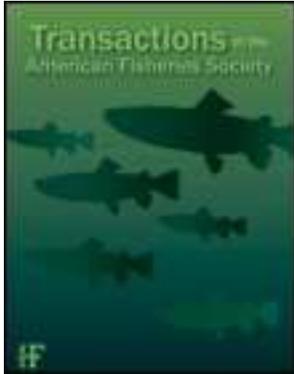


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## Passive Integrated Transponder (PIT) Tagging Did Not Negatively Affect the Short-Term Feeding Behavior or Swimming Performance of Juvenile Rainbow Trout

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**Abstract.**—Passive integrated transponder (PIT) tagging is a commonly used procedure to identify fish. However, there is a lack of research on the short-term effects of such tagging. The purpose of our study was to measure the short-term effects of PIT tagging on the feeding behavior and swimming performance of juvenile rainbow trout *Oncorhynchus mykiss*. Three experiments were conducted. The treatment groups in the first two experiments consisted of a control group and a PIT-tagged group. In the first experiment, we timed the latency to resume feeding before and after the experimental day in fish trained to feed with a light cue. In the second experiment, we recorded the amount of food ingested before and after the experimental day in fish that had been fed to satiation every day for a week prior to the experiment. We found no significant differences between control and PIT-tagged groups for either the latency to resume feeding (time from providing food to food intake) or the amount of food eaten. The third experiment consisted of a fixed-velocity swimming performance test in which fish that had been tagged 40 d before the test and whose wounds had healed were compared with fish that were tagged on the day of the experiment. We found no significant differences between the fish that had healed wounds and fish that had just been tagged.

Passive integrated transponder (PIT) tagging is a commonly used method to mark fish. For example, the Columbia River Basin's fisheries agencies have used PIT tags since 1987 as a research and management tool to mark and track anadromous fish, such as Chinook salmon *Oncorhynchus tshawytscha* and steelhead (anadromous rainbow trout) *O. mykiss* smolts during downstream migration (CBFWA 1999). Passive integrated transponder tags have been used in upstream-migrating adult steelhead to monitor their movements through the hydroelectric complex along the Columbia and Snake rivers (McCutcheon et al. 1994). In 1999, more than 1 million unique coded PIT tags were implanted (Ryan et al. 2001). The tagging procedures involve anesthetizing the fish, inserting the tag into the peritoneal cavity, and allowing the fish to recover for at least 30 min before its release into the wild (CBFWA 1999). Juvenile salmonids PIT-tagged in the field are often released in streams after as little as 30 min of recovery (CBFWA 1999); it is critical for their survival that they be able to feed and swim normally.

Little is known about the short-term effects of PIT tagging. Prentice et al. (1990) reported that PIT tags reduced growth during days 1–20 but did not affect swimming tail-beat frequency, respiration rate, or stamina in juvenile fall Chinook salmon. Other studies have investigated the long-term effects of PIT tagging

in salmonids, Nile tilapia *Oreochromis niloticus*, and Eurasian perch *Perca fluviatilis* (Ombredane et al. 1998; Baras et al. 1999, 2000). The growth rate in the Nile tilapia was initially slowed for the first few days, but after 2 weeks it had returned to normal. None of the studies above found significant long-term effects of PIT tagging on growth rate or survival.

Feeding is a sensitive behavioral measure (Bernier 2006). Ortega et al. (2005) exposed rainbow trout *Oncorhynchus mykiss* to different levels of ammonia (a stress factor) and monitored plasma cortisol and feeding behavior over a 96-h period. The ammonia caused an increase in plasma cortisol levels. These cortisol levels returned to pretreatment levels within 96 h. However, despite the return of plasma cortisol levels to control levels, the food intake remained significantly lowered. Stress is known to cause a reduction in food intake (Pickering 1993) and disrupts normal searching and feeding behaviors (Beitinger 1990). Handling is another important stress factor that decreases food intake (Pickering et al. 1982). Indeed, feeding behavior has been used as a measure of potentially painful procedures in fish (Sneddon et al. 2003). Sneddon et al. (2003) injected "painful" chemical stimuli into the lip of rainbow trout and monitored how long it took the fish to resume feeding.

Swimming performance is another critical behavior in fish for survival (Plaut 2001). Reduced swim performance can reduce fitness by decreasing the ability of individuals to capture prey and avoid predators (McDonald et al. 1998; Plaut 2001). Thus,

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the survival of PIT-tagged fish may depend on their ability to swim normally, even within hours of their release. We used a fixed-velocity test to determine the acute effects of PIT tagging on the swimming performance of juvenile trout. The fixed-velocity test measures swimming ability during short duration (20 s to 200 min), high-intensity swimming (Beamish 1978).

The purpose of our study was to investigate the short-term effect of PIT tagging on feeding behavior and prolonged swimming performance in juvenile rainbow trout. We used a series of three experiments. In the first experiment, we recorded the latency to resume feeding (time from providing food to food intake) after the PIT-tagging procedure. In the second experiment, we monitored the amount of food consumed before and after the PIT-tagging procedure. In the third experiment, we recorded the time to fatigue during a fixed-velocity test. In the first two experiments, the two treatment groups were a control group and a PIT-tagged group. In the third experiment, we compared the time to fatigue in trout PIT-tagged 30 min before the swim test (the “recently tagged” group) and in trout PIT-tagged 40 d prior to the swim test (the “pretagged–healed” group). Because we were interested in the acute effects of the PIT-tagging procedure itself, the pretagged–healed group was used as a control to account for any effect the tag itself might have on swimming performance. In a previous study on gilthead seabream (also known as gilthead bream) *Sparus auratus* fingerlings, the average time for a PIT-tagging wound to heal was 20 d (Navarro et al. 2006).

Even very short-term behavioral effects could have negative fitness consequences in the field. Thus, our study focuses on the short-term effects of PIT tagging on the behavior of juvenile rainbow trout, a commonly tagged fish species. We hypothesized that PIT tagging would affect neither the latency to resume feeding, the amount of food ingested, nor the time to fatigue.

## Methods

*Experimental subjects and treatment.*—Eighty-eight rainbow trout were obtained from Rainbow Springs Hatchery, Ontario, and housed in the Hagen Aqualab at the University of Guelph. Fish were held in flow-through tanks (70 cm in diameter, 110 cm high), supplied with Guelph well water. They were hand-fed trout pellets at a maintenance ration of 1% of their body weight every other day. This project was approved by the Animal Care Committee at the University of Guelph.

On the day of the experiment, the trout were anesthetized in benzocaine (ethyl-*p*-aminobenzoate; 2.5 g/L stock solution of benzocaine in ethanol, diluted

to yield a final benzocaine concentration of 0.03 mL/mL water), weighed (in experiment 3, the fork length was measured), and randomly assigned to one of two groups: a control group (the pretagged–healed group in experiment 3) and a PIT-tagged group (the recently tagged group in experiment 3).

The PIT tag treatment consisted of inserting a 12-mm (0.09-g) glass-coated PIT tag (Biomark, Boise, Idaho) into the peritoneal cavity of the fish using a 12-gauge needle mounted on a spring-loaded syringe. After tagging, the fish were placed in a chamber (12 × 7 × 7 cm) on a water table with flow-through Guelph well water (13 ± 1°C) and then allowed 30 min to recover from the anesthetic. The control group was anesthetized, handled, and treated in exactly the same manner as the PIT-tagged group except that they were not injected and thus did not receive a PIT tag. There were no mortalities during or after any of the experiments.

*Experiment 1: latency to resume feeding.*—Thirty trout (weight ± SE = 14.4 ± 0.9 g) were housed individually in chambers (12 × 7 × 7 cm) on a water table for 1 week before the experiment. They were conditioned, once a day for 7 days, to eat trout pellets within 5 min after the lid of the box was removed. At the end of the training period, all fish ingested their first pellet within 2 min. On the day before (day -1), the day of (day 0), and the day after treatment (day 1), the lid was removed and three floating pellets were given to each fish. The time to ingest the first pellet (latency to resume feeding) was recorded.

*Experiment 2: amount of food eaten.*—Another 30 trout (weight ± SE = 14.5 ± 0.9 g) were also placed individually in chambers (12 × 7 × 7 cm) on the water table 1 week before this experiment. The trout were conditioned, as above, but this time they were fed 4% of their body weight during each trial. After each 1-h training period, the uneaten pellets were removed and placed in an oven to determine dry weight. The food intake was calculated as the difference between the initial weight given to each fish and the dry weight of the uneaten food. The 1-h limit was chosen because Sneddon et al. (2003) presented rainbow trout with food every 30 min after injecting a “painful” stimulus into the lip. Therefore, a 1-h period is more than enough time for the fish to eat if it is going to eat. By the end of the training period, all fish ate at least 3% of their body weight within an hour. On the 2 d before (days -2 and -1), the day of (day 0), and 2 days after the treatment (days 1 and 2), fish were given pellets (4% of their body weight) and allowed 1 h to feed. We recorded the total amount of food ingested throughout the training and experimental periods.

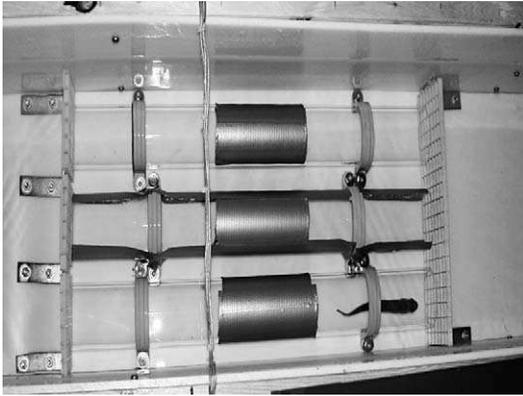


FIGURE 1.—Apparatus for the prolonged swim performance test of PIT-tagged juvenile rainbow trout.

*Experiment 3: fixed-velocity test.*—For the fixed-velocity swimming performance test, three clear polyvinyl tubes (55 cm long, 5.7 cm inside diameter) with a 15-cm  $\times$  8-cm shaded portion in the middle of the tube for cover and a 55-cm  $\times$  5-cm shaded portion on either side of the tube to visually isolate the fish from one another (Figure 1) were placed in a swim flume (420 cm in length, 28 cm wide, and 24 cm deep) with a water flow of 19 cm/s (16 cm deep). The water was pumped with a centrifugal pump (341A BF AP; Pentair Group, Aurora, Illinois). The flow velocity was controlled using a variable speed drive (E-trac WF2 sensorless vector drive; TB Wood's) and was powered by a Hubbell wiring device—Kellens circuit-lock manual controller.

In this experiment, 14 rainbow trout that were PIT-tagged 40 d before the experiment to allow adequate time for the incision to heal completely served as controls. This was done because we were interested in the short-term effects of the PIT-tagging procedure itself. PIT tag wounds in gilthead seabream fingerlings heal on average in 20 d (Navarro et al. 2006); moreover, previous long-term studies have shown that PIT tags do not affect a fish's swimming performance (Ombredane et al. 1998; Baras et al. 1999; Gibbons and Andrews 2004). On the day of the experiment, fish (weight  $\pm$  SE = 23.9  $\pm$  0.9 g; fork length  $\pm$  SE = 13.1  $\pm$  0.2 cm) were randomly assigned to a swim tube in the swim flume 30 min after anesthesia and treatment (handling of pretagged–healed fish and PIT tagging of recently tagged fish). After 5 min of orientation in the current (19 cm/s), the flow velocity was gradually increased over 5 min until the test velocity was reached (46 cm/s). We timed the entire trial, starting when all the fish were in the tube and ending when they fatigued. Fatigue was defined as the time when the fish

TABLE 1.—Weight (g; mean  $\pm$  SE) for control and PIT-tagged juvenile rainbow trout in each of the three experiments.

Experiment	Control fish	PIT-tagged fish
1 (latency to resume feeding)	12.87 $\pm$ 0.77	15.94 $\pm$ 1.67
2 (amount of food eaten)	14.85 $\pm$ 1.37	14.1 $\pm$ 1.17
3 (fixed-velocity test)	23.78 $\pm$ 0.96	26.2 $\pm$ 0.98

failed to swim off the screen after three stimulations with a metal rod. Fatigued fish were removed from the flume.

*Statistical analysis.*—Repeated-measures analysis of variance (ANOVA) was used to compare the two treatment groups in experiments 1 and 2; in this analysis,  $\alpha = 0.05$  was used to determine significance (SAS version 8.2). We used a *t*-test (Microsoft Excel 2003;  $\alpha = 0.05$ ) to compare latency in experiment 1 and food intake in experiment 2. In experiment 3, an analysis of covariance (ANCOVA) was used to compare the time to fatigue of the two treatment groups, fork length being used as a covariate (MINITAB version 12.1, Minitab, Inc.).

## Results

There were no significant differences between the weights of the control and the PIT-tagged groups (experiments 1 and 2) or those of the pretagged–healed and recently tagged groups (experiment 3) (Table 1). Also, we found no significant differences between the fork lengths of the two treatment groups in experiment 3.

### *Experiment 1: Latency to Resume Feeding*

All fish ate within 5 min. The latency to resume feeding on the day of the experiment for control and PIT-tagged groups was 1.29  $\pm$  0.33 and 0.47  $\pm$  0.13 min, respectively (mean  $\pm$  SE) after the 30-min recovery period. We found no significant differences in latencies between the control and the PIT-tagged groups on any given day (ANOVA;  $P = 0.18$ ; Figure 2). The latencies were not significantly different between the day before and the day of treatment or the day of and the day after treatment for the PIT-tagged group (paired *t*-test;  $P = 0.16$  and  $P = 0.25$ , respectively). Although the control group ate faster on the day after the treatment than on the day of the treatment (paired *t*-test;  $P = 0.03$ ), this behavior was not significantly different from that of the PIT-tagged group (*t*-test;  $P = 0.17$ ).

### *Experiment 2: Amount of Food Eaten*

We found no significant difference in the quantity of food intake between the control and the PIT-tagged groups (ANOVA;  $P = 0.62$ ; Figure 3). Both groups

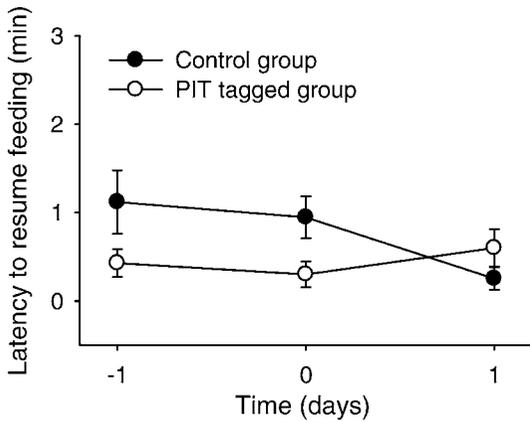


FIGURE 2.—Average  $\pm$  SE latency to resume feeding for the control ( $n = 15$ ) and PIT-tagged groups ( $n = 15$ ) in experiment 1. There were no significant differences between the groups (ANOVA;  $P = 0.18$ ).

consumed significantly less food on the day of the treatment (control = 17%, PIT-tagged fish = 31%), but both returned to pretreatment levels within 1 day ( $t$ -test;  $P = 0.72$ ).

#### Experiment 3: Fixed-Velocity Swimming Performance Test

There were no obvious effects of PIT tagging on the swimming behavior of the rainbow trout. All fish swam either at the front or in the middle of the swim tube until they fatigued and fell back to the rear of the tube. The times to fatigue for the pretagged-healed and the recently tagged groups were  $712.9 \pm 28.5$  s and  $730.9 \pm 30.3$  s, respectively. We found no significant differences between the two groups (ANCOVA;  $P =$

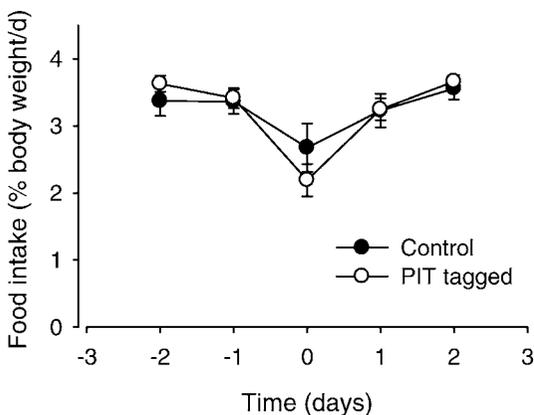


FIGURE 3.—Food intake of the control and PIT-tagged groups. There were no significant differences between the groups (ANOVA;  $P = 0.62$ ).

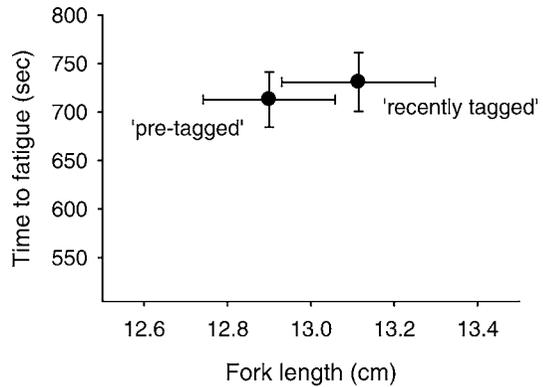


FIGURE 4.—Average  $\pm$  SE time to fatigue versus average  $\pm$  SE fork length for pretagged-healed and recently tagged fish. There were no significant differences in time to fatigue between the two groups ( $P = 0.64$ ), and fork length did not affect time to fatigue (ANCOVA;  $P = 0.37$ ).

0.64) nor an effect of fork length on time to fatigue (ANCOVA;  $P = 0.37$ ) (Figure 4).

#### Discussion

The present study investigated the short-term effects of PIT tagging on the feeding and the swimming behavior in juvenile rainbow trout. Tagging did not significantly affect the latency to resume feeding, nor the amount of food consumed. Similarly, we saw no effects of PIT tagging on the swimming performances between the tagged fish that had healed wounds and the fish with recent wounds. Gibbons and Andrews (2004) reported that PIT tags had virtually no negative long-term impact on animals. Ombredane et al. (1998) investigated the effect of PIT tags on survival and growth rate of juvenile brown trout *Salmo trutta* and concluded that the tags did not affect survival or growth rate. Although long-term effects are important, even short-term disruptions in feeding and swimming behavior could have negative fitness consequences in nature. Our study has shown that PIT tagging does not have any short-term effects on the feeding behavior and swimming performance of juvenile rainbow trout.

Both control and PIT-tagged trout decreased their food intake on the day of treatment, probably as a result of the stress of being anesthetized and handled. Food intake is reduced by stress (Pickering 1993), and handling is a stressful event for the fish (Pickering et al. 1982). Therefore, because all fish were handled on the day of the experiment, it was not surprising that the total amount of food intake decreased in a similar fashion for both treatment groups.

Sneddon et al. (2003) injected bee venom or acetic acid into the lip of rainbow trout and reported that the

latency to resume feeding was 3 h in these trout compared with 1 h for the control group, thus suggesting that feeding was delayed 2 h by the injection of a “painful stimulus” and that feeding latency can be used as a measure of pain in fish. Our results show that PIT tagging did not affect the latency to resume feeding between the control and the PIT-tagged groups, thereby suggesting that PIT tagging is not a painful stimulus. Furthermore, Narnaware and Peter (2001) performed brain surgery in goldfish and reported that fish resumed feeding 30 min after surgery.

We found no significant differences in the time to fatigue between the pretagged–healed and the recently tagged group during the fixed-velocity test. These times correspond to the time to fatigue of untagged juvenile rainbow trout of the same size recorded by McDonald et al. (1998) and extrapolated from their data. Our times to fatigue did not depend on size because we found no effects of fork length on time to fatigue. This is not surprising because McDonald et al. (1998) reported that individual swimming performance had very little to do with size in juvenile rainbow trout. Therefore, the short-term effect of PIT tagging did not affect swimming performance. Thus, trout that are PIT-tagged and allowed at least 30 min to recover should be able to effectively swim and escape predators in the wild.

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