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RESEARCH PAPER OPEN ACCESS

Effect of 12 mm PIT tags on the survival, growth, and ecologically significant behaviours of juvenile critically endangered endemic *Zingel asper*

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Abstract – Understanding the movement ecology and habitat use of freshwater fish is crucial for their conservation, with telemetry being an essential tool. Current tagging methods are restricted by the minimum body size of fish, as tags should not exceed 2% of the fish's body mass. This limitation hinders the study of juvenile small-bodied endangered species, as the reduced fitness of tagged individuals can negatively impact their populations. This study examines the feasibility of using 12-mm PIT tags on juvenile and subadult individuals (63–90 mm fork length) of the critically endangered Rhône streber, *Zingel asper*. Results indicate that tagging does not significantly affect survival, growth or behavioural traits, despite the tag weight being between 2.2% and 5.4% of the fish's weight. Tag retention was 100%, with 90% of individuals having the PIT tag fully encapsulated in the body cavity wall after 55 days. The only possible negative effect observed was a tendency, albeit statistically non-significant, for slower escape velocity in the smallest tagged individuals. These findings reduce the known minimum body size limit for 12-mm PIT tags for this species. However, the smallest individuals may be more sensitive to tagging, warranting careful consideration to avoid potential negative impacts on wild populations.

Keywords: Tag effects / passive integrated transponder / juvenile fish / mortality / swimming

1 Introduction

The decline in diversity of freshwater fish species has been accelerating over the last century and the rate of extinction of freshwater fishes is now higher than in other taxa (Reid *et al.*, 2019). Riverine fishes are at particularly high risk of extinction due to the numerous anthropogenic stressors affecting their ecosystems, including degradation of habitat quality and connectivity, biological invasions, and pollution (Su *et al.*, 2021). A critical step towards the conservation of freshwater fishes is a better understanding of their movement ecology and habitat use (Cooke *et al.*, 2013). Fish telemetry is an irreplaceable tool in achieving this goal (Spedicato *et al.*, 2005). The current tagging methods enabling the tracking of fish movements are limited by the minimum body size of individuals according to the rule of thumb that the tag size should not exceed 2% of the body mass of tagged fish (but see Jepsen *et al.*, 2005). The minimum body size rule thus significantly limits the capacity to learn about the ecology of juvenile small-bodied endangered species. Some studies however emphasize that it is possible to exceed the 2% rule without any major impact on survival, growth or swimming performances (Brown *et al.*, 1999; Richard *et al.*, 2013; Smircich and Kelly, 2014).

Radio frequency identification telemetry using passive integrated transponder tags (PIT tags) is the most suitable tool for tracking the movements of small bodied species given the

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small size of the tags. The minimum fish body size for application of the most common PIT tags (*i.e.*, 12-mm tags) has been studied mainly in juvenile salmonids (Vollset *et al.*, 2020) and cyprinids (Skov *et al.*, 2005; Bolland *et al.*, 2009). However, the negative effects of PIT tags can be specific to particular species (Skov *et al.*, 2020). Thus, extrapolating the minimum body size for tagging of endangered species from experimental studies using other fish taxa can lead to inaccurate conclusions on the behavioural, physiological and fitness consequences of tagging for small individuals.

An example of a critically endangered species with unknown minimum body size for PIT tag application is the endemic Rhône streber, *Zingel asper*. The range of this small-bodied species endemic to the Rhône basin has decreased by 83% during the last century (Keith *et al.*, 2020). Habitat fragmentation and degradation are suspected to be the main causes of this decline (Georget, 2021). Better information on its migration behaviour and habitat use are therefore critical for its effective conservation. However, this species has a slow growth rate, reaching its maximum fork length of only about 180 mm at the age of 4+ and with an estimated fork length at one year old ranging between 53 and 84 mm (Monnet *et al.*, 2022). To date, the impact of PIT tagging has not been assessed on *Z. asper* but *in situ* monitoring has already been carried out successfully on individuals larger than 100 mm (Labonne, 2002; Labonne and Gaudin, 2005). The effect of PIT tagging on the youngest individuals in the populations thus remains unexplored. Given that many riverine fish species undergo an ontogenetic shift in habitat and niche use (Gaudin, 2001), the lack of knowledge about the behaviour and ecology of juvenile *Z. asper* could lead to a serious underestimation of the species' ecological requirements. At the same time, for the Rhône streber, it is essential to thoroughly check that the tagging method selected for future telemetry studies does not affect the fitness of individuals, because any negative effects of tagging could have dramatic consequences on the population of this critically endangered and red-listed species (Crivelli, 2006).

Studies on the effects of PIT tagging often only examine impacts on survival, individual growth and tag retention (*e.g.* Baras *et al.*, 2000; Bolland *et al.*, 2009; Hopko *et al.*, 2010). Fewer studies investigate other possible impacts such as swimming capacity or the ability of tagged individuals to escape predators (Watson *et al.*, 2019). However, for studies in the wild it is critical to find out how the behaviour of the tagged fish might be affected (in particular at young stages) because differences in behaviour can have direct implication for the fitness and survival of individuals (Koeck *et al.*, 2013).

In this study, we tested the effect of PIT tagging with 12-mm tags on juvenile *Z. asper* of hatchery origin with fork lengths ranging from 63 to 90 mm (mean ± SD = 74 ± 4 mm). This body size corresponds to that of individuals at one year of age in wild populations (Monnet *et al.*, 2022). We examined tag retention and encapsulation in body tissues, and its effects on the survival and growth rate of individuals. In addition, we investigated the effects of PIT tags on ecologically significant behavioural traits, *i.e.*, activity (Závorka *et al.*, 2020) and escape response (Petersson and Järvi, 2006).

2 Methods and materials

2.1 Fish origin, tagging and storage

The *Z. asper* were provided by the Museum of the Citadel of Besançon participating in the reproduction programme for saving and restoring the species. All fish were born and raised in captivity. The fish were transported on May 3, 2021 to the ECP Facility (ECP, INRA, 2018) in the laboratory of the French National Research Institute for Agriculture, Food, and Environment (INRAE), accredited for animal experimentation (DDPP64/SPAE/2023-1767 establishment number C64-495-2). From their arrival until May 31, the fish were first held in an outdoor holding tank to acclimatize them to the quality of the local water, supplied from the Nivelle River.

The experiment started on May 31, 2021, when all individuals reached a total length of at least 69 mm (as recommended for juvenile salmonids by Vollset *et al.*, 2020). All fish were anesthetized with benzocaine (prepared as a 10% solution in ethanol, used at a concentration of 0.5 ml/L), and measured (fork length to nearest mm, body mass to nearest 0.1 g). Individuals were then alternately assigned to the control group ($n = 99$, mean fork length (mm) ± SD = 76.2 ± 4.9, mean weight (g) ± SD = 3.0 ± 0.7) or test group ($n = 101$, mean fork length (mm) ± SD = 76.4 ± 4.2, mean weight (g) ± SD = 3.0 ± 0.6). All individuals in the test group were then tagged with a 12-mm long and 2.12-mm wide PIT, inserted in the peritoneal cavity by hand via a surgical incision (2–3 mm, without suture) done with surgical mini scalpel blade on the ventral part (slightly offset from the ventral line, under the left pectoral and pelvic fins both situated in the front part of the body, see Suppl. Mat. Fig. 1). After the fish had recovered from anaesthesia in well-oxygenated recovery tanks, they were housed in 6 rectangular holding tanks (120 × 30 × 30 cm, 33–34 individuals per tank, 3 tanks with the test and 3 with the control individuals), supplied with water from the Nivelle River. The physicochemical conditions were monitored every day (temperature range: 18.0–24.2 °C, pH: 7.8–7.9, conductivity: 196–232 µS/cm). A gentle current was maintained in the holding tanks and roof tiles were added to provide refuges for fish. The tanks were covered with mesh to prevent any individuals escaping by jumping. The fish were fed to apparent satiation, twice a day with frozen chironomids twice a day, with the same quantity (10–25 g) distributed in each tank (uneaten food was removed from tanks every day). The artificial lighting in the laboratory was automatically adjusted to match the day length typical of the region and time of the year.

2.2 Fish survival and growth

Survival and growth were monitored for 50 days after tagging. Fish mortality was observed every day in the holding tanks (during the monitoring of animal welfare conditions). The body growth of individuals and healing of the incision after tag implantation were recorded on the 5th, 10th, 25th and 50th days after tagging, when all fish were anesthetized (following the protocol described above), and measurements of their fork length and body mass were taken. The tag weight (0.111g) was deducted from the weight of tagged individuals.

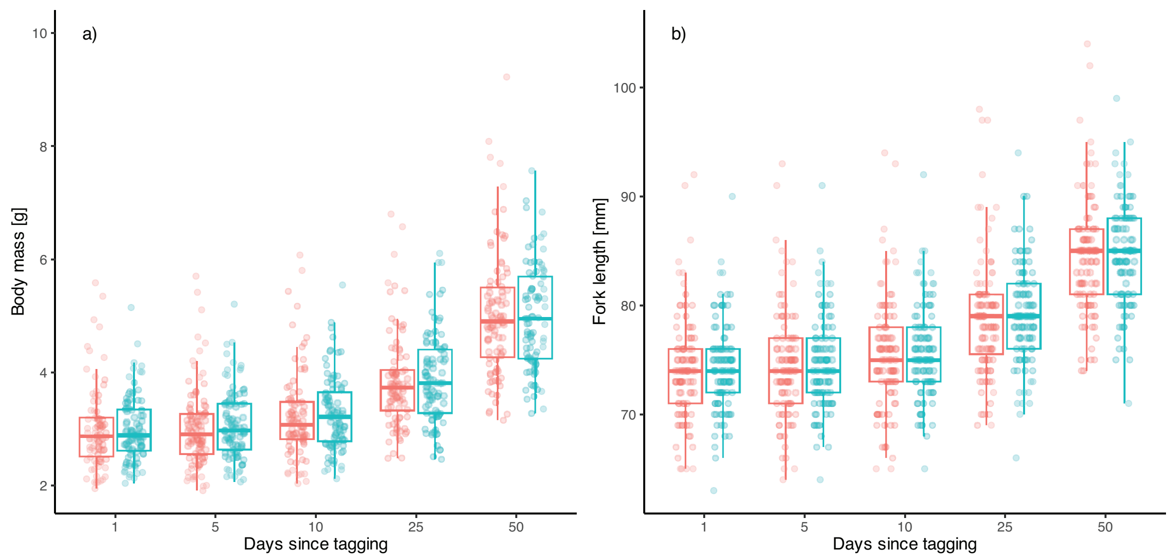

Fig. 1. Boxplot summarizing the distribution of body mass (a) and fork length (b) of *Z. asper* across the five body size measurements (Day 1, 5, 10, 25 and 50 post-tagging) between PIT-tagged (green) and control (red) groups.Box edge represents the 25th, 50th and 75th percentiles and whiskers cover the 95th percentiles.

2.3 Behavioural scoring

Two behavioural tests were carried out between July 20 and 28 (between the 51st and 59th days post-tagging) to check that the tag in the body cavity had no effect on individual behaviour: an activity test and an escape test.

Activity test – The activity test, previously used by Závorka *et al.* (2020), consisted in monitoring the movement of each fish in a new environment, over a defined time. The activity of 40 fish from the test group, and 40 fish from the control group, selected randomly and uniformly from all holding tanks, were recorded and analysed. Each fish was individually placed in a corner of a rectangular opaque white bare scoring tank (57.5 × 36.5 cm, 5.5 cm water depth), and filmed (top view, 10 images/s) for 30 minutes. Recording started immediately after placing individual in the scoring tank. Scoring tanks were rinsed and filled with fresh water before each trial. Four scoring tanks were recorded in parallel, two with tagged fish and two with control fish. The tests were carried out during the day, between 9:00 and 17:00. The lighting of the tanks was identical (or very close) to that in the holding tanks during the day. Two of the experimental tanks were illuminated by mistake with a stronger light intensity in 12 out of the 20 trials. However, we found no effect of light intensity on fish behaviour (see below). After each session, the fish were anesthetized, then euthanized using a lethal dose of benzocaine (3 ml/L) and measured (fork length and body mass). In addition, the position of the tag in the peritoneal cavity and the sex of individuals were determined by dissection.

The video recordings were analysed using the Lolitrack® software (version 4.1.0). We assessed the time until the first movement after being placed in the scoring tank (the first movement was defined as continuous swimming for at least 10 seconds). We choose the 10 seconds limit based on observation of fish behaviour during the experiment as the best cut-off that allowed us to filter out brief movements followed by an extended period of inactivity typical for benthic fish in stressful situations (*e.g.*, handling and exposure to a novel environment of the scoring box). Then, we measured the distance moved by each individual (using calibration landmarks placed in the tank) for 10 minutes, between the 15th and 25th minutes of the recording, as a proxy of activity (considering the initial 15 minutes as an acclimatization period).

Escape test – The escape test consisted in recording the startling response of fish (Tudorache *et al.*, 2008). Each of 60 fish (30 from the test and 30 from the control group, chosen randomly and uniformly from all the holding tanks) were placed, one by one, in an opaque vertical starting tube (10 cm in diameter), in the middle of a scoring tank (36 × 57 cm, translucent and top-lit, filled to a height of 5 cm with holding water). After 10 seconds, the tube was gently removed by hand, with the fish mostly remaining motionless (if an individual moved immediately after removing the tube, it was replaced, and the motion was repeated after another 10 seconds). Subsequently, an aquarium dip net (8.5 × 6 cm) attached to a string was released in the scoring tank (without falling completely), approximately 10 cm from the caudal fin of the individual, to frighten it and produce the startling reaction. Each fish was stimulated 4 times. Between trials the fish was placed back in the tube and allowed to recover for at least 10 seconds. At the end of the experiment, the fish was anesthetized, euthanized, measured, dissected and sexed. The water in the scoring tank was renewed for each new individual.

The video acquisition frequency was 45 frames/s. Each of the 4 trials was analysed separately using Lolitrack® software. Twenty frames per trial were selected for analysis, the first frame being when the dip net hit the water surface. The movement trajectory was reconstructed frame-by-frame, and the fish's escape velocity was calculated between consecutive

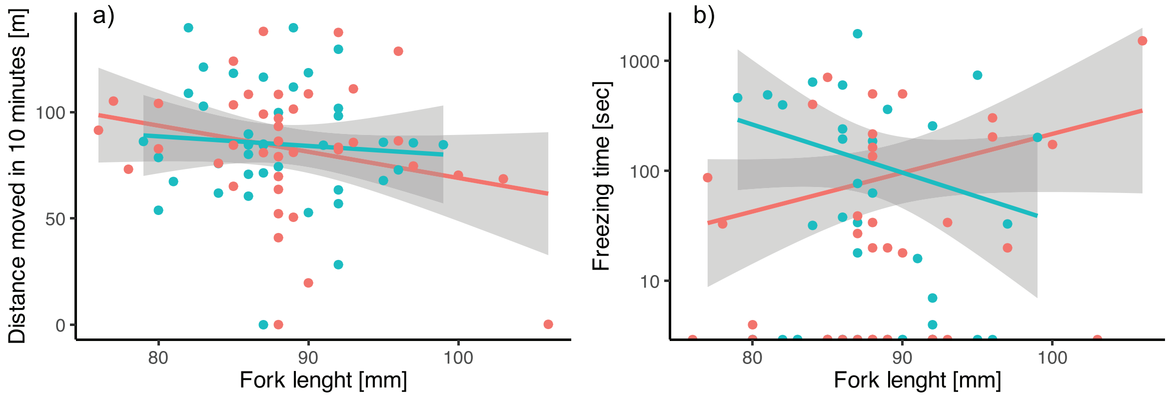

Fig. 2. The relationship between fork length and (a) distance moved and (b) freezing time in the open field test in control (red) and PIT-tagged (green) *Z. asper*. The shaded area surrounding the fitted lines indicates standard error.

frames (cm/s) using calibration landmarks placed in the tank. The 90th percentile of the escape velocity in each trial was used for further analysis (*i.e.*, 4 values per individual).

2.4 Data analysis

Statistical analyses were performed using R (R Core Team, 2019), 4.3.2 "Eye Holes" version. The effect of tagging on the evolution of body mass and fork length was tested by a linear model with log-transformed body mass and fork length as response variables, and tagging treatment (factor with two levels: tagged, control) and the number of days since tagging (factor with 5 levels: 1, 5, 10, 25, 50) as predictor variables. To test the effect of tagging on distance moved and time until recovery of movement, we used a linear model with the predictor variables tagging treatment, sex (factor with three levels: male, female, not developed) and fork length. We initially also ran the same model including the effect of trial tank ID (factor with four levels) and light intensity (factor with two levels: 30 and 100), but these variables had no significant effect (trial tank ID effect: $F_{1,75} = 1.75$, $p = 0.164$ for distance moved, $F_{1,75} = 0.73$, $p = 0.537$ for time until the movement recovery; light intensity effect: $F_{1,75} = 0.10$, $p = 0.752$ for distance; $F_{1,75} = 0.05$, $p = 0.824$ for time until the movement recovery), and were removed from the final model. The escape response of individuals was tested using a linear mixed effect model with the 90th percentile of the escape velocity as the response variable. The tagging treatment, sex, order of the escape trial (factor with four levels 1–4), and fork length were the main predictor (fixed) variables and the ID of individuals was a random intercept. Repeatability (R) of individual's escape response over the four trials was tested with adjustment for the order of the escape trial (Stoffel *et al.*, 2017). We observed that the escape velocity of individuals had significant but low repeatability ($R < 0.37$, Bell *et al.*, 2009), and that the escape velocity decreased from trial one to four. This indicates that while this measurement of escape response was robust, individuals were becoming habituated to the experimental setup with increasing number of test repetitions. Therefore, we ran an additional linear model with escape velocity only for the first trial (*i.e.*, before individuals were habituated to the setup) with interaction of tagging treatment, fork length and sex of individuals as predictor variables. The differences between the categories of predictor variables in all models were tested using Tukey's post-hoc HSD test. Deviations from the assumptions of the models were diagnosed by visual inspection of the distribution of model residuals. The assumptions of the models were satisfyingly met in all cases.

2.5 Ethical considerations

The study was validated by the French Ethic Committee N°073 (APAFIS#29260-2021012109399275 v2) and obtained the authorization of the French Ministry for Research. Restocking program being halted, hatchery individuals used in this experiment could not be released in the nature or returned to the hatchery and had to be euthanized at the end of the study.

3 Results

3.1 Body growth, tag retention, and mortality

We found no effect of PIT tagging on growth in body mass ($F_{1,983} = 0.36$, $p = 0.547$; Fig. 1a) and fork length ($F_{1,986} = 0.50$, $p = 0.478$; Fig. 1b). All individuals (tagged and control group) showed positive growth in both body mass and fork length over the total experimental period, as evidenced by significant increase in body mass ($F_{1,983} = 217$, $p < 0.001$; Fig. 1a), and fork length ($F_{1,986} = 155$, $p < 0.001$; Fig. 1b). However, during the first days a potential negative impact of the experimental treatment (including manipulation, anaesthesia and placement in new holding tanks) on body growth was observed in all individuals. This was indicated by the absence of a significant difference (*i.e.*, for both treatment groups together) in body mass between Day 1 and Day 5 (post-hoc $p = 0.983$, Fig. 1a), no significant change in fork length between Day 1 and Day 5 (post-hoc $p = 0.976$), and between Day 5 and Day 10 (post-hoc $p = 0.134$, Fig. 1b). In contrast, comparisons between all other days demonstrated a significant increase in both fork length and body mass over time.

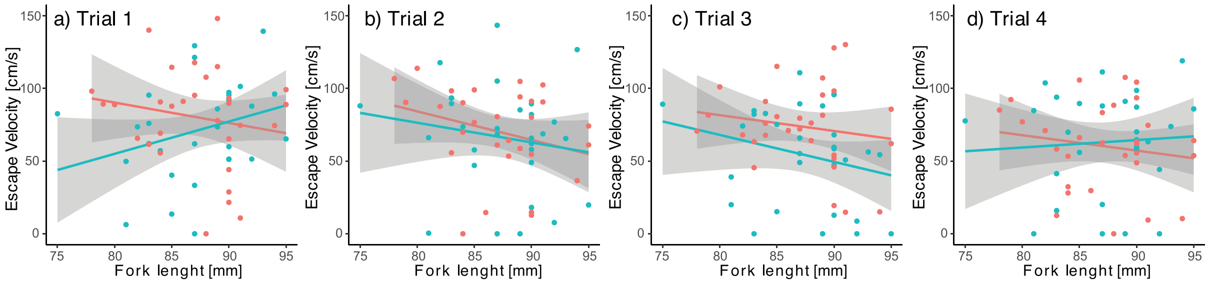

Fig. 3. The relationship between fork length and escape response velocity of control (red) and PIT-tagged (green) *Z. asper* in the first (a), second (b), third (c), and fourth (d) trial of the escape test. The shaded area surrounding the fitted lines indicates standard error.

PIT tag retention in this study was 100%. Upon dissection after the end of the study, we observed that in 76 individuals (76.8%), the PIT tag was located in the middle of the body cavity, while in 23 individuals (23.2%), it was situated at the posterior end of the body cavity. In 10 out of 99 individuals (10.1%), the tag was found freely moving in the body cavity, not encapsulated by tissue. The mortality associated with the tagging procedure was 1.98%. Specifically, there were only two fatalities: one individual failed to recover from the anaesthesia, and the other died within 24 hours after tagging.

3.2 Activity and escape response

Activity (distance moved) in the open field test did not differ between the control and PIT tagged individuals ($F_{1,75} = 0.001$, $p = 0.952$; Fig. 2a), between sexes ($F_{1,75} = 1.22$, $p = 0.301$) and it was not affected by the fork length of individuals ($F_{1,75} = 2.34$, $p = 0.130$; Fig. 2). We found no effect of PIT tagging ($F_{1,75} = 0.021$, $p = 0.883$; Fig. 2b), fork length ($F_{1,75} = 0.509$, $p = 0.478$), their interaction ($F_{1,74} = 1.34$, $p = 0.251$; Fig. 2b) or sex ($F_{1,75} = 1.280$, $p = 0.284$) on the time individuals remained frozen after release into the scoring tank.

The escape velocity of individuals was significantly repeatable over the four trials although the repeatability was relatively low $R_{adj.} = 0.266$, 95 % CI [0.13; 0.41]. Escape velocity did not differ between the control and PIT tagged individuals ($F_{1,56} = 1.61$, $p = 0.209$; Figs. 3a–3d), between sexes ($F_{1,56} = 0.061$, $p = 0.807$), and was not affected by the fork length of individuals ($F_{1,55} = 1.17$, $p = 0.284$; Figs. 3a–3d). However, the escape velocity of individuals decreased from the first to the last trial (mean T1 = 75.7 cm/s, T2 = 68.2 cm/s, T3 = 64.0 cm/s, T4 = 61.6 cm/s, $F_{1,174} = 2.67$, $p = 0.049$; Figs. 3a–3d) with a significantly lower average escape velocity in the last compared to the first trial (post-hoc $p = 0.0474$). Importantly, when considering the escape response of individuals in the first trial only, we found a trend, albeit non-significant, for an effect of the interaction between the tag treatment and fork length of individuals ($F_{1,55} = 3.38$, $p = 0.071$; Fig. 3a). This pattern indicates that among PIT-tagged individuals, the escape velocity tended to increase with increasing body size, while there was no effect of body size on the escape response in the control group. In particular, small tagged individuals tended to have a slower escape reaction in comparison with untagged fish of the same size.

4 Discussion

The results of our study indicate that tagging juvenile and subadult individuals (63–90 mm fork length) of critically endangered *Z. asper* with 12-mm PIT tags in the body cavity has no significant effect on growth, mortality or certain ecologically important behavioural traits. This is an encouraging finding, which reduces the previously known minimum body size limit for the use of 12-mm PIT tags for this species (Labonne, 2002; Labonne and Gaudin, 2005).

The mortality observed during this study is slightly less than 2% and this rate is one of the lowest mortalities obtained during PIT tagging tests on juvenile fish (see study reviews carried out by Musselman *et al.*, 2017; Vollset *et al.*, 2020; Swarr *et al.*, 2022). Our study showed that 12-mm PIT tagging has no significant negative effect on juvenile *Z. asper* although the tag weight exceeded the threshold of 2% of the fish weight (Winter, 1983), being between 2.2% and 5.4% (3.6% on average). This result confirms, in accordance with other previous studies (Brown *et al.*, 1999; Richard *et al.*, 2013; Smircich and Kelly, 2014), that in some species it is possible to exceed the 2% threshold without causing any significant harm to individuals. Note however that this study largely complied with the recommendations of Vollset *et al.* (2020), established for juvenile salmonids, *i.e.*, that the tag size should not exceed 17.5% of total fish length (the tag size in our study was between 12.8 and 18.5 %, 15.8% on average, of the total fish length, see Suppl. Mat. for illustration).

Previous studies have already reported relatively high retention rates of PIT tags, usually above 85–90% (see summary of Musselman *et al.*, 2017), but it can be lower, especially if the tagging is carried out on small individuals (Acolas *et al.*, 2007; Richard *et al.*, 2013). While tag retention was 100% in our study (without any method to close the incision), we found that the PIT tag was not encapsulated in the tissue of body cavity in 10% of cases. This could increase a potential risk of PIT tag loss (assumed to be equal to or lower than 10%), during spawning for example (Šmejkal *et al.*, 2019) in long-term monitoring studies.

We found a non-significant tendency for decreasing escape velocity with decreasing body size of tagged individuals in the first escape velocity test trial. This result could indicate that the capacity of individuals to evade predators might be negatively affected by the 12-mm PIT tag for small *Z. asper* and particularly below the fork length of 63 mm (Fuiman *et al.*, 2006). This is an important finding, potentially indicating a higher sensitivity of small *Z. asper* to PIT-tagging. In comparison, bullhead *Cottus* *gobio* as small as 50 mm tagged by 12-mm PIT tags showed no difference in survival, growth and swimming capacity compared to the control group (Knaepkens *et al.*, 2007). We also observed that all fish, both tagged and control groups, showed lower growth rate during the first days after the procedure when they were also transferred from the outdoor holding tanks to the indoor holding tanks. This negative effect was thus likely to be a consequence of changes in holding environment along with handling stress (Hoskonen and Pirhonen, 2006; Závorka *et al.*, 2019) rather than the effect of the tagging itself.

Our study has several methodological limits that should be taken in account when applying the results of our work. The effect of time since tagging is the first potential limit as we tested the effects of 12-mm PIT tags on behaviour traits only 50 days after tagging. Studies investigating the movement ecology and habitat use of wild fishes typically release tagged individuals shortly after tagging, once they have recovered from surgery (Labonne, 2002; Labonne and Gaudin, 2005). Therefore, our study does not provide any information on how the behaviour of tagged *Z. asper* is affected during the recovery period in the first days following tagging. Our study was also conducted on hatchery-reared individuals and under laboratory conditions, which may bias the results as the artificial selection pressures in the hatchery environment and laboratory conditions are known to alter the behaviour, growth, and survival of fishes (Adriaenssens and Johnsson, 2011; Tang *et al.*, 2017; Lieggi *et al.*, 2020; Skov *et al.*, 2020).

5 Conclusion

Despite some unavoidable methodological limitations, our study provides the first standardized medium-term assessment of the effect of PIT tagging on endangered *Z. asper*. The encouraging results of our test, conducted in the laboratory on juvenile fish, along with the results of *in situ* studies on adults (Labonne, 2002; Labonne and Gaudin, 2005), suggest that future monitoring studies using the PIT tagging of wild populations of *Z. asper* (individuals with fork length > 63 mm) should have no or a negligible effect on their survival, growth or behaviour. We however suspect some sensitivity of the smallest individuals (non-significant tendency in our study) so the use of 12-mm PIT tags should be carefully considered to avoid any potential negative effect on wild populations of this critically endangered species. In the case of very young individuals, the use of smaller, 9- and 8-mm PIT tags, although available only under full-duplex technology, could be more precautionary (if the performance of this technology is suitable for the purpose of a given study).

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Supplementary material

The Supplementary Material is available at https://www.kmae.org/10.1051/kmae/2024017/olm.

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