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Effects of passive integrated transponder tagging methods on survival, tag retention and growth of age-0 brown trout

Alexandre Richard[a,b,*], Jane O'Rourke[a], Arnaud Caudron[b,c], Franck Cattanéo[a]

[a] Hepia, University of Applied Sciences of Western Switzerland, Earth Nature Environment Institute, Route de Presinge 150, 1254 Jussy (GE), Switzerland
[b] INRA, UMR 0042 Carrtel, F-74203 Thonon, France
[c] Fédération de Haute-Savoie pour la Pêche et la Protection du Milieu Aquatique, Le Villaret, 2092 route des Diacquenods, 74370 St-Martin Bellevue, France

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ABSTRACT

We evaluated the effect of 12-mm passive integrated transponder (PIT) tag implantation on age-0 brown trout *Salmo trutta*. The effects of implantation method (i.e. surgical incision or injection) and individual tagger on survival, tag retention and growth were assessed during a 60-day hatchery experiment. Two size classes of fish (total length) were considered: small (50–55 mm) and large (56–63 mm). For fish ≤ 55 mm, survival rate at 60 days was lower for tagged than for control fish (80.7 *vs* 91.2%, respectively), varied between taggers, but was not affected by the implantation method. For this size class injection resulted in a higher retention rate than surgical implantation (89.4 *vs* 69.4%, respectively); tag retention also varied among the individual taggers. The growth in length and weight of fish from this class was significantly impaired by tagging at 30 and 60 days (e.g. mean ± SD length at 60 days = 76.5 ± 8.4 mm for tagged fish *vs* 81.2 ± 7.9 mm for control), and individual specific growth rates (SGR) of tagged fish differed between taggers. In contrast, for larger fish (>55 mm), neither implantation method nor tagger affected survival (mean = 93.2%), tag retention (mean = 86.6%), and growth rate (mean ± SD specific growth rate = 1.07 ± 0.48% during the first 30 days). A slight slowdown in growth (length) appeared within 30 days post-tagging but was compensated at 60 days. Results suggest that implanting 12-mm PIT tags in salmonids smaller than 55 mm (TL), by different taggers and using either surgery or injection, may have significant effects on survival, tag retention, and growth.

1. Introduction

Understanding the underlying regulating processes during early life stages is critical for sound ecological knowledge of population dynamics and for management purposes. However, few tagging techniques are currently available to investigate the behavior of young-of-the-year fish (Skalski et al., 2009). Passive integrated transponder (PIT) tags are commonly used to assess individual survival, migration and growth. For more than a decade, 12-mm tags have been tested on various salmonid species such as steelhead *Oncorhynchus mykiss* (Prentice et al., 1990a; Meyer et al., 2011), Chinook salmon *Oncorhynchus tshawytscha* (Prentice et al., 1990a; Knudsen et al., 2009), Atlantic salmon *Salmo salar* (Gries and Letcher, 2002), brook trout *Salvelinus fontinalis* (Dieterman and Hoxmeier, 2009) or brown trout *Salmo trutta* (Ombredane et al., 1998; Cucherousset et al., 2006; Acolas et al., 2007; Teixeira and Cortes, 2007). Though 9-mm tags are available, their limited detection range (i.e. 10–14 cm for underwater antennas) restricts their use to studies in shallow streams (Dixon and Mesa, 2011) and recapture experiments. The recent development of the half-duplex (HDX) technology enables 12-mm tags to be detected up to 60 cm (Texas Instrument, datasheet TRPGR30TGC), increasing their potential for studying fish behavior at early life stage *in natura* (e.g. Cucherousset et al., 2006; Teixeira and Cortes, 2007) with the use of fixed and/or mobile antennas.

The effects of PIT tagging have been well documented on salmonids larger than 55 mm (Prentice et al., 1990a; Ombredane et al., 1998; Dare, 2003; Cucherousset et al., 2005; Dieterman and Hoxmeier, 2009), but few studies focused on smaller fish. In a laboratory experiment on juvenile brown trout ranging between 41 and 70 mm fork length (FL), Acolas et al. (2007) showed a survival rate of 95%, a retention rate of 70%, and no growth alteration for fish larger than 52 mm FL. While tag injection has been favored in most studies on age-0 salmonids (Prentice et al., 1990a; Ombredane et al., 1998; Acolas et al., 2007; Brakensiek and Hankin, 2007; Acolas et al., 2011) surgical implantation was only reported on fish larger than 60 mm (Gries and Letcher, 2002; Sigourney et al., 2005). However, the potential effects of both implantation methods on survival and

* Corresponding author at: Hepia, University of Applied Sciences of Western Switzerland, Earth Nature Environment Institute, Route de Presinge 150, 1254 Jussy (GE), Switzerland. Tel.: +41 22 546 68 64; fax: +41 22 546 68 14.
E-mail address: alexandre.richard@hesge.ch (A. Richard).

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growth are not well known. For instance, surgical implantation was shown to induce lower mortality than injection on silvery minnow *Hybognathus amarus* (Archdeacon et al., 2009) ranging between 45 and 90 mm standard length.

For increasingly common large-scale studies, the required tagging effort can be very high and cannot be performed by a unique tagger. In a tagging project on 145,000 juvenile spring Chinook salmon, Dare (2003) related the higher tag loss rate at the start of the tagging process (1.15% after 48 h *vs* 0.06% in subsequent marking) with the initial lack of experience of the personnel. Meyer et al. (2011) showed that rainbow trout longer than 100 mm marked by experienced taggers had significantly higher retention rates than those marked by inexperienced ones, even if the retention rates remained high in both cases (98% and 95% respectively).

In this study, we simultaneously tested the effects of tag implantation method and tagger on survival, retention rate and growth rate of age-0 brown trout. Our results aimed at providing guidelines for an acceptable tagging protocol for small trout, which is a prerequisite to carry out large-scale tagging campaigns in the field.

2. Material and Methods

2.1. Experimental design

The experiment took place at the French hatchery of Rives (Thonon-les-Bains, France). Tagging started on 27 July 2011 on first hatched fry (17 February 2011, median hatching date). The minimum size for the experiment was 50 mm total length (TL), as preliminary trials highlighted the difficulty to implant 12-mm tags in smaller trout. A batch of 360 fingerlings was used, with TL ranging between 50 and 63 mm (mean ± SD = 55.6 ± 2.6 mm). Fish were sorted according to their size into two length classes (180 fish per class): 50–55 mm (mean ± SD = 53.6 ± 1.4 mm) and 56–63 mm (mean ± SD = 57.6 ± 1.8 mm). Mean weights were 1.57 g (range = 1.2–2.0, SD = 0.16) and 1.99 g (range = 1.6–2.9, SD = 0.25) for the small and large fish group respectively, and significantly differed ($t = 19.16$, $p < 0.001$). In each size class, one-third of the fish (i.e. $n = 60$ fish) was not tagged (control), one-third was tagged by surgical implantation ($n = 60$), and one-third was tagged by injection ($n = 60$). Two taggers (tagger1 and tagger2), having both tagged 200–300 fish in preliminary tests using both methods, each marked 30 fish per tagging procedure. In each length class, individuals were randomly assigned to one treatment, thereafter defined as a tagger × an implantation method (4 treatments). After tagging, fish were dispatched in four rectangular tanks (2.4 m × 0.55 m, Vol. = 0.2 m^3), with two tanks per length class, each one containing a mix of the different treatments (90 fish per tank, i.e. 15 fish per treatment, except 30 for the control).

2.2. Tagging method and rearing

All 360 fish were first anesthetized using a 10% clove oil stock solution (Keene et al., 1998), dissolved in water at a final eugenol concentration of 30–35 ppm. A maximum of five fish were bathed simultaneously for about 3 min, to prevent overexposure. Each one was measured (±1 mm) and weighed (±0.1 g). A total of 240 fish were implanted with half duplex PIT tags (Texas Instrument; model TRPGR30TGC; 134.6 kHz; 12 mm × 2.15 mm, 0.1 g in air), while the 120 remaining fish were kept for control. Direct injection (Prentice et al., 1990b) was done with a lock needle equipped with a plunger and mounted on a plastic injector. Surgical implantation (Baras et al., 1999) consisted of a preliminary short incision (2 mm max) with a scalpel, before introducing the tag with the lock needle. In this case, the needle was only used as a guide to ensure sterile conditions. Injection and incision were both done just posterior to the insertion of the pectoral fin, close to the mid-ventral line (Prentice et al., 1990b). All needles and tags were disinfected in a 70% ethanol solution for at least 10 min before operation and throughout the tagging (Wagner et al., 2011), therefore 10 different needles were used. The scalpel was also plunged in ethanol between two markings. Handling time varied according to fish size and tagger, but ranged between 30 and 60 s. After implantation, the wound was not sutured, and fish were immediately released in their final tank for recovery. At the start of the experiment, tag to body weight ratio in air ranged between 5.0 and 7.7% (mean ± SD = 6.5 ± 0.6%) for small fish, and between 3.4 and 6.3% (mean ± SD = 5.1 ± 0.6%) for large ones.

Fish were fed every 2 days with pellets (Inicio plus 801, 1.5 mm, BioMar, contents = 54% protein, 18% lipids, 11% N-free extract) slowly distributed by automatic feeders. Food ration was approximately 3.0% of total body weight during the first month (small ration so as to prevent disease proliferation), then *ad libitum* until the end of the experiment. Fish feeding was not interrupted before tagging to mimic eating habits of wild fish. Water was supplied from a natural spring, and did not re-circulate (flow = 1.1 m^3/h). Temperature was recorded every day and ranged between 13 °C and 14 °C over the period (mean = 13.35 °C). Oxygen concentration was regularly checked, and remained in the range of tolerance for brown trout (>8 mg/L). Tanks were cleaned every day, dead fish removed, measured and weighed, and assigned to their treatment. Furthermore, the bottom of each tank was screened for any lost tags. After 30 and 60 days, all fish were anesthetized, measured, weighed and scanned with a handheld tag detector. The presence of a scar was noted, allowing the distinction between control and fish that lost their tag.

2.3. Data analysis

Tag retention was calculated as the percentage of fish that retained their tag, relative to the number of live fish tagged. Survival was the percentage of live fish relative to the number of fish initially tagged. Because we mixed different treatments in each tank, we could not assign to their initial treatment fish that died but that had previously shed their tag. We chose not to account for those fish in survival calculation, as their low number only marginally affected the survival estimates (6 fish died over 35 fish that lost their tag). For tagged fish, specific growth rate (SGR) was individually computed over two periods (SGR1 from 0 to 30 days and SGR2 from 30 to 60 days post-tagging) using the following formula (Busacker et al., 1990): $\mathrm{SGR}\,(\%) = \log_e (W_{t2}/W_{t1})/(t2 - t1) \times 100$, with W_{t1} and W_{t2} the weights (g) of a fish at time $t1$ and $t2$. PIT tag weight (0.1 g) was removed from all fish weights at recapture.

As control fish were not individually identifiable, survival and growth of tagged fish were first compared with untagged fish. Survival was analyzed using 2 × 2 contingency tables and Barnard's unconditional tests with Wald (W) statistics (Barnard, 1945; "Barnard" R package), which are more powerful than Fisher's exact tests for two binomial proportions (Mehta and Senchaudhuri, 2003). Log-transformed TL and weights were considered as proxies for growth to compare tagged to control fish. For this purpose, we used analyses of variance (ANOVA), with tagging and tank as fixed effects at 0, 30 and 60 days after tagging.

Generalized linear mixed models (GLMM) on a binomial probability distribution (logit model) were implemented to analyze survival and tag retention. SGR was analyzed using linear mixed models (LMM) on repeated measures (0–30 days, and 30–60 days post-tagging). For both GLMM and LMM, tagger and implantation method (and time in LMM) were treated as fixed effects. Tank was considered as a random effect. The significance of the variables was tested using likelihood ratio tests, compared to a χ^2 distribution (LR tests, Pinheiro and Bates, 2000). Residuals for linear mixed