

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

Original

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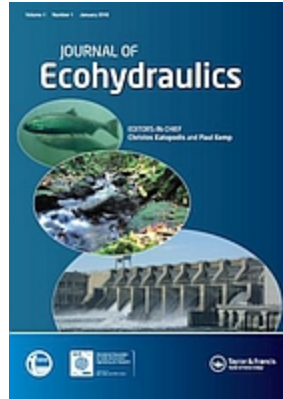
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Survival and Swimming Performance in Small-sized South European Cypriniformes tagged with Passive Integrated Transponders

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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
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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

1
2
3 103 bullheads (Knaepkens *et al.*, 2007), spiny loaches (Nyqvist *et al.*, 2022) and lampreys
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5 104 (Mueller *et al.*, 2006). Schiavon *et al.* (2023), however, did detect an effect of PIT-tags
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7 105 on the prolonged swimming performance, but not on maximum burst speeds, in Italian
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9 106 riffle dace.

10
11 107 An open field test, on the other hand, consists of letting an animal freely explore
12
13 108 an experimental arena, and consequently, when it comes to fish, measure a type of
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15 109 voluntary swimming behavior, typically within the sustained mode. Open field tests are
16
17 110 commonly used in behavioral ecology to test for individual animal's willingness to
18
19 111 explore an area, often as a proxy for activity, boldness or exploratory behavior
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21 112 (Mittelbach *et al.*, 2014; Perals *et al.*, 2017), and in the field of ecotoxicology to test for
22
23 113 chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*,
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25 114 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests,
26
27 115 volitional swimming in open field tests has been seen to correlate with activity (Závorka
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29 116 *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as
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31 117 downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to
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33 118 tag effects, open field tests have been used to study effects of PIT-tags on Italian spined
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35 119 loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

36
37 120 There is a general lack of information on the ecology and habitat preferences for
38
39 121 many fish species, in particular for small-sized fish with little economical interest
40
41 122 (Negro *et al.*, 2021; Smialek *et al.*, 2019). South European nase (*Protochondrostoma*
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43 123 *genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to
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45 124 streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation
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47 125 concern while confronted with a high number of in-stream barriers as well as
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49 126 anthropogenically induced habitat change such as increased temperatures and water
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51 127 scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et*
52
53 128 *al.*, 2017). The need to increase the knowledge of the ecology and behavior of these,
54
55 129 and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-
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57 130 telemetry offers a valuable research tool, but the need to evaluate for potential tagging
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59 131 effects beyond mere survival remains.

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61 132 Here, we study PIT-tagging effects on survival and a range of swimming
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63 133 behaviors – encompassing sustained, prolonged and burst swimming - in South
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65 134 European nase and brook barbel. Effects on volitional swimming activity (sustained
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67 135 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.

23
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
31
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).

35
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
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40 154 control group. Fish were tagged with a passive integrated transponder (PIT-tag; Biomark,
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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
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48 158 and forward in the abdominal cavity to align with the fish's body (Bolland *et al.*, 2009;
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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
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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
57
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
20
21 177 traditional swimming trials, and were instead tested in a barrier passage experiment.
22
23 178 The test arena within the flume had a cross-section of 30 cm by 30 cm and a length of
24
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
28
29 181 the test section and delimited the testing arena in the upstream direction. A downstream
30
31 182 fine-meshed grid delimited the downstream end of the arena. Water depth, temperature,
32
33 183 and flow were continuously monitored using dedicated sensors (Schiavon *et al.* 2023 for
34
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).

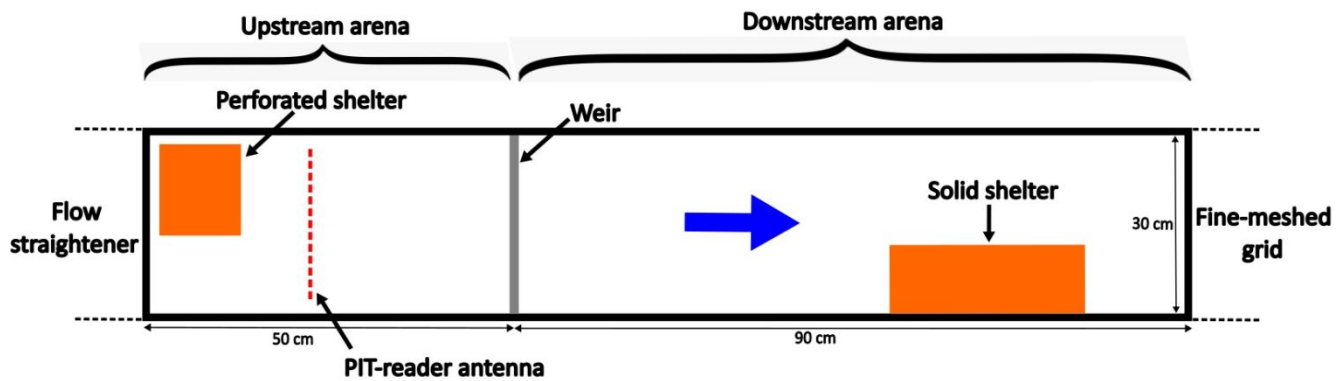
37 186 *Time-to-fatigue experiment*

38
39 187 The swimming performance of South European nase were tested in an increasing
40
41 188 velocity swimming test (Schiavon *et al.*, 2023) on 13-14 December, 24-25 days after
42
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
46
47 191 sized fish (≤ 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
50
51 193 gently released in the swimming arena. In the arena, fish were given 5 min to habituate
52
53 194 to the flume at a low flow velocity of 17-19 cm/s. At the start of the swimming trial,
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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
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9 203 holding tank.

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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
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17 207 groups of five (Amaral *et al.*, 2016). For the barrier passage experiment a wooden weir
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19 208 covered with black cloth was introduced in the flume. The barrier divided the flume in a
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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
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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
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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
2
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
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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
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35 266 typically depends on the length of the fish, the swimming speed was normalized to the
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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**
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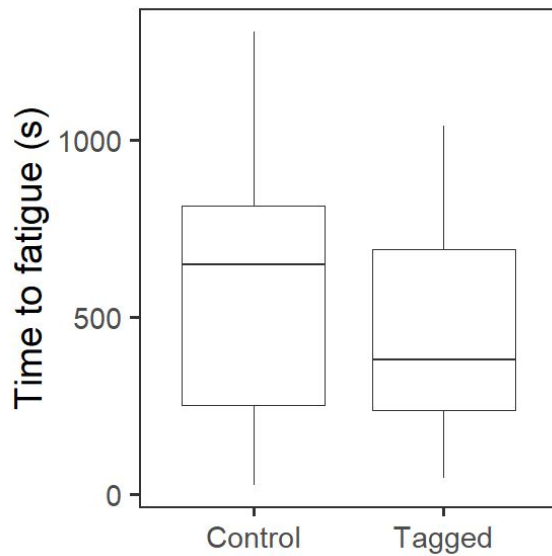
15 288 There was no difference in length or weight between tagged and control fish within any
16 289 of species (Mann-Whitney, $p > 0.1$).
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20 290 ***Survival and tag retention***
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22 291 Survival 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***
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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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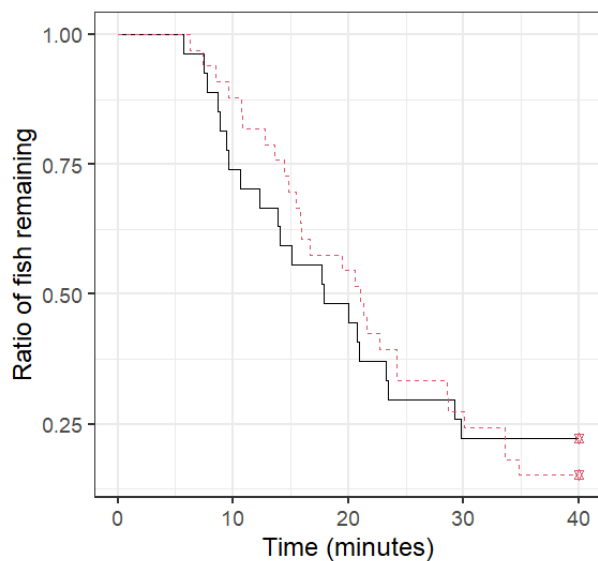


303

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.



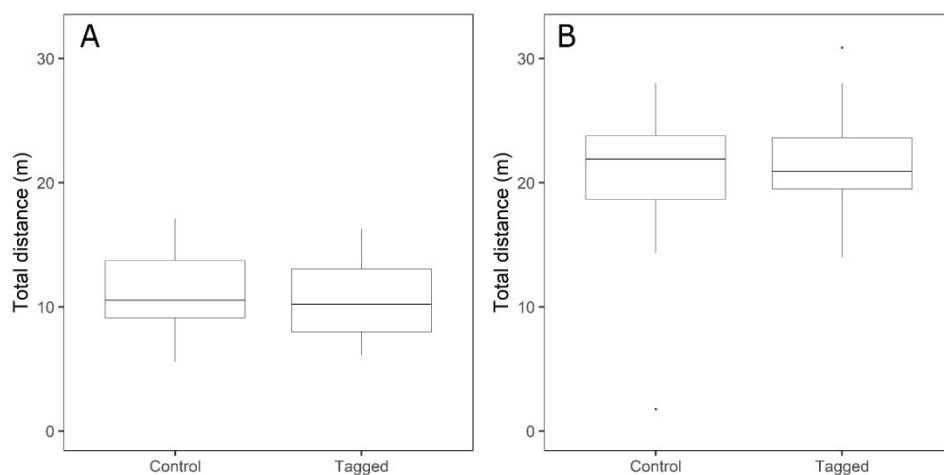
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318 Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line; $n = 27$)
 319 and tagged fish (dashed line; $n = 33$) remaining downstream the barrier over time.

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, $p = 1$).

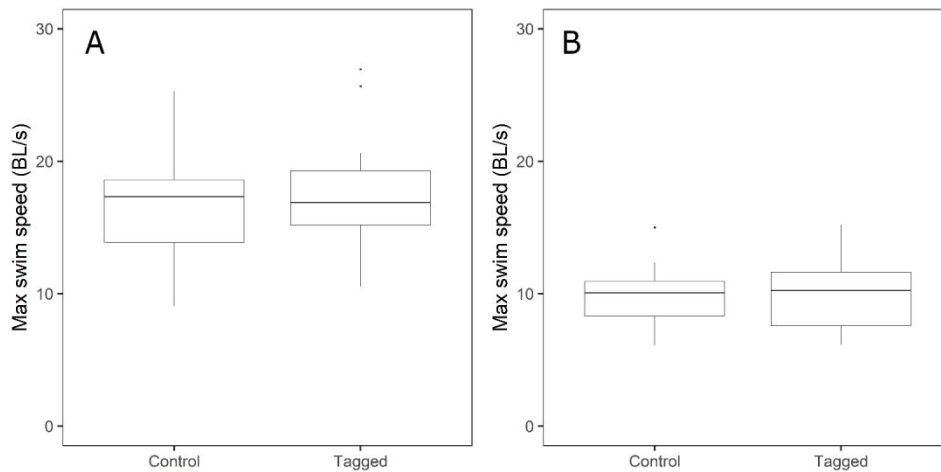


335

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
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9 376 swimming performance (Knaepkens *et al.*, 2007; Mueller *et al.*, 2006; Nyqvist *et al.*,
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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 &
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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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32
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
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43 392 nature (Hühn *et al.*, 2014; Ombredane *et al.*, 1998; Skov *et al.*, 2020), but not always
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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
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55 398 effects (Wilson *et al.*, 2017). Recently this approach was extended to PIT-telemetry,
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57 399 revealing effects of tagging and handling on fish passage performance of PIT-tagged
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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.

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403 The estimated maximum swimming speeds – as all swimming tests relying on
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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
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9 409 more habituated to the artificial hatchery environment. When a researcher approached
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11 410 their respective holding tanks, nase typically hid into their shelters while the barbel
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13 411 anticipated feeding. Perhaps a combination of natural behavior and partial habituation to
14
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
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18 414 detected. Both tagged and control fish passed the barrier by swimming over the
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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
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24 417 both natural and artificial barriers (Haugen *et al.*, 2008; Volpato *et al.*, 2009), and may
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26 418 be explained by differential swimming performance (Katopodis & Gervais, 2012). In
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28 419 our experiments, however, the length of the smallest fish to pass did not differ from the
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30 420 length of the smallest fish to fail, indicating a rather subtle selection process of this
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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
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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
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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
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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*
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49 430 *al.*, 2019; Dudgeon *et al.*, 2006). In this light, the present study encourages the use of
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51 431 PIT-telemetry to study, for example, movement dynamics, survival, habitat use,
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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-
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9 441 sized South European nase and brook barbel.

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25 26 450 **References**

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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~~

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

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

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

Conventional swimming tests constitute forced or provoked swimming in flumes or swim chambers where the fish swim to fatigue, and test prolonged or burst swimming capability while also deducing sustained swimming performance (Tudorache

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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 (≤ 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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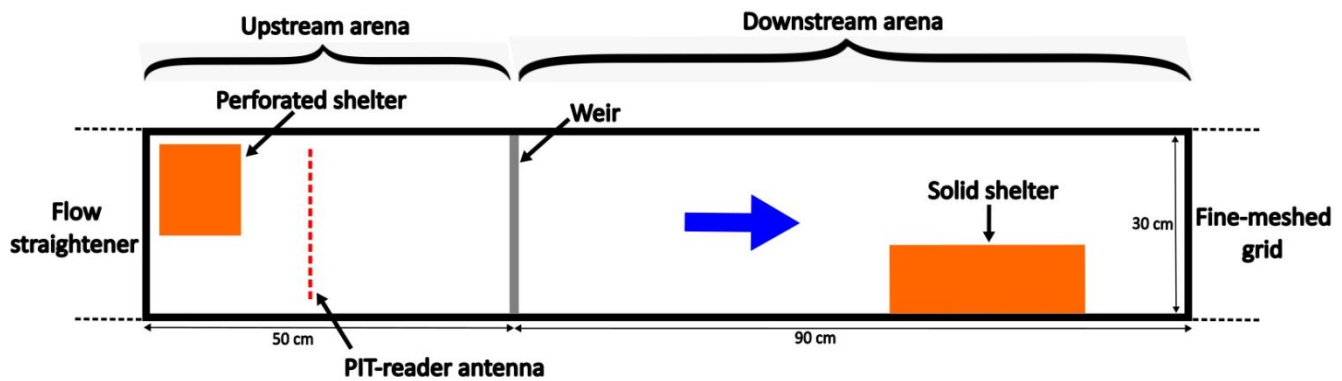


Figure 1. A scaled drawing of the experimental arena for the barrier passage experiment. For the Time-to-fatigue experiment the flow straightener delimited the experimental in an upstream direction while the fine-meshed grid did so in a downstream direction, with an arena length of 60 cm.

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 (χ^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, $p = 0.49$).

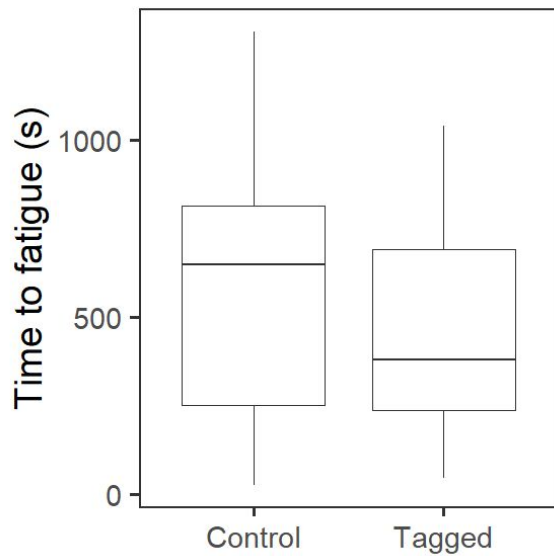


Figure 2. Time-to-fatigue for control (n =25) and tagged (n =16) South European nase. First ~~600s~~600 s in 45 cm/s, followed by 52 cm/s until fatigue. The horizontal line represents the median value, the box the interquartile range, the whiskers the range of data.

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.

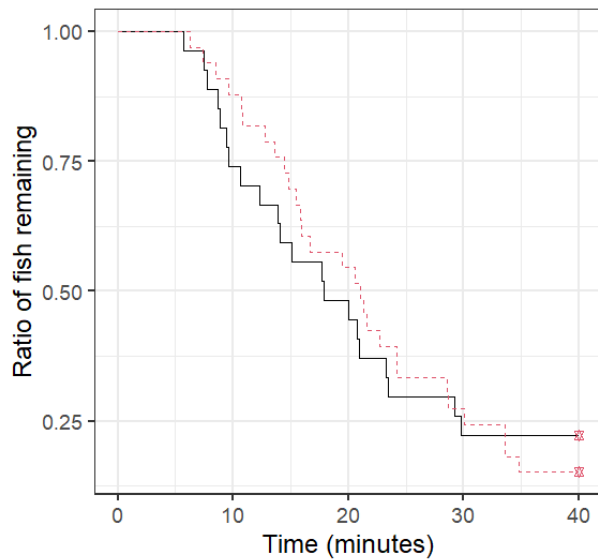


Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line; $n = 27$) and tagged fish (dashed line; $n = 33$) remaining downstream the barrier over time.

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, $p = 1$).

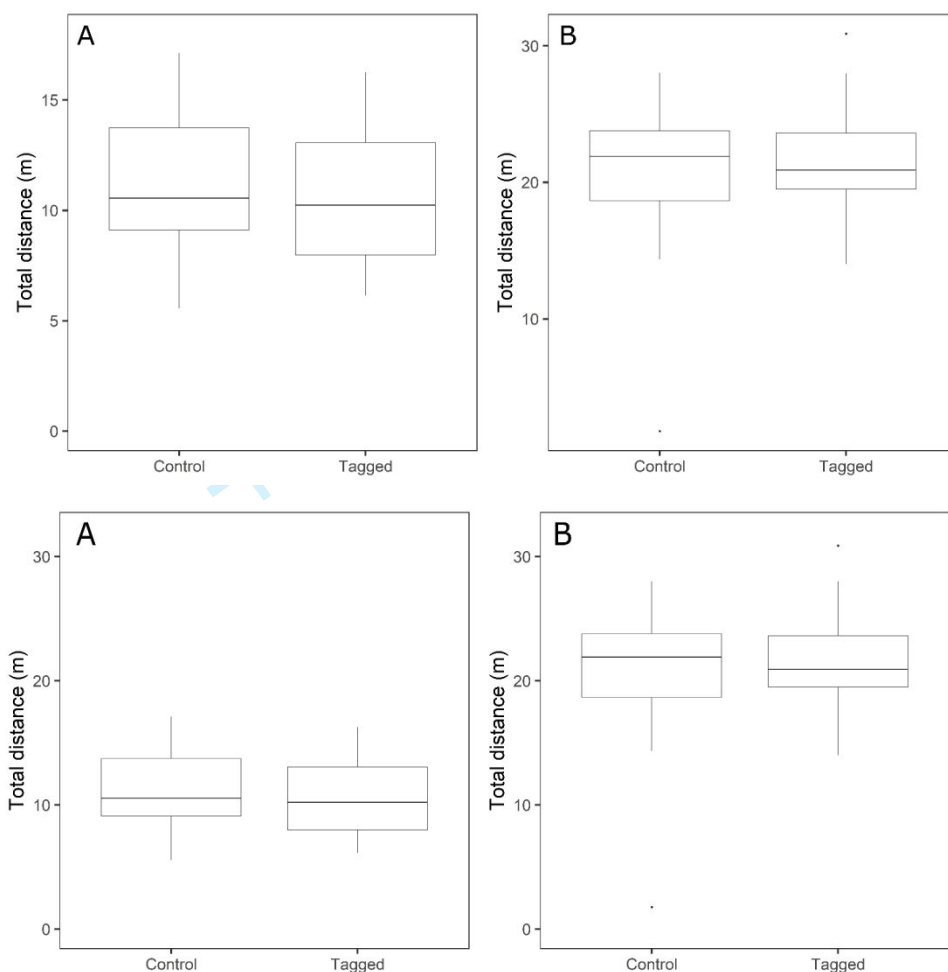


Figure 34. Total distance moved for control and tagged fish during the open field test for A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

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](#))

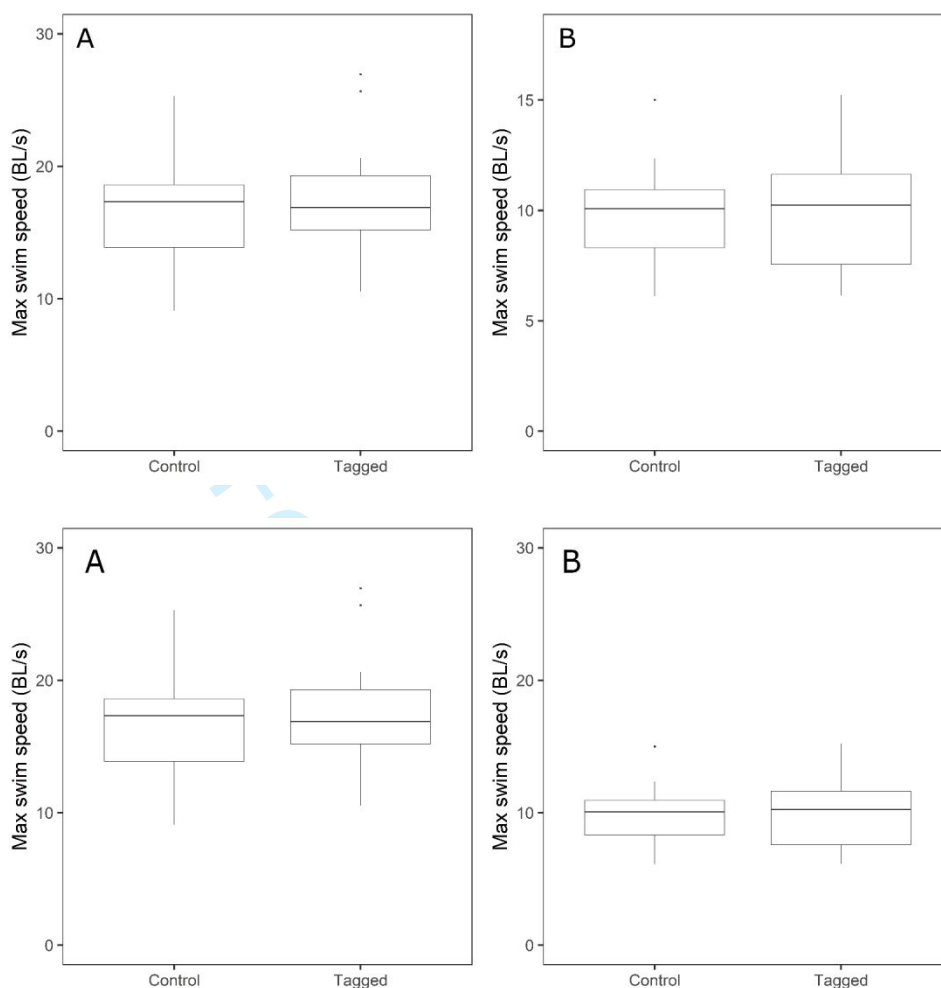


Figure 45. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment for A) South European nase (n = 34) and B) brook barbel (n = 54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are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