

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

Original

Survival and swimming performance in small-sized South European Cypriniformes tagged with passive integrated transponders / Nyqvist, D.; Schiavon, A.; Candiotta, A.; Tarena, F.; Comoglio, C.. - In: JOURNAL OF ECOHYDRAULICS. - ISSN 2470-5365. - (2024), pp. 1-11. [10.1080/24705357.2024.2306419]

Availability:

This version is available at: 11583/2985992 since: 2024-02-16T12:28:52Z

Publisher:

Taylor and Francis Ltd.

Published

DOI:10.1080/24705357.2024.2306419

Terms of use:

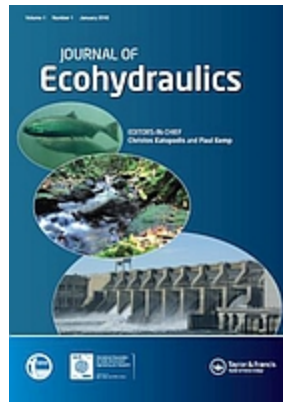
This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

Publisher copyright

Taylor and Francis postprint/Author's Accepted Manuscript

This is an Accepted Manuscript of an article published by Taylor & Francis in JOURNAL OF ECOHYDRAULICS on 2024, available at <http://www.tandfonline.com/10.1080/24705357.2024.2306419>

(Article begins on next page)



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

Journal:	<i>Journal of Ecohydraulics</i>
Manuscript ID	TJoE-2023-0024.R1
Manuscript Type:	Research Papers
Date Submitted by the Author:	06-Dec-2023
Complete List of Authors:	Nyqvist, Daniel; Politecnico di Torino Schiavon, Alfredo; Leibniz-Institute of Freshwater Ecology and Inland Fisheries in the Forschungsverbund Berlin eV; Free University of Berlin Candiotta, Alessandro; Ittiologo libero professionista Tarena, Fabio; Politecnico di Torino Comoglio, Claudio; Politecnico di Torino, DIATI
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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

	study the tested fish species.

SCHOLARONE™
Manuscripts

1
2
3 **1 Survival and Swimming Performance in Small-sized South European**
4 **2 Cypriniformes tagged with Passive Integrated Transponders**
5
6
7
8
9

10
11 4 Daniel Nyqvist^{a*}, Alfredo Schiavon^{b,c}, Alessandro Candiotto^d, Fabio
12
13 5 Tarena^a, and Claudio Comoglio^a
14

15
16 6 *^aDepartment of Environment, Land and Infrastructure Engineering, Politecnico di*
17 7 *Torino, Italy; ^bDepartment of Ecohydrology and Biogeochemistry, Leibniz Institute of*
18 8 *Freshwater Ecology and Inland Fisheries, Berlin, Germany; ^cDepartment of Biology,*
19 9 *Chemistry and Pharmacy, Free University of Berlin, Germany; ^dIttiologo libero*
20 10 *professionista, Predosa, Italy*
21
22
23
24

25
26
27
28 12 * Daniel Nyqvist, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino
29
30 13 daniel.nyqvist@polito.it
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

15 **Survival and Swimming Performance in Small-sized South European** 16 **Cypriniformes tagged with Passive Integrated Transponders**

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

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

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.

1
2
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
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
7
8 106 riffle dace.

10
11 107 An open field test, on the other hand, consists of letting an animal freely explore
12 108 an experimental arena, and consequently, when it comes to fish, measure a type of
13 109 voluntary swimming behavior, typically within the sustained mode. Open field tests are
14 110 commonly used in behavioral ecology to test for individual animal's willingness to
15 111 explore an area, often as a proxy for activity, boldness or exploratory behavior
16 112 (Mittelbach *et al.*, 2014; Perals *et al.*, 2017), and in the field of ecotoxicology to test for
17 113 chemically induced changes in animal behavior (Echevarria *et al.*, 2008; Gould *et al.*,
18 114 2009; Hong & Zha, 2019). Interestingly, and highlighting the relevance of the tests,
19 115 volitional swimming in open field tests has been seen to correlate with activity (Závorka
20 116 *et al.*, 2016) and movement (Fraser *et al.*, 2001; Watz, 2019) in nature, as well as
21 117 downstream by-pass passage at a hydropower dam (Haraldstad *et al.*, 2021). Relating to
22 118 tag effects, open field tests have been used to study effects of PIT-tags on Italian spined
23 119 loach, not finding tagging effects on swimming behavior (Nyqvist *et al.*, 2022).

33 120 There is a general lack of information on the ecology and habitat preferences for
34 121 many fish species, in particular for small-sized fish with little economical interest
35 122 (Negro *et al.*, 2021; Smialek *et al.*, 2019). South European nase (*Protochondrostoma*
36 123 *genei*) and brook barbel (*Barbus caninus*) are two small-sized cyprinids, native to
37 124 streams on the Italian peninsula. (Fortini, 2016) Both species are under conservation
38 125 concern while confronted with a high number of in-stream barriers as well as
39 126 anthropogenically induced habitat change such as increased temperatures and water
40 127 scarcity (Belletti *et al.*, 2020; Carosi *et al.*, 2019; Rondinini *et al.*, 2022; Skoulikidis *et*
41 128 *al.*, 2017). The need to increase the knowledge of the ecology and behavior of these,
42 129 and other small-sized previously neglected species, is pressing (Vollestad 2023). PIT-
43 130 telemetry offers a valuable research tool, but the need to evaluate for potential tagging
44 131 effects beyond mere survival remains.

54 132 Here, we study PIT-tagging effects on survival and a range of swimming
55 133 behaviors – encompassing sustained, prolonged and burst swimming - in South
56 134 European nase and brook barbel. Effects on volitional swimming activity (sustained
57 135 swimming) and maximum swimming speed (escape response; burst swimming) were

1
2
3 136 tested in arena trials. Tagging effects on the prolonged swimming performance was
4
5 137 tested in South European nase in an increasing velocity test, while a barrier passage test
6
7 138 was designed to further investigate tagging effects in brook barbel.
8
9

10 139 **Material and methods**

11
12 140 South European nase and brook barbel were caught in Lemme River, Italy using wading
13
14 141 electrofishing (direct current; ELT60IIGI, Scubla, Italy) on 15 November (UTM
15
16 142 484564E, 4947986N, zone 32T) and 16 November 2021 (UTM 487799E 4941784N,
17
18 143 zone 32T), respectively, and brought to Predosa Hatchery (Predosa, Alessandria, Italy).
19
20 144 Fish were kept in spring-fed flow-through tanks for two to four days before tagging. All
21
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
25
26 147 (56 tagged and 56 control) were included in the study. South European nase (60 tagged
27
28 148 and 60 control) had a median length of 9.9 cm (range 6.2 – 13.7 cm, interquartile range =
29
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
33
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;
37
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,
45
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
55
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
59
60 164 smaller tanks with higher water exchange rates for brook barbel (length*width*depth =
165
166 150 cm * 45 cm * 20 cm). Temperatures were kept stable around 13°C and the light
167
shelters comprised of perforated bricks. Fish were fed daily. Brook barbel were fed with

1
2
3 168 Sera Koi Royal pellets® while South European nase were fed Tetra TabiMin sinking
4
5 169 pellets containing a higher proportion of vegetarian content. For both species, the
6
7 170 commercial pellets were supplemented with wild-caught macrozoobenthos. The tanks
8
9 171 were inspected for mortalities daily, and missing tags were checked at the end of the
10
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
16
17 175 of plexiglass (Fig. 1). South European nase were tested in a traditional time to fatigue
18
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
36
37 185 TK-2000 chiller).

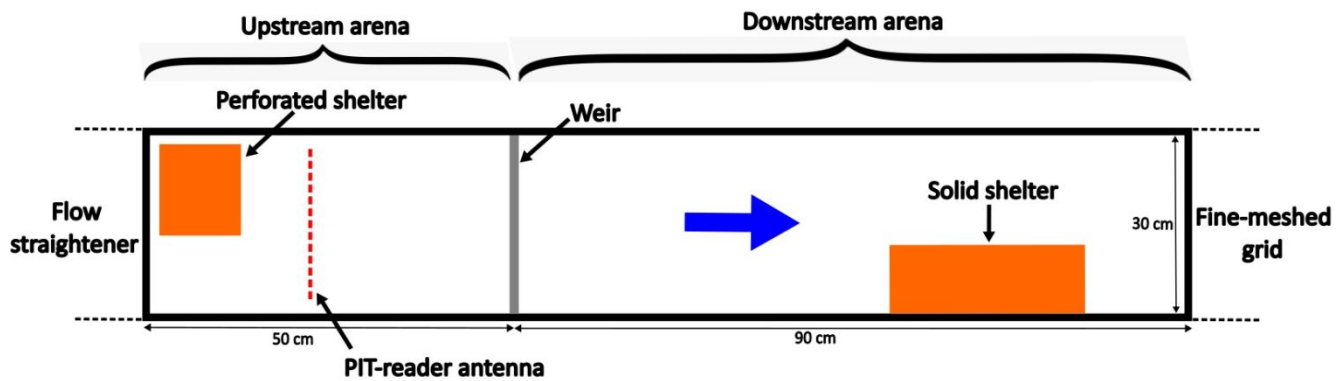
37 186 ***Time-to-fatigue experiment***

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,
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
60
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

1
2
3 200 swimming. A fish was defined as fatigued after resting on the grid despite poking, and
4
5 201 time to fatigue as the time from the start of testing velocity to the fish refusing to swim.
6
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*

12
13 205 The barrier experiment was conducted on 15-16 December, 27-28 days after tagging. A
14
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
48
49 223 experimental fish were returned to the original holding tanks.
50
51
52
53
54
55
56
57
58
59
60



224

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
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>).

1
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).
10
11
12

13 287 **Results**
14

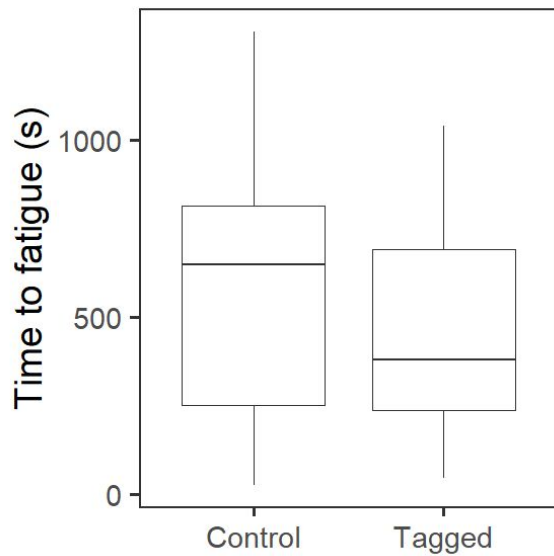
15 288 There was no difference in length or weight between tagged and control fish within any
16 289 of species (Mann-Whitney, $p > 0.1$).
17
18
19

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.
28
29
30
31
32
33

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, $p = 0.49$).
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

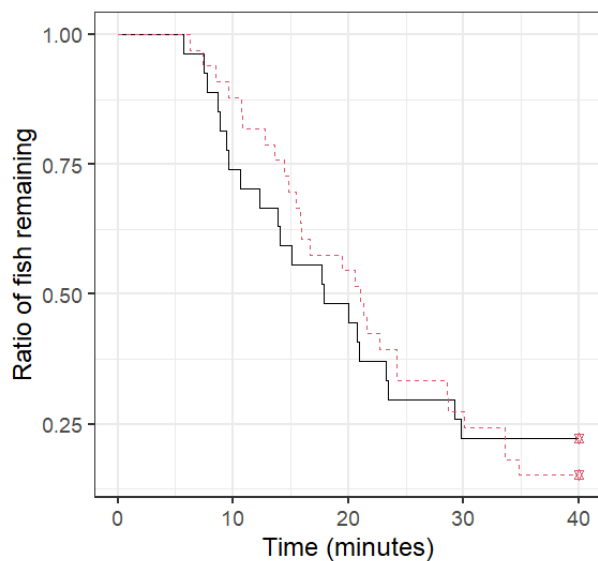


303

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.



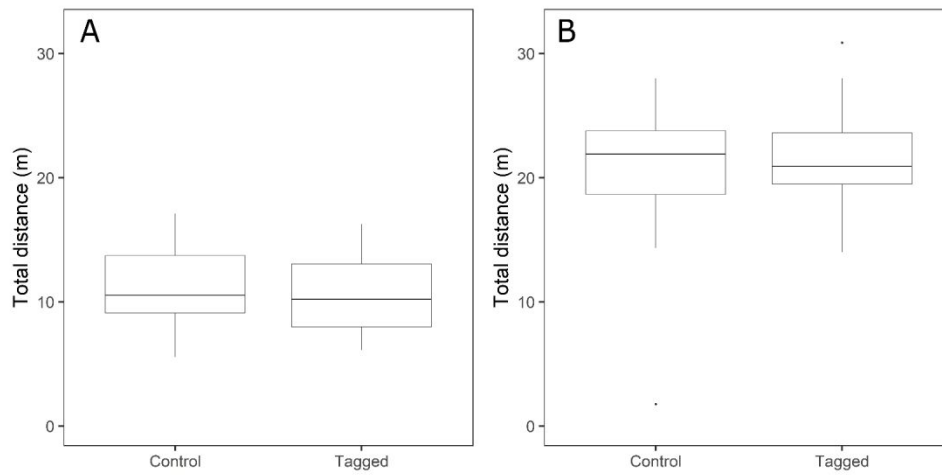
317

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, $p = 1$).

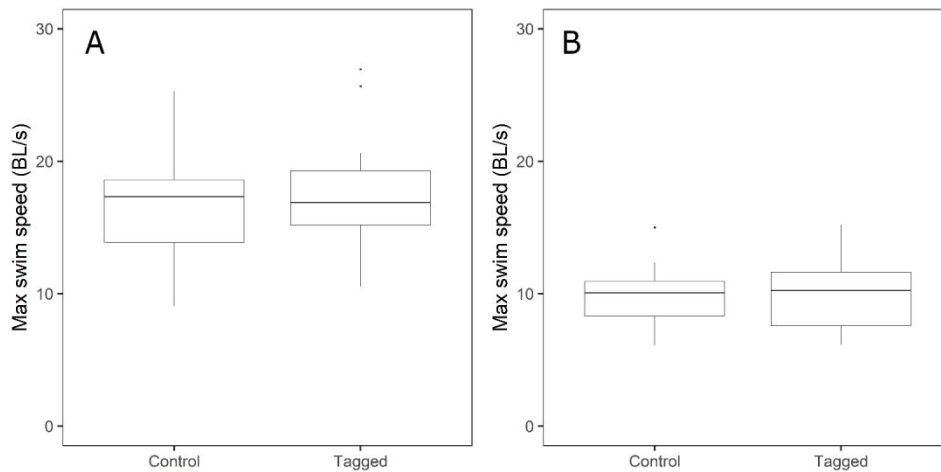


335

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

1
2
3 373 design (Castro-Santos *et al.*, 2022; Katopodis & Gervais, 2012; Tudorache *et al.*, 2008).
4
5 374 Previous studies have found no effects of PIT-tags on sustained and prolonged
6
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.
12
13 378 No effects of PIT-tagging were found on sustained swimming activity in the open field
14
15 379 test, burst swimming in the provoked escape response, or prolonged swimming in the
16
17 380 increased velocity test (nase) and barrier passage test (barbel). Sample sizes were
18
19 381 relatively modest, so the result should be taken with some caution. The distribution of
20
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
24
25 384 perhaps for nase in the increasing velocity test (similar to Italian ruffe; Schiavon
26
27 385 2023). Overall this constitutes encouraging results for PIT-tagging small sized
28
29 386 Cypriniformes fish.

30
31
32
33 387 The range of swimming behaviors investigated covers a wide range of behaviors
34
35 388 relevant to survival and movement of fish in their natural environment (Castro-Santos *et*
36
37 389 *al.*, 2022; Videler, 1993), but future studies may go a step further to investigate
38
39 390 potential tagging effects on the behavior in the wild. Studies on salmonids, pikes, and
40
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
46
47 394 studies, to some extent, summarize the consequences of behavior for wild fish, specific
48
49 395 studies on tagging effects on behavior in nature are scarce in the literature. For acoustic
50
51 396 tags, the behavior of fish tagged in previous years has been compared to recently tagged
52
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
60
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

1
2
3 406 windows using tracking technology within a flume (Schiavon *et al.*, 2023). The lower
4
5 407 swimming performance in barbel, might be due to them relying more on camouflage
6
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
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
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
21
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
33
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,
38
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
42
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
46
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-
54
55 433 Santos *et al.*, 1996; Keeler *et al.*, 2007; Watz *et al.*, 2019b) in these and similar species.

56 434

57 435 **Conclusions**

58 436 In agreement with many other studies on PIT-tagged fish, our results demonstrate high
59
60 437 survival and tag retention. In addition, we investigated potential tagging effects on a

1
2
3 438 range of fish swimming behaviors, relevant to survival and movement of fish in their
4
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.

10 442 **Acknowledgements**

11
12 443 This research work has been funded by the European Union Horizon 2020 Research and
13
14 444 Innovation Programme under the Marie Skłodowska-Curie Actions, Grant Agreement
15
16 445 No. 86080 and part of the work performed within LIFE21-NAT-IT-LIFE Minnow,
17
18 446 101074559 “Small fish, small streams, big challenges: conservation of endangered
19
20 447 species in tributaries of the upper Po River”. We acknowledge Florian Eggers, Gloria
21
22 448 Mozzi, Usama Ashraf, Costantino Manes, and Armando Piccinini for technical
23
24 449 assistance.

25 26 450 **References**

- 27
28 451 Amaral, S. D., Branco, P., da Silva, A. T., Katopodis, C., Viseu, T., Ferreira, M. T., ...
29 452 Santos, J. M. (2016). Upstream passage of potamodromous cyprinids over small
30 453 weirs: the influence of key-hydraulic parameters. *Journal of Ecohydraulics*, *1*,
31 454 79–89.
- 32 455 Baki, A. B. M., Zhu, D. Z., Harwood, A., Lewis, A., & Healey, K. (2020). Hydraulic
33 456 design aspects of rock-weir fishways with notch for habitat connectivity.
34 457 *Journal of Ecohydraulics*, *5*, 94–109.
- 35 458 Baras, E., Westerloppe, L., Mélard, C., Philippart, J.-C., & Bénech, V. (1999).
36 459 Evaluation of implantation procedures for PIT-tagging juvenile Nile tilapia.
37 460 *North American Journal of Aquaculture*, *61*, 246–251.
- 38 461 Bartoň, D., Brabec, M., Sajdlová, Z., Souza, A. T., Duras, J., Kortan, D., ... Šmejkal,
39 462 M. (2022). Hydropeaking causes spatial shifts in a reproducing rheophilic fish.
40 463 *Science of The Total Environment*, *806*, 150649.
- 41 464 Belletti, B., de Leaniz, C. G., Jones, J., Bizzi, S., Börger, L., Segura, G., ... Barry, J.
42 465 (2020). More than one million barriers fragment Europe’s rivers. *Nature*, *588*,
43 466 436–441.
- 44 467 Bolland, J. D., Cowx, I. G., & Lucas, M. C. (2009). Evaluation of VIE and PIT tagging
45 468 methods for juvenile cyprinid fishes. *Journal of Applied Ichthyology*, *25*, 381–
46 469 386.
- 47 470 Breen, M. J., Ruetz, C. R., Thompson, K. J., & Kohler, S. L. (2009). Movements of
48 471 mottled sculpins (*Cottus bairdii*) in a Michigan stream: how restricted are they?
49 472 *Canadian Journal of Fisheries and Aquatic Sciences*, *66*, 31–41.
- 50 473 Brönmark, C., Skov, C., Brodersen, J., Nilsson, P. A., & Hansson, L.-A. (2008).
51 474 Seasonal migration determined by a trade-off between predator avoidance and
52 475 growth. *PloS one*, *3*, e1957.
- 53 476 Brown, R. S., Eppard, M. B., Murchie, K. J., Nielsen, J. L., & Cooke, S. J. (2011). An
54 477 introduction to the practical and ethical perspectives on the need to advance and
55
56
57
58
59
60

- 1
2
3 478 standardize the intracoelomic surgical implantation of electronic tags in fish.
4 479 *Reviews in Fish Biology and Fisheries*, 21, 1–9.
- 5 480 Brown, R. S., Cooke, S. J., Anderson, W. G., & McKinley, R. S. (1999). Evidence to
6 481 challenge the “2% rule” for biotelemetry. *North American Journal of Fisheries*
7 482 *Management*, 19, 867–871.
- 8 483 Carosi, A., Padula, R., Ghetti, L., & Lorenzoni, M. (2019). Endemic freshwater fish
9 484 range shifts related to global climate changes: A long-term study provides some
10 485 observational evidence for the Mediterranean area. *Water*, 11, 2349.
- 11 486 Castro-Santos, T., Goerig, E., He, P., & Lauder, G. V. (2022). Applied aspects of
12 487 locomotion and biomechanics. *Fish Physiol. A*, 39, 91–140.
- 13 488 Castro-Santos, T., & Perry, R. (2012). Time-to-event analysis as a framework for
14 489 quantifying fish passage performance. *Telemetry techniques: a user guide for*
15 490 *fisheries research*. America Fisheries Society, Bethesda, Maryland, 427–452.
- 16 491 Castro-Santos, T., Haro, A., & Walk, S. (1996). A passive integrated transponder (PIT)
17 492 tag system for monitoring fishways. *Fisheries research*, 28, 253–261.
- 18 493 Clark, S. R. (2016). Effects of Passive Integrated Transponder Tags on the Physiology
19 494 and Swimming Performance of a Small-Bodied Stream Fish. *Transactions of the*
20 495 *American Fisheries Society*, 145, 1179–1192.
- 21 496 Connors, K., Scruton, D., Brown, J., & McKinley, R. (2002). The effects of surgically-
22 497 implanted dummy radio transmitters on the behaviour of wild Atlantic salmon
23 498 smolts. *Aquatic Telemetry* (pp. 231–237). Springer.
- 24 499 Cooke, S. J., Hinch, S. G., Wikelski, M., Andrews, R. D., Kuchel, L. J., Wolcott, T. G.,
25 500 & Butler, P. J. (2004). Biotelemetry: a mechanistic approach to ecology. *Trends*
26 501 *in ecology & evolution*, 19, 334–343.
- 27 502 Crossin, G. T., Heupel, M. R., Holbrook, C. M., Hussey, N. E., Lowerre-Barbieri, S. K.,
28 503 Nguyen, V. M., ... Cooke, S. J. (2017). Acoustic telemetry and fisheries
29 504 management. *Ecological Applications*, 27, 1031–1049.
- 30 505 Dieterman, D. J., & Hoxmeier, R. J. H. (2009). Instream evaluation of passive
31 506 integrated transponder retention in brook trout and brown trout: effects of
32 507 season, anatomical placement, and fish length. *North American Journal of*
33 508 *Fisheries Management*, 29, 109–115.
- 34 509 Domenici, P. (2010). Escape responses in fish: kinematics, performance and behavior.
35 510 *Fish locomotion: An eco-ethological perspective*, 123–170.
- 36 511 Domenici, P., & Blake, R. (1997). The kinematics and performance of fish fast-start
37 512 swimming. *Journal of Experimental Biology*, 200, 1165–1178.
- 38 513 Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J.,
39 514 Lévêque, C., ... Stiassny, M. L. (2006). Freshwater biodiversity: importance,
40 515 threats, status and conservation challenges. *Biological reviews*, 81, 163–182.
- 41 516 Echevarria, D. J., Hammack, C. M., Pratt, D. W., & Hosemann, J. D. (2008). A novel
42 517 behavioral test battery to assess global drug effects using the zebrafish.
43 518 *International Journal of Comparative Psychology*, 21.
- 44 519 Eilam, D. (2005). Die hard: A blend of freezing and fleeing as a dynamic defense—
45 520 implications for the control of defensive behavior. *Neuroscience &*
46 521 *Biobehavioral Reviews*, 29, 1181–1191.
- 47 522 Ficke, A. D., Myrick, C. A., & Kondratieff, M. C. (2012). The effects of PIT tagging on
48 523 the swimming performance and survival of three nonsalmonid freshwater fishes.
49 524 *Ecological Engineering*, 48, 86–91.
- 50 525 Fortini, N. (2016). Nuovo atlante dei pesci delle acque interne italiane: guida completa
51 526 ai pesci, ciclostomi e crostacei decapodi di acque dolci e salmastre. Aracne.

- 1
2
3 527 Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N., & Skalski, G. T. (2001). Explaining
4 528 leptokurtic movement distributions: intrapopulation variation in boldness and
5 529 exploration. *The American Naturalist*, *158*, 124–135.
- 6 530 Gibbons, W. J., & Andrews, K. M. (2004). PIT tagging: simple technology at its best.
7 531 *Bioscience*, *54*, 447–454.
- 8 532 Gould, T. D., Dao, D. T., & Kovacsics, C. E. (2009). The Open Field Test. In T. D.
9 533 Gould (Ed.), *Mood and Anxiety Related Phenotypes in Mice* (pp. 1–20). Totowa,
10 534 NJ: Humana Press/Neuromethods.
- 11 535 Gries, G., & Letcher, B. (2002). Tag retention and survival of age-0 Atlantic salmon
12 536 following surgical implantation with passive integrated transponder tags. *North*
13 537 *American Journal of Fisheries Management*, *22*, 219–222.
- 14 538 Haraldstad, T., Haugen, T. O., Olsen, E. M., Forseth, T., & Höglund, E. (2021).
15 539 Hydropower-induced selection of behavioural traits in Atlantic salmon (*Salmo*
16 540 *salar*). *Scientific Reports*, *11*, 16444.
- 17 541 Harbicht, A. B., Watz, J., Nyqvist, D., Virmajä, T., Carlsson, N., Aldvén, D., ... Calles,
18 542 O. (2022). Guiding migrating salmonid smolts: Experimentally assessing the
19 543 performance of angled and inclined screens with varying gap widths. *Ecological*
20 544 *Engineering*, *174*, 106438.
- 21 545 Haro, A., Castro-Santos, T., Noreika, J., & Odeh, M. (2004). Swimming performance of
22 546 upstream migrant fishes in open-channel flow: a new approach to predicting
23 547 passage through velocity barriers. *Canadian Journal of Fisheries and Aquatic*
24 548 *Sciences*, *61*, 1590–1601.
- 25 549 Haugen, T. O., Aass, P., Stenseth, N. C., & Vøllestad, L. A. (2008). Changes in
26 550 selection and evolutionary responses in migratory brown trout following the
27 551 construction of a fish ladder. *Evolutionary Applications*, *1*, 319–335.
- 28 552 Hong, X., & Zha, J. (2019). Fish behavior: A promising model for aquatic toxicology
29 553 research. *Science of The Total Environment*, *686*, 311–321.
- 30 554 Hosmer, D. W., Lemeshow, S., & May, S. (2008). *Applied Survival Analysis:*
31 555 *Regression Modeling of Time-to-Event Data, Second Edition*. John Wiley &
32 556 Sons, Inc., Hoboken, New Jersey, USA.
- 33 557 Hühn, D., Klefoth, T., Pagel, T., Zajicek, P., & Arlinghaus, R. (2014). Impacts of
34 558 external and surgery-based tagging techniques on small northern pike under
35 559 field conditions. *North American journal of fisheries management*, *34*, 322–334.
- 36 560 Jepsen, N., Thorstad, E. B., Havn, T., & Lucas, M. C. (2015). The use of external
37 561 electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal*
38 562 *Biotelemetry*, *3*, 49.
- 39 563 Jepsen, N., Schreck, C., Clements, S., & Thorstad, E. B. (2005). A brief discussion on
40 564 the 2% tag/body mass rule of thumb. *Aquatic telemetry: advances and*
41 565 *applications*, 255–259.
- 42 566 Jones, P. E., Champneys, T., Vevers, J., Börger, L., Svendsen, J. C., Consuegra, S., ...
43 567 Garcia de Leaniz, C. (2021). Selective effects of small barriers on river-resident
44 568 fish. *Journal of Applied Ecology*, *58*, 1487–1498.
- 45 569 Katopodis, C., & Gervais, R. (2012). ECOHYDRAULIC ANALYSIS OF FISH
46 570 FATIGUE DATA: ECOHYDRAULIC ANALYSIS OF FISH FATIGUE
47 571 DATA. *River Research and Applications*, *28*, 444–456.
- 48 572 Keeler, R. A., Breton, A., Peterson, D. P., & Cunjak, R. A. (2007). Apparent survival
49 573 and detection estimates for PIT-tagged slimy sculpin in five small New
50 574 Brunswick streams. *Transactions of the American Fisheries Society*, *136*, 281–
51 575 292.

- 1
2
3 576 Knaepkens, G., Maerten, E., Tudorache, C., De Boeck, G., & Eens, M. (2007).
4 577 Evaluation of passive integrated transponder tags for marking the bullhead
5 578 (Cottus gobio), a small benthic freshwater fish: effects on survival, growth and
6 579 swimming capacity. *Ecology of Freshwater fish*, 16, 404–409.
- 8 580 Miklósi, A., Topal, J., & Csányi, V. (1992). Development of open-field and social
9 581 behavior of the paradise fish (*Macropodus opercularis* L.). *Developmental*
10 582 *Psychobiology: The Journal of the International Society for Developmental*
11 583 *Psychobiology*, 25, 335–344.
- 12 584 Mittelbach, G. G., Ballew, N. G., Kjølvik, M. K., & Fraser, D. (2014). Fish behavioral
13 585 types and their ecological consequences. *Canadian Journal of Fisheries and*
14 586 *Aquatic Sciences*, 71, 927–944.
- 16 587 Moser, M. L., Corbett, S. C., Keefer, M. L., Frick, K. E., Lopez-Johnston, S., & Caudill,
17 588 C. C. (2019). Novel fishway entrance modifications for Pacific lamprey. *Journal*
18 589 *of Ecohydraulics*, 4, 71–84.
- 19 590 Mueller, R. P., Moursund, R. A., & Bleich, M. D. (2006). Tagging juvenile Pacific
20 591 lamprey with passive integrated transponders: methodology, short-term
21 592 mortality, and influence on swimming performance. *North American Journal of*
22 593 *Fisheries Management*, 26, 361–366.
- 24 594 Mulligan, K. B., Haro, A., & Noreika, J. (2021). Effect of backwatering a streamgage
25 595 weir on the passage performance of adult American Shad (*Alosa sapidissima*).
26 596 *Journal of Ecohydraulics*, 0, 1–13.
- 27 597 Nagel, C., Droll, J., Kroemer, K., Pander, J., & Geist, J. (2023). Testing the effects of
28 598 passive integrated transponder (PIT) tags on survival, growth, and tag retention
29 599 of common nase (*Chondrostoma nasus* L.) and European barbel (*Barbus barbus*
30 600 L.). *Animal Biotelemetry*, 11, 33.
- 32 601 Newby, N. C., Binder, T. R., & Stevens, E. D. (2007). Passive Integrated Transponder
33 602 (PIT) Tagging Did Not Negatively Affect the Short-Term Feeding Behavior or
34 603 Swimming Performance of Juvenile Rainbow Trout. *Transactions of the*
35 604 *American Fisheries Society*, 136, 341–345.
- 36 605 Nyqvist, D., Schiavon, A., Candiotti, A., Mozzi, G., Eggers, F., & Comoglio, C.
37 606 (2022). PIT -tagging Italian spined loach (*Cobitis bilineata*) – methodology,
38 607 survival, and behavioral effects. *Journal of Fish Biology*, jfb.15289.
- 40 608 Nyqvist, D., Hedenberg, F., Calles, O., Österling, M., von Proschwitz, T., & Watz, J.
41 609 (2020). Tracking the movement of PIT-tagged terrestrial slugs (*Arion vulgaris*)
42 610 in forest and garden habitats using mobile antennas. *Journal of Molluscan*
43 611 *Studies*.
- 44 612 Ombredane, D., Bagliniere, J. L., & Marchand, F. (1998). The effects of Passive
45 613 Integrated Transponder tags on survival and growth of juvenile brown trout
46 614 (*Salmo trutta* L.) and their use for studying movement in a small river.
47 615 *Hydrobiologia*, 371, 99–106.
- 49 616 Ovidio, M., Dierckx, A., & Benitez, J.-P. (2023). MOVEMENT BEHAVIOUR AND
50 617 FISHWAY PERFORMANCE FOR ENDEMIC AND EXOTIC SPECIES IN A
51 618 LARGE ANTHROPIZED RIVER. *Limnologica*, 126061.
- 53 619 Perals, D., Griffin, A. S., Bartomeus, I., & Sol, D. (2017). Revisiting the open-field test:
54 620 what does it really tell us about animal personality? *Animal Behaviour*, 123, 69–
55 621 79.
- 56 622 Quintella, B. R., Andrade, N. O., Espanhol, R., & Almeida, P. R. (2005). The use of PIT
57 623 telemetry to study movements of ammocoetes and metamorphosing sea
58 624 lampreys in river beds. *Journal of Fish Biology*, 66, 97–106.

- 1
2
3 625 Rondinini, C., Battistoni, A., & Teofili, C. (2022). Lista Rossa IUCN dei Vertebrati
4 626 Italiani 2022. *Comitato Italiano IUCN e Ministero dell’Ambiente e della Tutela*
5 627 *del territorio e del mare: Roma, Italy.*
- 6 628 Schiavon, A., Comoglio, C., Candioto, A., Hölker, F., Ashraf, M. U., & Nyqvist, D.
7 629 (2023). Survival and swimming performance of a small-sized Cypriniformes
8 630 (*Telestes muticellus*) tagged with passive integrated transponders.
9 631 *Journal of Limnology*, 82.
- 10 632 Schwinn, M., Baktoft, H., Aarestrup, K., & Koed, A. (2017). A comparison of the
11 633 survival and migration of wild and F1-hatchery-reared brown trout (*Salmo*
12 634 *trutta*) smolts traversing an artificial lake. *Fisheries Research*, 196, 47–55.
- 13 635 Skoulidakis, N. T., Sabater, S., Datry, T., Morais, M. M., Buffagni, A., Dörflinger, G.,
14 636 ... Tockner, K. (2017). Non-perennial Mediterranean rivers in Europe: Status,
15 637 pressures, and challenges for research and management. *Science of The Total*
16 638 *Environment*, 577, 1–18.
- 17 639 Skov, C., Hansen, J. H., Baktoft, H., Brönmark, C., Brodersen, J., Chapman, B. B., ...
18 640 Nilsson, P. A. (2020). A field evaluation of long-term effects of PIT tagging.
19 641 *Journal of Fish Biology*, 96, 1055–1059.
- 20 642 Šmejkal, M., Blabolil, P., Bartoň, D., Duras, J., Vejřík, L., Sajdlova, Z., ... Kubečka, J.
21 643 (2019). Sex-specific probability of PIT tag retention in a cyprinid fish. *Fisheries*
22 644 *Research*, 219, 105325.
- 23 645 Smialek, N., Pander, J., Mueller, M., van Treeck, R., Wolter, C., & Geist, J. (2019). Do
24 646 We Know Enough to Save European Riverine Fish?—A Systematic Review on
25 647 Autecological Requirements During Critical Life Stages of 10 Rheophilic
26 648 Species at Risk. *Sustainability*, 11, 5011.
- 27 649 Sullivan, K. M., Bailey, M. M., & Berlinsky, D. L. (2023). Passage Efficiency of
28 650 Alewife in a Denil Fishway Using Passive Integrated Transponder Tags. *North*
29 651 *American Journal of Fisheries Management*, 43, 772–785.
- 30 652 Thorstad, E. B., Rikardsen, A. H., Alp, A., & Okland, F. (2013). The use of electronic
31 653 tags in fish research: an overview of fish telemetry methods. *Turkish Journal of*
32 654 *Fisheries and Aquatic Sciences*, 13, 881–896.
- 33 655 Tudorache, C., De Boeck, G., & Claireaux, G. (2013). Forced and preferred swimming
34 656 speeds of fish: a methodological approach. *Swimming physiology of fish* (pp. 81–
35 657 108). Springer.
- 36 658 Tudorache, C., Viaene, P., Blust, R., Vereecken, H., & De Boeck, G. (2008). A
37 659 comparison of swimming capacity and energy use in seven European freshwater
38 660 fish species. *Ecology of freshwater fish*, 17, 284–291.
- 39 661 Videler, J. J. (1993). *Fish swimming*. Springer Science & Business Media. Vol. 10.
- 40 662 Vøllestad, L. A. (2023). A paradoxical bias in knowledge about Norwegian freshwater
41 663 fishes: research efforts during 1980-2020. *Fauna norvegica*, 42, 6–30.
- 42 664 Vollset, K. W., Lennox, R. J., Thorstad, E. B., Auer, S., Bär, K., Larsen, M. H., ...
43 665 Dohoo, I. (2020). Systematic review and meta-analysis of PIT tagging effects on
44 666 mortality and growth of juvenile salmonids. *Reviews in Fish Biology and*
45 667 *Fisheries*, 30, 553–568.
- 46 668 Volpato, G. L., Barreto, R. E., Marcondes, A. L., Andrade Moreira, P. S., & de Barros
47 669 Ferreira, M. F. (2009). Fish ladders select fish traits on migration—still a growing
48 670 problem for natural fish populations. *Marine and Freshwater Behaviour and*
49 671 *Physiology*, 42, 307–313.
- 50 672 Wargo Rub, A. M., Sandford, B. P., Butzerin, J. M., & Cameron, A. S. (2020). Pushing
51 673 the envelope: Micro-transmitter effects on small juvenile Chinook salmon
52 674 (*Oncorhynchus tshawytscha*). *PloS one*, 15, e0230100.

- 1
2
3 675 Watson, J. R., Goodrich, H. R., Cramp, R. L., Gordos, M. A., & Franklin, C. E. (2019).
4 676 Assessment of the effects of microPIT tags on the swimming performance of
5 677 small-bodied and juvenile fish. *Fisheries Research*, 218, 22–28.
6 678 Watz, J. (2019). Structural complexity in the hatchery rearing environment affects
7 679 activity, resting metabolic rate and post-release behaviour in brown trout *Salmo*
8 680 *trutta*. *Journal of Fish Biology*, 95, 638–641.
9 681 Watz, J., Bergman, E., Piccolo, J. J., & Greenberg, L. (2016). Ice cover affects the
10 682 growth of a stream-dwelling fish. *Oecologia*, 1–13.
11 683 Watz, J., Otsuki, Y., Nagatsuka, K., Hasegawa, K., & Koizumi, I. (2019a).
12 684 Temperature-dependent competition between juvenile salmonids in small
13 685 streams. *Freshwater Biology*, 64, 1534–1541.
14 686 Watz, J., Calles, O., Carlsson, N., Collin, T., Huusko, A., Johnsson, J., ... Nyqvist, D.
15 687 (2019b). Wood addition in the hatchery and river environments affects
16 688 post-release performance of overwintering brown trout. *Freshwater biology*.
17 689 Wilson, A. D., Hayden, T. A., Vandergoot, C. S., Kraus, R. T., Dettmers, J. M., Cooke,
18 690 S. J., & Krueger, C. C. (2017). Do intracoelomic telemetry transmitters alter the
19 691 post-release behaviour of migratory fish? *Ecology of Freshwater Fish*, 26, 292–
20 692 300.
21 693 Winter, J. (1983). Underwater biotelemetry. *Fisheries techniques. American Fisheries*
22 694 *Society, Bethesda, Maryland*, 371–395.
23 695 Zakeś, Z., Demska-Zakeś, K., Rożyński, M., Gomułka, P., & Rożyński, R. (2022).
24 696 Influence of intraperitoneal implantation of 12 mm PIT on the welfare of
25 697 juvenile brown trout (*Salmo trutta*). *Fisheries Research*, 255, 106458.
26 698 Závorka, L., Aldvén, D., Näslund, J., Höjesjö, J., & Johnsson, J. I. (2016). Inactive trout
27 699 come out at night: behavioral variation, circadian activity, and fitness in the
28 700 wild. *Ecology*, 97, 2223–2231.
29 701
30
31
32
33
34
35 702
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Survival and Swimming Performance in Small-sized South European**
4 **Cypriniformes tagged with Passive Integrated Transponders**
5
6
7
8
9

10 Daniel Nyqvist^{a*}, Alfredo Schiavon^{b,c}, Alessandro Candiotta^d, Fabio
11 Tarena^a, and Claudio Comoglio^a
12
13
14

15 *^aDepartment of Environment, Land and Infrastructure Engineering, Politecnico di*
16 *Torino, Italy; ^bDepartment of Ecohydrology and Biogeochemistry, Leibniz Institute of*
17 *Freshwater Ecology and Inland Fisheries, Berlin, Germany; ^cDepartment of Biology,*
18 *Chemistry and Pharmacy, Free University of Berlin, Germany; ^dIttiologo libero*
19 *professionista, Predosa, Italy*
20
21
22
23
24
25
26
27

28 * Daniel Nyqvist, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino
29 daniel.nyqvist@polito.it
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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~~

1
2
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.
7
8
9

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).
19
20
21
22
23
24
25

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.
43
44
45
46
47
48
49
50
51
52
53
54
55

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
59
60

1
2
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
11 riffle dace.
12
13
14
15
16
17
18

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).
32
33
34
35
36
37
38
39
40
41

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-
52
53
54
55
56
57
58
59
60

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

1
2
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*

27
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*

50
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
56
57
58
59
60

1
2
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.
15
16
17
18
19
20
21
22
23
24

25 *Barrier passage experiment*

26
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.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

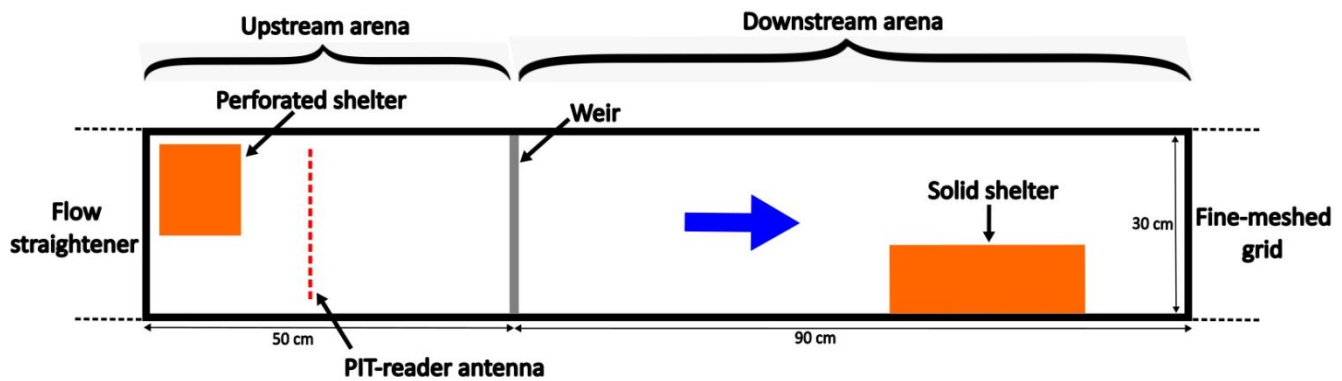


Figure 1. A scaled drawing of the experimental arena for the barrier passage experiment. For the Time-to-fatigue experiment the flow straightener delimited the experimental in an upstream direction while the fine-meshed grid did so in a downstream direction, with an arena length of 60 cm.

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

1
2
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).
5
6

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).
23
24
25
26
27
28
29
30
31
32
33
34
35

36 *Statistics*

37
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.
41
42 Difference in survival between tagged and control fish was tested using chi2-tests.
43
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>).
53
54
55
56
57
58
59
60

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, $p = 0.49$).

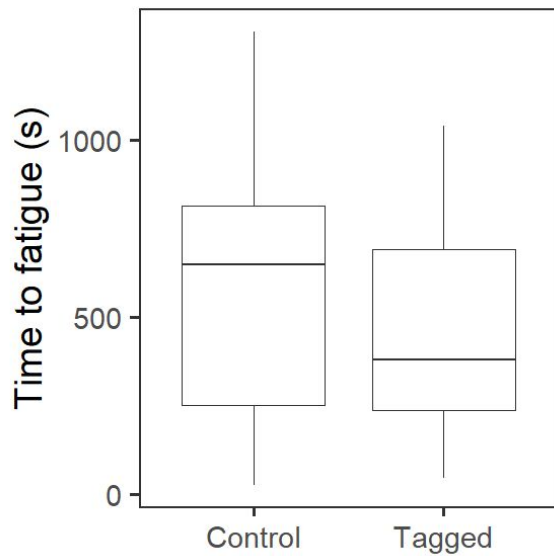


Figure 2. Time-to-fatigue for control (n =25) and tagged (n =16) South European nase. First ~~600s~~600 s in 45 cm/s, followed by 52 cm/s until fatigue. The horizontal line represents the median value, the box the interquartile range, the whiskers the range of data.

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.

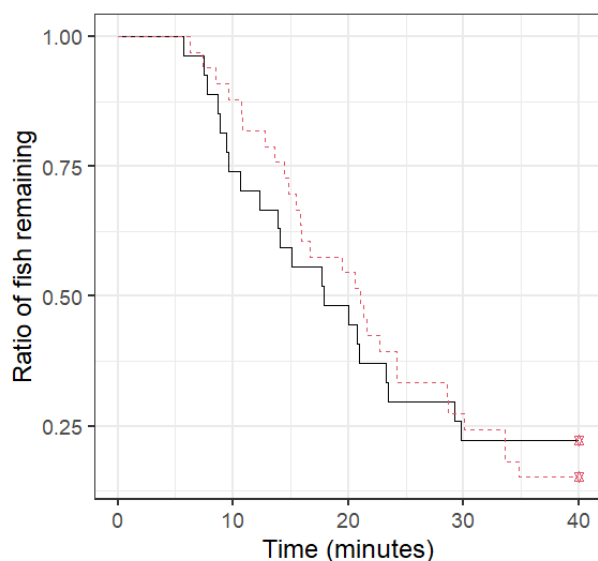


Figure 3. Kaplan-Meier curve representing the ratio of control fish (solid line; $n = 27$) and tagged fish (dashed line; $n = 33$) remaining downstream the barrier over time.

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, $p = 1$).

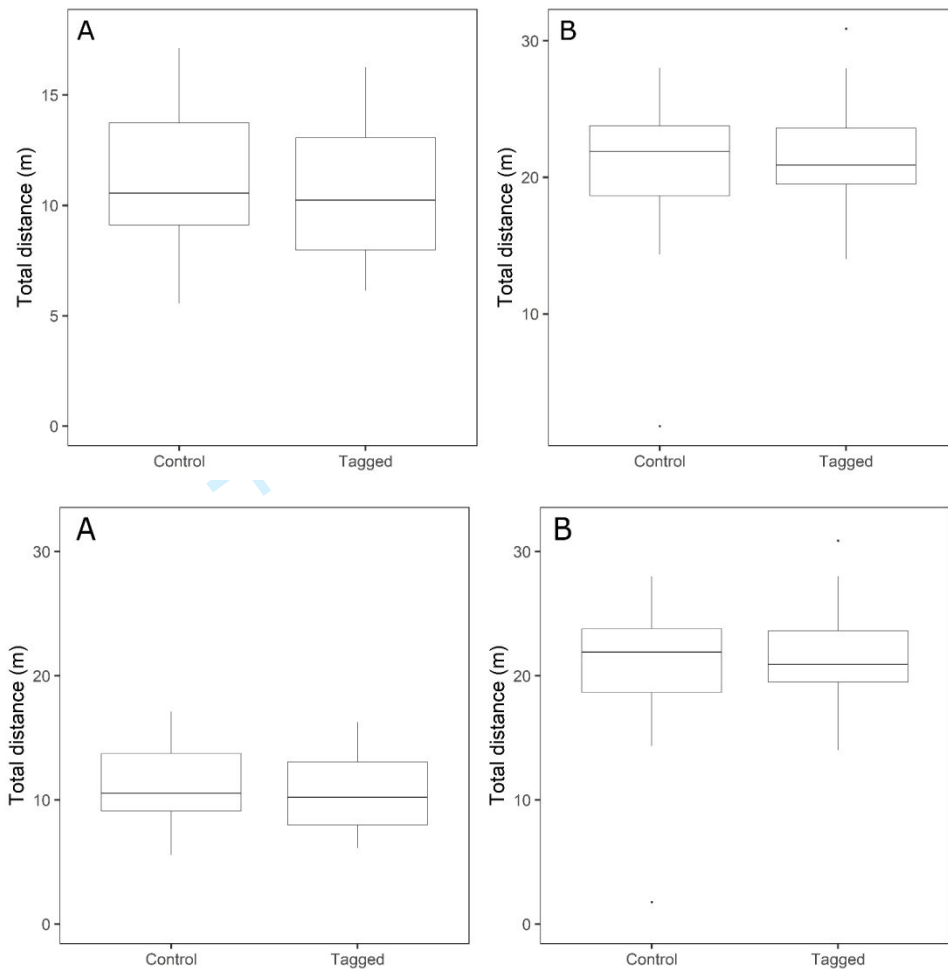


Figure 34. Total distance moved for control and tagged fish during the open field test for A) South European nase (204 s, n = 34) and B) brook barbel (230 s, n =54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are outliers.

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](#))

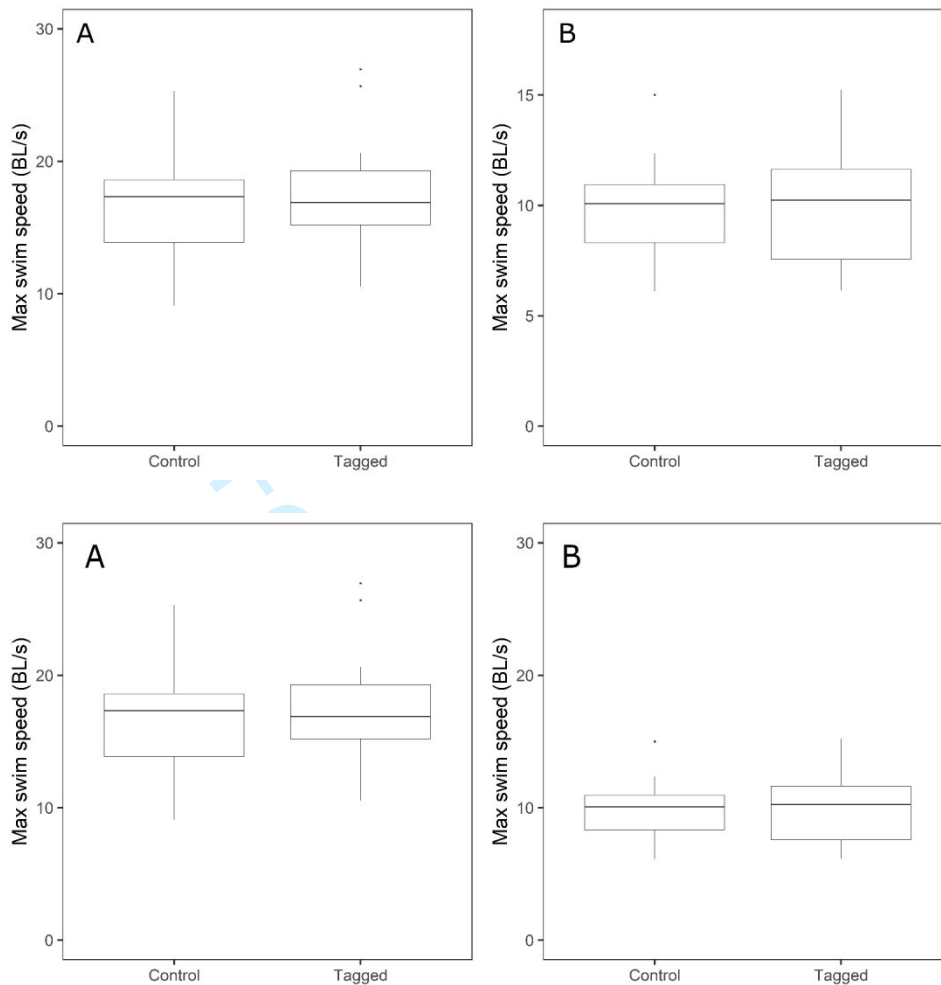


Figure 45. Maximum swimming speed recorded over 400 ms for control and tagged fish in a provoked escape experiment for A) South European nase (n = 34) and B) brook barbel (n = 54). The horizontal line represents the median value, the box the interquartile range, the whiskers 1.5 IQR, and the point are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

1
2
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.

10
11
12
13
14
15
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.

32
33
34
35
36
37
38
39
40
41
42
43
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
54
55
56
57
58
59
60

1
2
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.~~
9

10
11
12
13
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.
25
26
27
28
29
30
31
32

33
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).
45
46
47
48
49
50
51
52

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
58
59
60

1
2
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.
8
9
10
11
12
13
14

15 **Conclusions**

16
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.
23
24
25
26
27

28 **Acknowledgements**

29
30 This research work has been funded by the European Union Horizon 2020 Research and
31 Innovation Programme under the Marie Skłodowska-Curie Actions, Grant Agreement
32 No. 86080 and part of the work performed within LIFE21-NAT-IT-LIFE Minnow,
33 101074559 “Small fish, small streams, big challenges: conservation of endangered
34 species in tributaries of the upper Po River”. We acknowledge Florian Eggers, Gloria
35 Mozzi, Usama Ashraf, Costantino Manes, and Armando Piccinini for technical
36 assistance.
37
38
39
40
41
42
43

44 **References**

- 45
46 Amaral, S. D., Branco, P., da Silva, A. T., Katopodis, C., Viseu, T., Ferreira, M. T., ...
47 Santos, J. M. (2016). Upstream passage of potamodromous cyprinids over small
48 weirs: the influence of key-hydraulic parameters. *Journal of Ecohydraulics*, 1,
49 79–89.
50
51 Baki, A. B. M., Zhu, D. Z., Harwood, A., Lewis, A., & Healey, K. (2020). Hydraulic
52 design aspects of rock-weir fishways with notch for habitat connectivity.
53 *Journal of Ecohydraulics*, 5, 94–109.
54 Baras, E., Westerloppe, L., Mélard, C., Philippart, J.-C., & Bénech, V. (1999).
55 Evaluation of implantation procedures for PIT-tagging juvenile Nile tilapia.
56 *North American Journal of Aquaculture*, 61, 246–251.
57 Bartoň, D., Brabec, M., Sajdlová, Z., Souza, A. T., Duras, J., Kortan, D., ... Šmejkal,
58 M. (2022). Hydropeaking causes spatial shifts in a reproducing rheophilic fish.
59 *Science of The Total Environment*, 806, 150649.
60

- 1
2
3 [Belletti, B., de Leaniz, C. G., Jones, J., Bizzi, S., Börger, L., Segura, G., ... Barry, J. \(2020\). More than one million barriers fragment Europe's rivers. *Nature*, 588, 436–441.](#)
- 4
5
6
7 Bolland, J. D., Cowx, I. G., & Lucas, M. C. (2009). Evaluation of VIE and PIT tagging methods for juvenile cyprinid fishes. *Journal of Applied Ichthyology*, 25, 381–386.
- 8
9
10 Breen, M. J., Ruetz, C. R., Thompson, K. J., & Kohler, S. L. (2009). Movements of mottled sculpins (*Cottus bairdii*) in a Michigan stream: how restricted are they? *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 31–41.
- 11
12
13 Brönmark, C., Skov, C., Brodersen, J., Nilsson, P. A., & Hansson, L.-A. (2008). Seasonal migration determined by a trade-off between predator avoidance and growth. *PloS one*, 3, e1957.
- 14
15
16
17 Brown, R. S., Eppard, M. B., Murchie, K. J., Nielsen, J. L., & Cooke, S. J. (2011). An introduction to the practical and ethical perspectives on the need to advance and standardize the intracoelomic surgical implantation of electronic tags in fish. *Reviews in Fish Biology and Fisheries*, 21, 1–9.
- 18
19
20
21 Brown, R. S., Cooke, S. J., Anderson, W. G., & McKinley, R. S. (1999). Evidence to challenge the “2% rule” for biotelemetry. *North American Journal of Fisheries Management*, 19, 867–871.
- 22
23
24
25 [Carosi, A., Padula, R., Ghetti, L., & Lorenzoni, M. \(2019\). Endemic freshwater fish range shifts related to global climate changes: A long-term study provides some observational evidence for the Mediterranean area. *Water*, 11, 2349.](#)
- 26
27
28
29 Castro-Santos, T., Goerig, E., He, P., & Lauder, G. V. (2022). Applied aspects of locomotion and biomechanics. *Fish Physiol. A*, 39, 91–140.
- 30
31
32 Castro-Santos, T., & Perry, R. (2012). Time-to-event analysis as a framework for quantifying fish passage performance. *Telemetry techniques: a user guide for fisheries research. America Fisheries Society, Bethesda, Maryland*, 427–452.
- 33
34
35 Castro-Santos, T., Haro, A., & Walk, S. (1996). A passive integrated transponder (PIT) tag system for monitoring fishways. *Fisheries research*, 28, 253–261.
- 36
37
38 Clark, S. R. (2016). Effects of Passive Integrated Transponder Tags on the Physiology and Swimming Performance of a Small-Bodied Stream Fish. *Transactions of the American Fisheries Society*, 145, 1179–1192.
- 39
40
41 Connors, K., Scruton, D., Brown, J., & McKinley, R. (2002). The effects of surgically-implanted dummy radio transmitters on the behaviour of wild Atlantic salmon smolts. *Aquatic Telemetry* (pp. 231–237). Springer.
- 42
43
44
45 Cooke, S. J., Hinch, S. G., Wikelski, M., Andrews, R. D., Kuchel, L. J., Wolcott, T. G., & Butler, P. J. (2004). Biotelemetry: a mechanistic approach to ecology. *Trends in ecology & evolution*, 19, 334–343.
- 46
47
48
49 Crossin, G. T., Heupel, M. R., Holbrook, C. M., Hussey, N. E., Lowerre-Barbieri, S. K., Nguyen, V. M., ... Cooke, S. J. (2017). Acoustic telemetry and fisheries management. *Ecological Applications*, 27, 1031–1049.
- 50
51
52
53 Dieterman, D. J., & Hoxmeier, R. J. H. (2009). Instream evaluation of passive integrated transponder retention in brook trout and brown trout: effects of season, anatomical placement, and fish length. *North American Journal of Fisheries Management*, 29, 109–115.
- 54
55
56
57 Domenici, P. (2010). Escape responses in fish: kinematics, performance and behavior. *Fish locomotion: An eco-ethological perspective*, 123–170.
- 58
59
60 Domenici, P., & Blake, R. (1997). The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology*, 200, 1165–1178.

- Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., ... Stiassny, M. L. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews*, 81, 163–182.
- Echevarria, D. J., Hammack, C. M., Pratt, D. W., & Hosemann, J. D. (2008). A novel behavioral test battery to assess global drug effects using the zebrafish. *International Journal of Comparative Psychology*, 21.
- Eilam, D. (2005). Die hard: A blend of freezing and fleeing as a dynamic defense—implications for the control of defensive behavior. *Neuroscience & Biobehavioral Reviews*, 29, 1181–1191.
- Ficke, A. D., Myrick, C. A., & Kondratieff, M. C. (2012). The effects of PIT tagging on the swimming performance and survival of three nonsalmonid freshwater fishes. *Ecological Engineering*, 48, 86–91.
- Fortini, N. (2016). Nuovo atlante dei pesci delle acque interne italiane: guida completa ai pesci, ciclostomi e crostacei decapodi di acque dolci e salmastre. Aracne.
- Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N., & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *The American Naturalist*, 158, 124–135.
- Gibbons, W. J., & Andrews, K. M. (2004). PIT tagging: simple technology at its best. *Bioscience*, 54, 447–454.
- Gould, T. D., Dao, D. T., & Kovacsics, C. E. (2009). The Open Field Test. In T. D. Gould (Ed.), *Mood and Anxiety Related Phenotypes in Mice* (pp. 1–20). Totowa, NJ: Humana Press/Neuromethods.
- Gries, G., & Letcher, B. (2002). Tag retention and survival of age-0 Atlantic salmon following surgical implantation with passive integrated transponder tags. *North American Journal of Fisheries Management*, 22, 219–222.
- Haraldstad, T., Haugen, T. O., Olsen, E. M., Forseth, T., & Höglund, E. (2021). Hydropower-induced selection of behavioural traits in Atlantic salmon (*Salmo salar*). *Scientific Reports*, 11, 16444.
- Harbicht, A. B., Watz, J., Nyqvist, D., Virmajä, T., Carlsson, N., Aldvén, D., ... Calles, O. (2022). Guiding migrating salmonid smolts: Experimentally assessing the performance of angled and inclined screens with varying gap widths. *Ecological Engineering*, 174, 106438.
- Haro, A., Castro-Santos, T., Noreika, J., & Odeh, M. (2004). Swimming performance of upstream migrant fishes in open-channel flow: a new approach to predicting passage through velocity barriers. *Canadian Journal of Fisheries and Aquatic Sciences*, 61, 1590–1601.
- Haugen, T. O., Aass, P., Stenseth, N. C., & Vøllestad, L. A. (2008). Changes in selection and evolutionary responses in migratory brown trout following the construction of a fish ladder. *Evolutionary Applications*, 1, 319–335.
- Hong, X., & Zha, J. (2019). Fish behavior: A promising model for aquatic toxicology research. *Science of The Total Environment*, 686, 311–321.
- Hosmer, D. W., Lemeshow, S., & May, S. (2008). *Applied Survival Analysis: Regression Modeling of Time-to-Event Data, Second Edition*. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.
- Hühn, D., Klefoth, T., Pagel, T., Zajicek, P., & Arlinghaus, R. (2014). Impacts of external and surgery-based tagging techniques on small northern pike under field conditions. *North American journal of fisheries management*, 34, 322–334.
- Jepsen, N., Thorstad, E. B., Havn, T., & Lucas, M. C. (2015). The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry*, 3, 49.

- 1
2
3 Jepsen, N., Schreck, C., Clements, S., & Thorstad, E. B. (2005). A brief discussion on
4 the 2% tag/body mass rule of thumb. *Aquatic telemetry: advances and*
5 *applications*, 255–259.
- 6 Jones, P. E., Champneys, T., Vevers, J., Börger, L., Svendsen, J. C., Consuegra, S., ...
7 Garcia de Leaniz, C. (2021). Selective effects of small barriers on river-resident
8 fish. *Journal of Applied Ecology*, 58, 1487–1498.
- 9 Katopodis, C., & Gervais, R. (2012). ECOHYDRAULIC ANALYSIS OF FISH
10 FATIGUE DATA: ECOHYDRAULIC ANALYSIS OF FISH FATIGUE
11 DATA. *River Research and Applications*, 28, 444–456.
- 12 Keeler, R. A., Breton, A., Peterson, D. P., & Cunjak, R. A. (2007). Apparent survival
13 and detection estimates for PIT-tagged slimy sculpin in five small New
14 Brunswick streams. *Transactions of the American Fisheries Society*, 136, 281–
15 292.
- 16 Knaepkens, G., Maerten, E., Tudorache, C., De Boeck, G., & Eens, M. (2007).
17 Evaluation of passive integrated transponder tags for marking the bullhead
18 (*Cottus gobio*), a small benthic freshwater fish: effects on survival, growth and
19 swimming capacity. *Ecology of Freshwater fish*, 16, 404–409.
- 20 Miklósi, A., Topal, J., & Csányi, V. (1992). Development of open-field and social
21 behavior of the paradise fish (*Macropodus opercularis* L.). *Developmental*
22 *Psychobiology: The Journal of the International Society for Developmental*
23 *Psychobiology*, 25, 335–344.
- 24 Mittelbach, G. G., Ballew, N. G., Kjelvik, M. K., & Fraser, D. (2014). Fish behavioral
25 types and their ecological consequences. *Canadian Journal of Fisheries and*
26 *Aquatic Sciences*, 71, 927–944.
- 27 Moser, M. L., Corbett, S. C., Keefer, M. L., Frick, K. E., Lopez-Johnston, S., & Caudill,
28 C. C. (2019). Novel fishway entrance modifications for Pacific lamprey. *Journal*
29 *of Ecohydraulics*, 4, 71–84.
- 30 Mueller, R. P., Moursund, R. A., & Bleich, M. D. (2006). Tagging juvenile Pacific
31 lamprey with passive integrated transponders: methodology, short-term
32 mortality, and influence on swimming performance. *North American Journal of*
33 *Fisheries Management*, 26, 361–366.
- 34 Mulligan, K. B., Haro, A., & Noreika, J. (2021). Effect of backwatering a streamgage
35 weir on the passage performance of adult American Shad (*Alosa sapidissima*).
36 *Journal of Ecohydraulics*, 0, 1–13.
- 37 Nagel, C., Droll, J., Kroemer, K., Pander, J., & Geist, J. (2023). Testing the effects of
38 passive integrated transponder (PIT) tags on survival, growth, and tag retention
39 of common nase (*Chondrostoma nasus* L.) and European barbel (*Barbus barbus*
40 L.). *Animal Biotelemetry*, 11, 33.
- 41 Newby, N. C., Binder, T. R., & Stevens, E. D. (2007). Passive Integrated Transponder
42 (PIT) Tagging Did Not Negatively Affect the Short-Term Feeding Behavior or
43 Swimming Performance of Juvenile Rainbow Trout. *Transactions of the*
44 *American Fisheries Society*, 136, 341–345.
- 45 Nyqvist, D., Schiavon, A., Candiotta, A., Mozzi, G., Eggers, F., & Comoglio, C.
46 (2022). PIT -tagging Italian spined loach (*Cobitis bilineata*) – methodology,
47 survival, and behavioral effects. *Journal of Fish Biology*, jfb.15289.
- 48 Nyqvist, D., Hedenberg, F., Calles, O., Österling, M., von Proschwitz, T., & Watz, J.
49 (2020). Tracking the movement of PIT-tagged terrestrial slugs (*Arion vulgaris*)
50 in forest and garden habitats using mobile antennas. *Journal of Molluscan*
51 *Studies*.
- 52
53
54
55
56
57
58
59
60

- Ombredane, D., Bagliniere, J. L., & Marchand, F. (1998). The effects of Passive Integrated Transponder tags on survival and growth of juvenile brown trout (*Salmo trutta* L.) and their use for studying movement in a small river. *Hydrobiologia*, 371, 99–106.
- Ovidio, M., Dierckx, A., & Benitez, J.-P. (2023). MOVEMENT BEHAVIOUR AND FISHWAY PERFORMANCE FOR ENDEMIC AND EXOTIC SPECIES IN A LARGE ANTHROPIZED RIVER. *Limnologica*, 126061.
- Perals, D., Griffin, A. S., Bartomeus, I., & Sol, D. (2017). Revisiting the open-field test: what does it really tell us about animal personality? *Animal Behaviour*, 123, 69–79.
- Quintella, B. R., Andrade, N. O., Espanhol, R., & Almeida, P. R. (2005). The use of PIT telemetry to study movements of ammocoetes and metamorphosing sea lampreys in river beds. *Journal of Fish Biology*, 66, 97–106.
- [Rondinini, C., Battistoni, A., & Teofili, C. \(2022\). Lista Rossa IUCN dei Vertebrati Italiani 2022. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del territorio e del mare: Roma, Italy.](#)
- Schiavon, A., Comoglio, C., Candioto, A., Hölker, F., Ashraf, M. U., & Nyqvist, D. (2023). Survival and swimming performance of a small-sized Cypriniformes (*Telestes muticellus*) tagged with passive integrated transponders. *Journal of Limnology*, 82.
- Schwinn, M., Baktoft, H., Aarestrup, K., & Koed, A. (2017). A comparison of the survival and migration of wild and F1-hatchery-reared brown trout (*Salmo trutta*) smolts traversing an artificial lake. *Fisheries Research*, 196, 47–55.
- [Skoulikidis, N. T., Sabater, S., Datry, T., Morais, M. M., Buffagni, A., Dörflinger, G., ... Tockner, K. \(2017\). Non-perennial Mediterranean rivers in Europe: Status, pressures, and challenges for research and management. Science of The Total Environment, 577, 1–18.](#)
- Skov, C., Hansen, J. H., Baktoft, H., Brönmark, C., Brodersen, J., Chapman, B. B., ... Nilsson, P. A. (2020). A field evaluation of long-term effects of PIT tagging. *Journal of Fish Biology*, 96, 1055–1059.
- Šmejkal, M., Blabolil, P., Bartoň, D., Duras, J., Vejřík, L., Sajdlova, Z., ... Kubečka, J. (2019). Sex-specific probability of PIT tag retention in a cyprinid fish. *Fisheries Research*, 219, 105325.
- [Smialek, N., Pander, J., Mueller, M., van Treeck, R., Wolter, C., & Geist, J. \(2019\). Do We Know Enough to Save European Riverine Fish?—A Systematic Review on Autecological Requirements During Critical Life Stages of 10 Rheophilic Species at Risk. Sustainability, 11, 5011.](#)
- [Sullivan, K. M., Bailey, M. M., & Berlinsky, D. L. \(2023\). Passage Efficiency of Alewife in a Denil Fishway Using Passive Integrated Transponder Tags. North American Journal of Fisheries Management, 43, 772–785.](#)
- Thorstad, E. B., Rikardsen, A. H., Alp, A., & Okland, F. (2013). The use of electronic tags in fish research: an overview of fish telemetry methods. *Turkish Journal of Fisheries and Aquatic Sciences*, 13, 881–896.
- Tudorache, C., De Boeck, G., & Claireaux, G. (2013). Forced and preferred swimming speeds of fish: a methodological approach. *Swimming physiology of fish* (pp. 81–108). Springer.
- Tudorache, C., Viaene, P., Blust, R., Vereecken, H., & De Boeck, G. (2008). A comparison of swimming capacity and energy use in seven European freshwater fish species. *Ecology of freshwater fish*, 17, 284–291.
- Videler, J. J. (1993). *Fish swimming*. Springer Science & Business Media. Vol. 10.

- 1
2
3 Vøllestad, L. A. (2023). A paradoxical bias in knowledge about Norwegian freshwater
4 fishes: research efforts during 1980-2020. *Fauna norvegica*, 42, 6–30.
5
6 Vollset, K. W., Lennox, R. J., Thorstad, E. B., Auer, S., Bär, K., Larsen, M. H., ...
7 Dohoo, I. (2020). Systematic review and meta-analysis of PIT tagging effects on
8 mortality and growth of juvenile salmonids. *Reviews in Fish Biology and*
9 *Fisheries*, 30, 553–568.
- 10 Volpato, G. L., Barreto, R. E., Marcondes, A. L., Andrade Moreira, P. S., & de Barros
11 Ferreira, M. F. (2009). Fish ladders select fish traits on migration—still a growing
12 problem for natural fish populations. *Marine and Freshwater Behaviour and*
13 *Physiology*, 42, 307–313.
- 14 Wargo Rub, A. M., Sandford, B. P., Butzerin, J. M., & Cameron, A. S. (2020). Pushing
15 the envelope: Micro-transmitter effects on small juvenile Chinook salmon
16 (*Oncorhynchus tshawytscha*). *PloS one*, 15, e0230100.
- 17 Watson, J. R., Goodrich, H. R., Cramp, R. L., Gordos, M. A., & Franklin, C. E. (2019).
18 Assessment of the effects of microPIT tags on the swimming performance of
19 small-bodied and juvenile fish. *Fisheries Research*, 218, 22–28.
- 20 Watz, J. (2019). Structural complexity in the hatchery rearing environment affects
21 activity, resting metabolic rate and post-release behaviour in brown trout *Salmo*
22 *trutta*. *Journal of Fish Biology*, 95, 638–641.
- 23 Watz, J., Bergman, E., Piccolo, J. J., & Greenberg, L. (2016). Ice cover affects the
24 growth of a stream-dwelling fish. *Oecologia*, 1–13.
- 25 Watz, J., Otsuki, Y., Nagatsuka, K., Hasegawa, K., & Koizumi, I. (2019a).
26 Temperature-dependent competition between juvenile salmonids in small
27 streams. *Freshwater Biology*, 64, 1534–1541.
- 28 Watz, J., Calles, O., Carlsson, N., Collin, T., Huusko, A., Johnsson, J., ... Nyqvist, D.
29 (2019b). Wood addition in the hatchery and river environments affects
30 post-release performance of overwintering brown trout. *Freshwater biology*.
- 31 Wilson, A. D., Hayden, T. A., Vandergoot, C. S., Kraus, R. T., Dettmers, J. M., Cooke,
32 S. J., & Krueger, C. C. (2017). Do intracoelomic telemetry transmitters alter the
33 post-release behaviour of migratory fish? *Ecology of Freshwater Fish*, 26, 292–
34 300.
- 35 Winter, J. (1983). Underwater biotelemetry. *Fisheries techniques. American Fisheries*
36 *Society, Bethesda, Maryland*, 371–395.
- 37 Zakeś, Z., Demska-Zakeś, K., Rożyński, M., Gomułka, P., & Rożyński, R. (2022).
38 Influence of intraperitoneal implantation of 12 mm PIT on the welfare of
39 juvenile brown trout (*Salmo trutta*). *Fisheries Research*, 255, 106458.
- 40 Závorka, L., Aldvén, D., Näslund, J., Höjesjö, J., & Johnsson, J. I. (2016). Inactive trout
41 come out at night: behavioral variation, circadian activity, and fitness in the
42 wild. *Ecology*, 97, 2223–2231.
- 43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60