Tag retention in and effects of passive integrated transponder tagging on survival and swimming performance of a small-bodied darter

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Abstract
Fisheries biologists have been hesitant to use passive integrated transponder (PIT) tags in small-bodied fishes (40–200 mm TL) such as darters (Percidae: Etheostomatinae) because of the fishes' size and potential effect on swimming performance. The authors used constant acceleration trials to evaluate the swimming performance of Arkansas darters *Etheostoma cragini* in control (no incision or tag), sham (incision and suture) or PIT tagged (surgically implanted 8 × 1.4 mm intra-peritoneal PIT tag) treatments. Tag retention and fish survival were monitored for up to 199 days post-tagging. Maximum swimming velocity did not differ between control, sham and PIT tag treatments, nor was maximum swimming velocity affected by the tagging procedure. Tag retention was 100%, and the overall survival of tagged fish was 88% in the swimming study, and 100% in the long-term study, suggesting that small PIT tags are suitable for use in darters. The authors include a brief meta-analysis on the results reported by 20 studies that PIT tagged small-bodied fishes, representing 38 species and nine families of freshwater fish.

1 | INTRODUCTION

Conservation and management of native fishes requires an understanding of their life history, ecology and habitat preferences, and population status (Bestgen et al., 2007; Fausch et al., 2002), information that can often be gleaned through mark-recapture or mark-resight studies. Nonetheless, such studies are challenging for species whose body size or morphology precludes them from consideration for most mark or tag types, such as darters (Percidae: *Etheostomatinae*). Darters are disproportionately imperilled compared to other groups of North American fishes (Helfman, 2007). Of the 203 recognized species of darters, 54 (26%) are designated as critically Endangered, Endangered or Vulnerable to extinction (IUCN, 2019). Because the group is of growing conservation concern, the development of effective monitoring methods is crucial.

Passive integrated transponder (PIT) tags are widely used to individually identify and discretely monitor the movement of fishes. When coupled with fixed or mobile antenna arrays (Fetherman et al., 2014; Ficke, 2015), fisheries biologists can identify individual fish and collect spatiotemporal and movement information. Large fishes (> 200 mm) can be tagged externally (Castro-Santos et al., 1996; Haro et al., 2004), or internally (Lee et al., 2009). Smaller fishes are generally tagged intraperitoneally to reduce impacts on swimming ability caused by external tags or tags in the axial swimming muscles (Clark, 2016; D’Amico et al., 2021; Ficke et al., 2012).

The authors of this study are aware of three published studies that describe attempts to PIT tag darters. Baxter (2015) tagged Kentucky arrow darters *Etheostoma spilotum* Gilbert 1887 using 8.4 mm PIT tags in the field. *E. spilotum* is a relatively large species of darter with adults ranging from 60 to 125 mm TL. Baxter (2015) did not report fish
survival or tag retention rates due to the nature of the study. Musselman (2007) PIT tagged limited numbers of orangethroat darter *Etheostoma spectabile* (Agassiz 1854) (*n* = 9) and greenside darter *Etheostoma blennioides* Rafinesque 1819 (*n* = 3) using 12 mm PIT tags and reported survival exceeding 80% 60 days post-tagging. The survival of *E. spectabile* in the study drastically declined after 60 days to 56% survival, which was attributed to a poor diet. Schumann et al. (2020) tagged Johnny darter *Etheostoma nigrum* Rafinesque 1820 with 8 mm tags in a 42 d study and reported 63% survival and 70% retention.

Tagging methodology can drastically affect survival. Baxter (2015) and Musselman (2007) both used handheld tagged guns to inject tags into the body cavity of the fish, as did Ruetz III et al. (2006) with mottled sculpins *Cottus bairdi* Girard 1850, Pennock et al. (2016) with southern redbelly dace *Chrosomus erythrogaster* (Rafinesque 1820), and Johnston and Smithson (1999) with bluntface shiner *Cyprinella camura* (Jordan & Meek 1884) and Creek chub *Semotilus atromaculatus* (Mitchell 1818). The injection approach is commonly used for larger species. A number of studies on tagging survival and tag retention with small-bodied fishes such as striped jumprock *Moxostoma rupiscartes* Jordan & Jenkins 1889, bullhead *Cottus gobio* L. 1758, red shiner *Cyprinella lutrensis* (Baird and Girard 1853), Roach *Rutilus rutilus* (L. 1758) and dace *Leuciscus leuciscus* (L. 1783) have used a surgical approach (Bolland et al., 2009; Bruymdonx et al., 2002; Cary et al., 2017; Pennock, 2017; Pennock et al., 2016; Skov et al., 2005). Archdeacon et al. (2009) reported that 64–67 mm TL Rio Grande silvery minnow *Hybognathus amarus* (Girard 1856) experienced higher survival when 12 mm PIT tags were surgically implanted (>87%) rather than injected (50%). Similarly, Baras et al. (1999) reported higher survival (71%–100%) in small (1.9–13.7 g) Nile tilapia *Oreochromis niloticus* (L. 1758) with surgically implanted tags vs. those with injected tags (10%–50%). Moreover, Ficke (2015) noted that Iowa darters *Etheostoma exile* (Girard 1859) and *Etheostoma nigrum* have a relatively small peritoneal cavity; the pilot dissections of preserved *E. nigrum*, *E. exile* and Arkansas darters *Etheostoma cragini* Gilbert 1885 confirmed that this is similar for these three darter species, suggesting that a surgical approach to tagging is warranted.

Determining whether PIT tags impact darter health and behaviour is an important step in their potential adoption as a monitoring method, as one of the key assumptions of any tagging approach is that the tags do not affect the survival or behaviour of the tagged organisms (Guy et al., 1996). The authors used this study to develop a surgical technique for PIT tagging *E. cragini* and evaluated tag retention, fish survival post-tagging and the effects of PIT tags on swimming performance. In addition, they evaluated survival and tag retention in fish whose incisions were sutured following tag insertion when compared to fish with un-sutured incisions.

## 2 | MATERIALS AND METHODS

### 2.1 | Source of fish and fish care

The care and use of experimental animals complied with United States animal welfare laws, guidelines and policies as approved by Colorado State University Institutional Animal Care and Use Committee (protocol reference number 15-5712A). Hatchery-reared *E. cragini* (mean ± S.E. TL: 51 ± 3 mm; mean wet weight: 1.40 ± 0.28 g) from the Colorado Parks and Wildlife Native Aquatic Species Restoration Facility (Alamosa, CO) were held in a 340 l round polyethylene tank receiving 5–10 l min⁻¹ of air-saturated water at 20 ± 0.5°C through a spray bar that produced a current of 0.05–0.10 m s⁻¹ along the periphery of the tank. The laboratory was kept under a natural photoperiod for Fort Collins, Colorado, USA (40.581° N, 105.138° W). A cover was provided in the form of PVC pipe, PVC sheets and artificial aquatic plants. Fish were fed daily satiation rations of thawed bloodworms.

Individual fish were taken from the holding tank and placed in a 0.9 l tank for 24 h prior to the first measurement of swimming performance and treatment application. Fish were randomly assigned to one of three treatments: control (handled, but no surgery or tag), sham (surgery and suture without a PIT tag) and PIT tagged (8 mm PIT tag surgically implanted into the fish’s body cavity and sutured closed) with a sample size of 15 darters per treatment.

### 2.2 | Swimming performance measurement

The constant acceleration test (CAT) swimming methodology was used to measure the maximum swimming velocities (*V*<sub>max</sub>) of *E. cragini* (Leavy & Bonner, 2009). The constant acceleration approach was used because it requires fish to use a full range of swimming gaits and should indicate whether one particular gait was more susceptible to the effects of PIT tag insertion than the others. Secondarily, the high relative velocities reached in a CAT trial force fish to use their fastest-swimming gait and require the use of most of the axial swimming muscles.

Individual fish *V*<sub>max</sub> was measured at three time points to evaluate the short-term effects of PIT tagging on fish swimming ability:

- T<sub>C</sub>: Immediately prior to surgical treatment application to determine baseline swimming ability;
- T<sub>1</sub>: One day following the surgical treatment; and
- T<sub>7</sub>: Seven to eight days after the treatment.

Fish were swum in a Loligo Model 32 swim flume (32 l volume, 55 × 14 × 14 cm test section; velocity range of 3–110 cm s⁻¹; Loligo Systems, Denmark). Fish were given 1 h to become familiar with the flume with a 11 cm s⁻¹ current for rheotaxis. At the beginning of the constant acceleration trial, water velocity increased from the starting velocity by 5 cm s⁻¹ every 5 s until exhaustion, defined as partial or full-body impingement for more than 5 s on the rear screen of the swimming chamber. The velocity at exhaustion was defined as the maximum exposure velocity (*V*<sub>max</sub>) and was recorded in both cm s⁻¹ and body lengths per second (BL s⁻¹). If fish were found to be “cheating,” defined as resting on the rear screen of the flume or resting in a low velocity area of the flume, the current was momentarily reversed to encourage swimming behaviours. Non-performing fish, or fish that refused to swim in one of the trials, were removed from the study (Table 1).
Table 1: Effects of tagging procedure on the survival, tag retention rate and baseline swimming ability (T0), and 1- and 7 day post-tagging swimming performance (T1 and T7–8) of Etheostoma cragini

| Treatment       | n  | TL (mm) | Wt (g)  | Survival rate (%) | Tag retention rate (%) | Maximum swimming velocity (BL/s) | Non-performers |
|-----------------|----|---------|---------|-------------------|------------------------|---------------------------------|----------------|
| Swim-control    | 15 | 51 (3)  | 1.40 (0.26) | 100               | -                      | 13.1 (1.8)                      | 1              |
| Swim-sham       | 15 | 52 (3)  | 1.41 (0.28) | 100               | -                      | 11.9 (1.8)                      | 4              |
| Swim-tag        | 17 | 52 (3)  | 1.41 (0.26) | 88                | 100                    | 12.8 (1.6)                      | 3              |
| Suture          | 16 | 51 (3)  | 1.42 (0.31) | 100               | 100                    | -                               | -              |
| No suture       | 29 | 53 (3)  | 1.50 (0.20) | 100               | 100                    | -                               | -              |

Note: Tagged fish had an 8.0 × 1.4 mm PIT tag surgically implanted in their peritoneal cavity. Survival and tag retention were monitored for 199 days post-treatment application for control, sham and tag groups. Values are means with S.D. in parentheses. Incisions of PIT tagged fish were sutured for this portion of the study. There were no statistically significant differences in maximum swimming velocity within or between treatments (RMANOVA; P > 0.05). Additionally, survival and tag retention were monitored for 243 days for E. cragini tagged with 8 mm PIT tags where incisions were sutured closed or left open. There were no statistically significant differences between suture and no suture treatments (X²; P > 0.05).

2.3 Tagging procedure

After the initial swimming trial, all darters were anaesthetised using 40 mg l⁻¹ of tricaine methanesulfonate, weighed (g) and measured (TL, mm). Control fish were placed in a recovery tank supplied with air-saturated fresh water for approximately 5 min until equilibrium was regained. Once fish had gained equilibrium, they were returned to their individual holding tank, and Kordon® Pond Fish Protector™ was added at a concentration of 0.4 ml l⁻¹ to ease stress and maintain the fish’s mucous coating (Swanson et al., 1996). Fish in the sham and PIT tag treatments were weighed and measured, and then a #12 scalpel blade was used to make a 2 mm long ventral incision oriented along the anterior–posterior axis, slightly offset from the ventral midline, just anterior of the vent to prevent the incision from becoming irritated as the fish rubbed on the bottom of the tank. The incision was closed using Braunamid DS24 polyamide monofilament suture attached to a 24 mm curved suture needle. Once fish recovered from the anaesthesia, they were returned to their individual 0.9 l tank with the same post-handling treatment as the control fish.

Darters in the PIT tag treatment underwent the same procedures as the fish in the sham surgery treatment, with the additional step of having an 8.0 × 1.4 mm PIT tag (FDX-B, 134.2 kHz ISO, 0.027 g; Oregon RFID, Portland, OR, USA) inserted anteriorly into the incision prior to the suturing of the incision. These tags weighed less than 2% of the total weight of the fish, following the Cooke et al. (2011) suggestion that tags should weigh less than 12% of the fish’s body weight.

Fish were swum at T1 and T7–8 using the same procedures as before. Survival of all groups and tag retention of fish in the tagged treatment were monitored daily for up to 199 days. Repeated-measures ANOVAs were used to determine if there were significant differences in \( V_{\text{max}} \) in body lengths per second (BL s⁻¹) between treatments (Control, Sham or Tag) and swimming trial (Pre-treatment, 1 day post-tagging, and 7–8 days post-tagging).

2.4 Suture vs. non-suture comparison

During the study, it became apparent that suturing the fish posed three challenges: (a) it was challenging to suture such small fish; (b) the probability of harming the fish was high if body wall was improperly pierced with the suture needle; (c) suturing added 30–45 s to each tagging event. To address these challenges, the authors conducted a follow-up study comparing the tag retention and survival of sutured vs. un-sutured E. cragini.

Randomly selected individual darters were placed in 9 l holding tanks and fasted for 24 h prior to surgery. Fish were randomly assigned to either the sutured or un-sutured group. A PIT tag was inserted into the peritoneal cavity as described above, with fish receiving a suture (\( n = 16 \)) or no suture (\( n = 29 \)). Fish were inspected daily for the presence of a suture and closure of the incision. Fish remained in the individual tanks for 21 days post-tagging and were then moved to a communal 340 l holding tank. Tag retention and survival were monitored daily for up to 221 additional days.

2.5 Survey of small-bodied fish tagging literature

The authors reviewed 20 published studies that focused on PIT tagging small-bodied fishes. Studies were included in the review if the study species was a non-salmonid and if the study included fish under 150 mm TL. The authors made note of the species, fish size (or size range), tag size, tagging method, survival and tag retention percentages, sample size and study duration. They used this data set to identify common trends in the use of PIT tags with small-bodied fish. These included identifying the most commonly used tag sizes, evaluating the ratio of tag size to fish size, and determining the most relative frequency of different tagging approaches (e.g., surgery vs. injection). They also conducted a series of simple analyses (one-way ANOVA) to see whether there were broad trends with respect to the effects of tag size and tagging method on fish survival rates and tag retention.
| Family           | Species                  | Common name                     | Fish size (various units) | Tag size (mm) | Tagging method | n  | Survival (%) | Retention (%) | Study duration (days) | Reference      |
|------------------|--------------------------|---------------------------------|---------------------------|---------------|----------------|----|--------------|---------------|-----------------------|----------------|
| Catostomidae     | Catostomus commersoni    | White sucker                    | 118 mm TL                 | 12.5          | S              | 19 | 32           | 100           | 30                    | Ficke et al., 2012 |
|                  | Catostomus commersoni    | White sucker                    | 139 mm TL                 | 23            | S              | 18 | 44           | 100           | 30                    | Ficke et al., 2012 |
|                  | Catostomus commersonii   | White sucker                    | 52–169 mm TL              | 8 × 1.4       | S              | 30 | 90           | 90            | 42                    | Schumann et al., 2020|
|                  | Hypentelium nigricans    | Northern hog sucker             | 47–97 mm TL               | 8 × 1.4       | S              | 19 | 100          | 100           | 7                     | Cary et al., 2017  |
|                  | Moxostoma rupiscartes    | Striped jumprock                | 59–81 mm TL               | 8 × 1.4       | S              | 4  | 100          | 100           | 7                     | Cary et al., 2017  |
| Centrarchidae    | Lepomis megalotis        | Longear sunfish                 | 56–131 mm SL              | 14            | I              | 57 | 61           | 70            | 180                   | Johnston & Smithson, 1999 |
| Cichlidae        | Micropterus dolomieu     | Smallmouth bass                 | 148 mm TL                 | 23            | I              | 25 | 96           | 100           | 60                    | Musselman, 2007    |
|                  | Oreochromis niloticus    | Nile tilapia                    | < 3 g                     | 10.3 × 2.1    | I              | 10 | 0            | N/A           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 3–4 g                     | 10.3 × 2.1    | I              | 10 | 20           | 50            | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 4–7 g                     | 10.3 × 2.1    | I              | 10 | 30           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 7–15 g                    | 10.3 × 2.1    | I              | 10 | 50           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 15–20 g                   | 10.3 × 2.1    | I              | 10 | 90           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 20–25 g                   | 10.3 × 2.1    | I              | 10 | 100          | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | < 3 g                     | 10.3 × 2.1    | S              | 12 | 83           | 90            | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 3–4 g                     | 10.3 × 2.1    | S              | 14 | 71           | 90            | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 4–7 g                     | 10.3 × 2.1    | S              | 7  | 100          | 86            | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 7–15 g                    | 10.3 × 2.1    | S              | 7  | 100          | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | < 3 g                     | 10.3 × 2.1    | S w/suture     | 8  | 75           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 3–4 g                     | 10.3 × 2.1    | S w/suture     | 11 | 73           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 4–7 g                     | 10.3 × 2.1    | S w/suture     | 12 | 92           | 100           | 49                    | Baras et al., 1999  |
|                  | Oreochromis niloticus    | Nile tilapia                    | 7–15 g                    | 10.3 × 2.1    | S w/suture     | 24 | 100          | 100           | 49                    | Baras et al., 1999  |
| Cottidae         | Misgurnus anguillicaudatus| Oriental weather Loach          | 84 mm TL                  | 12.5 × 2.07   | I              | 32 | 93           | 97            | 30                    | Kano et al., 2013   |
|                  | Misgurnus anguillicaudatus| Oriental weather Loach          | 84 mm TL                  | 12.5 × 2.07   | S              | 32 | 97           | 97            | 30                    | Kano et al., 2013   |
| Cottidae         | Cottus bairdii           | Mottled sculpin                 | 50–80 mm TL               | 8 × 1.4       | I              | 43 | 97.7         | 100           | 7                     | Cary et al., 2017   |
|                  | Cottus bairdii           | Mottled sculpin                 | 55–59 mm TL               | 12 × 2.1      | I              | 9  | 100          | 100           | 28                    | Ruetz et al. 2006   |
|                  | Cottus bairdii           | Mottled sculpin                 | 60–69 mm TL               | 12 × 2.1      | I              | 8  | 88.9         | 88.9          | 28                    | Ruetz et al. 2006   |
|                  | Cottus bairdii           | Mottled sculpin                 | ≥ 70 mm TL                | 12 × 2.1      | I              | 9  | 100          | 100           | 28                    | Ruetz et al. 2006   |
| Family      | Species                  | Common name       | Fish size (various units) | Tag size (mm) | Tagging method | n    | Survival (%) | Retention (%) | Study duration (days) | Reference                  |
|------------|--------------------------|-------------------|----------------------------|---------------|----------------|------|--------------|---------------|------------------------|-----------------------------|
| Cottidae   | Cottus gobio             | Bullhead          | 70 mm TL                   | 12 × 2.1      | S              | 6    | 100          | 100           | 28                     | Bruyndoncx et al., 2002    |
|            | Cottus gobio             | Bullhead          | 50–60 mm                   | 12 × 2.1      | S              | 13   | 100          | 100           | 49                     | Knaepkens et al., 2007     |
|            | Cottus gobio             | Bullhead          | 65–79 mm                   | 12 × 2.1      | S              | 21   | 100          | 90            | 49                     | Knaepkens et al., 2007     |
|            | Cottus gobio             | Bullhead          | 80–94 mm                   | 12 × 2.1      | S              | 10   | 90           | 100           | 49                     | Knaepkens et al., 2007     |
| Cyprinidae | Campostoma anomalum      | Central stoneroller | 94 mm TL                   | 12            | I              | 8    | 88           | 100           | 90                     | Musselman, 2007             |
|            | Chrosomus erythrogaster  | Southern redbelly dace | 48–71 mm TL               | 8 × 1.4       | I              | 30   | 97           | 86            | 30                     | Pennock et al., 2016       |
|            | Chrosomus erythrogaster  | Southern redbelly dace | 48–71 mm TL               | 9 × 2.15      | I              | 30   | 80           | 40            | 30                     | Pennock et al., 2016       |
|            | Cyprinella camura        | Bluntface shiner  | 62–95 mm SL                | 14            | I              | 31   | 87           | 6             | 80                     | Johnston & Smithson, 1999  |
|            | Cyprinella lutrensis     | Red shiner        | 48–74 mm TL                | 9 × 2.15      | S              | 40   | 97           | 82            | 48                     | Pennock, 2017              |
|            | Hybognathus amarus       | Rio Grande silvery minnow | 67 mm SL              | 12.5 × 2.07   | I              | 80   | 50           | 89            | 32                     | Archdeacon et al., 2009    |
|            | Hybognathus amarus       | Rio Grande silvery minnow | 65 mm SL              | 12.5 × 2.07   | S              | 80   | 87           | 90            | 32                     | Archdeacon et al., 2009    |
|            | Squalius cephalus        | Chub              | 113 mm TL                   | 12 × 2.1      | S              | 80   | 97.5         | 100           | 182                    | Bolland et al., 2009       |
|            | Squalius cephalus        | Chub              | 167 mm TL                   | 23 × 3.4      | S              | 101  | 98           | 100           | 182                    | Bolland et al., 2009       |
|            | Squalius cephalus        | Chub              | 169 mm TL                   | 23 × 3.4      | S w/glue       | 100  | 100          | 100           | 182                    | Bolland et al., 2009       |
|            | Leuciscus leuciscus      | Dace              | 115 mm TL                   | 12 × 2.1      | S              | 80   | 96.3         | 100           | 182                    | Bolland et al., 2009       |
|            | Leuciscus leuciscus      | Dace              | 121 mm TL                   | 23 × 3.4      | S w/glue       | 80   | 72.5         | 96.6          | 182                    | Bolland et al., 2009       |
|            | Luxilus cardinalis       | Cardinal shiner  | 91 mm TL                    | 12            | I              | 12   | 100          | 100           | 90                     | Musselman, 2007            |
|            | Luxilus cornutus         | Common shiner     | 44–123 mm TL               | 8 × 1.4       | S              | 30   | 100          | 60            | 42                     | Schumann et al., 2020      |
|            | Nocomis leptcephalus     | Bluehead chub     | 40–100 mm TL               | 8 × 1.4       | S              | 144  | 96.5         | 99            | 7                      | Cary et al., 2017          |
|            | Notropis girardi         | Arkansas river shiner | 51 mm TL              | 8 × 1.4       | I              | 15   | 53           | 50            | 120                    | Moore & Brewer, 2021       |
|            | Notropis girardi         | Arkansas river shiner | 51 mm TL              | 8 × 1.4       | I              | 15   | 47           | 57            | 120                    | Moore & Brewer, 2021       |
|            | Notropis lutpinnis       | Yellowfin shiner  | 51–87 mm TL                 | 8 × 1.4       | S              | 89   | 93.2         | 99            | 7                      | Cary et al., 2017          |
|            | Notropis stramineus      | Sand shiner       | 52–67 mm TL                 | 9 × 2.15      | S              | 38   | 97           | 51            | 48                     | Pennock, 2017              |
|            | Notropis stramineus      | Sand shiner       | 47–71 mm TL                 | 8 × 1.4       | S              | 30   | 90           | 76.7          | 42                     | Schumann et al., 2020      |
|            | Oregonichthys crameri    | Oregon chub       | 54.9                        | 8.4 × 1.4     | I              | 120  | 94           | 95            | 150                    | Bangs et al., 2013         |
| Family        | Species                        | Common name          | Fish size (various units) | Tag size (mm) | Tagging method | n  | Survival (%) | Retention (%) | Study duration (days) | Reference                      |
|--------------|--------------------------------|----------------------|--------------------------|---------------|----------------|----|---------------|---------------|------------------------|--------------------------------|
| Cyprinidae   | Oregonichthys crameri          | Oregon chub          | 55.3                     | 9 × 2.15      |                | 1  | 100           | 82            | 150                    | Bangs et al., 2013            |
|              | Platygobio gracilis            | Flathead chub        | 119 mm TL                | 12.5          |                | 23 | 100           | 100           | 20                      | Ficke et al., 2012            |
|              | Rhinichthys atratulus          | Blacknose dace       | 46–90 mm TL              | 8 × 1.4       |                | 30 | 100           | 100           | 40                     | Schumann et al., 2020         |
|              | Rutilus rutilus               | Roach                | 116 mm TL                | 12.1 × 3.85   |                | 21 | 100           | 100           | 120                    | Bolland et al., 2009         |
|              | Rutilus rutilus               | Roach                | 133 mm TL                | 23.1 × 3.85   |                | 16 | 100           | 100           | 120                    | Bolland et al., 2009         |
|              | Rutilus rutilus               | Roach                | 104 mm TL                | 23.1 × 3.85   |                | 14 | 100           | 100           | 140                    | Bolland et al., 2009         |
|              | Semotilus atromaculatus        | Creek chub           | 39–101 mm TL             | 8 × 1.4       |                | 30 | 100           | 100           | 20                     | Cary et al., 2017             |
|              | Fundulus sciadiceus            | Plains topminnow     | 39–66 mm TL              | 8 × 1.4       |                | 14 | 100           | 96.7          | 42                     | Schumann et al., 2020         |
|              | Noturus exilis                 | Slender madtom       | 77 mm TL                 | 12 × 2.15     |                | 12 | 100           | 100           | 100                    | Musselman, 2007               |
|              | Noturus flavus                 | Stonecat             | 143 mm TL                | 8 × 1.4       |                | 14 | 100           | 100           | 100                    | D’Amico et al., 2021          |
|              | Noturus gyrinus                | Talbot madtom        | 175 mm TL                | 12.1 × 2.12   |                | 23 | 100           | 100           | 100                    | D’Amico et al., 2021          |
|              | Noturus phaeus                 | Brown madtom         | 38–95 mm TL              | 8 × 1.4       |                | 14 | 100           | 100           | 31                     | Johnston & Smithon, 1999     |

**Notes:**
- TL = Total length
- SL = Standard length
- I = Implant
- C2 = Suture
- S = Suture
- w/glue = With glue
- w/suture = With suture
- P = Permanently implanted

**References:**
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- Ficke et al., 2012
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- Schumann et al., 2020
- Johnston & Smithon, 1999
## TABLE 2 (Continued)

| Family         | Species                     | Common name         | Fish size (various units) | Tag size (mm) | Tagging method | n   | Survival (%) | Retention (%) | Study duration (days) | Reference                  |
|----------------|-----------------------------|---------------------|---------------------------|---------------|----------------|-----|--------------|---------------|-------------------------|-----------------------------|
| Percida        | Etheostoma blennioides      | Greenside darter    | 95 mm TL                  | 12            | I              | 3   | 100          | 100           | 60                      | Musselman, 2007             |
|                | Etheostoma cragini          | Arkansas darter     | 53 mm TL                  | 8.0 × 1.4     | S              | 29  | 100          | 100           | 243                     | This study                  |
|                | Etheostoma cragini          | Arkansas darter     | 52 mm TL                  | 8.0 × 1.4     | S w/suture     | 15  | 100          | 100           | 199                     | This study                  |
|                | Etheostoma cragini          | Arkansas darter     | 51 mm TL                  | 8.0 × 1.4     | S w/suture     | 16  | 100          | 100           | 243                     | This study                  |
|                | Etheostoma nigrum           | Johnny darter       | 36–69 mm TL               | 8 × 1.4       | S              | 30  | 63.3         | 70            | 42                      | Schumann et al., 2020       |
|                | Etheostoma specabile        | Orangethroat darter| > 50 mm TL                | 8.4 × 1.4     | I              | 9   | 56           | 88            | 60                      | Musselman, 2007             |
|                | Etheostoma spilotum         | Kentucky arrow darter|                           | 60 mm T       | 12             | 121 | N/A          | N/A           | N/A                     | Baxter, 2015                |
|                | Perca fluviatilis           | Eurasian perch      | 65–90 mm FL               | 11 × 2.2      | I              | 30  | 60           | 77            | 28                      | Baras et al., 2000          |
|                | Perca fluviatilis           | Eurasian perch      | 65–90 mm FL               | 11 × 2.2      | S              | 30  | 77           | 80            | 28                      | Baras et al., 2000          |

Abbreviations: n.r., not reported; P, polymer.

Note: Fish sizes are presented in the units provided in the reference; tagging methods are coded as S = surgery, I = injection.

### RESULTS

#### 3. Differences in total lengths and wet weights of E. cragini used in the various treatments

- E. cragini swimming performance measurements (PERMANOVA; P = 0.12; df = 1) were not statistically significant for control and sham groups, and 88% for the tag group in the swimming portion of the study. The lower survival was caused by the insertion approach, except for surgery with sutures, where reported as non-performers in the swimming study. The mortalities were thought to have resulted from injuries incurred during suturing. Sutures were also inserted using a surgical approach without sutures (45.5% of studies), followed by the larger 23 mm tags (14.8% of the time). The most commonly used (54.5%) tags were in the 10–14 mm length range, with 8.9% PIT tags being used 30.7% of the time.

- The summary of studies that focused on tagging small-bodied fishes is shown in Table 2. This table includes information from 38 species of small-bodied fishes (36–20 mm total length) and includes 17 reported cases of small-bodied fishes (36–174 mm mean TL at 80 mm in nine families, tagged with PIT tags ranging from 8 to 23 mm in length. The studies took 40–60 s depending on the experience of the tagger: suturing took 45–60 s and 60 s depending on the experience of the tagger: suturing closed 3–5 days post-surgery for unfixed fish. The mortalities were thought to have resulted from injuries incurred during suturing. Sutures were also inserted using a surgical approach without sutures (45.5% of studies), followed by the larger 23 mm tags (14.8% of the time). The most commonly used (54.5%) tags were in the 10–14 mm length range, with 8.9% PIT tags being used 30.7% of the time.

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(83.1%). Fish size did affect retention, wherein larger fish generally had higher retention rates (ANOVA, F = 10.60; P = 0.002). Increased tag retention had a slight positive correlation with tag size (ANOVA, F = 4.442; P = 0.038), and a stronger negative correlation with greater tag-to-fish ratios (ANOVA; F = 8.77; P = 0.004).

4 | DISCUSSION

The results show that it is possible to tag E. crugini ≥ 48 mm TL with 8 mm PIT tags without significantly affecting swimming ability or survival. Despite the relatively large size of the PIT tags (up to 16% of the fish’s TL, but <2% of their weight), the surgical approach allowed the authors to successfully tag the fish and, importantly, did not violate two of the key assumptions of any marking or tagging operation—there was no significant difference in survival between tagged and untagged individuals and that the tags did not affect the physical performance of the fish. Indeed, the continued growth of some individuals of E. crugini (up to 12 mm during 199 d post-tagging period) and the sexual maturation of male and female darters were further evidence that the tags had little impact on the fish.

In this study, the use of sutures did not improve tag retention and sutures were identified as the likely cause of mortality for two of the fish. Additionally, sutures remained in the body for 4–9 days after the incision healed in non-sutured fish. This extended timeframe where sutures are present, especially in benthic species like darters, increases the likelihood of issues in the wild where the suture could get caught on external objects.

4.1 | Review of small-bodied fish PIT tagging

Surgical technique and tag size can affect the survival of small-bodied fishes post-surgery. Four studies on small-bodied fish PIT tagging reported better survival following surgical implantation when compared to injection (Archdeacon et al., 2009; Baras et al., 1999; Baras et al., 2000; Kano et al., 2013; Table 2). Tag size relative to fish body size also affects survival. Acolas et al. (2007) tagged brown trout Salmo trutta L. 1758 of varying sizes with 11.5 × 2.1 mm PIT tags and found that larger fish had higher survival. Baras et al. (1999) tagged Oreochromis niloticus using a variety of surgical techniques and at different sizes with 10.3 × 2.1 mm PIT tags and found that survival was almost always higher for larger fish for any given surgical procedure (surgery, surgery and suture, and injection) (Table 2). Ficke et al. (2012) also noted that survival decreased (68.4% to 62.5%) for Semotilus atromaculatus of similar size when a larger tag (12 mm vs. 23 mm) was used (Table 2). Because larger fish have a larger peritoneal cavity, it is intuitive that their bodies can accommodate a larger tag. Future studies tagging small-bodied fish should consider body cavity size before choosing a tag size, remembering the functional limitations of smaller tags with regard to tag type (full duplex vs. half duplex) and tag detection range.

Suturing small-bodied fish is not recommended due to the increased likelihood of accidental injury during the suturing process. Baras et al. (1999) noted lower survival rates 49 days after surgery on two size (weight) classes of Oreochromis niloticus (< 3 g and 4–7 g) for sutured fish (75%; 91.7%) compared to non-sutured fish (83.3%; 100%) showing the potential for mortality when suturing small-bodied fish. A similar result was reported by Skov et al. (2005) in their study on the survival of Rudd Scardinius erythrophthalmus (L.1758) and Rutilus rutillus with 23 mm tags that were surgically implanted with and without sutures. Mortality of R. rutillus and S. erythrophthalmus only occurred in the suture group, and because of this, the authors recommended avoiding sutures in small-bodied cyprinids. Interestingly, D’Amico et al. (2021) used sutures to close PIT tagging incisions in the Stonecat Noturus flavus (Rafinesque 1818) and had survival rates of 89% over 840 days for regular 12 mm PIT tags and survival rates of 99% over 751 days for fish tagged with polymer-coated 12 mm tags (Table 2). The results from the sutured vs. non-sutured comparison indicate that tag retention and survival were comparable to that of sutured fish, so there appears to be no benefit to the use of sutures for darters. One possible approach for situations where there is a desire to close the incision would be to use surgical glue. Bolland et al. (2009) reported that the survival of R. rutillus and Chub Squalius cephalus with surgically implanted tags was high (>97%) and largely unaffected by using surgical glue to close the incision, whereas Leuciscus leuciscus with glued incisions survived at a lower rate (72.5% vs. 96.3%) over the course of a 182-day study (Table 2).

The results of this study show that E. crugini ≥ 48 mm TL can successfully be tagged with 8 mm PIT tags without impairing their swimming ability and long-term survival. A study on blackspotted topminnow Fundulus olivaceus (Storer 1845) reported similar findings, and also found PIT tags impacted neither gonadal development nor swimming kinematics (Clark, 2016). The authors successfully applied the same tagging technique to other small-bodied fishes including flathead chub Platygobio gracilis (Richardson 1836) and Noturus flavus with success, supporting the view that the technique is applicable to other taxa (D’Amico et al., 2021; Swarr, 2018). More broadly, the results of the studies included in Table 2 and the meta-analysis suggest that the use of 12 mm or smaller PIT tags is appropriate for a number of small-bodied species, provided that the PIT tags selected for use are no more than 17% of the fish’s total length.

The ability to use PIT tags in small-bodied fishes could improve monitoring and conservation efforts for these smaller species by allowing fisheries biologists to monitor their movements with passive or mobile antenna arrays placed in stream networks, at fish passage structures, in laboratory studies, or by allowing rapid broodstock identification in conservation hatcheries. For smaller fish, it may be possible to use alternative technologies, such as the p-Chips evaluated by Moore and Brewer (2021), though that technology may not be suitable for remote detection of fish in the field, at least using current techniques.

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AUTHOR CONTRIBUTIONS
T. R. S.: Ideas, data generation, experimental design, data analyses, fish husbandry and manuscript preparation. C. A. M. Ideas, manuscript preparation, data analyses, fish husbandry and funding. R. M. F. Manuscript preparation, fish acquisition and funding.

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