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Survival and Swimming Performance of Small-Sized Gobiidae Implanted with Mini Passive Integrated Transponders (PIT-Tags)

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Abstract: Telemetry techniques are important tools in freshwater fish ecology but are limited by the size of the fish in relation to the size of the electronic tags. The emergence of very small PIT tags (8 mm, mini PIT tags) opens the door to study the individual movement and behavior of small-sized fish species and life stages previously outside the scope of fish telemetry. Although high survival from mini PIT tags have been shown in some groups of fish, suitability assessments are lacking for many taxa, and potential behavioral effects have rarely been evaluated. Here, we evaluate the survival tagging effects in small-sized (35–76 mm) Padanian goby (*Padogobius bonelli*) implanted with mini PIT tags. PIT-tagging was associated with high survival and tag retention in the tagged fish. No effects of PIT-tagging on volitional swimming activity nor on maximum swimming speed were found. Similar results were obtained implanting larger tags (12 mm) in gobies down to 50 mm in length. Our results indicate that PIT telemetry—using mini PIT tags—is applicable for the study of behavior and movement in small-sized gobies.

Keywords: fish telemetry; passive integrated transponders; tagging effects; goby; escape response; open field test

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1. Introduction

Telemetry techniques are widely used to study the movement and behavior of individual animals. The methodology is, however, limited by the size of the animal in relation to the size of the electronic tags [1,2]. For small animals, Passive Integrated Transponders (PIT tags; 7–32 mm) are commonly used. PIT tags transmit a unique ID code when activated by the electromagnetic field of a reader antenna. Although the detection range is typically short (<1 m), the tags last indefinitely, and the technology is effectively used to identify recaptured animals or track free-roaming animals with stationary or mobile antennas [3,4]. In fish ecology, PIT tags are used, for example, to study survival [5], growth [6], habitat use [7,8], home range [9], migration patterns [10,11], activity [12], fish passage performance [13–15], and the effects of habitat restoration measures [8,16]. Thus, they constitute an important tool both for fundamental and applied research [17].

In animal telemetry, a fundamental assumption is that the performance of a tagged animal is not substantially different from that of a similar untagged individual [17,18]. For fish, a 2% tag-to-fish weight ratio, originally based on the swim bladders capacity to compensate for the tag burden, is often used as a rule of thumb to avoid tagging effects [19–21]. For salmonids, a meta-study recommends a tag-to-fish length ratio of 17.5% or lower to avoid effects on growth and survival [22]. Loosely applying these rules of

thumb, high survival and tag retention are typically achieved, but high tagging mortalities, at least under some conditions, have been observed in some species [23,24]. Hence, ideally, the tagging of new fish sizes or species should be preceded by evaluations of potential tagging effects [25]. Another research gap concerns potential effects of emerging mini tags, i.e., ≤8 mm tags [26]. Also, sub-lethal effects, including behavioral changes, while ecologically important, are much less studied [27].

Fish swimming involves both behavior and capability, and is central for the ecology of fish, shaping movements, reproduction, and predator–prey interactions [28,29]. The effects of PIT tags on fish swimming performance have been tested in swim chambers, flumes, or through provoked escape responses in standing water. No effects on prolonged (time to fatigue) or burst swimming are reported for Cypriniformes [30,31], salmonids [32], lampreys [33], bullheads [34], and loaches [27]. Further, no tagging effects on volitional swimming activity (distance moved in an open field test) in spined loaches [27] and small-sized Cypriniformes [31] have been reported. On the other hand, an effect of PIT tags on the prolonged swimming performance, but not on maximum burst speeds, has been reported for tagged Italian riffle dace [35].

The emergence of very small PIT tags (≤8 mm, mini PIT tags) opens the door to study individual movement and behavior of small-sized (35–100 mm) fish species and life stages previously outside the scope of fish telemetry [36,37]. The suitability of mini PIT tags (8 mm tags) for small-sized fish has been studied to some degree, mainly in pelagic or actively swimming species, and often with relatively modest sample sizes. High survival and tag retention have been reported for darters [26], salmonids [36,38], catfishes [24], and several *Cypriniformes* fish [25,39]. For shiners (*Cypriniformes*), however, both high and low survival, and low tag retention rates have been observed [40,41]. Survival and the potential effects of mini PIT tags on swimming performance has been studied in topminnows, Asiatic glassfishes, silversides, sleeper gobies, and a set of Australian *Perciformes* fish, showing no difference between tagged and control fish [23,24]. Regarding benthic fish, mottled sculpins (*Cottus bairdii*) displayed high survival and tag retention rates and no effect of tagging on recapture probabilities in nature [39]. In banded sculpin (*Cottus carolinae*), on the other hand, survival was low in small fish while relatively high in larger fish [42].

Gobies (*Gobidae*) are a diverse family of mainly small-sized, typically benthic, fish spread across the whole world, in both freshwater and in the marine environment. Gobies display elaborate parental care, constitute important predator and prey species, sometimes partake migrations, and include species of concern both for conservation and invasion biology [43]. High survival and tag retention after PIT-tagging (12 mm tags) has been observed in the relatively robust (median size of groups 67–132 mm), and highly invasive, round goby (*Neogobius melanostomus*; [44], and this species has subsequently also been tracked in a semi-natural environment [45]. Regarding smaller tags, survival was evaluated for naked goby (*Gobiosoma bosc*; 40–56 mm SL), a marine benthic fish: a quarter of tagged fish died within two weeks of tagging [46]. This study, however, lacked a non-tagged control, making it impossible to distinguish holding from tagging mortality and leaving the feasibility of tagging small-sized gobies an open question.

The Padanian goby (*Padogobius bonelli*, Bonaparte, 1846) is a relatively small-sized (typically 7–8 cm) riverine goby. It is a benthic species found in streams with moderate flow, with the presence of pebbles. The species display territorial behavior for feeding and spawning purposes, competing aggressively for control of the pebbles, which are used as shelters for avoidance behavior and as substrates for eggs [47,48]. The species is native to Padano-Venetian district in northern Italy and classified as Least Concern on the IUCN Red List [47,49]. It is morphologically similar to the smaller and endangered Italian spring goby (*Orsinigobius punctatissimus*) [47]. As a preparation for field studies on both Padanian goby and Italian spring goby, we here evaluate the effects of the surgical implantation of mini PIT tags on the survival of small-sized (35–76 mm TL) Padanian goby. To evaluate the potential behavioral and performance effects, we compared volitional swimming activity (i.e., distance moved in an open field test) and maximum swimming speed in a subsequent

escape response test between tagged and control fish [27,31]. For larger fish (>50 mm), survival and behavioral effects were also evaluated for fish tagged with 12 mm PIT tags. Tag retention proportions were quantified for both tag sizes.

2. Materials and Methods

Padanian goby (n = 272) were caught in the Lemme River (Italy) using wading electrofishing (direct current; ELT60IIGI, Scubla, Remanzacco, Italy) on 19 March 2024 (UTM 478725E, 4952707N, zone 32T) and brought to the Alessandria Province Hatchery (Predosa, Italy). Fish were left to recuperate overnight and tagged the following day.

2.1. Tagging

Fish were tagged with 8 mm (8.4 mm × 1.4 mm, 0.03 g) or 12 mm (12 mm × 2.1 mm; 0.10 g) passive integrated transponders (PIT tags; Biomark, Boise, USA). Before tagging, fish were anesthetized in clove oil (Aromalabs, Lacour, France; approximately 0.2 mL clove oil/L water). Fish smaller than 50 mm were randomly assigned to either a tagging (8 mm tag) or control group, while fish larger than 50 mm were tagged with 8 mm tags, 12 mm tags, or assigned to a control group. The tagging procedure consisted of making a 2–3 mm incision on the ventral side of the fish, offset slightly from the center and anterior to the pelvic fins, after which the tag was pushed in and forward in the abdominal cavity to align with the fish's body [35,50]. The tagger was aided by magnifying glasses. Fish were then weighed and measured for fork length before being left to recover in aerated tanks. Control fish were subject to the same anesthetic treatment but were only measured and weighed before being left to recover in the aerated tanks. Among fish smaller than 50 mm, 76 were tagged with 8 mm tags and 76 were control fish. For fish 50 mm long or larger, 38 were tagged with 8 mm tags, 42 were tagged with 12 mm tags, and 40 subject to control treatment (Table 1). There were no differences in length or weight between treatment groups for small (Wilcoxon Mann–Whitney test, p = 0.56) or large (Kruskal–Wallis test, p = 0.81) fish. A sham control, performing surgery without inserting a tag, was not included in the study design as the focus was on differences between tagged fish and untagged conspecifics.

Table 1. Sample size and length and weight distributions of the tagged and control small (<50 mm) and large (≥50 mm) Padanian gobies included in the study.

Size	Group	*n*	Length (mm)				Weight (g)			
			Median	IQR	Min	Max	Median	IQR	Min	Max
Small	8 mm	76	45	41–47	35	49	0.9	0.7–1.1	0.3	2.2
	control	76	44	41–46	35	49	0.9	0.7–1.0	0.4	1.4
Large	8 mm	38	57	53–64	50	76	2.3	0.8	1.3	4.4
	12 mm	42	57	54–60	50	73	2.0	0.9	0.8	4.1
	control	40	57	53–61	50	76	2.2	1.0	1.1	5.8

2.2. Fish Holding

After having recovered from anesthesia, fish were placed in a spring-fed flow-through tank (length × width × depth = 200 cm × 200 cm × 60 cm). An array of shelters (perforated bricks and shingles) was present in the holding tank. Temperature was stable at 14 ± 0.5 °C (mean ± sd) and light condition followed the natural cycle (ceiling lighting and windows in the hatchery). Fish were regularly fed with wild caught macroinvertebrates and cultured freshwater *Daphnia magna*. The tank was inspected for dead fish every 1–3 days (shelters were only moved once a week to reduce the risk of injury to the experimental fish). At the end of the experiment, remaining fish were caught, scanned for tags, inspected, and measured for length. The tank was emptied and thoroughly checked for lost tags.

2.3. Behavioral Tests

Potential tagging effects on fish swimming behavior were tested using two subsequent tests. An open field test quantified volitional swimming behavior (activity) while a provoked escape response test was used for quantifying maximum swimming speed. The former test concerned mainly swimming in the sustained mode, while the latter constituted a burst swimming test [31]. On 17–19 April (28–30 days after tagging), a random subset of fish was tested for volitional activity and maximum swimming speed in an arena trial. Individual fish were gently netted from the holding tank, and placed into an experimental arena (length × width × depth = 565 mm × 365 mm × 100 mm). Fish were left in the arena for five minutes to habituate to the new environment [51], while the following five minutes were allocated to an open field test [27,52,53]. After this time, ten minutes after having been introduced to the experimental arena, an escape response was provoked by dropping a spherical weight near 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 provoked by dropping another spherical weight near the fish. In total, three escape responses were provoked [27,34,54]. After stopping for the third time, the fish was netted, anesthetized, checked for presence of a tag, and measured for fork length. Any sign of tag loss (i.e., a scar without tag detection) was noted, and this fish was excluded from the subsequent analysis. Four trials were run in parallel. Water temperature was measured in a separate tank, identical to the test tank, and water was changed regularly to maintain a stable temperature across all trials.

The experimental arena was video recorded with an overhead camera (Sony 4K, FDR-AX43, 50 fps, Minato City, Tokyo, Japan). Video recordings were then used to track fish. For the open field test, videos were trimmed to obtain the five-minute open field test recording. TREX (version 1.1.9, August 2022), a software designed to track individual moving objects using computer vision and machine learning, was then used to generate fish trajectories during the open field test [55]. The entire tracking process consisted of two phases. First, the video was segmented into background and foreground objects (blobs) and results were saved into a non-proprietary video format (PV) file. Next, the fish was tracked by following its movement across frames, estimating future positions based on previous velocity and angular speed [55]. The tracking data were saved in a CSV file with columns containing frame number and fish body centroid position in X and Y coordinates. As a measure of volitional swimming activity, total distance moved during the five minutes of the open field test was quantified. For the provoked escape response test, water surface disturbances did not allow automatic tracking. Instead, a manual tracking MATLAB (R2021b; The Math-Works Inc., Natick, MA, USA) script (https://github.com/SilverFox275/manual-point-tracking) (accessed on 1 April 2024) was used to track fish positions at 10 frames per second. Distances in pixels was transformed in to distance in meters using the known dimensions of the arena. Maximum swimming speed was estimated by extracting the fastest 400 ms during the provoked escape response [27,34,54]. The swimming speed was normalized to the length of the fish, as maximum swimming speed typically scales with fish length [56].

2.4. Statistics

As most of the data did not fulfill the assumptions for parametric tests, the difference between groups were compared using Wilcoxon Mann–Whitney tests (small fish) and Kruskal–Wallis tests (large fish). To test for potential effects of fish size, the correlation between fish length and behavioral score was tested with Spearman's rank correlation separately for fish tagged with 8 mm and 12 mm tags. The failure proportion (observed mortality + tag loss) between tagging groups was compared using Fisher's exact test [57] with Bonferroni correction for multiple comparisons between groups.

3. Results

3.1. Survival and Tag Retention

During the course of the experiment, three large fish (≥50 mm) were found dead: one tagged with 8 mm tag (2.6% mortality) and two tagged with 12 mm tags (4.7% mortality). No small fish nor any control fish were found dead during the experiment (Table 2).

Table 2. Number and proportions of mortalities and tag losses in the treatment groups. Failure proportion represents the combination of tag losses and mortalities in the given group.

Size	Group	*n*	Dead (*n*)	Mortality (%)	Loss (*n*)	Loss (%)	Failure (%)
Small	8 mm	76	0	0	3	3.9	3.9
	control	76	0	0	-	-	0
Large	8 mm	38	1	2.6	0	0	2.6
	12 mm	42	2	4.7	4	9.5	14.3
	control	40	0	0	-	-	0

Seven tags (four 12 mm tags and three 8 mm tags) were found at the bottom of the tank at the end of experiment. All three 8 mm tags belonged to small fish (<50 mm). This corresponded to a tag loss of 3.9% among small fish tagged with 8 mm tags and 9.5% for fish tagged with 12 mm tags. Scars, indicating tag loss, were observed on three fish without tags. By the end of the experiment, five fish were not accounted for: one tagged fish (8 mm tag, small fish) and four control fish (or fish having lost their tag). These fish were potentially lost during the transfer to the tank, escaped through the exit pipe of the holding tank, jumped out of the tanks at some point of the experiment, or died and was eaten by the other fish. Given their low number and the uncertainty of their fate, these fish were ignored in the calculation of mortalities and failure proportions.

Among the small fish, no difference in failure proportion was detected between tagged and control groups (Fisher's exact test, $p = 0.25$). For large fish, although a substantially higher proportion of fish tagged with large tags lost their tag or died, the differences in failure proportions were not statistically significant between any of the three groups (Fisher's exact test, $p > 0.08$).

3.2. Behavioral Tests

A random subset of large and small fish was tested for tagging effects on volitional swimming activity and maximum swimming speed. For the small fish, 61 tagged fish (8 mm tag) and 74 control fish were tested. Among the large fish, 33 fish tagged with 12 mm tags, 35 fish tagged with 8 mm tags, and 30 control fish were tested. There was no difference in length between the groups of small (Wilcoxon Mann–Whitney, $p = 0.59$) nor large (Kruskal–Wallis, $p = 0.71$) fish.

Fish moved on average 5.9 m (median, IQR = 4.1–7.1 m; Figure 1) during the open field test. Swimming activity did not differ between tagged and control fish among small (Wilcoxon Mann–Whitney, $p = 0.09$) or large (Kruskal–Wallis, $p = 0.28$) fish (Figure 1). No correlation between fish length and maximum swimming speed was seen for fish tagged with 8 mm (Spearman, $p = 0.41$) or 12 mm tags (Spearman, $p = 0.40$).

The median maximum swimming speed in the provoked escape response test was 11.6 BL/s (IQR = 9.8–14.4 BL/s; Figure 2), corresponding to absolute maximum swimming speeds of 0.57 m/s (IQR = 0.46–0.71 m/s). No difference in the maximum swimming speed between tagged and control fish among the large (Kruskal–Wallis, $p = 0.29$) or small (Wilcoxon-Mann–Whitney, $p = 0.59$) fish was detected (Figure 2). No correlation between the fish length and maximum swimming speed was seen for fish tagged with 8 mm (Spearman, $p = 0.63$) or 12 mm tags (Spearman, $p = 0.10$).

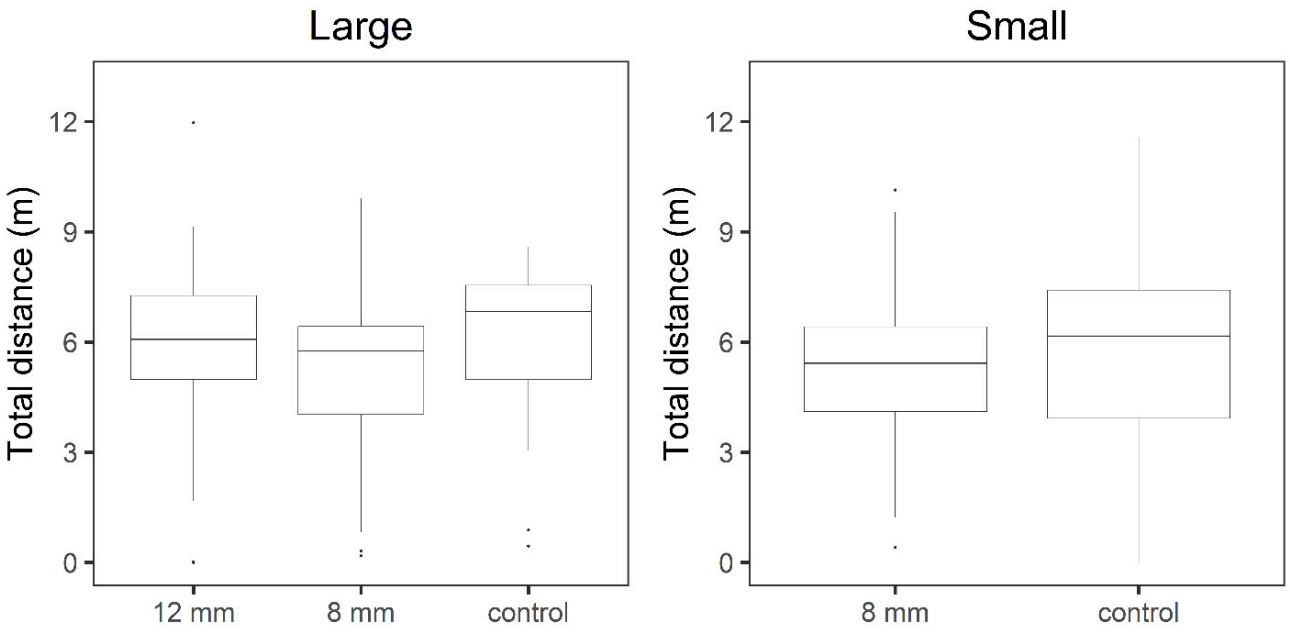


Figure 1. Swimming activity as total distance moved (m) in the open field test for large fish (≥50 mm; (**left**)) tagged with 12 mm tags, 8 mm tags, or belonging to the control group and small fish (<50 mm; (**right**)) tagged with 8 mm tags or belonging to the control group. The solid black horizontal line inside the bounding box is the median of total distance moved. The black dots represent the outliers, whereas the bounding box defines the Interquartile Range (IQR). The vertical solid black lines mark Q1–1.5·IQR (bottom end) and Q3 + 1.5·IQR (top end), where Q1 and Q3 are the 25th and 75th percentiles, respectively. Dots represent outliers.

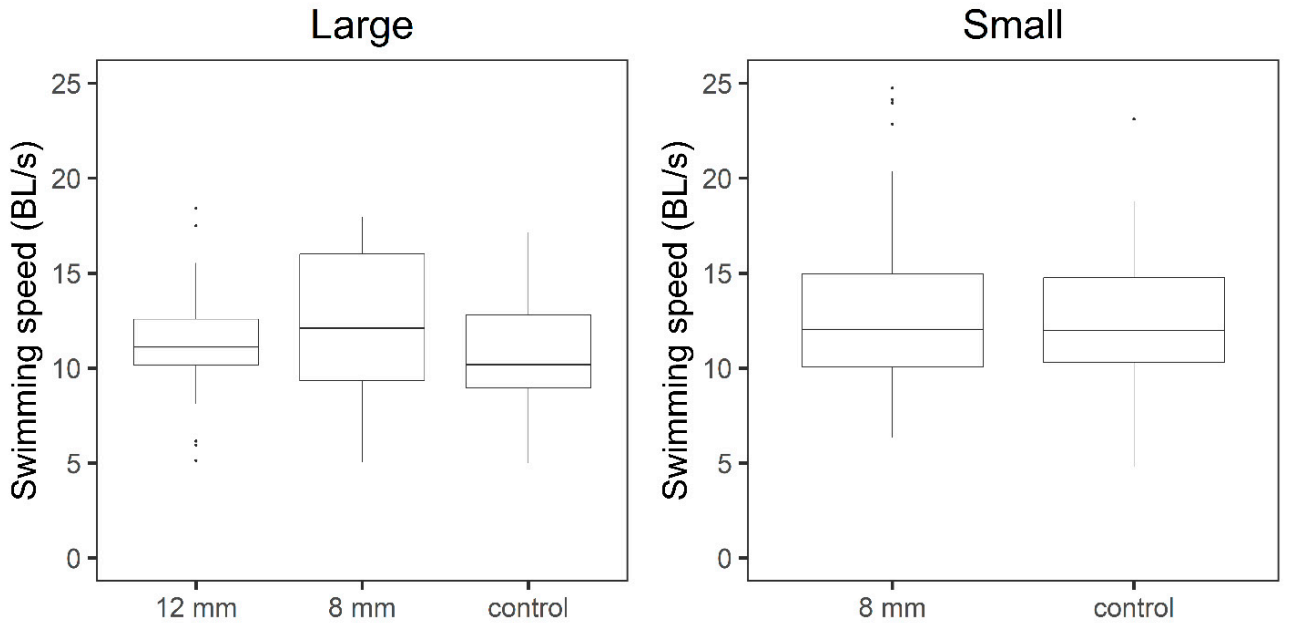


Figure 2. Maximum swimming speed (expressed in body length per second, BL/s) in the provoked escape response test for large fish (≥50 mm; (**left**)) tagged with 12 mm tags, 8 mm tags, or belonging to the control group and small fish (<50 mm; (**right**)) tagged with 8 mm tags or belonging to the control group. The solid black horizontal line inside the bounding box is the median of swimming speed. The black dots represent the outliers, whereas the bounding box defines the Interquartile Range (IQR). The vertical solid black lines mark Q1–1.5·IQR (bottom end) and Q3 + 1.5·IQR (top end), where Q1 and Q3 are the 25th and 75th percentiles, respectively. Dots represent outliers.

4. Discussion

The implantation of 8 mm PIT tags (mini PIT tags) was associated with high survival and tag retention in small-sized (35–76 mm) Padanian goby. No effects of PIT-tagging on volitional swimming activity nor on maximum swimming speed were found in subsequent laboratory trials. Similar results were obtained from implanting larger tags (12 mm) in gobies down to 50 mm in length.

The high survival and tag retention mirrors the results of many previous PIT tag effect studies from other fish taxa and a variety of sizes (e.g., [23,31,50]).

The average tag-to-fish weight ratio in our study was 3% for small fish tagged with 8 mm tags and 5% for large fish tagged with 12 mm tags. The corresponding tag-to-fish length ratios were 18% and 21% for the same groups. These values are higher than both the 2% tag-to-fish weight ratio threshold often referred to in the literature [19–21] and the tag-to-fish length ratio threshold established for salmonids [22], underlining the need for flexibility concerning these thresholds [20,58]. The larger tag burden among the fish tagged with 12 mm tags, however, may have contributed to the non-significant tendency of a higher failure proportion in this group.

Beyond survival, the open field test and the provoked escape response test evaluate swimming behavior and performance in a controlled environment. While both tests were performed in an artificial environment, potentially influencing the absolute test scores, both tests still evaluated behaviors that are relevant to their performance in nature [29,59,60]. Activity in the open field test has been reported to correlate with dispersal and activity in nature [12,53,61] and fish passage rates [62]. The maximum swimming performance, on the other hand, is of high importance for predator–prey interactions and for passing velocity barriers [28,63]. In the present study, no effect on volitional swimming activity (i.e., total distance moved in an open field test) or the maximum swimming speed was detected. Similar results, using the same methodology, have been found for both Cypriniformes [31] and spined loaches [27]. Also, no PIT-tagging effects on swimming performance have been reported for salmonids [32], lampreys [33], and bullheads [34].

The Padanian gobies displayed median maximum swimming speeds of 12 BL/s (0.57 m/s), with a few individuals registering speeds of >20 BL/s (>1 m/s). This is within the range of what has been reported for small-sized riverine Cypriniformes and loaches from other studies [27,31,54]. For round gobies, escape velocities of 16 BL/s have been observed [64]. In fishery management, fish swimming capability is important for the design of fishways [28,65]. In relation to any fish passage application, however, it is important to note that we quantified maximum swimming speeds over 400 ms in the context of a provoked escape response, not during continuous swimming against a current. On the other hand, many gobies, including the Padanian goby, have modified their pelvic fins into a pelvic sucker, allowing them to keep station against strong flow, and to potentially move upstream against velocity barriers using burst-and-hold swimming [47,64]. Future studies need to further explore the swimming capacity and behavior of small-sized gobies.

To conclude, this is, to our knowledge, the first study on effects of mini PIT tags on the survival and behavior of a small-sized goby. We demonstrate high survival and tag retention, and no effect on volitional swimming activity or the maximum swimming speed was detected under controlled laboratory conditions. Our results indicate that PIT telemetry—using mini PIT tags—is applicable for the study of behavior and movement of small-sized gobies. Additional studies investigating potential tagging effects in different temperature regimes [66,67], in the presence of predators [68] and in the fish' natural environment [69,70], may also safeguard against potential unwanted tagging effects. Although larger gobies have been tagged and tracked previously [45], the successful tagging of gobies down to 35 mm in length opens the door to PIT telemetry studies on small-sized fish, including both small-sized species and younger life stages [36,37]. Future studies may include general movement ecology, behavior in relation to restoration efforts or environmental stressors, and the passage of in-stream barriers [8,13,71]. In gobies, nest-guarding dynamics, previously studied using visual identification [72], would also be a relevant area

for the application of PIT telemetry, possibly using an array of small stationary antennas. Regarding the potential tagging effects, a controlled field study, including predation risk and the possibility to disperse, may complement and corroborate our results [73].

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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