

Brief Report

# Effects of Predation Tags on Growth and Stress Response in Juvenile Rainbow Trout *Oncorhynchus mykiss*

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**Abstract:** Acid-sensitive predation tags have recently been introduced to fisheries management. The objective of this study was to ascertain the impact of these tags on juvenile rainbow trout *Oncorhynchus mykiss* physiology and growth after implantation. Twenty approximately 13 g rainbow trout were placed into each of five tanks, with ten surgically implanted with dummy acid-sensitive predation tags and ten control fish not subjected to surgical procedures. Glucose, hematocrit, tag-retention, survival, and growth metrics were collected for 30 days post-surgery. Four tagged fish died while no control fish died. Tag retention was 76%, with tags lost in weeks 2, 3, and 4. Control fish were significantly longer and gained significantly more weight at the end of the experiment. Hematocrit levels for the tagged fish dropped significantly over the course of the trial and were 30% lower than those of the untagged control fish at the end of 30 days. Glucose levels were highly variable for both treatments. The results of this study indicate the negative impacts of predation tagging on the physiology of juvenile rainbow trout. Results from predation field trials should be interpreted with caution because the tagged fish are likely at a competitive disadvantage compared to their untagged conspecifics.

**Keywords:** rainbow trout; *Oncorhynchus mykiss*; acoustic transmitter; surgery; predation sensor



**Citation:** Millsap, E.K.; Huysman, N.; Gravenhof, D.A.; Fincel, M.J.; Barnes, M.E. Effects of Predation Tags on Growth and Stress Response in Juvenile Rainbow Trout *Oncorhynchus mykiss*. *Hydrobiology* **2023**, *2*, 467–474. <https://doi.org/10.3390/hydrobiology2030031>

Academic Editors: Constanze Pietsch and Lucian Pârvulescu

Received: 19 June 2023

Revised: 14 July 2023

Accepted: 17 July 2023

Published: 20 July 2023



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## 1. Introduction

Biotelemetry technology continues to advance, with a relatively new type of tag, the acid-sensitive predation sensor, recently introduced. This tag is designed to report a predation event due to contact with acids in the stomach of a predator [1]. These predation tags give the advantage over other tags in knowing whether the fish has been consumed by a predator whereas traditional tags simply show that the tagged fish is still moving, giving it the illusion that it is still alive [2,3]. Using predation tags can help eliminate the observation bias of seeing a moving tag and assuming that it is the original fish [4].

The ability of acid-sensitive predation tags to detect a predation event has been studied with mixed results. Lennox et al. [3] had a 50% false predation detection rate in laboratory trials and 30% false predation in field trials. In a field trial, Daniels et al. [4] had 24 out of 41 tags detect a predation event but could only declare that 5 of the tags were exclusively detected as post-predated. In this same experiment, nine tags were never detected, and 63% of tags signaling predation were only detected at one receiver. In another field trial, Weinz et al. [5] were able to positively assign 15 out of 19 fish as being predated. The remaining four fish had unclear fates. Halfyard et al. [1] had a 94 and 95% success rate of predation tags triggering a predation response in staged predation events.

Because of the impact of tagging data on fisheries management decisions, and the labor and cost associated with tagging, it is essential that the behavior, growth, and physiology of tagged fish be similar to that of untagged conspecifics [6–8]. Also, there is currently no standard protocol for recovery time post-surgery before stocking into the desired waterbody.

The USGS recommends holding salmonoids for 18–36 h post-surgery to lessen stress levels before transport and stocking [9]. However, the stress levels of fish can remain high for over a week post-stocking, which increases the chances of mortalities and tag expulsion [10].

There is a large need for information on the potential impact predation sensors may be having on fish physiology and growth. Thus, the objective of this study was to determine how the surgical implantation of dummy predation tags affects the stress, growth, and survival of juvenile rainbow trout *Oncorhynchus mykiss*.

## 2. Materials and Methods

This experiment occurred at McNenny State Fish Hatchery, Spearfish, South Dakota, U.S.A., using de-gassed and aerated well water (11 °C; total dissolved hardness 360 mg/L CaCO<sub>3</sub>; alkalinity as CaCO<sub>3</sub>, 210 mg/L; pH 7.6; total dissolved solids 390 mg/L). Five 190 L, flow-through, semi-square tanks were used, with each tank nearly fully covered with black corrugated plastic [11]. Each tank contained 20 Shasta strain rainbow trout *Oncorhynchus mykiss* (mean initial length 105 ± 0.7 mm and weight 13.3 ± 0.2 g,  $n = 100$ ), with 10 fish receiving an Innovasea V5 (Bedford, NS, Canada) dummy transmitter (0.65 g in air, 12.7 mm length, and 4.3 × 5.73 mm diameter) and 10 control fish. The V5 transmitter is designed so that it can either function as a traditional acoustic transmitter, depth sensor, or predation tag depending on the user's specifications. This transmitter tag burden (±SE) on the fish was 4.8 ± 0.09%. All fish were fed 1.5 mm floating feed (Protec FW Skretting; Tooele, Utah) daily to satiation from vibrating feeders (Sweeny AVF6; Cary, NC, USA). Just prior to the start of the experiment and approximately every week thereafter, each fish was measured (total length) to the nearest millimeter and weighed to the nearest 0.1 g (tagged fish had 0.65 g subtracted from their total weight to account for the tag). The experiment lasted for a total of 30 days.

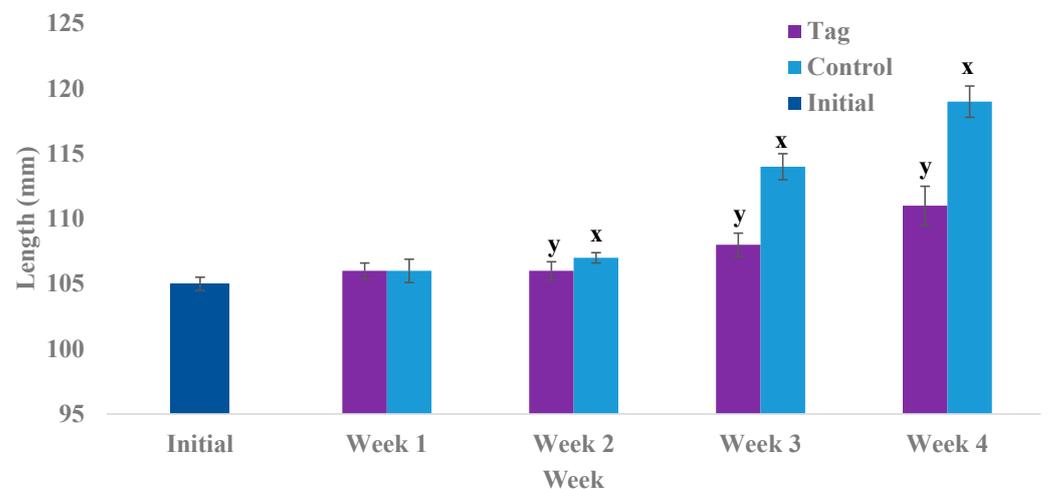
To surgically implant the dummy tags, each fish was anesthetized to stage IV using tricaine methane sulfonate (Tricaine-S MS-222, Syndel; Ferndale, WA, USA) [12]. After anesthetization, control fish were handled and then placed in their respective tanks. Experimental fish were placed ventral side upwards in a grooved sponge. A small incision, just large enough to insert the tag, was then made along the mid-ventral line. Iodine-soaked dummy acid-sensitive tags were then inserted into the peritoneal cavity. A singular suture (Securocryl Poliglecaprone 25 Monofilament, Riverpoint Medical; Portland, Oregon) was made to close the incision site. After suturing, the tagged fish were placed in their respective tanks for recovery.

Glucose and hematocrit data were collected from the common pool of fish initially and then from one tagged and one untagged rainbow trout from each tank at 2, 24, 48, 96, and 168 h and 30 days after the start of the experiment. To collect the blood sample, fish were euthanized using a lethal dose of tricaine methane sulfonate, and blood was collected by severing the caudal fin. Glucose was recorded using a blood glucose monitor (Accu-Check Guide Me, Roche Diabetic Care; Indianapolis, Indiana). Hematocrit was measured by first collecting a blood sample in a heparinized microhematocrit capillary tube (Fisher Scientific; Pittsburg, PA, USA) sealed with Critoseal (Oxford Labware; St. Louis, MO, USA) and placing it in a centrifuge for 10 min at 11,500 rpm. The percentage of red blood cells in relation to total blood volume was then recorded. Tanks were checked daily for ejected tags or mortality.

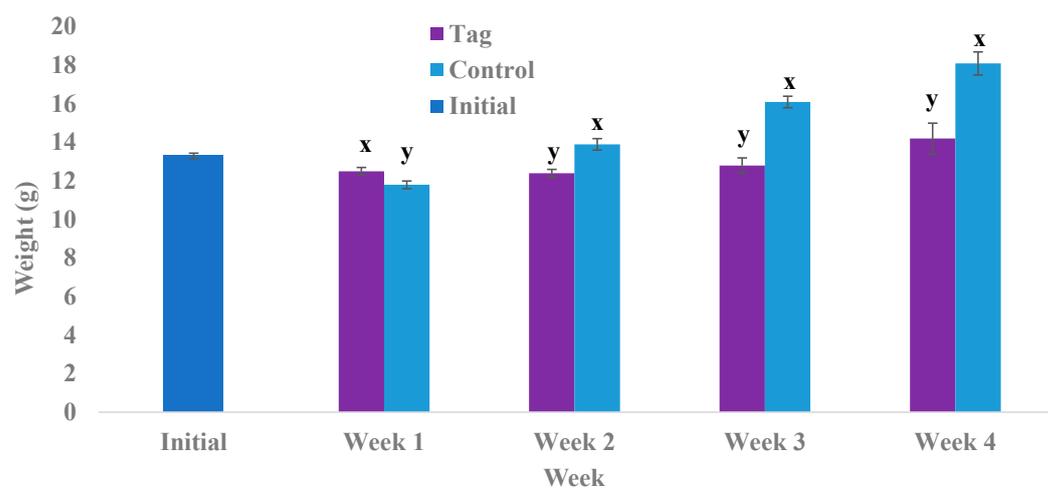
Data were analyzed using the SPSS (24.0) statistical analysis program (IBM, Armonk, NY, USA) with significance predetermined at  $p < 0.05$ . A repeated measures ANOVA was used to determine if differences occurred over the course of the study for glucose, hematocrit, weight, and length. If a significant difference was detected, then a one-way ANOVA on each timepoint was run as a post hoc test. Chi-square was used to determine if there was a significant difference in survival.

### 3. Results

Survival was significantly different between treatments at 100% in the untagged controls compared to 92% in the tagged fish ( $p = 0.041$ ). All mortality occurred in the first week. Tag retention was 76%, with two tags lost in the second week, three tags lost in the third week, and one tag lost in the fourth week. Mean lengths were significantly different between the tagged and untagged fish over the course of the trial ( $F_{2,38, 19.02} = 13.998$ ,  $p = 0.0001$ , Figure 1). Subsequent one-way ANOVA indicated that the control fish were significantly longer beginning in the second week and continuing to the end of the experiment. Similarly, untagged fish were also significantly heavier over the course of the trial ( $F_{1,36, 10.91} = 19.365$ ,  $p = 0.001$ , Figure 2). Beginning at week 2, the control fish began gaining weight significantly faster. Over the course of the trial, the control fish grew  $14 \pm 1$  mm and gained  $4.8 \pm 0.6$  g while the tagged fish grew  $6 \pm 1$  mm and gained  $0.9 \pm 0.9$  g.

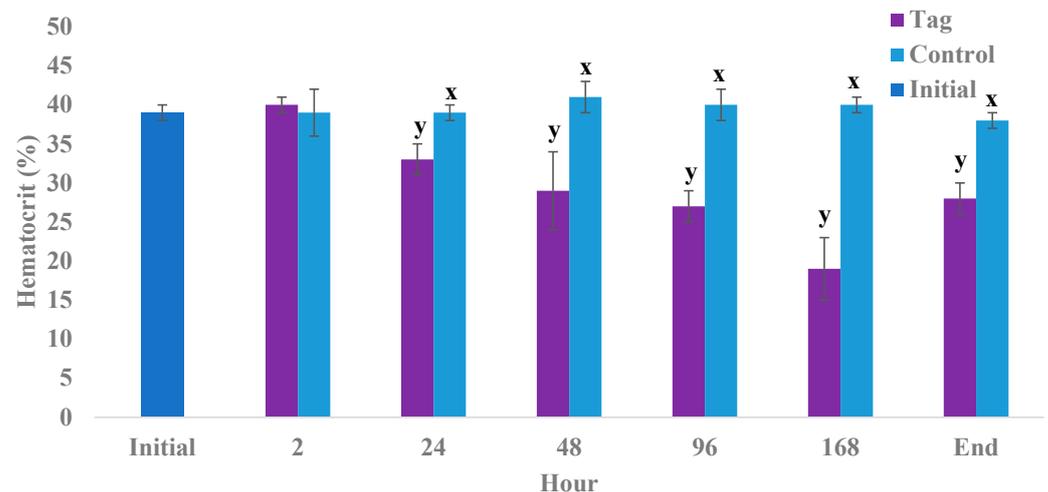


**Figure 1.** Mean total length (mm) of rainbow trout *Oncorhynchus mykiss* subjected to surgical implantation of a dummy acid-sensitive acoustic tag and a control group over a four-week experimental trial. The control group had significantly longer mean lengths over the four-week period ( $F_{2,38, 19.02} = 13.998$ ,  $p = 0.0001$ ). Means in a week with different letters above are significantly different from each other ( $p < 0.05$ ).

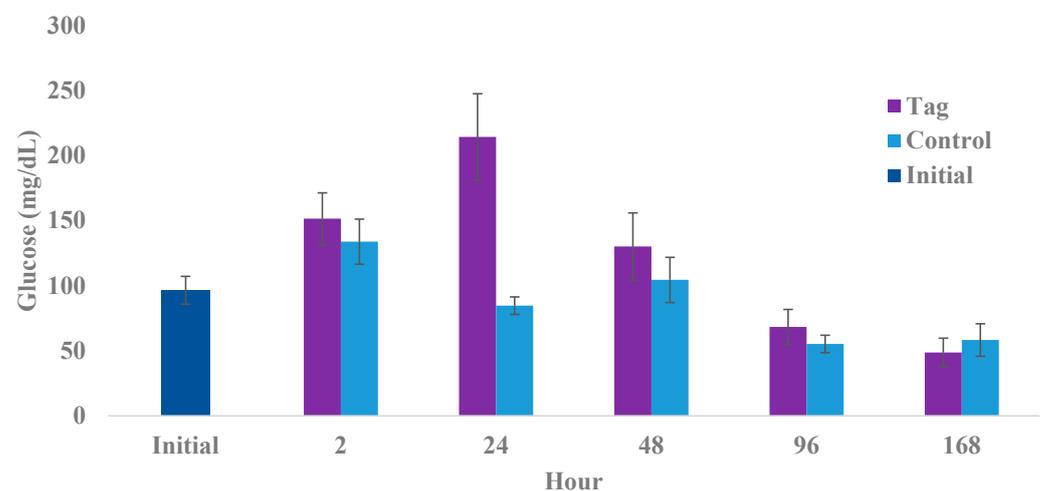


**Figure 2.** Mean total weight (g) of rainbow trout *Oncorhynchus mykiss* subjected to surgical implantation of a dummy acid-sensitive acoustic tag and a control group over a four-week experimental trial. The control group had significantly greater mean weights over the four-week period ( $F_{1,36, 10.91} = 19.365$ ,  $p = 0.0001$ ). Means in a week with different letters above are significantly different from each other ( $p < 0.05$ ).

Hematocrit levels were significantly different between the tagged and untagged fish throughout the course of the trial ( $F_{2,9, 23,19} = 5.360, p = 0.006$ , Figure 3). Beginning at 24 h and extending to the end of the study, control fish had similar hematocrit levels to basal levels. However, hematocrit decreased by up to 50% in the tagged fish. By the end of the trial, tagged fish hematocrit began to increase slightly (30% reduction compared to control) but never attained levels close to basal levels. Glucose showed no significant difference over the course of the trial ( $F_{2,16, 17,29} = 2.601, p = 0.1$ , Figure 4).



**Figure 3.** Mean hematocrit levels of rainbow trout *Oncorhynchus mykiss* subjected to surgical implantation of a dummy acid-sensitive acoustic tag and a control group over a four-week experimental trial. The control group had significantly higher hematocrit levels over the four-week period ( $F_{2,9, 23,19} = 5.360, p = 0.006$ ). Means in a week with different letters above are significantly different from each other ( $p < 0.05$ ).



**Figure 4.** Mean glucose levels (mg/dL) of rainbow trout *Oncorhynchus mykiss* subjected to surgical implantation of a dummy acid-sensitive acoustic tag and a control group over a four-week experimental trial. There was no significant difference over the course of the trial ( $F_{2,16, 17,29} = 2.601, p = 0.1$ ).

#### 4. Discussion

The results of this study displayed the negative short-term impacts of transmitter surgery on juvenile rainbow trout. Fish that underwent surgery grew significantly less, had reduced survival, and had significantly lower hematocrit levels compared to control fish. Growth and behavioral results from acoustic and predation transmitter surgeries have been inconsistent. Urbaniak et al. [13] found that acoustically tagged rainbow trout grew slower for 38 days compared to untagged rainbow trout. Similarly, acoustically

tagged juvenile Atlantic salmon *Salmo salar* have been shown to have reduced growth performance [14,15]. Contrarily, Brown et al. [16] found no difference in the growth of sock-eye salmon *Oncorhynchus nerka*, but tagged fish did have reduced swimming performance compared to control and sham fish. Smircich and Kelly [17] did not witness a difference in swimming performance but found reduced growth of brook trout *Salvelinus fontinalis* in their heaviest tag treatment. The inconclusive nature of the results of these studies shows the difficulty in determining just how impactful these types of surgeries and tags are on the fish being studied.

Hematocrit is a measure of the capacity of red blood cells to carry oxygen through the body. Thus, a reduction in hematocrit means a reduced ability to effectively function at optimum levels [18]. The untagged fish in this study maintained a 40% hematocrit level throughout the trial, which is well within the normal range of 30–40% [19]. However, the tagged fish were clearly anemic. Hematocrit levels of tagged fish began lowering within 48 h after surgery and continued to decrease for 168 h (7 days), finally reaching a nearly 50% reduction. By the end of the trial (30 days), hematocrit levels of tagged fish were still approximately 30% lower than the initial values and those of the control group. Reduced hematocrit (anemia) in fish is usually associated with infection [20], parasites [21], or toxins in the diet or water [22,23]. Rainbow trout with as little as a 22% reduction in hematocrit have been shown to have significant reductions in critical swimming velocity and maximal oxygen uptake [24].

High-stress environments should trigger an increase in hematocrit to enhance the blood's ability to carry oxygen under the high energy demand of stress [25]. For example, Fazio et al. [26] found an increase in hematocrit in sea bream faced with multiple acute handling stresses. Smircich and Kelly [17] did not find an increase in hematocrit with swimming trials in tagged brook trout but also did not have a control group that did not have surgery for comparison or a basal hematocrit reading prior to surgery. The fact that tagged fish in this study had reduced hematocrit levels that acted more like infection suggests that the body treated the tags and sutures as an infection. A long-term 30% reduction in hematocrit would likely impair the ability of the tagged fish to function after release into the wild. Thus, compared to the untagged fish without anemia, the tagged fish would likely be more susceptible to predation and have a hindered ability to feed.

The anemic response as seen in this study is not unprecedented. It has been shown that post-operative salmonids may have reduced hematocrit levels for up to 3 weeks [26,27]. Also, it is unknown exactly how long it takes for a juvenile rainbow trout to replace its total blood volume following surgery. It has been shown that rainbow trout can begin increasing total blood volume as soon as 30 min post-injection of labeled red blood cells [28].

Increases in glucose, a secondary stress response, are not as immediate as primary stress markers [29]. As expected, both the tagged and control groups' glucose levels fluctuated at similar rates despite the relatively high variance throughout the experiment. The glucose levels observed were relatively lower than the normative values of  $108 \pm 9.98$  mg/dL reported for rainbow trout [30]. However, glucose levels in both the tagged and untagged control fish rose and fell along the same points throughout the experiment. The initial glucose stress response and return to basal levels found in this experiment were similar to other studies also using rainbow trout [31–34].

Tag retention, after the exclusion of euthanized fish for glucose and hematocrit data collection, was 19/25 fish, or 76%. This is similar to the 73 and 78% tag retention for hydroacoustic tags in rainbow trout reported by Urbaniak et al. [13] and Kientz et al. [35]. Tags were lost either through expulsion at the incision site or through the nearby skin, both of which involve the proliferation of tissue at the site of least resistance [36]. Fish that expelled tags survived for the remainder of the study.

It is unknown if the 5% tag burden used in this study could have affected the results. While Winter [37] initially stated that the tag should not exceed 2% of the fish body weight, subsequent studies have successfully implanted tags well above that level. Lennox et al. [38] showed no effect on the migration or behavior of Atlantic salmon sub-

jected to a 5.2% predation tag burden. Brown et al. [39] showed no effect on swimming performance for rainbow trout with a tag burden of 6 to 12%. Salmonids in general have fared well with increased tag-to-body ratios [17,40–42].

## 5. Conclusions

The results of this study show the negative impacts of predation tag implantation on juvenile rainbow trout physiology and growth. Of particular concern is the relatively long-term anemia associated with predation tags combined with the fact that rainbow trout that undergo surgery are known to have chronic inflammation up to 10 weeks after surgery [43]. While individual fish behavior was not evaluated in this study, based on the negative impacts associated with the implantation and retention of predation tags, assuming identical behavior to untagged conspecifics may not be correct. If surgeries are performed “in the field” with fish being released shortly after surgery, it is likely that implanted fish are at a competitive disadvantage compared to untagged conspecifics. Considerable additional controlled research in a closed environment is needed to determine post-surgery recovery times, particularly in relation to tag burden, and investigate techniques to minimize the negative effects of predation tag surgery and retention on fish physiology, growth, and survival.

**Author Contributions:** Conceptualization, N.H., E.K.M., D.A.G., M.J.F. and M.E.B.; methodology, N.H., D.A.G. and M.E.B.; formal analysis, N.H.; investigation, N.H. and E.K.M.; data curation, N.H. and E.K.M.; writing—original draft preparation, E.K.M. and N.H.; writing—review and editing, N.H., E.K.M., D.A.G., M.J.F. and M.E.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** This experiment was performed within the guidelines set out by the Aquatics Section Research Ethics Committee of the South Dakota Department of Game, Fish and Parks (approval code, SDGFPARC20233), and within the guidelines for the Use of Fishes in Research set by the American Fisheries Society.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available from authors upon request.

**Acknowledgments:** We would like to thank Jaid Freestone, Molly Gross, and Natalie Geary for their assistance in this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

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