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Testing the effects of passive integrated transponder (PIT) tags on survival, growth, and tag retention of common nase (*Chondrostoma nasus* L.) and European barbel (*Barbus barbus* L.)

Christoffer Nagel[1], Jan Droll[1], Katharina Kroemer[1], Joachim Pander[1] and Juergen Geist[1*]

Abstract

Freshwater fish populations are in steep decline, prompting conservation measures and a need for their evaluation. Fish are increasingly monitored with passive integrated transponders (PIT), although the suitability of this tagging technique has not yet been validated for most European target species of conservation. Consequently, this study tested the effect of commonly used 12 mm full-duplex (FDX) PIT tags implanted into the abdominal cavity of common nase (*Chondrostoma nasus* L.) and European barbel (*Barbus barbus* L.). A controlled laboratory setup was used to compare survival, growth (both length and weight) and tag retention for two different size classes of sub-adults over 61 days. Survival in the treatment groups was high (96.7–100%) and not statistically different from the control groups (97.5–100%). Highest mortality occurred in small tagged barbel (*n* = 4; 96.7% survival), while no mortality occurred in large tagged nase. Mean growth rates for tagged fish (2.28 mm, 3.26 g) were similar to those of control fish (2.77 mm, 3.59 g). Overall tag retention rate was 99.0% and tag loss only occurred in nase. The results of this study demonstrate the suitability of PIT injection in the body cavity of nase and barbel > 100 mm TL, which is of high methodological importance given the increasing role these species play in PIT tag-based assessments of freshwater fish conservation in European rivers.

Keywords Fish tagging, Fish monitoring, Freshwater fish conservation, Fish biology, Cypriniformes

Introduction

Monitoring aquatic ecosystems and their biota is challenging, yet crucial considering vanishing aquatic biodiversity [1]. This especially accounts for the group of freshwater fish that face steep population declines [2, 3] and high extinction rates [4]. Among the great variety of monitoring techniques available for the observation of freshwater fish populations [5, 6], tagging fish with passive integrated transponders (PIT tags) became increasingly important and evolved to the gold standard of fish monitoring when it comes to the assessment of fish bypass facilities [7]. However, the field of application for PIT tags in the monitoring of freshwater fish populations in the wild is diverse, ranging from tracking spatiotemporal habitat use to the estimation of growth rates, demographic parameters and abundances [8].

PIT tags usually allow a lifelong and individual observation of fish while requiring only minimal surgical

*Correspondence:
Juergen Geist
geist@tum.de
[1] Aquatic Systems Biology Unit, TUM School of Life Sciences, Technical University of Munich, Mühlenweg 22, 85350 Freising, Germany

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procedure [9]. Although the use of PIT tags is generally considered a relatively low invasive fish tagging technique, potentially harmful effects on the respective target species are important to know, analogously to the application of visible implant elastomers and other techniques [10]. Considering such effects is critical concerning a robust interpretation of field data, as wrong assumptions on retention rates and PIT tag-induced mortality may lead to biased and thus false management decisions [11, 12]. It is further important to reliably evaluate effects on fish vitality, particular in rare and endangered species, as tagging can be a source of stress in fishes, which has been shown to have negative effects including reduced growth and condition [13]. Changes in growth are known to have further consequences related to recruitment dynamics, survival, and the sensitivity to environmental changes [14]. There is a wealth of studies that address the applicability of PIT tagging, e.g., by analyzing the minimal, suitable fish size [15, 16] and tag bodyweight-ratios [17] as well as by comparing different application techniques and tag sizes [18, 19]. However, the great majority of these studies worked with economically important salmonid species [15–19]. Much less is known about economically less important target species of conservation, such as the rheophilic Cypriniformes common nase (*Chondrostoma nasus* L.; termed nase hereafter) and European barbel (*Barbus barbus* L.; termed barbel hereafter), which used to be very common in streams of central and eastern Europe. Both species are considered potamodromous fish with reported average home ranges of 20–35 km [20] and well-documented spawning migrations, often located in tributaries of large rivers [21–23]. Habitat loss and fragmentation are therefore considered the main cause for the steep population declines of nase and barbel [21, 24]. Consequently, conservation efforts to support these species often target the restoration of key habitats [23] and migration routes, e.g., by constructing fish bypass facilities [25, 26]. The evaluation of these

measures is increasingly based on PIT tag monitoring programs [20, 25–27], although the applicability of PIT tags on these species remains largely unknown.

To tackle this knowledge gap, this study tested the effect of commonly used 12 mm full-duplex (FDX) PIT tags implanted in the abdominal cavity of two different size classes of nase and barbel in a controlled laboratory experiment. Effects on survival, growth (both length and weight), and tag retention were studied over a period of 61 days in comparison to untagged control groups. In line with a broad range of evidence on other species, we hypothesized that (i) implanting PIT tags in the body cavity of nase and barbel does not result in reduced survival and growth compared to untagged control fish, and (ii) tag retention is higher in larger fish compared to smaller ones.

Study design

This study was conducted at the fish breeding facility of the Aquatic Systems Biology Unit (Technical University of Munich, Freising, Germany). The experimental duration was 61 days from October 20 to December 19, 2022. To test the applicability of PIT tags for nase and barbel, two size classes (termed "small" and "large" hereafter) of each species were selected, resulting in four treatment groups in total (Table 1). To minimize the number of experimental fish while retaining statistical robustness, each treatment group was replicated three times with 40 individuals, resulting in 120 fish per treatment group. Each replicate ($n = 40$ fish) was held in a separate through-flow tank. Analogously to the treatment groups, control groups were selected, consisting of 120 fish for each group, subdivided into three replicates of 40 fish each (Table 1). Tagging of treatment fish and initial data collection of weight [g] and total length [TL; mm] of treatment and control fish were conducted on the first day of the experiment. Weight and TL of all fish were

Table 1 Overview of all treatment and control groups. Initial total length [TL] and initial weight [g] are given as mean values ± standard deviation

Species	Size class	Group	ID	*n*	Initial TL [mm]	Initial weight [g]
Nase	Small	Treatment	TNS	120 (3×40)	141.7±9.0	20.3±3.8
	Small	Control	CNS	120 (3×40)	142.9±9.1	21.7±3.9
	Large	Treatment	TNL	120 (3×40)	164.6±9.8	32.6±5.5
	Large	Control	CNL	120 (3×40)	163.6±8.8	31.9±5.0
Barbel	Small	Treatment	TBS	120 (3×40)	124.2±8.1	17.7±3.1
	Small	Control	CBS	120 (3×40)	118.4±11.2	15.9±4.2
	Large	Treatment	TBL	120 (3×40)	153.2±11.2	31.7±6.9
	Large	Control	CBL	120 (3×40)	154.2±13.6	32.4±8.9

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again measured after 28 days (intermediate) and on the last day of the experiment after 61 days (termination).

Fish tagging procedure

Before tagging, fish were sorted into two different size classes for each species (nase: 110–150 mm, 151–180 mm; barbel: 110–140 cm, 141–180 mm) and visually assessed for their health status. Only healthy fish were used for the experiment. Small batches of 20 fish were anesthetized simultaneously with MS222 following the recommended dose for cyprinids in Adam et al. [28]. Anaesthetized fish were tagged with 12 mm 134.2 kHz FDX tags (Biomark, Boise, USA) in the abdominal cavity close to the posterior end of the pelvic fin (see Fig. 1 for detailed tagging position). Fish tagging was performed at a water temperature of 12.3 °C. To minimize the risk of contamination and infection during tag injection, separate pre-loaded needles attached to an implanter gun (Biomark, Boise, USA) were used individually for each fish. After determining the PIT tag ID using a handheld HPR light reader (Biomark, Boise, USA), fish weight was determined to the nearest 0.1 g with an electronic scale (Ohaus Scout SPX), and fish were measured to the nearest millimeter using an electronic measuring board (Biomark, Boise, USA). All measuring devices were connected via Bluetooth to a tablet, and data were automatically stored in a digital database. This setup allowed a quick tagging procedure and data collection, which took 10–15 s for each fish individual. Fish of the control

groups were treated likewise, without receiving needle puncturing and PIT tag injection. The same experienced person tagged all fish.

Rearing and monitoring

Rearing of fish was conducted in a flow-through system consisting of 12 separate tanks (GRP, internal dimensions: $3 \times 0.7 \times 0.7$ m, external dimensions: $3.1 \times 0.8 \times 0.9$ m; Aquacultur Fischtechnik GmbH, Nienburg, Germany). Water was supplied directly from the Moosach River without recirculation to mimic natural exposure conditions. The through-flow was set to 0.5 l/s and water depth in each tank was 30 cm, which corresponds to a water volume of ~ 630 l per tank. Flumes were covered with fabric-lined frames to minimize fish stress. Eighty fish were held in each tank, comprising a mix of 40 barbel and 40 nase of the same size class and treatment (tagged or control). Pre-trials revealed that both species could be reared in the same tank without negative effects on food uptake and survival. Fish were fed daily with commercial diet pellets for cyprinids (Intensive 2 mm Alltech Coppens; content = 40% protein, 10% fat, 1.4% crude fiber, 6.1% ash, 1.10% total P). Food was dispersed slowly and evenly during daylight hours with automatic feeders. A daily feed dose of 1.0% of total fish weight was applied for the first four weeks of the experiment. Subsequently, this dose was reduced to 0.5% of total fish weight for the remaining duration of the experiment, accounting for the relatively low and decreasing water temperatures during
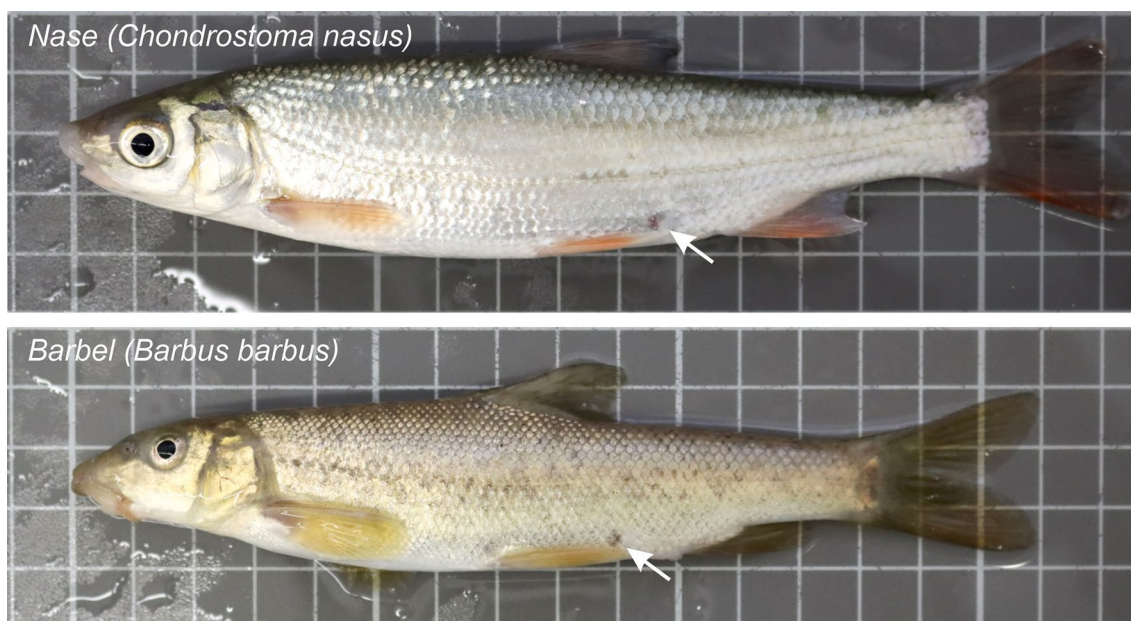

Fig. 1 Photographs of tagged nase (total length: 178 mm) and barbel (total length: 157 mm) taken at the intermediate measurement after 28 days post-tagging. White arrows indicate the point of tag injection

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this time of the year. Two days before the intermediate measurements of weight and length on day 28 and at the termination of the experiment on day 61, feeding was stopped. Cleaning of the experimental tanks was performed three times a week.

Fish were visually inspected each day, dead fish were immediately removed and, if tagged, scanned with a handheld reader to allow individual assignment. During the daily inspections, each tank bottom was checked for shed tags, which were also scanned and recorded for an individual assignment. After 28 as well as 61 days, fish were again anesthetized, measured, and weighted, and fish in the treatment tanks scanned for retained PIT tags. The scar of each tagged fish was also checked for signs of inflammation or infection. No signs of inflammation or infection were observed in the area of tag injection in any of the tagged fish, both at the intermediate measurement and at the termination of the experiment. Treatment fish that lost their tag were noted, removed from the experimental tank, and identified by comparing initial tag IDs in each tank with corresponding IDs from days 28 and 61.

Abiotic variables in each experimental tank were documented every 2–4 days. Temperature [℃], electric conductance [µS/cm, related to 20 ℃], oxygen content [mg/L], and pH were measured using a handheld multiprobe meter (Multi 3430, WTW, Weilheim, Germany). Abiotic measurements throughout the experimental period showed a mean water temperature of 9.9 ± 2.2 ℃, a mean oxygen concentration of 9.9 ± 2.2 mg/L, a mean electric conductance of 757 ± 7 µS/cm, and a mean pH of 8.1 ± 0.1. Turbidity [NTU] was measured using a Photo-Flex Turb handheld field measurement unit (WTW) and revealed a mean of 5.6 ± 1.9 NTU.

Data analysis

Survival rates were calculated separately for each experimental flume as the percentage of fish alive in treatment and control groups at the termination of the experiment in relation to the respective initial numbers of fish at the start of the experiment. Growth rates (both length and weight) were determined for each experimental flume and the two periods of the experiment separately, by subtracting the initial mean values from mean values at the intermediate measurement (period 1) and by subtracting the mean values at the termination of the experiment from those at the intermediate measurement, respectively (period 2). Tag weight (0.1 g) was deduced from the weight of all tagged fish prior to further analysis. Dead tagged fish were excluded from the dataset and only used for the calculation of survival rates. Dead fish from control groups could not be individually assigned and therefore not be excluded. Tag retention was analyzed separately for each experimental flume as the number of fish that retained their tag at the termination of the experiment related to the number of fish initially tagged. Survival and retention rates were visualized in cumulative curves. The development of growth rates was displayed separately for each group and experimental flume as mean values, including standard deviation as whiskers. All figures were computed in R [29] using the tidyverse [30] and ggbump [31].

Univariate statistics were used to test for differences in survival and growth between treatment and the respective control groups and for differences in tag retention between the two size classes. Prior to the statistical significance tests, Shapiro–Wilk and Levene tests were applied to check for normal distribution and homogeneity of variances. In case requirements for parametric testing were met, differences in mean values of each experimental flume for survival and growth rates between tagged and control groups as well as tag retention between the two size classes were tested with t-tests. If requirements for parametric testing were not met, pairwise comparisons were tested with Kruskal–Wallis tests. To not only account for differences in growth rates, length–weight relationships were analyzed. Given the different data structure, this dataset's assumptions of normality and homoscedasticity were visually tested in diagnostic plots using the package ggfortify [32]. For an easier analysis with linear regression, the allometric data were log-transformed. The regression coefficients estimated from the log-transformed data were used to compare the relative condition of treatment and control group. To test for any differences in the regression lines, an analysis of variance (ANOVA) was used. The ANOVA can control for the effects of a differing size range.

All statistical analyses were computed in R [29]. Significance levels were set to $p < 0.05$.

Results

Survival

Survival was very high across all groups. Including treatment and control groups - a total of 14 fish died during the experiment ($\sim 1.5\%$), resulting in an overall survival of 98.5%. In treatment groups, survival was equally high (96.7–100%) as survival in the control groups (97.5–100%). Highest mortality occurred in small tagged barbel ($n = 4$; 96.7% survival), while no mortality occurred in large tagged nase. All mortalities in the treatment groups occurred within the first 28 days after tagging (Fig. 2). In the second half of the experiment (day 29–day 61), only two fish from the control groups died. Mortality was slightly higher in small fish of treatment groups (small: 2.1%; large: 0.4%), while no differences were observed in control groups (small: 1.3%; large: 1.3%). No statistical
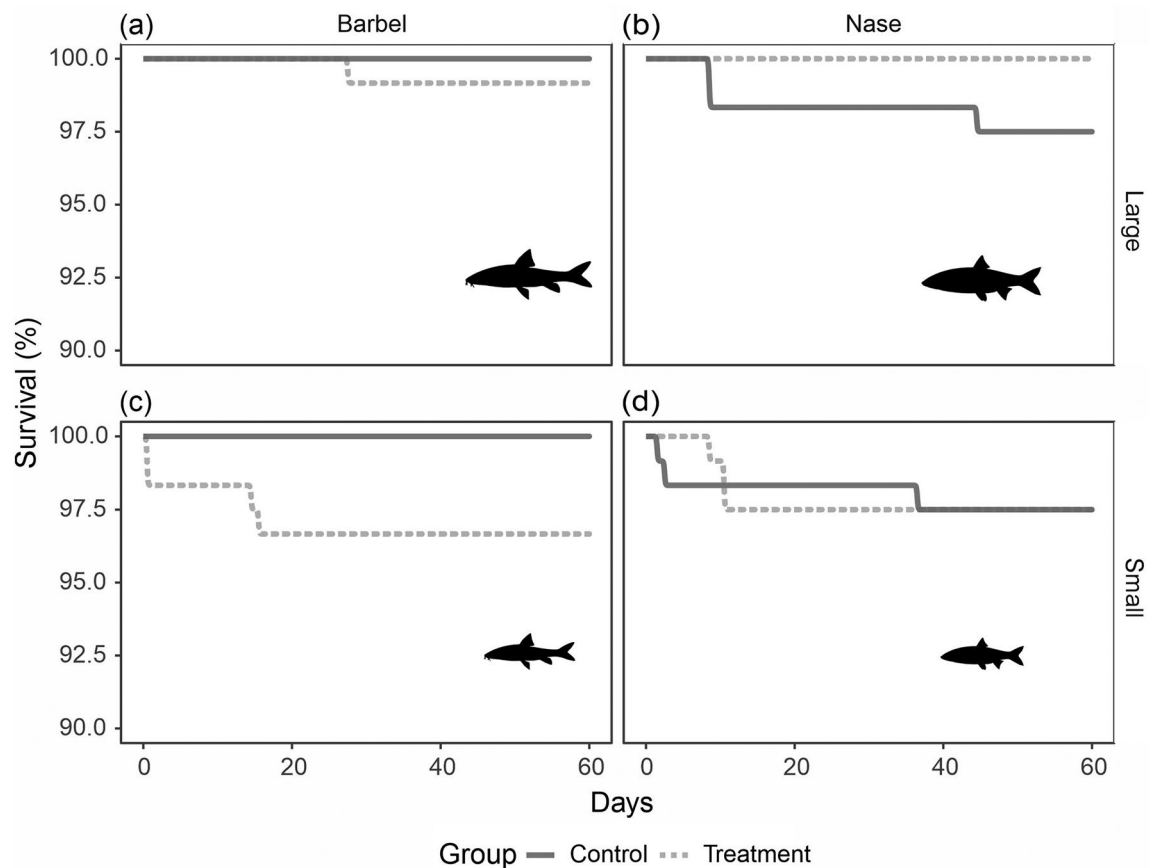
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Fig. 2 Cumulative survival rates for tagged and control groups of small and large barbel (**a**, **c**) and small and large nase (**b**, **d**) between the start of the experiment (day 0) and its termination (day 61). Tagged groups are represented by dashed light grey lines, control groups by solid dark grey lines

differences were found in survival rates between the tagged and the respective control groups (see Additional file 1 for results of pairwise comparisons).

Growth

At the termination of the experiment, mean growth rates were slightly lower in tagged fish (weight = 3.26 g; TL = 2.28 mm) compared to control fish (weight = 3.59 g; TL = 2.77 mm), but this trend was not statically significant (weight: $p = 0.71$; TL: $p = 0.30$). In pairwise comparisons of growth rates between tagged groups and the respective control groups, no differences were observed in terms of gained weight (Table 2). Significant differences were only found for TL of large nase (difference = 0.8 mm) and barbel (difference = 0.7 mm) in period 1 (day 0–day 28), which showed significantly higher growth rates in tagged fish (nase = t-test: t = − 5.00; *d.f.* = 2.74; p < 0.05; barbel = t-test: t = − 3.09; *d.f.* = 3.29; $p < 0.05$; Table 2, Fig. 3). Moreover, analysis of length–weight relationships revealed no differences between treatment and respective control groups at the

intermediate measurement and the termination of the experiment (Additional file 2). In both treatment and control groups, small fish showed lower growth rates compared to larger fish and reduced growth in period 1 compared to period 2 (Table 2, Fig. 3).

Length–weight relationship

The linear models that were fitted to the log-transformed length, and weight data did not show significant differences between the control and the treatment groups (Fig. 4). Alpha and beta values of the control and treatment groups of the regressions were close together at all the measuring points (Additional file 2) and showed little variation throughout the experiment.

The ANOVAs for the length–weight relationship for small, and large fish throughout all measurements showed no statistically significant difference ($p > 0.05$; Additional file 2) between the control and the treatment groups over all measurement points and all size classes.

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Table 2 Growth rate for tagged and control fish given as total length [TL] and weight [g] between the start of the experiment (day 0) and the intermediate measurement (day 28) as well as between the intermediate measurement (day 29) and the termination of the experiment (day 61)

Species	Size class	Group	ID	Growth [g] day 0–day 28	Growth [g] day 29–day 61	Growth [mm] day 0–day 28	Growth [mm] day 29–day 61
Nase	Small	Treatment	TNS	0.8 ± 0.4[a]	1.4 ± 0.4[a]	0.3 ± 0.4[a]	0.4 ± 0.3[a]
	Small	Control	CNS	0.6 ± 0.3[a]	2.0 ± 0.3[a]	0.2 ± 0.4[a]	1.7 ± 0.9[a]
	Large	Treatment	TNL	3.8 ± 0.2[a]	2.2 ± 0.3[a]	0.9 ± 0.2[a]	1.7 ± 0.5[a]
	Large	Control	CNL	3.6 ± 0.6[a]	2.9 ± 0.7[a]	0.1 ± 0.1[b]	3.0 ± 1.2[a]
Barbel	Small	Treatment	TBS	0.3 ± 0.1[a]	0.5 ± 0.3[a]	0.8 ± 0.2[a]	1.8 ± 0.5[a]
	Small	Control	CBS	0.1 ± 0.0[a]	1.1 ± 0.3[a]	0.8 ± 0.3[a]	2.4 ± 0.3[a]
	Large	Treatment	TBL	2.7 ± 0.3[a]	1.3 ± 0.3[a]	1.5 ± 0.3[a]	1.8 ± 0.6[a]
	Large	Control	CBL	2.6 ± 0.2[a]	1.4 ± 0.4[a]	0.8 ± 0.5[b]	1.9 ± 0.5[a]

Values are given as means ± standard deviation. Different small uppercase letters (a, b) indicate significantly different groups based on statistical testing of pairwise comparisons of treatment groups and the respective control groups

Retention

A total of 5 fish out of 480 shed their tag, resulting in an overall retention rate of 99.0%. Tag retention differed between species and size classes and ranged from 96.7% in large nase (TNL), to 99.2% in small nase (TNS) and 100% in both small and large barbel (TBS, TBL). Although tag loss was slightly higher in large nase compared to smaller ones, no statistical difference was found for this comparison ($p = 0.24$). All tag rejections occurred within the first 28 days of the experiment, from which 4 of 5 shed tags were detected at the intermediate control after 28 days post-tagging (Fig. 5).

Discussion

The results of this experiment indicate that implantation of 12 mm PIT tags into the abdominal cavity of sub-adult nase and barbel is well suited as a tagging technique for these species, as it had no detrimental effects on survival and growth compared to the untagged control groups while achieving high retention rates. This adds further important target species of conservation to the existing body of knowledge on the applicability of PIT tags [see e.g., 12, 16, 18, 19, 33, 34].

The high survival rates of nase and barbel observed in this study are consistent with findings from Bolland et al. [34] for similar sized Cypriniformes chub (*Squalius cephalus* L.), dace (*Leuciscus leuciscus* L.), and roach (*Rutilus rutilus* L.), which were tagged in a comparable approach. Mortality in our study was highest in the first days post-tagging, and no tagged fish died after 28 days post-tagging. This observation is in line with several other studies on salmonids, in which 80–90% [16, 18] of all mortalities occurred in the first weeks after tag implantation, indicating that long-term PIT-related survival effects are unlikely and that an observation period of ~60 days is suited to detect survival effects sufficiently. Moreover, the higher survival rates of untagged barbel (100%) compared to untagged nase (97.5%) may indicate a higher sensitivity of nase to handling stress, which was also demonstrated in other experimental studies by Pander et al. [35], who detected higher handling susceptibility of nase during a standardized catch efficiency experiment in the context of fish damage at hydropower stations. Yet, this effect was not visible in tagged fish (nase: 98%, barbel 97.5%), demonstrating that the tested tagging approach is equally suited for both species.

No negative effects of PIT tagging on the growth of nase and barbel were observed in this study, which is in concordance with findings from Acolas et al. [15], who did not find any significant effect on the growth of juvenile brown trout (*Salmo trutta* L.) 27 days post-tagging. In contrast, Richard et al. [16] revealed a lower TL and weight for small brown trout, which was explained by a relatively high tag–bodyweight ratio of 6.3% in air, exceeding the recommended threshold value of 2% by Winter [36]. Minimizing adverse effects of tagging on growth is particularly critical in endangered species as impaired growth can reduce fecundity and may thus result in recruitment problems of tagged fish [14]. Growth rates were higher in the experiment's second period than the first, which was especially notable in small fish (both control and treatment). A similar observation was made in other studies, which documented a slightly reduced growth rate in the days following PIT implantation, which was compensated in the following weeks [37–39]. Since our study observed this pattern also
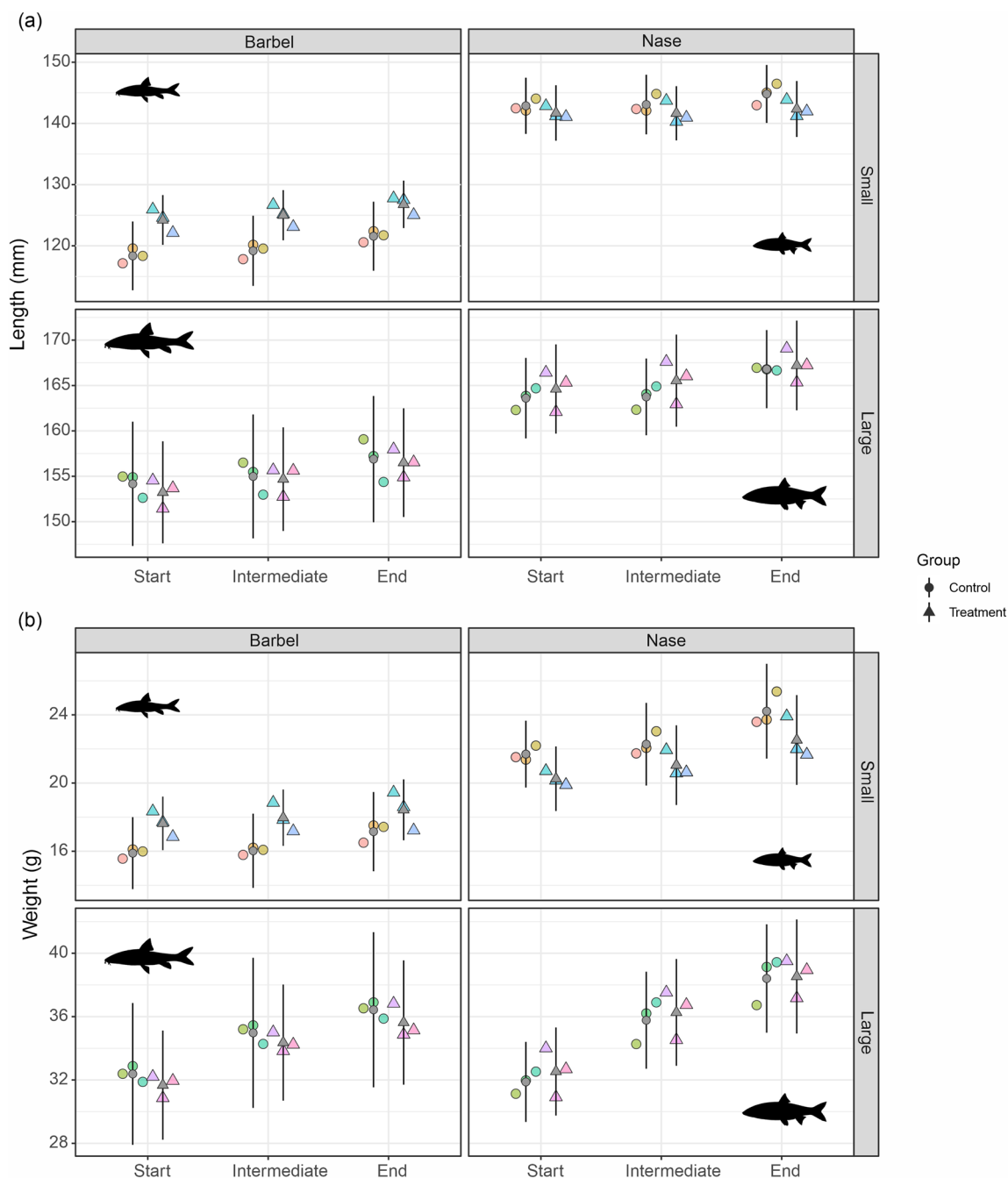
Fig. 3 Time line analyses of total length (**a**) and weight (**b**) for tagged and control groups of small and large barbel (left) and small and large nase (right) between the start of the experiment (day 0), the intermediate measurement (day 28) and the termination of the experiment (day 61). Each colored symbol indicates the mean value of a separate experimental flume; the range is indicated with whiskers. Treatment groups are marked with dots, control groups are marked with triangles

in control fish, it is likely that this effect was caused by the change in rearing conditions rather than by tag injection.

Retention rates were very high and comparable to those revealed for other Cypriniformes tagged in the body cavity by Bolland et al. [34]. Similar to the

observed mortality rates, tag loss occurred preliminarily in the first 4 weeks after tagging, which concurs with Richard et al. [16]. This study also reported that retention of 12 mm PIT tags decreases with fish size [16]. However, our study could not observe such an
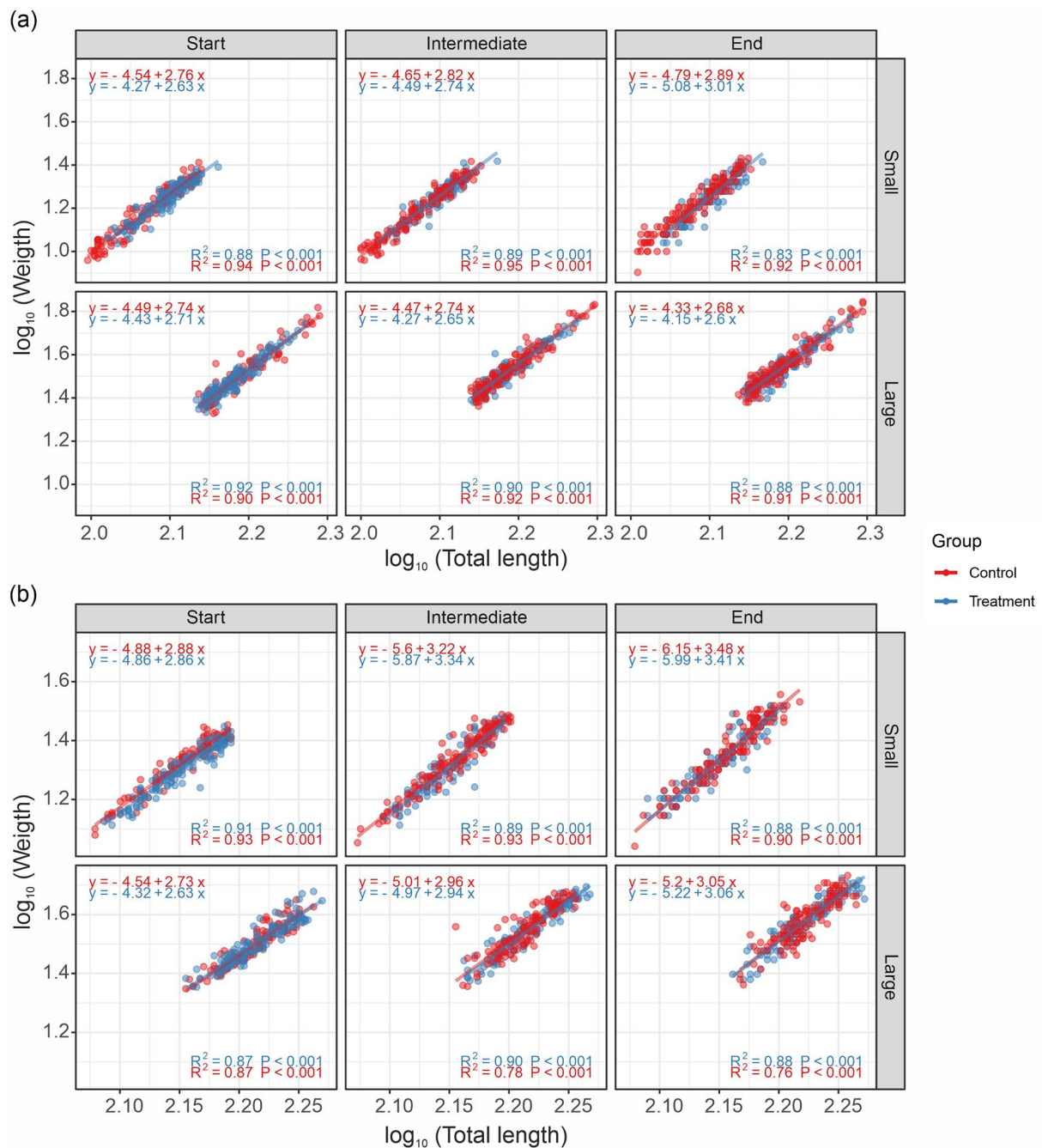
Fig. 4 Length–weight relationship of small and large barbel (**a**) and nase (**b**) comparing control (red) and treatment (blue) groups for the different points of measurements (start, intermediate, and end)

effect, indicating that the threshold for increased shedding rates is below 100 mm TL. As evident from our data, maintaining of tagged fish for a few weeks after tagging will largely minimize the risk of tag loss. This is probably most relevant if hatchery-reared fish are to be marked. However, it is very likely that retention rates

in the wild are lower than those observed in a laboratory environment. A study by Dieterman and Hoxmeier [40] suggests that tag expulsion rates are closely related to swimming effort, which increases with higher flow velocities. Since both target species of our study, nase and barbel, have a strong preference for habitats with medium to high current (classified rheophilic according

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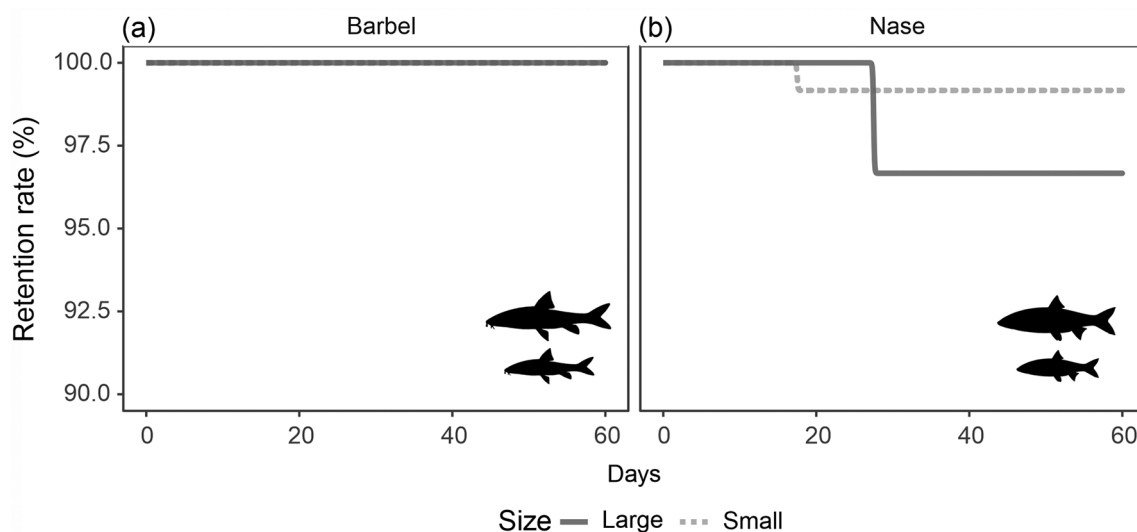
Fig. 5 Cumulative tag retention rates for tagged groups of small and large barbel (**a**) and small and large nase (**b**) between the start of the experiment (day 0) and its termination (day 61). Treatment groups of small fish are represented by dashed light grey lines, treatment groups of large fish by solid dark grey lines

to Zauner and Eberstaller [41]), tag expulsion in the wild might be higher than those observed in our study. Furthermore, results from the Cypriniformes species asp (*Leuciscus aspius* L.) indicate that even in cystovarian species, tags may be expulsed during spawning, which may particularly increase tag loss in females [12].

Conclusions

Studies on the suitability of PIT tags for Cypriniformes are scarce (but see [12, 34, 42]), compared to the wealth of knowledge on their applicability to salmonid species [16, 18, 19], highlighting the need to fill this knowledge gap. The results obtained in this study demonstrate the suitability of PIT tagging in the body cavity of nase and barbel > 100 mm TL, which is of high methodological importance given the increasing role these species play in PIT tag-based assessments of fish bypass facilities in European rivers [20, 25–27]. The evaluated tagging approach may also contribute to the monitoring of supportive breeding initiatives, which constitute an increasingly used tool to support weak populations of nase and barbel, although its contribution to the conservation of these species remains largely unknown [43, 44].

Abbreviations
ANOVA   Analysis of variance
FDX   Full-duplex
PIT   Passive integrated transponder
TL   Total length

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40317-023-00344-z.

Additional file 1: Results of statistical testing of survival rates in pairwise comparisons between treatment and the respective control groups. Note: Only survival data of small nase met the requirements for parametric testing.

Additional file 2: Results of ANOVA testing of length-weight relationships in comparisons between treatment and the respective control groups at the three assessed time points (start, intermediate, end).

Availability of data and materials
Data are available from the corresponding author upon reasonable request.

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Declarations

Ethics approval and consent to participate
All the work conducted in this study passed an intense ethical review and was authorized by the government authorities (government of Upper Bavaria—field 54—consumer protection, veterinary affairs) under license ROB-55.2-2532.Vet_02-21-173. The study design was chosen with a concern for using a minimal number of fish while maintaining statistical robustness. Tagging procedure was applied in close consultation with experts from MSD Animal Health. Separate, pre-loaded needles were used for each individual fish, and fish were held and reared in low densities. The same trained and experienced person tagged all fish.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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