

## Survival and Growth of Age-0 Steelhead after Surgical Implantation of 23-mm Passive Integrated Transponders

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**Abstract.**—Little information is available on the effects of implanting 23-mm passive integrated transponder (PIT) tags in salmonids less than 90 mm fork length (FL). Using juvenile steelhead *Oncorhynchus mykiss* (range, 73–97 mm FL), we compared instantaneous growth rates and survival among three experimental groups: control, surgery with no tag, and surgery with tag. Survival rate was lower for tagged fish (86%) than for control and surgery–no tag fish (virtually 100% in each group). Approximately 90% of the mortalities occurred during days 1–3. Growth rate for the tagged group was lower for the first two 10-d measurement intervals; however, during the third 10-d interval, growth rates for tagged fish equaled or exceeded values for the other groups. These results suggest that tagged fish recovered by day 20. Growth rates for the control and surgery–no tag groups did not differ from one another during any measurement interval. Tag retention rate was 97% over the 30-d period of the study. It appears that the combination of fish length and tag size in this study resulted in short-term negative effects on growth rate and survival; however, 23-mm PIT tags may still be useful for studies of salmonids 80–90 mm FL when survival is not the parameter of interest.

Passive integrated transponder (PIT) tags have been used successfully to evaluate the movement, growth, and survival of a variety of fishes. These tags have many desirable qualities, including the identification of individuals, tag life equal to that of the tagged individual, and the ability to be detected remotely. These attributes provide a cost-effective way to gather behavioral and survival information on fishes. Passive integrated transponder tags come in a variety of sizes and, in general, larger PIT tags have greater detection distances (with other parameters held constant). In applications where fish are not recaptured but are remotely detected via fixed or mobile antenna, PIT-tag

size becomes an important consideration with regard to sampling efficiency.

The effects of PIT tags on fish survival (Prentice et al. 1990; Peterson et al. 1994; Ombredane et al. 1998; Gries and Letcher 2002), growth (Prentice et al. 1990; Peterson et al. 1994; Ombredane et al. 1998; Zydlewski et al. 2003), behavior (Zydlewski et al. 2003), and performance (Prentice et al. 1990) have been studied extensively. Although Prentice et al. (1990) and Zydlewski et al. (2003) report observing short-term reductions in growth relative to controls immediately after tagging, significant differences between tagged and untagged fish were not detected over the course of the studies. Similarly, no significant effects on fish survival rate have been reported from studies in which a control was employed. For example, in studies where 23-mm PIT tags were used, no detectable tag effect on growth or survival was reported for Atlantic salmon *Salmo salar* larger than 90 mm (Zydlewski et al. 2001), coho salmon *Oncorhynchus kisutch* larger than 100 mm, and steelhead *Oncorhynchus mykiss* larger than 100 mm (Zydlewski et al. 2003; all references to length in this paper are fork length). These findings suggest that it may be possible to use 23-mm PIT tags in salmonids equal to or smaller than 90 mm. Although Roussel et al. (2000) report the use of 23-mm PIT tags in salmonids smaller than 90 mm (range, 64–94 mm), sample size was small ( $n = 33$ ), a control was not used, nor was growth evaluated. These preliminary findings suggest that 23-mm PIT tags may indeed be useful for smaller fish, but a controlled experiment evaluating the effects of 23-mm PIT tags on fish smaller than 90 mm is a preliminary step. To address this need, we implanted 23-mm PIT tags into juvenile steelhead smaller than 90 mm and monitored growth and survival for 30 d. We specifically targeted fish within the 80- to 90-mm size range because age-0 salmonids in western Oregon can often attain this size by the end of their first summer of growth.

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### Methods

Juvenile steelhead were collected from the North Fork Alsea River Hatchery (Oregon Department of Fish and Wildlife) on July 9, 2003, and approximately 1,800 were transported in an aerated, insulated live-tank 67 km east to the Fish Performance Laboratory (Oregon State University, Department of Fisheries and Wildlife) in Corvallis. In the laboratory, fish were placed in two 1.8-m-diameter tanks each containing approximately 1,600 L of water. Water temperatures fluctuated on a daily basis within a range between 12.8°C and 14.0°C. Fish were held until July 27, 2003, when it was judged that fish size was close to the desired range (80–90 mm). At this time, 600 fish were removed from the holding tanks and placed in equal numbers in 1 of 24 smaller tanks. Small tanks were 0.92 m in diameter and contained 386 L of water. Each tank was assigned one of three possible treatments and each fish (1–600) was assigned to a tank. The net result was 8 tanks of 25 fish assigned to each treatment. Treatments were control, surgery with no tag (hereafter, surgery), or surgery with PIT tag (hereafter, tagged). Throughout the holding and experimental periods, fish were fed BioDiet Grower semimoist pellets (Bio-Oregon, Inc., Warrenton, Oregon) morning and evening ad libitum. Water temperatures fluctuated on a daily basis within a range between 12.8°C and 14°C. After 30 d, the experiment was terminated and the surviving fish were euthanized.

Initially, fish were removed from the large central tanks in small batches of approximately 5–15 fish and transported to the processing area. Individual fish were placed in a solution composed of 10 mL of a 10:1 mixture of 100% ethanol : clove oil (Keene et al. 1998) diluted in 8 L of water. Fish, regardless of treatment, were left in the solution until locomotion ceased. Fish were then removed from the solution by a processor; a data recorder would assign the fish a number in sequential order (i.e., 1, 2, 3, . . . , 600) and the appropriate treatment and tank number. All numbers (i.e., 1–600) had previously been randomly assigned to specific tanks, and treatments were subsequently assigned to tanks by random draw. Fish were weighed to the nearest 0.1 g and measured for length to the nearest millimeter. Control fish were then placed in a recovery bucket. Surgery and tagged fish each received an incision approximately the diameter of the PIT tag (Gries and Letcher 2002) just lateral to the midline on the ventral surface anterior to the pelvic fins. All tagged fish were implanted with a 23.00-mm × 3.85-mm half-duplex glass encapsulated PIT tag weighing 0.6 g (air) that was manufactured by Texas Instruments, Inc. (Dallas, Texas). After the incision and

insertion of the PIT tag, a topical antibiotic cream was applied with a swab to the incision and the fish was placed in a recovery bucket. We recorded the length of time required to anesthetize and handle each fish (weigh, measure, and make incision), as well as the individual performing the surgery. Surgeons represented a range of different experience levels, but all new surgeons were required to perform a minimum of 10 surgeries under the guidance of an experienced surgeon before the initiation of treatments. Recovery buckets were labeled with the number of the corresponding tank and color coded by treatment. After recovery, fish were removed from the buckets and placed in assigned tanks.

Each tank was monitored daily for dead fish and rejected tags. Every 10 d, all fish in all tanks were measured and weighed. A computer malfunction corrupted data files and resulted in the loss of length and weight data from the last 10-d period in 14 of the 24 tanks (data on survival and tag loss were unaffected by the loss of growth data). Because tanks were processed individually, the remaining 10 tanks represented intact replicates divided among treatments (i.e., 3 tanks each for control and surgery groups and 4 tanks with tagged fish). The weights of tagged fish were corrected by subtracting 0.6 g (approximate tag weight) from the recorded weight after tagging. Fish that died or failed to retain the tag were only used in analyses of survival and rates of tag retention.

Equality of means (among tanks and treatments) for weight and length at the initiation of treatment was evaluated with a one-way analysis of variance (ANOVA). The instantaneous growth rate ( $G$ ) was calculated by tank for three 10-d time intervals. Intervals 1–3 are defined as follows: (1) initial measurement to day 10, (2) days 11–20, and (3) days 21–30. For both weight and length, the following formula was used:

$$G = (\log_e Y_2 - \log_e Y_1) / (t_2 - t_1).$$

(Busacker et al. 1990), where  $t_1$  represents the time at the beginning of the interval and  $t_2$  the time at the end, and where  $Y_1$  and  $Y_2$  are either mean weight or length for fish in the respective tank at those times. Repeated-measures ANOVA was used to evaluate values of  $G$  for weight and length among treatments through time. Treatment and measurement interval were the two main factors included in the model; tank was included as the subject variable. The interaction between measurement interval and treatment was also evaluated. A Newman–Keuls test was used to conduct multiple comparisons among factors and two-factor interactions. For all multiple comparison tests,  $\alpha = 0.05$ . Because the loss of data created an unbalanced design with regard to

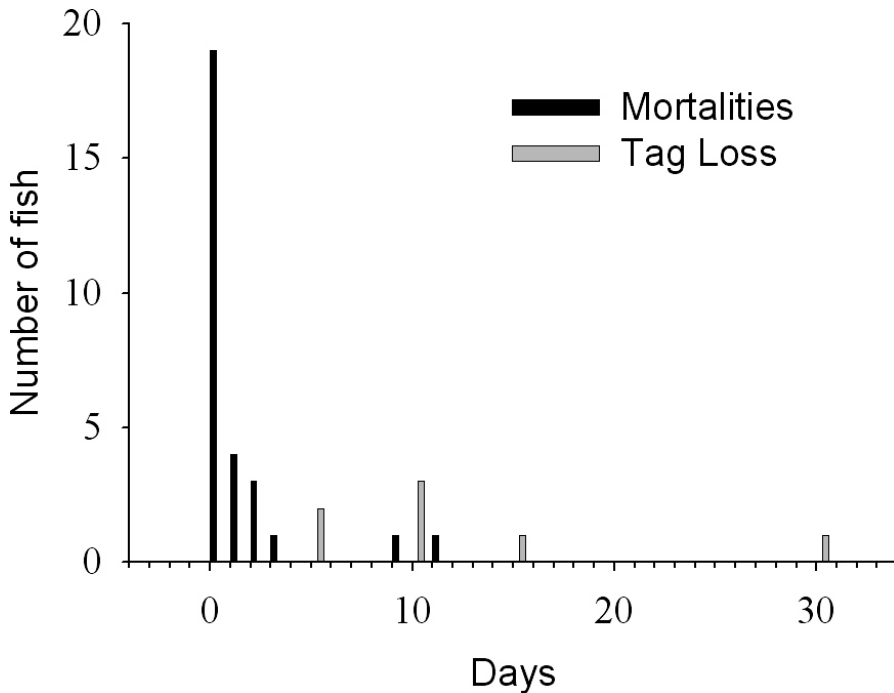


FIGURE 1.—Frequency distribution of fish mortality and PIT-tag loss for days 1–30 during a laboratory evaluation in July 2003 of the effects of 23-mm PIT tags on growth and survival of steelhead smaller than 90 mm.

growth, separate repeated-measures analyses were conducted for data from all 24 tanks for measurement intervals one and two and for the 10 tanks that had data for all four measurement events (i.e., days 1–30). Logistic regression was used to evaluate the effects of surgeon, handling time, and fish size on survival within the tagged group. Nonsignificant terms (i.e.,  $P > 0.05$ ) were removed from the model. All statistical analyses were performed with number cruncher statistical systems 2001 (J. Hintze, Kaysville, Utah).

### Results

Differences in survival rates were observed between the tagged group and the other two treatments. Of the fish receiving a PIT tag, 171 out of 200 (86%) survived, but the surgery and control groups had virtually 100% survival. Of the fish that died, 90% did so within the first 3 d (Figure 1), including a single mortality from the surgery group. These initial mortalities appeared to be caused by perforation of the stomach or intestine when the tag was inserted. The single fish from the surgery group died from excessive scalpel penetration. We could not determine cause of death for fish that died 5–13 d postsurgery. Seven fish did not retain the PIT tag (97% tag retention rate).

Timing of tag loss was evenly distributed throughout the study period (Figure 1).

Handling time (coefficient =  $-0.009$ ; SE = 0.018;  $P = 0.630$ ) and length (coefficient =  $-0.030$ ; SE = 0.025;  $P = 0.230$ ) were not significant predictors of survival and were removed from the logistic regression model. Surgeon was the only significant ( $P = 0.008$ ) predictor of survival; however, this variable explained only a small proportion of the variation in survival ( $R^2 = 0.12$ ). A total of five different surgeons participated, and surgeon-specific mortality rates ranged from 3% to 35%. The individuals with the single lowest and highest mortality rate were the two least experienced surgeons. The single worst surgeon tagged 13% of the fish and was associated with 31% of the mortalities.

Mean values for fish weight and length at initiation of treatments did not differ among tanks (weight:  $F = 1.04$ ,  $df = 23, 576$ ,  $P = 0.4$ ; length:  $F = 1.42$ ,  $df = 23, 576$ ,  $P = 0.1$ ) or treatments (Table 1). The initial mean tag-to-body weight ratio in air for all tagged fish was 9% (range, 6.5–12.5%). Using data from all 24 tanks for measurement intervals one and two, mean values of  $G$  for weight differed among treatment groups ( $F = 106.23$ ;  $df = 2, 21$ ;  $P < 0.01$ ) and between measurement intervals ( $F = 8.31$ ;  $df = 1, 21$ ;  $P < 0.01$ ). No interaction between treatment group and

TABLE 1.—Mean and range for weight (g) and fork length (mm) of juvenile steelhead by treatment group and 10-d measurement interval during a laboratory evaluation of the effects of 23-mm PIT tags on growth and survival of steelhead smaller than 90 mm in July 2003.

Time	Treatment	Weight (g)		Length (mm)	
		Mean	Range	Mean	Range
Day 1	Control	6.6	4.4–9.8	82	73–97
	Surgery–not tagged	6.6	4.8–9.3	83	75–93
Day 10	Surgery–tagged	6.7	4.8–9.2	83	74–91
	Control	8.0	5.2–11.4	87	77–99
Day 20	Surgery–not tagged	7.9	5.5–11.4	87	78–99
	Surgery–tagged	7.1	3.9–11.2	83	74–95
Day 30	Control	9.9	6.2–14.4	93	80–107
	Surgery–not tagged	10.0	6.7–15.9	94	79–107
Day 30	Surgery–tagged	8.3	4.0–14.5	88	73–104
	Control	12.6	8.4–17.8	101	88–115
Day 30	Surgery–not tagged	11.9	7.8–17.6	101	89–115
	Surgery–tagged	11.2	5.0–18.4	95	77–112

measurement interval was detected ( $F = 1.24$ ;  $df = 2, 21$ ;  $P = 0.31$ ). Results from a multiple comparison test indicated that the tagged group differed from the surgery and control groups, but surgery and control groups did not differ from one another (Figure 2a). For length, an interaction was detected between treatment group and measurement interval ( $F = 45.22$ ;  $df = 2, 21$ ;  $P < 0.01$ ). The multiple comparison test indicated that the mean value of  $G$  for the tagged group for measurement interval one differed from all other combinations of treatment group and measurement interval. The tagged group in measurement interval two differed from all other combinations of treatment group and time, except the control from measurement interval one (Figure 2c). Control and surgery groups did not differ within measurement intervals (Figure 2c).

When the results from just the 10 tanks that provided data for the entire 30-d study period were used, significant interactions between treatment group and measurement interval were observed for mean values of  $G$  for both weight and length (weight:  $F = 4.25$ ,  $df = 4, 14$ ,  $P = 0.02$ ; length:  $P < 0.01$ ). For weight, the tagged group from measurement interval one differed only from the tagged group from measurement interval three. No other differences among treatment groups and measurement intervals were detected (Figure 2b). For length, the tagged group differed from both the control and surgery groups at measurement intervals one and two. There were no detectable differences among treatment groups for measurement interval three. Control and surgery groups did not differ from one another within any measurement interval (Figure 2d).

**Discussion**

Tagging had a negative effect on survival. Although survival rates were similar to those of Roussel et al.

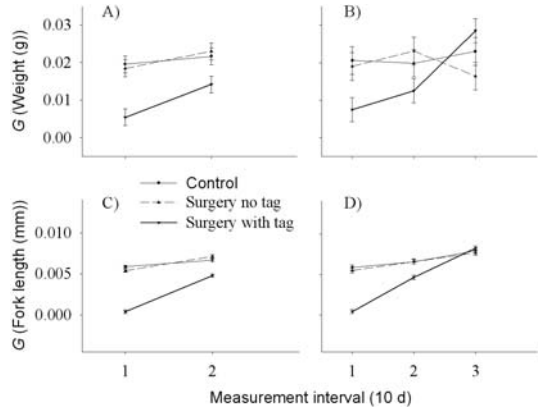


FIGURE 2.—Mean instantaneous growth rate ( $G$ ) and associated SE by treatment for each measurement interval during a laboratory evaluation of the effects of 23-mm PIT tags on growth and survival of steelhead smaller than 90 mm. Measurement interval 1 corresponds to days 1–10, interval 2 to days 11–20, and interval 3 to days 21–30. Panels (A) and (C) display mean values of  $G$  for weight and length derived from all 24 tanks. Panels (B) and (D) display mean values derived from the 10 tanks in which data were collected over the entire study.

(2000), it is difficult to compare because Roussel et al. (2000) did not use a control. In previous studies, salmonid survival rates for PIT-tagged fish range from 30% to 100% for time periods ranging from 14 d to more than 200 d; however, in all cases, survival of tagged fish did not differ from survival in control groups, regardless of tag, fish size, or time interval (Prentice et al. 1990a; Peterson et al. 1994; Ombredane et al. 1998; Zydlewski et al. 2003). These studies represent both injection and surgical implantation techniques. Because only one fish from the surgery group died, our data suggest that mortality in the tagged group was caused by organ damage incurred during tag insertion rather than from excessive scalpel penetration related to incision. Apparently, mortality resulted from the combination of fish length and tag size for the implantation technique used.

Initially, instantaneous growth rate was negatively affected by PIT tagging. The relationship between treatment groups and instantaneous growth rate varied among the three measurement intervals. Although control and surgery groups were not detectably different within any measurement interval, growth rates for the tagged group varied. Growth rates for this group were low relative to other treatments for intervals one and two (except for weight, interval two with the reduced sample size); however, by interval three, growth rates of tagged fish either exceeded or were not detectably different from the other treatment

groups. This indicates that fish in the tagged group began to grow at rates similar to those of the control and surgery groups sometime between day 10 and day 20. The resumption of growth coincided closely with the cessation of mortality.

Prentice et al. (1990a) reported reduced growth in juvenile Chinook salmon *Oncorhynchus tshawytscha* of similar size to the fish in this study during the first 20 d after receiving 12-mm PIT tags. Baras et al. (2000) observed reduced growth of Eurasian perch *Perca fluviatilis* for days 11–21 posttagging when tag-to-body weight ratios (in air) were greater than 2.4%. The greatest tag-to-body weight ratio reported by Baras et al. (2000) was 4.5%, or one-half that in the current study. Together, these studies suggest that a short period of reduced growth after tagging may be common when tag-to-body weight ratios are greater than 2.4% and that the recovery period may be similar across a range of tag-to-body weight ratios.

Additional reductions in survival related to implanting PIT tags in fish smaller than 90 mm may occur in the wild because of factors such as increased vulnerability to predation or disease during the recovery period. These relationships have not been investigated. Additional studies are needed, such as those of Peterson et al. (1994) and Ombredane et al. (1998) in which PIT tags and a second marking technique are used to compare long-term survival among tag groups. We recommend that any field experiments using 23-mm PIT tags in salmonids smaller than 90 mm be designed so that survival can be evaluated against a control group. Furthermore, by tagging and immediately releasing one group and then tagging and holding a second group for 10–15 d before release would provide insight into mortality directly related to tagging.

Although we recorded the rate and timing of tag loss in this study, it is difficult to explicitly address how fish size may affect this factor. Previous researchers have reported tag retention rates, but results are confounded by the environment, tag size, implantation technique, study duration, species, and surgical technique. In general, however, retention rates have been high. Of the 30 estimated retention rates we compiled, only 3 were less than 90%. In one of these studies, Roussel et al. (2000) monitored Atlantic salmon (length range, 64–94 mm) implanted with 23-mm PIT tags (surgery without suture) for 32 d. Mean retention rate calculated from their data for tagged fish without sutures was only 72%. Using the same-sized tag and implantation technique, Zydlewski et al. (2003) report retention rates of 89, 98, and 100%, respectively, for hatchery and wild steelhead and coho salmon, all of which were greater than 100 mm in length. Together, these data

suggest that tag retention rate may be influenced by an interaction between fish size and tag size.

The tag retention rate in this study (97%) was well within the range reported in the literature, and the results did not appear to be adversely affected by the specific combination of fish size and tag size. Because the duration of the study was short (30 d), it is possible that additional tag loss could occur; however, we do not believe that is likely. By day 30, most tagged fish had died, lost the tag, or completely healed.

Our results suggest that care in training or selection of surgeons could reduce acute mortality but not eliminate it. Exploring modified surgical techniques might also be a useful endeavor. The majority of observed mortalities in this study were caused by internal organ damage. It may be possible to avoid this damage by making larger incisions and placing tags into the body cavity parallel to the long axis of the body. For the technique used in this study, the tag was inserted through a small incision at a steep angle relative to the long axis of the body, and because of the limited depth of the body cavity, it was necessary to subsequently rotate tags to a position parallel to the long axis. It is possible that the act of rotating the tag trapped organs between the body wall and the tag. In larger fish, rotation of the tag is either unnecessary, or there is space within the body cavity and organs are not compressed against the spine. A larger incision would require sutures and, if effective in reducing acute mortality, it would present trade-offs between survival rates and cost of tagging.

Although it is often difficult to anticipate whether the results from the laboratory studies will be applicable in field situations, the results of this study and those of Zydlewski et al. (2001, 2003) suggest that mortality increases when 23-mm PIT tags are implanted in salmonids smaller than 90 mm. However, in situations where survival is not the parameter of interest, or in situations where fish could be tagged and held before release, mortality rates similar to those observed in this study may be acceptable. It appears that effects of 23-mm PIT tags on growth of salmonids similar in size to those in this study are probably minimal after 20 d.

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