

Micro-acoustic Transmitter Implantation Impacts Juvenile Rainbow Trout *Oncorhynchus mykiss* Growth, Hematocrit and Splenosomatic Index

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Abstract

Micro-acoustic transmitters are becoming increasingly popular in fisheries management. This study examined the short-term effects of micro-transmitter surgical insertion on juvenile rainbow trout *Oncorhynchus mykiss* (mean (SE) initial weight=23.9 (1.8) g, length=124 (2) mm). One group of trout were anesthetized and surgically implanted with micro-acoustic transmitters (tag-to-body ratio=2.94 (0.07) %). A second control group only underwent anesthesia. Ten fish from each group were placed in one of five experimental tanks for eight weeks, with weight, length, hematocrit, hepatosomatic index, viscerosomatic index and splenosomatic index data collected weekly. Survival in the untagged control group was 100%, which was significantly greater than 91.8% in the tagged group. Tag retention was 71%. Total lengths and weights were significantly less for the first six weeks after surgery in tagged fish compared to the control fish. Hematocrit was significantly lower and splenosomatic index was significantly higher in the tagged trout for the first three weeks. Hepatosomatic index and viscerosomatic index were not significantly different between the groups throughout the study. This study provides additional documentation of the potential negative effects of micro-acoustic tag implantation on juvenile rainbow trout. A minimum three-week recovery period is recommended for juvenile fish tagged at a 2.9% tag-to-body ratio.

Keywords: Rainbow trout; *Oncorhynchus mykiss*; Micro-acoustic transmitter; Surgery

Cameron et al. reported significantly reduced survival for sub-yearling Chinook salmon *Oncorhynchus tshawytscha* tagged with micro-acoustic transmitters [10]. Micro-acoustic tagged Chinook salmon exposed to rapid decompression experienced significantly reduced survival compared to untagged counterparts [11]. Furthermore, migrating Chinook salmon with micro-acoustic tags have reduced survival, longer downstream migration times and experience heightened inflammation inside the body cavity compared to untagged fish from the same group [9]. Lastly, a significant and large decrease in hematocrit, indicating an anemic response, was observed in juvenile rainbow trout *Oncorhynchus mykiss* for at least 30 days after implantation of dummy micro-acoustic tags [12].

The duration of anemia in micro-acoustic-tagged trout is unknown. Millsap et al. [12] experiment lasted only 30 days and hematocrit in the tagged trout did not return to basal levels by the end of the experiment. However, the mean tag-to-body ratio used by Millsap et al. [12] was 4.8%. Although this is more than the 2% ratio recommended by Winter over 25 years ago, it is well within the higher tag-to-body ratios currently being used with small salmonids [13-21]. It is unknown if these negative effects of tagging on hematocrit occur in trout at lower tag-to-body ratios.

This study had two objectives involving rainbow trout closer to a 2% tag-to-body ratio. The first objective was to determine the time required for complete recovery of anemia in micro-acoustically tagged rainbow trout. The second objective was to assess the potential impacts of micro-acoustic tagging on relatively larger juvenile rainbow trout to growth and morphological indices.

Introduction

Acoustic transmitters have been used in fisheries management to study behavior, survival and migration patterns of numerous fish species [1-6]. For acoustic transmitter data to be accurate and reliable, post-tagging behavior, physiology, growth and immune function of acoustically tagged fish must be similar to untagged fish [7-9]. However, this has not been observed in several studies.

Materials and Methods

All experimentation occurred at McNenny State Fish Hatchery, rural Spearfish, South Dakota, USA, using degassed and aerated well water (11°C; total hardness 360 mg/L CaCO₃; alkalinity as CaCO₃ 210 mg/L; pH 7.6, total dissolved solids 390 mg/L). This study used 98 Arlee strain rainbow trout. These fish arrived at the hatchery as eyed eggs on 23 November 2022 and had been on feed for approximately 150 days prior to use in the

experiment. Experimentation occurred in five 190-L semi square tanks. The top of each tank was partially covered with corrugated black plastic and the remaining opening covered with netting to prevent fish escapement [22].

All the fish were anesthetized with 60 mg/L tricaine methane sulfonate (MS-222, Syndel; Ferndale, Washington, USA). After approximately five minutes in anesthetic solution, each fish was weighed to the nearest 0.1 g and measured (total length) to the nearest mm. One-half of the fish (n=49) were then surgically implanted with a dummy acoustic transmitter (Innovasea V5, Nova Scotia, Canada; weight=0.7 g, length=12.7 mm, diameter=4.3 × 5.7 mm). To minimize potential surgeon bias, a single, experienced surgeon conducted all the surgeries [23]. Fish undergoing surgery were placed ventral side up in a grooved sponge and an incision was made just large enough for transmitter insertion. The location of the incision was beside the mid-central line and anterior to the pelvic girdle (Figure 1).

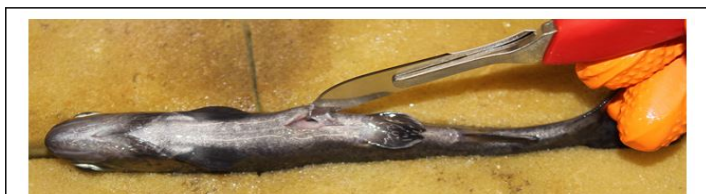


Figure 1: Size and location of incision for surgical implantation of a dummy micro-acoustic tag into juvenile rainbow trout.

After dummy transmitter insertion (Figures 2 and 3), the incision was closed with one suture (PDO II Violet monofilament absorbable polydioxanone suture, Oasis; Mettawa, Illinois, USA). After surgery, the tagged fish were placed in a recovery tank of fresh water. Anesthetized-only control fish were also placed in a recovery tank after five minutes of anesthesia, which was the typical duration of anesthesia for the tagged fish. After the fish recovered from anesthesia and were freely swimming, they were placed into one of the five tanks. Because of a limited number of dummy transmitters, four of the five tanks received 20 fish (10 tagged, 10 untagged), with one tank receiving 18 fish (9 tagged, 9 untagged).



Figure 2: Insertion of dummy micro-acoustic tag into the coelomic cavity through incision of a juvenile rainbow trout.

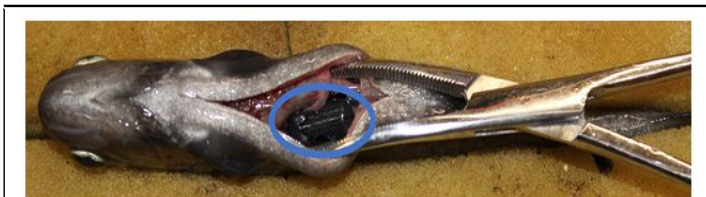


Figure 3: Location of a dummy micro-acoustic tag in the coelomic cavity of a juvenile rainbow trout. The incision was enlarged with forceps to allow the tag (in the blue circle) location to be clearly seen.

Every seven days and at the end of the experiment (day 56), all the fish were anesthetized, measured to the nearest mm (total length) and weighed to the nearest 0.1 g. One randomly selected experimental fish and one randomly selected control fish from each tank were euthanized with a lethal dose of 200 mg/L MS-222. Following euthanasia, the caudal fin was severed. Blood was collected in a heparinized microhematocrit capillary tube (Fisher Scientific; Pittsburg, Pennsylvania) and sealed with sealant (Critoseal, Oxford Labware, Sherwood Medical Products, St. Louis, Missouri, USA). The capillary tube was then centrifuged at 11,500 rpm for 10 minutes to separate the volume of red blood cells from blood plasma. A digital caliper was used to measure the red blood cell and the total blood volume in the capillary tube to the nearest 0.01 mm [24]. These values were used to directly calculate hematocrit [25]. The liver, viscera and spleen were removed from the fish and weighed to the nearest 0.0001 g. Hematocrit, Hepatosomatic Index (HSI), Viscerosomatic Index (VSI), Splenosomatic Index (SSI) and Specific Growth Rate (SGR) values were obtained using the following formulas:

$$\text{Hematocrit (\%)} = (\text{red/whole blood}) \times 100$$

$$\text{Hepatosomatic Index (HSI)} = (\text{liver weight (mg)/total weight (mg)}) \times 100$$

$$\text{Viscerosomatic Index (VSI)} = (\text{viscera weight (mg)/total weight (mg)}) \times 100$$

$$\text{Splenosomatic Index (SSI)} = (\text{spleen weight (mg)/total weight (mg)}) \times 100$$

$$\text{Specific Growth Rate (SGR)} = \left[\frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}} \right] \times 100$$

Each tank of fish received 1.5 mm extruded feed (Protec, Skretting; Tooele, Utah, USA) using automatic feeders (Pentair AES AVF6; Cary, North Carolina, USA). Fish were fed to apparent satiation. Feeding did not occur on the day of data collection each week. The experiment lasted for a total of eight weeks post-surgery.

Data were analyzed using the SPSS (24.0) statistical analysis program (IBM, Armonk, New York, USA) with significance predetermined at $P < 0.05$. A repeated measures analysis of variance ANOVA was used to determine if differences occurred between the untagged (control) and tagged groups over the course of the study for weight, length, hematocrit, hepatosomatic index, viscerosomatic index and splenosomatic index. The tanks were the experimental unit and the fish were a fixed effect. Mauchly's sphericity test was used to test for equal variances. If variances were unequal, a Huynh-Feldt correction was used. If the repeated measures ANOVA indicated overall significant differences between the tagged and untagged fish, a post-hoc test t-test was conducted at each weekly time point. Specific growth rate values were negative for the first week of the experiment, negating the use of repeated measures ANOVA. Instead, a t-test was used to analyze specific growth rate data for each week. *Chi-square* analysis was used to determine if there was a significant difference in percent survival between the tagged and untagged groups.

Results

At 91.8%, survival was significantly lower ($P=0.041$) in the tagged fish compared to the 100% survival in the untagged control fish. Tag retention was 71%, with three tags lost in the second week, one tag lost in the fourth week, four tags lost in the fifth week, three tags lost in the sixth week and three tags lost in the seventh week.

The initial mean (SE) weight and total length of the rainbow trout used in this study were 23.9 (1.8) g and 124 (2) mm, respectively. This fish size in relation to transmitter size resulted in an initial mean (SE) tag-to-body ratio of 2.94 (0.07) % in the surgically implanted trout. There was a significant difference in length ($F_{4.54, 36.28}=4.72$, $P=0.003$) and weight ($F_{4.87, 38.92}=2.56$, $P=0.044$) between the tagged and untagged groups of trout over the course of the trial (Figures 4 and 5). The control, anesthetized-only fish weighed significantly more than fish implanted with dummy acoustic transmitters at the end of the first, second, third, fifth and sixth weeks. Similarly, control fish were significantly longer than tagged fish at the end of the first, third, fifth and sixth weeks.

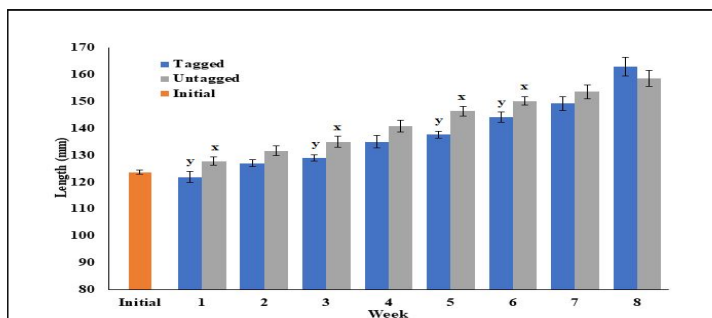


Figure 4: Mean (SE bars) total length of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. The control group was significantly longer over the eight weeks ($F_{4.54, 36.28}=4.72$, $P=0.003$). Means in a week with different letters above are significantly different from each other (P values for each week are 1=0.046, 2=0.075, 3=0.031, 4=0.109, 5=0.005, 6=0.042, 7=0.249, 8=0.362).

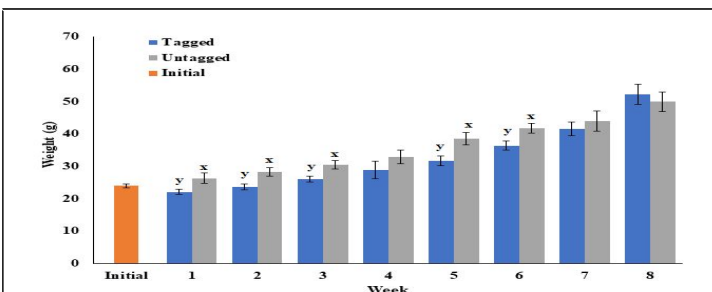


Figure 5: Mean (SE bars) total weight of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. The control group was significantly heavier over the course of the trial ($F_{4.87, 38.92}=2.56$, $P=0.044$). Means in a week with different letters above are significantly different from each other (P values for each week are 1=0.046, 2=0.022, 3=0.028, 4=0.288, 5=0.022, 6=0.033, 7=0.521, 8=0.617).

Specific growth rate was highly variable, with large standard errors at each sampling period (Figure 6). It was significantly different between the groups at the end of the first week, with negative values for the surgically implanted fish compared to positive values for the untagged control fish. While specific growth rate was positive for both groups of fish through the remainder of the experiment, there were no significant differences observed between the tagged and untagged fish.

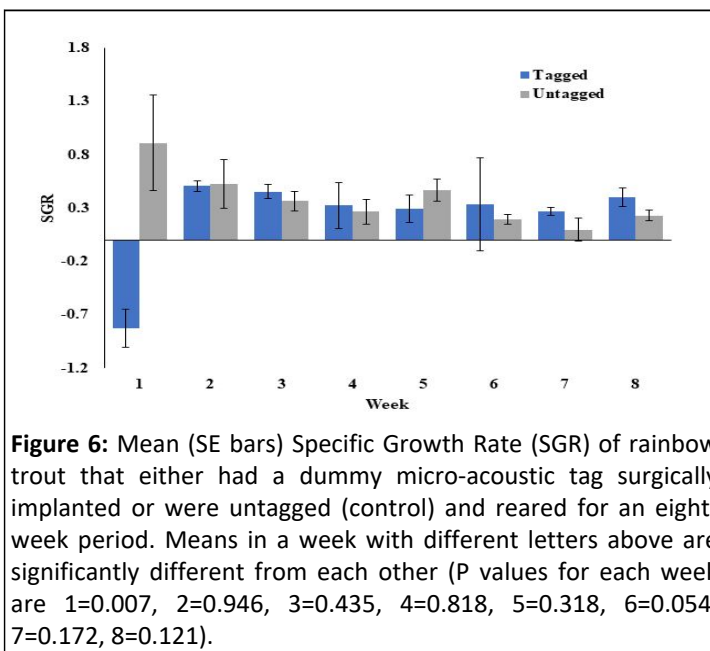


Figure 6: Mean (SE bars) Specific Growth Rate (SGR) of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. Means in a week with different letters above are significantly different from each other (P values for each week are 1=0.007, 2=0.946, 3=0.435, 4=0.818, 5=0.318, 6=0.054, 7=0.172, 8=0.121).

Hematocrit was significantly lower in the tagged fish compared to the untagged fish over the course of the trial ($F_{8,0, 40}=2.82$, $P=0.01$) (Figure 7). Significant differences occurred at the end of the first, second and third weeks of the experiment. Hematocrit was not significantly different between the two groups during the remainder of the experiment with levels stabilizing near basal for both treatments.

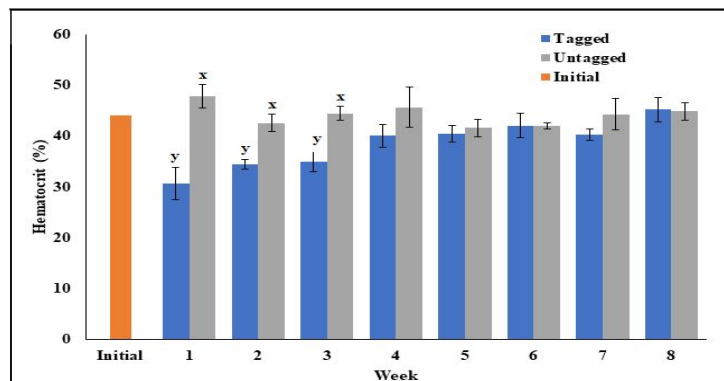


Figure 7: Mean (SE bars) hematocrit of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. The control group had significantly higher hematocrit levels over the course of the trial ($F_{8,0, 40}=2.82$, $P=0.01$). Means in a week with different letters above are significantly different from each other (P values for each week are 1=0.002, 2=0.003, 3=0.004, 4=0.227, 5=0.653, 6=0.994, 7=0.219, 8=0.924).

Neither hepatosomatic index ($F_{8,0, 64}=0.91$, $P=0.51$) nor viscerosomatic index ($F_{8,0, 64.0}=0.59$, $P=0.78$) were significantly different between the tagged and untagged fish throughout the experiment (Figures 8 and 9). However, splenosomatic index was significantly different between the groups ($F_{5,48, 43.85}=4.59$, $P=0.001$) (Figure 10). At the end of the first, second and third weeks, splenosomatic index was significantly higher in the fish with surgically implanted transmitters compared to untagged control fish. In the fourth, fifth, sixth and seven weeks after the start of the experiment, splenosomatic index was not significantly different between the tagged and untagged fish. However, at the end of the experiment (eighth week), the splenosomatic index was again significantly higher in the tagged group compared to the control group. In general, over the duration of the experiment, splenosomatic index in the tagged fish group was highly variable and never appeared to stabilize.

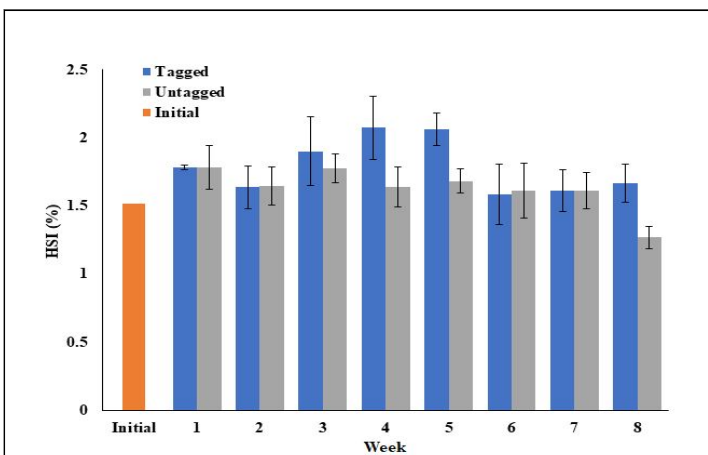


Figure 8: Mean (SE bars) Hepatosomatic Index (HSI) of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. There was no significant difference over the course of the trial ($F_{8,0, 64}=0.91$, $P=0.51$).

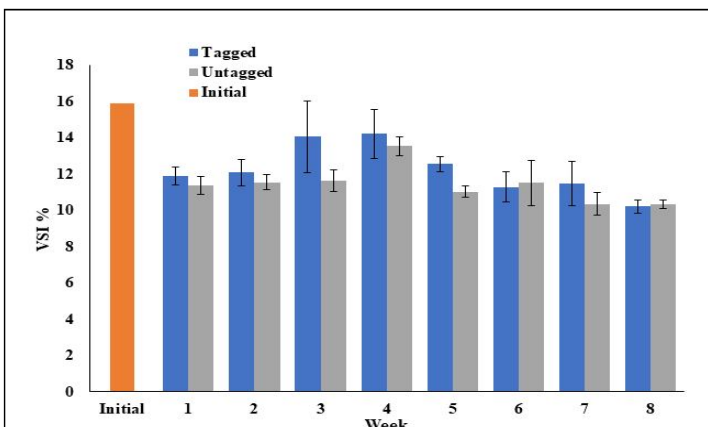


Figure 9: Mean (SE bars) Viscerosomatic Index (VSI) of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. There was no significant difference over the course of the trial ($F_{8,0, 64.0}=0.59$, $P=0.78$).

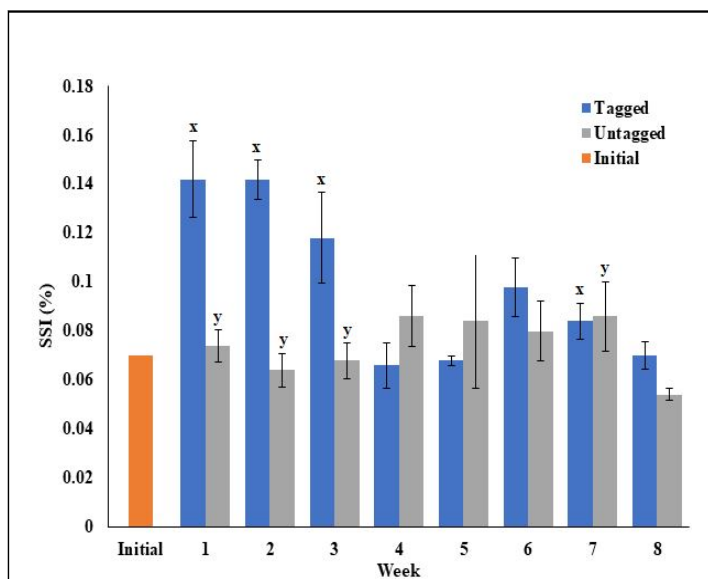


Figure 10: Mean (SE bars) Splenosomatic Index (SSI) of rainbow trout that either had a dummy micro-acoustic tag surgically implanted or were untagged (control) and reared for an eight-week period. The tagged group had significantly higher SSI levels over the course of the trial ($F_{5,48, 43.85}=4.59$, $P=0.001$). Means in a week with different letters above are significantly different from each other (P values for each week are 1=0.004, 2=0.001, 3=0.037, 4=0.235, 5=0.575, 6=0.324, 7=0.903, 8=0.029).

Discussion

The results of this study showing reduced hematocrit, increased splenosomatic index and reduced growth in juvenile rainbow trout for three weeks after tag implantation indicates that micro-acoustic tagging can have substantial negative short-term effects. These results support those of Millsap et al., who observed a similar large decrease in hematocrit in a smaller size class of rainbow trout [12]. Heightened inflammatory responses and poor body-conditions have also been reported in juvenile Chinook salmon tagged with micro-acoustic transmitters [9].

Hematocrit is the ratio of red blood cells to whole blood volume. A reduced hematocrit is indicative of anemia, which results in a reduced capacity to transport oxygen and subsequent negative impacts on energy utilization [26]. Transmitter implantation is highly invasive [27]. The 25% reduction in hematocrit observed in tagged fish for 21 days in this study indicates micro-acoustic tag implantation was an acute stress event for the fish [28,29]. Thus, it is problematic to assume that recently tagged fish are similar to untagged conspecifics. A 22% reduction in hematocrit has been shown to significantly reduce critical swimming velocities and maximal oxygen uptakes in fish [30]. Millsap et al. reported a 50% reduction in hematocrit a week after implanting micro-acoustic tags in rainbow trout, with hematocrit levels never reaching control fish levels during the four-week experiment. Because

predation tags have a relatively short battery life, implantation typically occurs shortly before release of the fish, at a time when the fish are most likely still anemic [6,21,31].

It is unknown if the large decrease in hematocrit observed in this study is from blood loss during the surgical process or is a stress response from the tag itself. However, surgery of pikeperch *Sander lucioperca* insertion of radio transmitters with a tag burden of less than 1.2% body weight did not result in a reduction of hematocrit compared to control fish [32]. Typically, hematocrit reductions in fish are due to parasites, infections, toxins or heavy metals in the water [33-39]. It is possible that the rainbow trout used in this study acted as if the tag was a foreign parasite or infection and increased white blood cell production to counteract this perceived threat.

In simplest terms, specific growth rate is the percentage increase in weight per day based on the exponential growth typically observed in smaller fish, like those used in this study [40]. Negative specific growth rates indicate weight loss [41]. Weight loss during the first week after tag implantation surgery in fish has been previously reported [42]. It is possible the negative specific growth rate observed in the tagged trout during the first week of this study could be because the fish either ceased or decreased food consumption because of the post-surgery inflammatory response or a combination of these factors [9,43]. However, Robertson et al. reported decreased growth but no difference in food consumption after transmitter implantation surgery in Atlantic salmon *Salmo salar* parr, indicating that it is more likely weight loss in the current study was a direct result of the post-surgery healing process [44].

Other studies using acoustic tags have observed adverse impacts on the growth of fish. For example, acoustically tagged rainbow trout grew slower for at least 38 days compared to untagged rainbow trout [45]. A similar decrease in growth was observed in acoustically tagged juvenile Atlantic salmon [44,46] and brook trout *Salvelinus fontinalis* [20]. Growth was not reduced in tagged sockeye salmon *Oncorhynchus nerka*, but swimming performance was reduced compared to sham and control treatments [47]. Similar results have been documented in coho salmon *Oncorhynchus kisutch* [16], Chinook salmon [17] and Atlantic salmon [44]. In a four-week study with juvenile rainbow trout, growth rates were lower throughout the study in the tagged fish compared to untagged controls [12].

The spleen in fish is directly involved in immune function and is the site of antibody production [48]. As such, the splenosomatic index is an indicator of both the immune status of the fish and its hematopoietic capacity [49-51]. Splenosomatic index values of the control (untagged) fish in the current study were within the range reported by numerous other studies involving rainbow trout [52-56]. However, the splenosomatic index of the tagged fish at both the start and end of the current study are much higher than those previously reported, indicating physiological stress in the fish receiving predation tags. It is unknown if the increase in relative spleen size in the tagged fish was a response to anemia or an indicator of challenges to the immune system.

Nearly 30 years ago, Winter [13] recommended a maximum 2% tag burden in relation to the total weight of the fish. Subsequent studies have successfully pushed well beyond that limit and it is no longer well-supported [9,15,19-21,57,58]. Thus, it is not surprising that the 2.9% tag-to-body ratio used in this study produced similar results to the 4.8% tag-to-body ratio used by Millsap et al [12]. It was also within the range of tag sizes used by Wargo Rub et al. [9] who observed similar results. It should be noted however, that there have been conflicting results associated with similar tag-to-body ratios. Lennox et al. [21] found no significant difference in migration for Atlantic salmon smolts at 5.8% tag-to-body ratio, but a highly extensive study by Wargo Rub et al. [19] found lower survival and increased migration times for acoustically tagged Chinook salmon with a mean tag-to-body ratio of 2.3%. Similarly, Smircich and Kelly [20] reported no difference in swimming performance in brook trout with tag burdens up to 7%, while Perry et al. [59] reported reduced swimming performance of juvenile Chinook salmon at a tag burden range from 3.4%-4.0%.

The lack of significant differences in hepatosomatic index between the tagged and untagged fish in this study indicates that tag implantation and surgery did not impact subsequent energy partitioning. Hepatosomatic index indirectly measures glycogen and carbohydrate levels and indicates the nutritional status of the fish [50,60-62]. The similar viscerosomatic index levels in the tagged and untagged fish indicate that tag implantation and surgery did not affect lipid metabolism [63-66].

It is possible, but unlikely, that the co-rearing of tagged and untagged fish may have influenced the results of this study. Rearing both groups of fish in the same tank was done to provide replication and because it is how tagged fish would typically be handled in a hatchery setting. Production hatcheries usually do not have the space to maintain tagged fish in a separate rearing unit or place individually tagged fish in their own discrete rearing units after surgery. Thoreau and Baras [67] rejected the idea that untagged fish somehow impaired the recovery of tagged tilapia *Oreochromis aureus*. In contrast to domesticated rainbow trout, Tilapia are much more territorial [68,69]. In addition, the rearing densities used in the current study were low. The maximum density index was only 0.32, which is well below the typical recommendations of 0.5-1.0 for rainbow trout [70].

Lietdtke et al. [71] recommending holding fish for up to 36 hours after surgical tag implantation before transport or stocking. Thoreau and Baras [67] recommended doubling the recovery period to 72 hours. However, both time frames are likely much too soon to release micro-acoustic tagged fish. Mortality and tag expulsion could occur well after this period, with fish stress remaining high for up to 168 hours post-stocking [72]. In addition, the stressful effects of loading and stocking [73,74] in combination with post-tagging anemia would be very problematic. Thus, the behavior and survival [74] of any fish released less than 30 days after tagging would most likely not be representative of untagged individuals, rendering any tagging data collected prior to 30 days inaccurate and unreliable [12].

The 75% tag retention observed in this study was nearly identical to the 76% retention reported by Millsap et al. [12] in a similar study using smaller rainbow trout. Tag retention in this study was also similar to the 73% reported by Urbaniak et al. [45] and the 78% reported by Kientz et al [76]. Tag expulsion occurred through the incision site, where it was likely enabled by the loss of skin integrity and inflammation [77]. Just as Millsap et al. [12] reported, the fish that expelled their tags remained alive for the duration of the experiment. In the current study only tagged fish died, with the mortality primarily occurring within the first two weeks after surgery. The 91.8% survival of the tagged fish and 100% survival of the control fish was also nearly identical to the 92% and 100% survival rates reported with smaller rainbow trout by Millsap et al [12].

The constant 11°C water temperature used in this study produced a very favorable tagging environment for the rainbow trout. Warmer temperatures, particularly above 17°C, have led to decreased survival, poorer surgical wound healing and poorer tag retention in tagged trout [9,78,79]. Higher temperatures increase the inflammatory response and may impact the intensity and longevity of the anemia observed in this study [30,80,81].

The Innovasea V5 dummy acoustic tag used in this study can be customized to collect different types of data. They are increasingly being used as acid-sensitive predation sensors to evaluate the survival of fish for a short time period after stocking [6,82-84]. The results of this study, along with those of Wargo Rub et al. and Millsap et al., [12] strongly suggest that the information [9] obtained from these transmitters should be used with caution. For example, Gravenhof et al. [6] estimated predation rates for juvenile Chinook salmon stocked at either 5-6 days or 19-20 days after surgical implantation of predation tags physically identical to those used in this and the Millsap et al. [12] study. The short-term anemic response, which appears to last longer in smaller fish, increased splenosomatic index and weight loss or reduced growth would likely make the tagged fish more vulnerable to predation, thereby negating the assumption that they are representative of untagged fish. It cannot be assumed that implanting micro-acoustic transmitters has a negligible effect on the tagged fish.

Conclusion

This study documented the negative effects of decreased hematocrit, reduced growth and potential immunological issues associated with predation tag implantation on juvenile rainbow trout. These results appear to invalidate the assumption that untagged and tagged fish behave and survive similarly after tagging. A minimum three-week recovery period is needed after surgery for the recovery of fish surgically implanted with acoustic tags. For wild fish tagged and immediately released, any data collected for the first three weeks should either be disregarded or used with extreme caution. More research in a controlled environment is needed to determine the post-implantation recovery times required for the additional species and sizes of fish receiving acoustic tags.

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