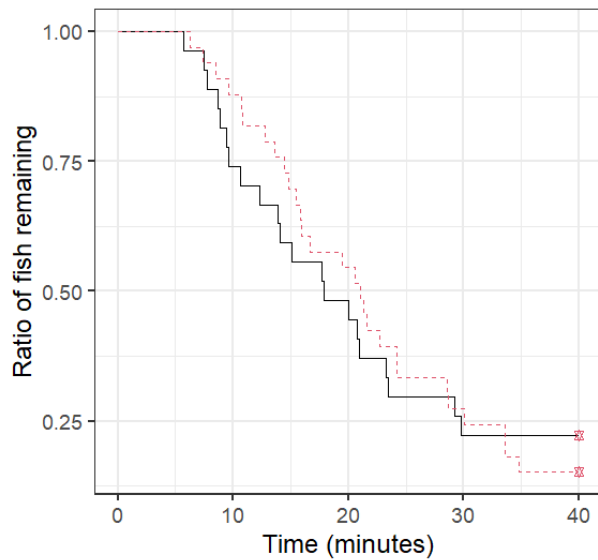


Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line;  $n = 27$ ) and tagged fish (dashed line;  $n = 33$ ) remaining downstream the barrier over time.

### *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,  $p = 1$ ).

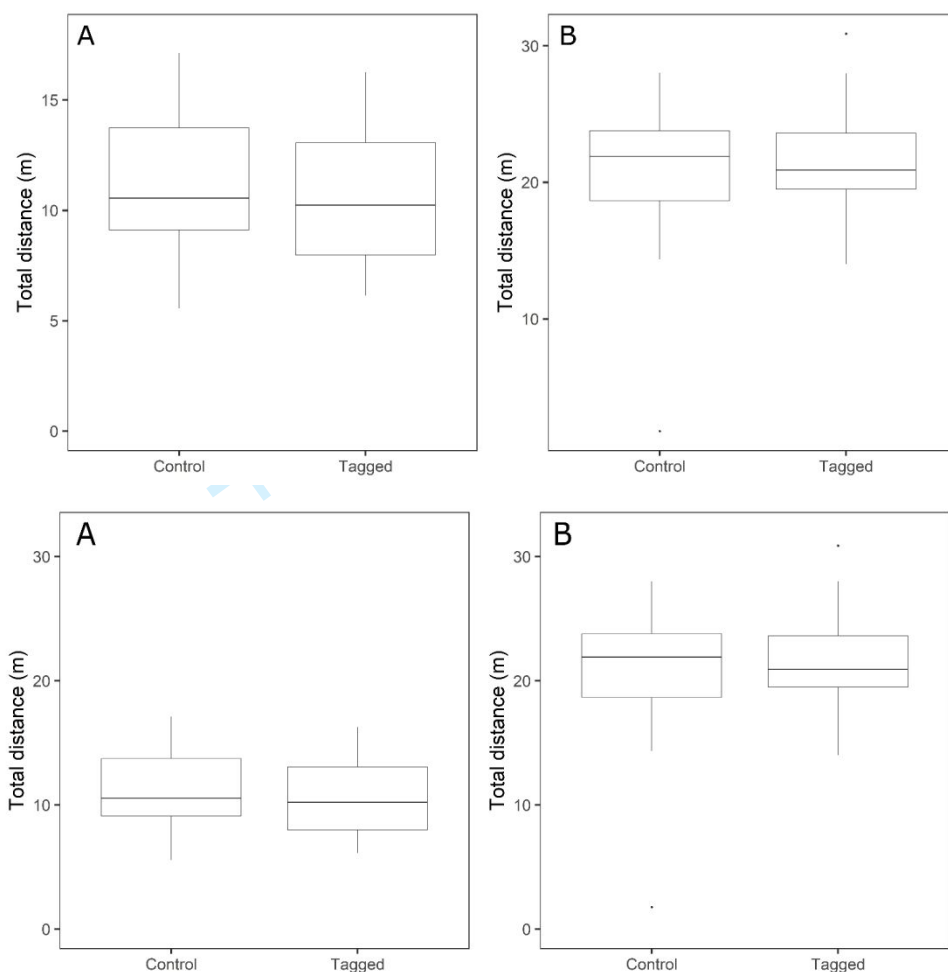


Figure 34. Total distance moved for control and tagged fish during the open field test for A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

### ***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](#))

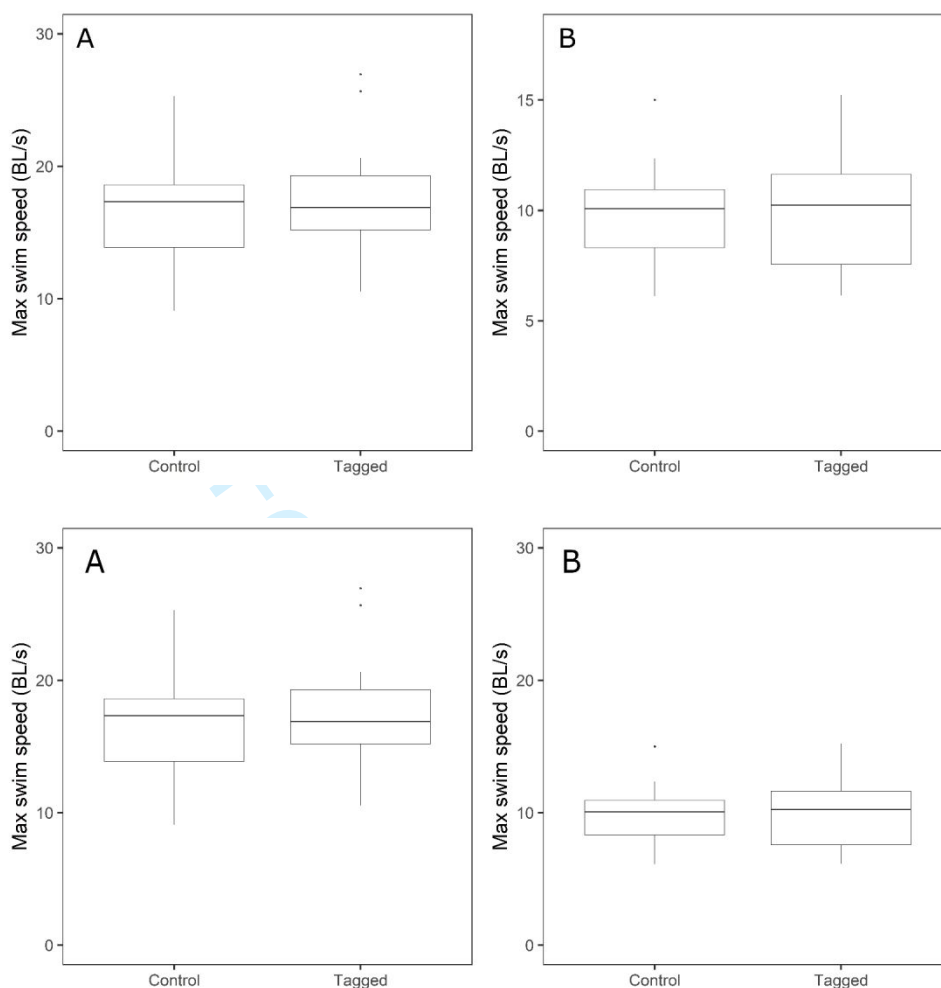


Figure 45. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment for A) South European nase (n = 34) and B) brook barbel (n = 54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are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

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3 survival and high retention rates after PIT-tagging (Nagel *et al.*, 2023). In relation to the  
4 often cited 2%-rule, recommending tag-to-fish-weight not to exceed 2%, our tag-to-fish  
5 ratios were higher than this in 30% of the nase and 9% of the barbel, in keeping with  
6 studies relativizing this rule (Brown *et al.*, 1999; Jepsen *et al.*, 2005). Corresponding  
7 proportions of fish exceeding the 17.5% tag-to-fish length ratio derived from a meta-  
8 analysis on salmonids (Vollset *et al.*, 2020), were 9% and 5% for nase and barbel,  
9 respectively. Potential tagging effects, however, may go beyond mere survival.

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16 Swimming is a central part of a fish biology and of particular importance for  
17 migration, habitat selection, and predator-prey interaction, as well as for fish passage  
18 design (Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012; Tudorache *et al.*, 2008).  
19 Previous studies have found no effects of PIT-tags on sustained and prolonged  
20 swimming (Ficke *et al.*, 2012; Mueller *et al.*, 2006; Newby *et al.*, 2007) or burst  
21 swimming performance (Knaepkens *et al.*, 2007; Mueller *et al.*, 2006; Nyqvist *et al.*,  
22 2022). Here we strengthen these results studying a large range of swimming behaviors.  
23 No effects of PIT-tagging were found on sustained swimming activity in the open field  
24 test, burst swimming in the provoked escape response, or prolonged swimming in the  
25 increased velocity test (nase) and barrier passage test (barbel). Sample sizes were  
26 relatively modest, so the result should be taken with some caution. The distribution of  
27 data, however although displaying an expected large spread of performances (Katopodis  
28 & Gervais, 2012), does not show any tendency of potential tagging effects, except  
29 perhaps for nase in the increasing velocity test (similar to Italian ruffe; Schiavon  
30 2023). Overall this constitutes encouraging results for PIT-tagging small sized  
31 Cypriniformes fish.

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44 The range of swimming behaviors investigated covers a wide range of behaviors  
45 relevant to survival and movement of fish in their natural environment (Castro-Santos *et al.*  
46 *et al.*, 2022; Videler, 1993), but future studies may go a step further to investigate  
47 potential tagging effects on the behavior in the wild. Studies on salmonids, pikes, and  
48 cyprinids show high survival and tag retention rates and no effect on growth also in  
49 nature (Hühn *et al.*, 2014; Ombredane *et al.*, 1998; Skov *et al.*, 2020), but not always  
50 (Dieterman & Hoxmeier, 2009; Šmejkal *et al.*, 2019). While growth and survival  
51 studies, to some extent, summarize the consequences of behavior for wild fish, specific  
52 studies on tagging effects on behavior in nature are scarce in the literature. For acoustic  
53 tags, the behavior of fish tagged in previous years has been compared to recently tagged  
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3 fish, assuming diminishing tagging effect over time, to check for behavioral tagging  
4 effects (Wilson *et al.*, 2017). ~~A similar approach should be applicable also for PIT-~~  
5 ~~telemetry. Recently this approach was extended to PIT-telemetry, revealing effects of~~  
6 ~~tagging and handling on fish passage performance of PIT-tagged alewife (Sullivan *et*~~  
7 ~~*al.*, 2023). A similar approach should be applicable also in a more natural context for~~  
8 ~~PIT-telemetry.~~  
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14 The estimated maximum swimming speeds – as all swimming tests relying on  
15 both behavior and capability - were 17 BL/s for nase and 10 BL/s for barbel. This is  
16 within the range of what has been reported for other Cypriniformes fish with similar  
17 methodology (Nyqvist *et al.*, 2022; Tudorache *et al.*, 2008), and over longer time  
18 windows using tracking technology within a flume (Schiavon *et al.*, 2023). The lower  
19 swimming performance in barbel, might be due to them relying more on camouflage  
20 than escape in their natural environment (Eilam, 2005), but perhaps also on having been  
21 more habituated to the artificial hatchery environment. When a researcher approached  
22 their respective holding tanks, nase typically hid in their shelters while the barbel  
23 anticipated feeding. Perhaps a combination of natural behavior and partial habituation to  
24 a predator free environment among the barbel explains their lower performance.  
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34 In the barrier test, most barbel did pass the barrier with no tagging effects  
35 detected. Both tagged and control fish passed the barrier by swimming over the  
36 streaming flow. Interestingly, larger barbel – independent of tagging – passed the  
37 barrier at a higher rate than smaller fish. Selection against shorter fish are known from  
38 both natural and artificial barriers (Haugen *et al.*, 2008; Volpato *et al.*, 2009), and may  
39 be explained by differential swimming performance (Katopodis & Gervais, 2012). In  
40 our experiments, however, the length of the smallest fish to pass did not differ from the  
41 length of the smallest fish to fail, indicating a rather subtle selection process of this  
42 barrier type on brook barbel. Further studies on the barrier passage capabilities of brook  
43 barbel, and other small stream fish, can help inform fish passage and barrier design for  
44 fish conservation (Jones *et al.*, 2021).  
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53 Both South European nase and brook barbel are species under conservation concern,  
54 endemic to the Italian peninsula, and listed as endangered on the regional IUCN redlist  
55 (Rondinini *et al.*, 2022). As for many other freshwater fish species with little direct  
56 economic value (Smialek *et al.*, 2019; Vøllestad, 2023), there is a lack of knowledge  
57 about their ecology and behavior. This is particularly pressing given that they are  
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3 subject to range of anthropogenic stressors requiring efficient management (Carosi *et*  
4 *al.*, 2019; Dudgeon *et al.*, 2006). In this light, the present study encourages the use of  
5 PIT-telemetry to study, for example, movement dynamics, survival, habitat use,  
6 restoration success, and fish passage performance (eg. Brönmark *et al.*, 2008; Castro-  
7 Santos *et al.*, 1996; Keeler *et al.*, 2007; Watz *et al.*, 2019b) in these and similar species.  
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## 15 **Conclusions**

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

29  
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35 Mozzi, Usama Ashraf, Costantino Manes, and Armando Piccinini for technical  
36 assistance.  
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