PNNL-20330

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Contract DE-AC05-76RL01830

Design, Production, and Assessment of a Neutrally Buoyant Externally Attached Acoustic Transmitter for Studying Behavior and Survival of Juvenile Salmonids

Final Report

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ZD Deng
RS Brown
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JJ Martinez
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October 2011

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Chapter 3:

Brown RS, BD Pflugrath, TJ Carlson, and ZD Deng. 2011. The effect of an externally attached neutrally buoyant transmitter on mortal injury during simulated turbine passage. Chapter 3 in *Design, Production, and Assessment of a Neutrally Buoyant Externally Attached Acoustic Transmitter for Studying Behavior and Survival of Juvenile Salmonids*, TJ Carlson, ZD Deng, RS Brown, AH Colotelo, JJ Martinez, BD Pflugrath, TK Abel, JM Janak, AP LeBarge, JR Stephenson, RP Mueller, and AG Seaburg. PNNL-20330, Pacific Northwest National Laboratory, Richland, Washington.

Summary

To develop a method to monitor the passage and survival of juvenile salmonids without bias through turbines at all dams within the Federal Columbia River Power System, the U.S. Army Corps of Engineers (USACE), Portland District, contracted Pacific Northwest National Laboratory (PNNL) to develop and assess the use of an externally attached neutrally buoyant transmitter for juvenile Chinook salmon (*Oncorhyncus tshawytscha*). PNNL design engineers and fisheries scientists developed multiple designs of neutrally buoyant transmitters and conducted tests to determine the effectiveness of each design.

This study consisted of three main thrusts:

- designing, developing, and assessing attachment of a neutrally buoyant external transmitter
- assessing swimming performance and predator avoidance of juvenile Chinook salmon with externally attached neutrally buoyant transmitters
- assessing the effects of an externally attached neutrally buoyant transmitter on mortal injury during rapid decompression associated with simulated turbine passage.

The details of these three related efforts are documented in the three chapters of this report.

Chapter 1

In this chapter, we describe the design and construction of three neutrally buoyant externally attached transmitters. Based on preliminary tests, it was concluded that attachment of a tag to the pelvic girdle of fish would not be effective. Further testing was conducted using two basic tag designs—Type A (sutured to the dorsal musculature of the fish anterior to the dorsal fin) and Type B (two-part design attached with wire pushed through the dorsal musculature, ventral to the dorsal fin). Both tags were constructed with a mock single-battery acoustic transmitter, similar to the current JSATS tag design. Mock transmitters were then coated in a specific mixture of resin and glass bubbles that made the tags neutrally buoyant in water and molded to one of the tag designs.

To determine the efficacy of these tags under non-turbine passage-related conditions, fish had one of the tags attached and were held for 14 days to determine any effects that the presence of the tag may have on growth, survival and tissue damage. We also evaluated the attachment method by monitoring tag retention. These two neutrally buoyant tag designs were compared to nontagged individuals and those surgically implanted with current Juvenile Salmon Acoustic Telemetry System (JSATS) transmitters and passive integrated responder (PIT) tags. In addition, two suture materials (Monocryl and Vicryl Rapide) were tested for attachment of Type A tags.

Throughout the holding period, tag retention was high; only three Type A tags were lost within the final 2 days of the study. Although no mortality was observed, two Type A tags attached with Vicryl Rapide sutures were lost (one on Day 13 and one on Day 14), and one Type A tag attached with Monocryl sutures was lost (on Day 13). Fish with Type B tags had significantly lower percentages of length and weight increase over the holding period when compared to all other treatment groups. In addition, Type B tags generally caused more negative tissue reaction than Type A tags.

The efficacy of Type A and Type B tag designs was also compared to nontagged individuals under shear exposure. Fish were exposed to one of three nozzle velocities (3.0 m/s, 9.1 m/s and 12.2 m/s) in a fiberglass flume to simulate turbine conditions within the Columbia River basin. Immediate mortality rates, tag retention, and the occurrence of shear injuries all were observed for test fish. Fish were then held for 4 days post-exposure to monitor delayed mortality and tissue damage associated with the shear exposure.

Throughout the shear exposure study, no mortalities or tag loss were observed. There was also no significant difference in the rates of shear injury between fish tagged with Type A and Type B tags. Injuries due to the shear forces were observed on 6.0% (n = 9/151) of individuals. Tissue damage rates observed at necropsy were higher for fish tagged with Type B tags when compared to those tagged with Type A tags. However, as nozzle velocity increased, this difference decreased.

Overall, these studies demonstrated that when compared with nontagged individuals, fish tagged with Type A tags did not differ significantly with respect to growth or mortality over a 14-day holding period. However, fish tagged with Type B transmitters had lower growth than the nontagged controls or other tag treatments. When tissue damage was assessed for tagged individuals exposed to shear forces, those tagged with Type A tags showed lower rates and severity of injury when compared to Type B-tagged fish. These results suggest that Type A tags may be a viable tag design for juvenile Chinook salmon passing through hydropower facilities. However, further testing is necessary.

Chapter 2

The evaluation of the effects on swimming performance and predator avoidance due to the presence of a neutrally buoyant externally attached transmitter is described in this chapter. The critical swimming speed (U_{crit}) for juvenile Chinook salmon tagged with two different neutrally buoyant external transmitters was measured and compared to nontagged individuals and those surgically implanted with the current JSATS acoustic transmitter. Based on these trials, it was determined that fish tagged with the Type A and B designs had lower swimming performance when compared to nontagged individuals. However, there was no difference in swimming performance among fish tagged with Type A or B designs or those with surgically implanted tags.

Further testing was then conducted to determine if predator avoidance ability was affected due to the presence of Type A tags when compared to nontagged fish. Tagged and nontagged individuals were simultaneously released into a tank containing predatory rainbow trout. The tank was monitored until 50% of the prey had been consumed; all prey then were removed, and the ratio of tagged to nontagged was assessed. We determined that there was no significant difference in the number of tagged and nontagged fish consumed throughout the predation trials. These results supported the efficacy of the neutrally buoyant externally attached Type A tag design.

Chapter 3

The investigation described in this chapter shows the effects of the Type A tag design on mortal injury rates for fish exposed to rapid decompression associated with turbine passage. Based on the results of the studies detailed in Chapters 1 and 2, it was determined that Type A tag design should be the focus of further studies. Therefore, tagged and nontagged individuals acclimated to a pressure of 21.2 psia

(15-ft water depth equivalent) were exposed to a nadir pressure of 1.6 to 11.6 psia in the Mobile Aquatic Barotrauma Laboratory hypo/hyperbaric chambers. Rates of mortal injury were then assessed for all individuals.

The rates of mortal injury were not significantly different for tagged and nontagged juvenile Chinook salmon, indicating that juvenile Chinook salmon tagged with Type A tags are not at a greater risk for mortal injury than nontagged conspecifics. This contrasts with previous research that has shown the negatively buoyant surgically implanted tags or injected PIT tags increase the rates of mortal injury for juvenile Chinook salmon experiencing rapid decompression. Our results further support the efficacy of this novel tag design for use in turbine passage survival studies in the Columbia River basin.

Overall Conclusions and Recommendations

Each year, the USACE surgically implants millions of fish with telemetry tags (PIT, acoustic and radio combined) to assess their passage and survival through hydropower facilities. Of particular concern is the passage of fish through turbines where they may be exposed to a plethora of forces that can result in injuries and/or mortality. Recent research has shown that the presence of a negatively buoyant surgically implanted tag may increase the rate of mortal injury for turbine-passed fish when compared to nontagged individuals, and this has direct influence on the reliability of survival studies. Therefore, there is a need for innovative tag designs to eliminate or reduce this bias. Our team of design engineers and fisheries biologists has designed, manufactured, and tested the efficacy of a neutrally buoyant externally attached tag that may be an effective design for future turbine passage survival studies throughout the Columbia River basin.

Based on the results presented in this report, our conclusions and recommendations for future research are as follows:

- Future research should be conducted to test the efficacy of this tag design in field conditions. Despite the positive results seen in these laboratory tests, field conditions present a range of conditions and exposure to stressors simultaneously, all of which influence the efficacy of the tag design. Comparison of current tagging strategies (surgical implantation) to our proposed method is therefore warranted.
- In comparison to current tagging methods (surgical implantation of negatively buoyant transmitters), our novel tag design has eliminated the excess mass of the transmitter and the volume of the body cavity occupied by the tag. This neutrally buoyant externally attached tag did not result in increased rates of mortal injury associated with rapid decompression, as was seen for the current JSATS tag design and PIT tags. The next evolution of tag design should include investigation into the mechanisms behind this lack of bias.

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The PNNL animal facilities used in this research are accredited by AAALAC International, the Association for Assessment and Accreditation of Laboratory Animal Care. Fish were handled in accordance with federal guidelines for the care and use of laboratory animals, and protocols for our study were approved by the Institutional Animal Care and Use Committee at Battelle–Pacific Northwest Division. The Pacific Northwest National Laboratory is operated by Battelle for the U.S. Department of Energy under Contract DE-AC05-76RL01830.

Abbreviations and Acronyms

3D	three-dimensional
AIC	Akaike information criterion
ANODEV	analysis of deviance
ANOVA	analysis of variance
ARL	Aquatic Research Laboratory
cm	centimeter(s)
d	day(s)
dB	decibel(s)
dB re 1 μPa at 1 m	transmitter source level
df	degree(s) of freedom
F	variance ratio (*F*-test)
FCRPS	Federal Columbia River Power System
FL	fork length
g	gram(s)
gal	gallon(s)
h	hour(s)
in.	inch(es)
kPa	kilopascal(s)
L	liter(s)
μ	micron(s)
μPa	micropascal(s)
m	meter(s)
mL	milliliter(s)
mm	millimeter(s)
mmHg	millimeters mercury
MSE	mean square error

N	population
n	sample
P	probability
PIT	passive integrated transponder
PNNL	Pacific Northwest National Laboratory
psia	pounds per square inch–atmospheric
RMS	root mean square
s	second(s)
SD	standard deviation
STP	simulated turbine passage
TDG	total dissolved gas
U_{crit}	critical swimming speed

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Chapter 1

Development of External and Neutrally Buoyant Acoustic Transmitters for Turbine Passage Evaluation

Z. Daniel Deng, Jayson J. Martinez, Alison H. Colotelo, Tylor K. Abel, Andrea P. LeBarge, Richard S. Brown, Brett D. Pflugrath, Robert P. Mueller, Thomas J. Carlson, Adam G. Seaburg

Introduction

Biotelemetry is commonly used to monitor the passage and survival of juvenile salmonids at hydroelectric facilities throughout the Columbia River basin (Steig 1999; Matter and Sandford 2003; McMichael et al. 2010). Data collected in these studies are used to determine passage routes taken and associated survival of fish; that information is extrapolated to the general population. A basic assumption of these studies is that the surgery process and the presence of the telemetry tag do not influence the behavior or survival of the individual (Nielsen 1992; Baras and Lagardère 1995; Bégout Anras et al. 1998). Violation of this assumption leads to inaccurate information being used for the management of these hydroelectric facilities.

Fish passing through hydroelectric facilities in the Columbia River basin may take three basic routes on their seaward migration—through a juvenile bypass system, over a spillway, or through a hydroelectric turbine. Passage through a turbine may expose fish to a number of different forces that can lead to injury (e.g., shear force, blade strike). However, all fish are exposed to rapid decompression as they pass by the turbine blade. This rapid decompression causes gases in the swim bladder and tissues to expand, which can result in numerous injuries, including ruptured swim bladder, exophthalmia, and internal hemorrhaging. In a recent laboratory study, Carlson et al. (2010) demonstrated that the presence of a telemetry tag inside the body of a juvenile salmon increased the likelihood of these injuries occurring. The increased excess mass (weight in water) of the tag requires fish to uptake more molecules of gas in the swim bladder to maintain neutral buoyancy, resulting in increased trauma during rapid decompression. The presence of the transmitter in the coelom during rapid decompression could also increase the likelihood of compression-related injuries. Carlson et al. (2010) outlined the need for improvements in telemetry technology that would minimize bias for fish carrying a tag, improving the accuracy of telemetry studies.

Carlson et al. (2010) found that tag burden was a predictor of mortal injury (mortality or injury leading to mortality; McKinstry et al. 2007) among fish exposed to rapid decompression. As tag burden increased, so too did the rates of mortality injury (Carlson et al. 2010). By reducing the excess mass of the tag, it is suggested that the bias associated with mortality injury rates would also be reduced. Although reduction of the mass of telemetry tags used in turbine survival studies is a viable path for future research, the addition of positively buoyant materials to reduce the excess wet mass of current tags may also be a practical option. Due to the increase in size of tags with the addition of positively buoyant materials, external attachment would be favored over internal implantation even though internal implantation would not increase excess mass for neutrally buoyant tags.

External attachment of telemetry tags is commonly used throughout fisheries research. This method has a number of advantages when compared to surgical implantation, including less time required for attachment (Jepsen et al. 2002; Cooke et al. 2003), potential less invasiveness (Lucas et al. 1993), and tag shedability after the conclusion of the study (Bégout Anras et al. 1998). These advantages would benefit turbine survival studies by potentially reducing bias associated with the tagging process and carrying the tag.

In addition to advantages associated with external attachment of telemetry tags, a number of concerns have been outlined. Externally attached tags are affixed directly on the skin of fish, which is covered by a protective layer of epithelial cells and mucus (Shephard 1994) and can become irritated due to the presence of the tag (Lonsdale and Baxter 1968; Roberts et al. 1973a, 1973b; Yeager 1985; Kalpers et al. 1989). External tags also alter the profile of the fish, potentially affecting their swimming performance, particularly under highly turbulent flow conditions. Due to these concerns, novel tag designs need to be evaluated to determine any potential bias they may create in telemetry studies.

Transmitters have been found to often increase the likelihood of mortality and decrease growth, and external transmitters can get fouled with debris, reduce swimming performance, and become dislodged during the study period. The goal of this research was to design a neutrally buoyant externally attached transmitter that would not influence the survival or behavior of fish and also stay attached when exposed to the high-velocity, high-turbulence conditions present during turbine passage. To accomplish this, a group of design engineers teamed with biologists to design an externally attached transmitter that would be hydraulically streamlined and have limited negative influence to fish (survival, growth, tissue damage). This chapter describes the design and construction of this transmitter and evaluation of the tagging effects and tag retention with juvenile Chinook salmon.

Transmitter Design and Fabrication

Three locations on a fish's body were originally proposed for the external transmitter. The first was anterior to the dorsal fin; the second was beneath the dorsal fin, where each half of a two-part transmitter would be placed; and the third was on the pelvic girdle. To investigate each of the attachment locations, the team constructed mock external transmitters from clay. The mock transmitters were attached using Ethicon Monocryl 5-0 absorbable monofilament sutures (Ethicon, Inc., New Brunswick, New Jersey) with and without moleskin (Dr. Scholl's Super Moleskin Plus; Schering-Plough Healthcare Products, Inc., Berkeley Heights, New Jersey) under the mock transmitter. The mock transmitters were also glued to the fish using Tissumend (Veterinary Products Laboratories) and Vetbond (3M Company, St. Paul, Minnesota), but neither glue was able to retain the mock transmitter for an extended period. Fish with the mock transmitters were kept in a holding tank for 8 d before researchers removed the transmitters and checked for injuries. The pelvic girdle attachment point was eliminated as a candidate attachment location because of its poor holding performance and higher injury rates.

Of the remaining two designs, the design with the attachment anterior to the dorsal fin is termed Type A and the two-part tag design with attachment beneath the dorsal fin is termed Type B. To manufacture the external transmitters, molds were created for each design. For the initial prototype molds, a three-dimensional printer (Stratasys FDM Vantage rapid prototyper, Stratasys Inc., Eden Prairie, Minnesota) was used with the fine (T10) tip. This tip is capable of depositing 0.005-in.-thick layers of polycarbonate. For the final testing, molds were CNC machined from aluminum bar stock. The machined aluminum molds produced external transmitters with a much smoother surface finish.

Type A Tag Design

The Type A tag is designed to be attached anterior to the dorsal fin using two sutures. Each suture rests in a notch that is 0.9 mm deep and 1.3 mm wide. The portion of the tag that contacts the juvenile salmonid's back has an interior angle of 90 degrees with a 2.1-mm-radius fillet (Figure 1.1). The tag extends 4.7 mm above the fish's back and 3.8 mm below. The maximum width of the tag is 9.3 mm, the maximum length is 18.5 mm, and the volume is 0.60 cm^3. Neutral buoyancy is achieved by using an epoxy with a density of 0.68 g/cm^3. For the acoustic portion of the Type A tag, an acoustic transmitter with one size 337 silver oxide button cell battery is used.

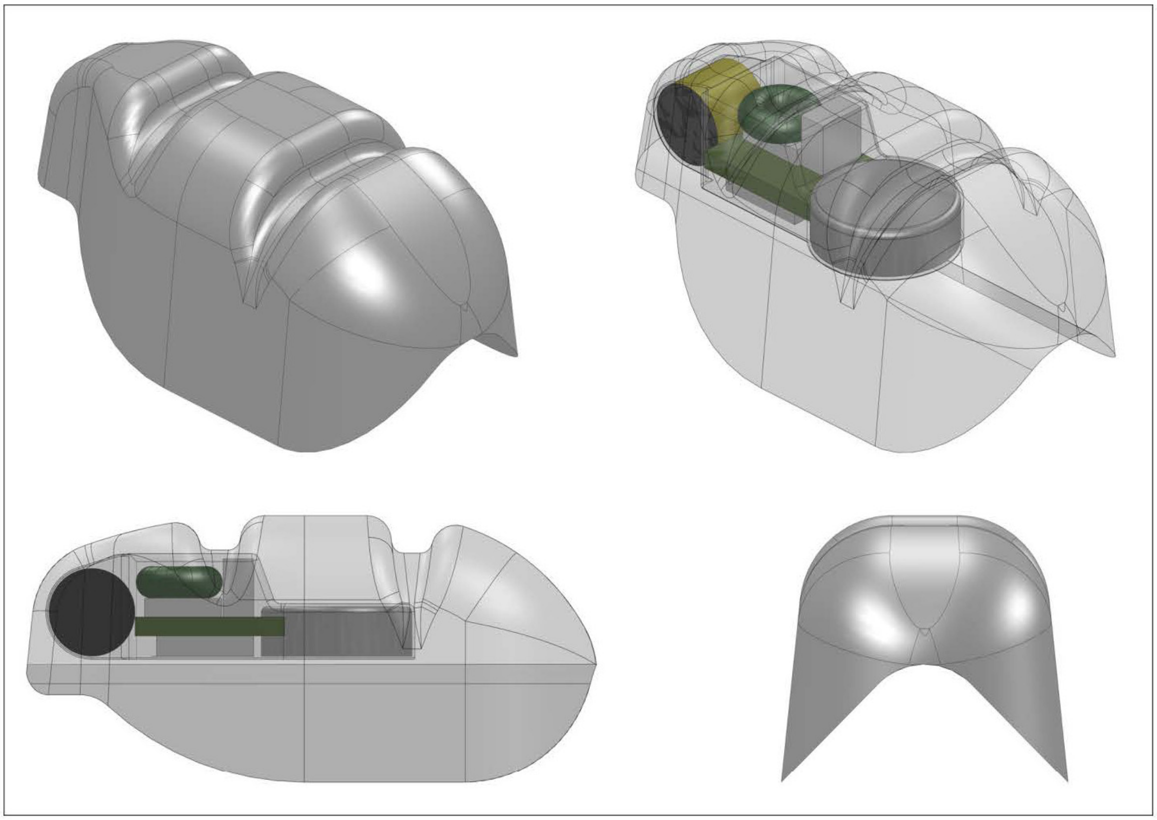

Figure 1.1. Tag A design CAD model drawings.

To manufacture the Type A tags, a two-part mold was machined from aluminum bar stock using a CNC mill (Figure 1.2). The lower portion of the mold contains 10 cavities. Each cavity has a 2.4-mm hole that was drilled through the mold to allow a steel pin to be placed in the mold for ejecting the tags after the epoxy has cured. The smallest tool used to machine the mold was a 1/16-in.-diameter ball-nosed end mill.

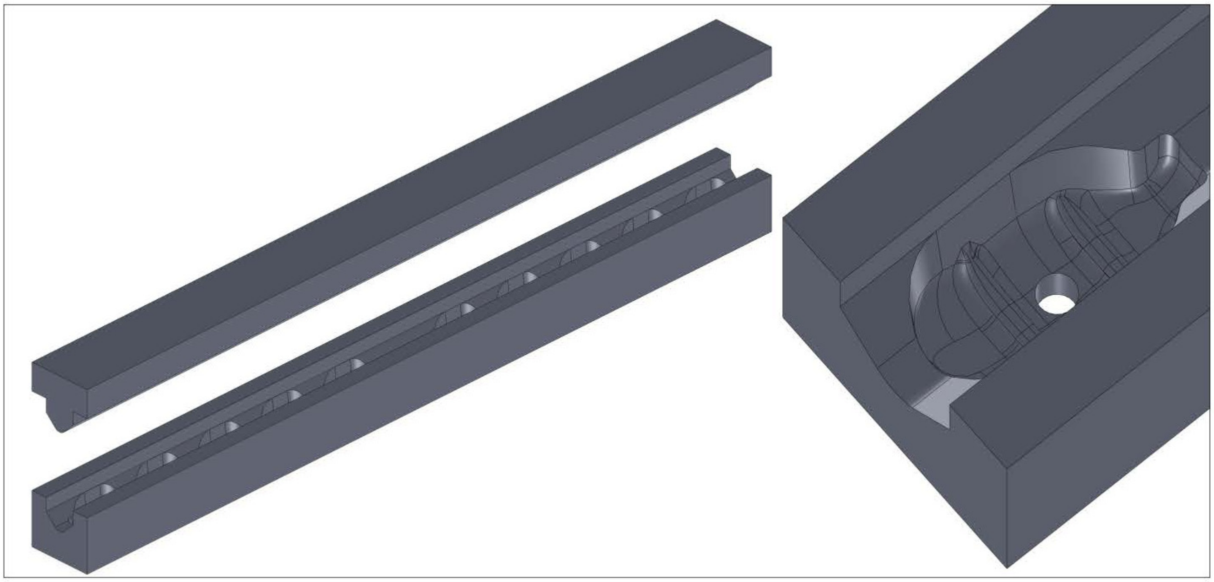

Figure 1.2. Molds for Type A tag.

Type B Tag Design

The Type B tag is a two-part design; each half of the transmitter is attached beneath the dorsal fin (Figure 1.3). One part of the tag contains a Size 337 silver oxide button cell battery with two electrical leads consisting of 25-gauge enamel-coated magnet wire protruding from the flat side (toward the fish). The other part of the tag contains electronics needed to drive the piezoelectric transducer. The transducer side of the tag also contains two locking electrical terminals (KS964-49GG, Advanced Interconnections, West Warwick, Rhode Island) spaced 7.5 mm apart. These terminals provide a retention force of 150 g each. In addition to providing an electrical connection between the two parts of the Type B tag, the electrical leads and terminals provide a mechanical connection to keep the tag attached to the juvenile salmonid. Each part of the Type B tag is based on an ellipsoidal cap shape with a 16.5-mm × 9-mm base and a height of 5.3 mm. The half of the tag containing the transducer has a flat surface at the top of the ellipsoidal cap, making the height only 4.4 mm. The transducer side also contains a protrusion on the side where the piezoelectric transducer extends out 2.2 mm beyond the basic ellipsoidal shape, making the maximum width 11.2 mm. The volume of the half containing the battery is 0.41 cm^3, and the half with the transducer is 0.43 cm^3. Neutral buoyancy for each half of the Type B tag is achieved by using an epoxy with a density of 0.70 g/cm^3.

To manufacture the Type B tags, a mold was machined from aluminum bar stock using a CNC mill. The mold contains a total of 30 cavities—15 battery sides and 15 transducer sides (Figure 1.4). Each cavity has a 2.4-mm hole that was drilled through the mold to allow a steel pin to be placed in the mold for ejecting the tags after the epoxy has cured. The cavities for the transducer sides of the tags also contain two 0.74-mm holes for holding the electrical terminals while the mold is being filled with epoxy. The smallest tool used to machine the mold was a 1/16-in.-diameter ball-nosed end mill.

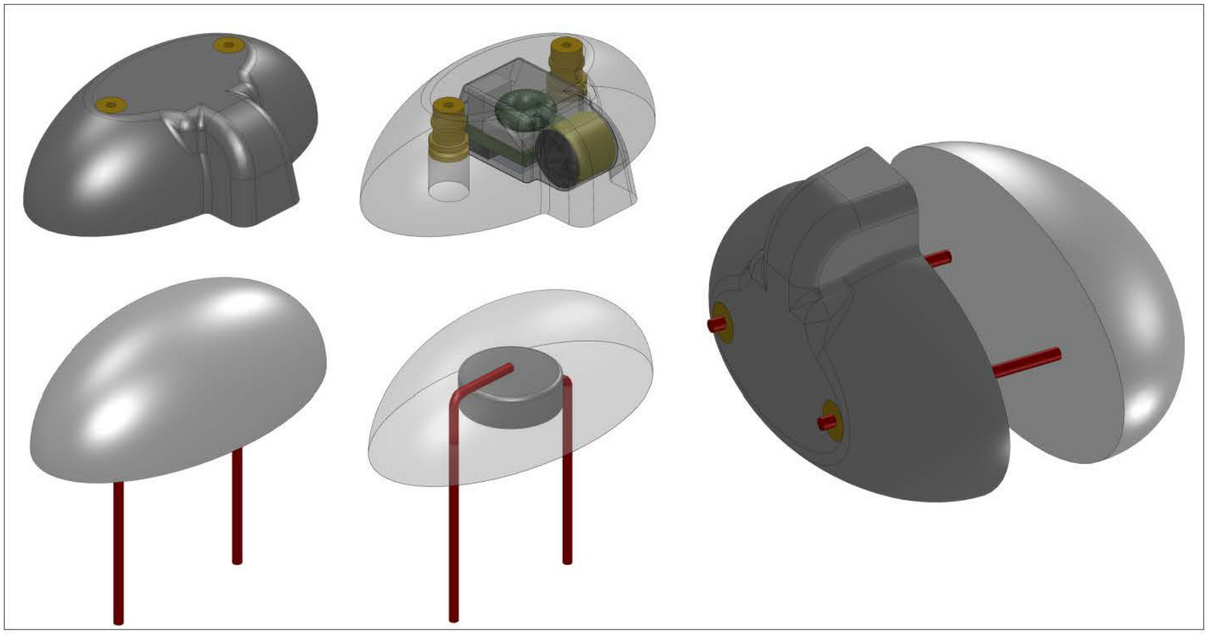

Figure 1.3. Type B design CAD drawings.

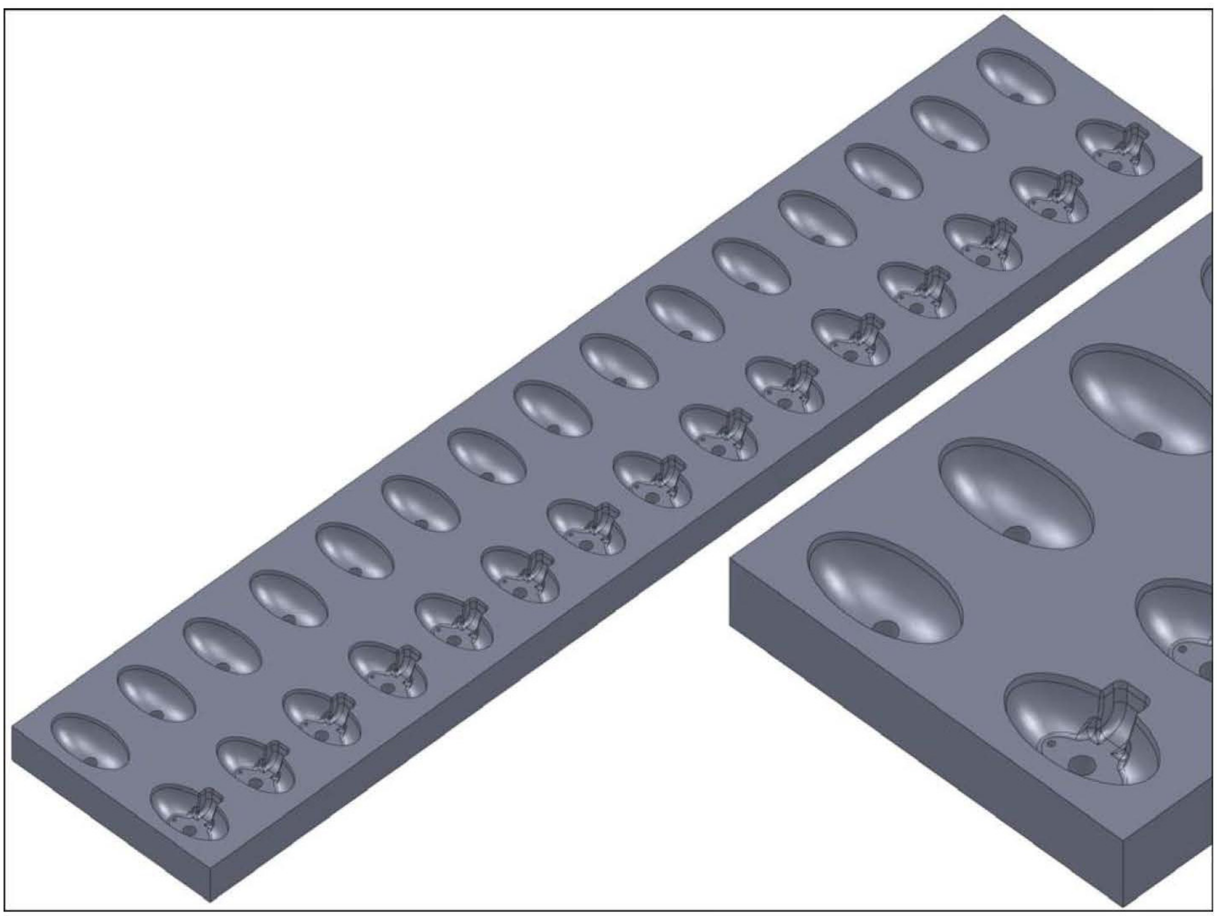

Figure 1.4. Mold for Type B design tag.

Tag Fabrication

The material used for prototyping the external transmitters was a mixture of Scotchcast Electric Resin 5 and A16/500 Glass Bubbles (3M Company, St. Paul, Minnesota). The resin is a two-part epoxy with a mixing ratio of 2:1 to equal the density of 1.16 g/cm^3. The glass bubble product is a powder composed of hollow glass spheres with a mean diameter of 60 µ and a typical density of 0.16 g/cm^3.

Varying the proportions of the resin and glass bubbles altered the density of the resulting mixture to produce neutrally buoyant external transmitters for each of the proposed designs. Testing several mixture ratios found that a mass ratio of 5.13 parts resin to 1 part glass bubbles (combined density of 0.575 g/cm^3) would produce a mixture that could be injected into a mold. This mixture was found to become very viscous approximately 18 min after it was mixed, making it unable to be injected into a mold. Increasing the proportion of the resin created a mixture that was less viscous but with a higher density. Mixture ratios of 5.13:1 (0.575 g/cm^3), 5.70:1 (0.6 g/cm^3), 6.97:1 (0.65 g/cm^3), 8.51:1 (0.7 g/cm^3), and 10.43:1 (0.75 g/cm^3) were allowed to fully cure and were tested in a water-filled pressure chamber. Weighing the samples before and after their placement in the pressure chamber found that these mixture ratios produced a material that was not impregnable by water.

To allow more external transmitter prototypes to be created for testing, mock acoustic transmitters were created to eliminate the need for actual acoustic transmitters. The actual acoustic transmitter was used to create a three-dimensional (3D) model for a single-battery acoustic transmitter to use with the Type A design and an acoustic transmitter without a battery to use with the Type B design. The 3D models of the acoustic transmitters were used to create molds that were manufactured using a rapid prototyper. To create a material that would give the mock acoustic transmitters the correct mass, tungsten powder (Technon Ultra Powder; Tungsten Heavy Powder, Inc., San Diego, California) was combined with 30-min epoxy.

Mixing Epoxy and Glass Bubbles

The epoxy has a mixing ratio of 2:1 with its hardener. The easiest way to achieve this when mixing small quantities was by using weight in grams. By using each product's known specific gravity and the volume of the mold, we could easily calculate the required mass of each component to obtain the desired final weight. Placing the mixing cup on a scale and adding each part individually, then slowly mixing the solution to minimize breakage of the glass bubbles, created a homogeneous mixture of glass bubbles and epoxy. This mixture was poured into a syringe ready for mold injection.

Preparing Aluminum Molds for Injection

Although the aluminum molds are extremely smooth, the application of a mold release was required to ensure maximum success during the ejection phase. Before the epoxy/bubbles mixture was injected into the castings, a healthy coat of wax-style mold release was applied to all surfaces that would encounter the epoxy.

Pouring Molds

For the Type B design, the locking socket electrical connectors were installed into their predrilled holes on the transducer side. The epoxy was then added to each side, respective to its desired density requirements. The subcomponents then were added to each casting and allowed to settle in the desired location within the mold. The electrical wires were then placed and secured into proper position on the battery side.

For the Type A design, a shaped lid was required to create the correct radius of the fish's back. With the proper density of epoxy mixture injected into the mold, the regular internal transmitter tag was added and pressed into its desired location. When the lid was installed, special care was needed so that there was no undesired movement inside the mold itself caused by the evacuated excess epoxy. We implemented the procedure of pressing the lid down one side at a time so that all the excess epoxy would escape from one side to reinforce the placement of the transmitter inside the enclosure. During curing, the mold was also placed on end so that the transmitters settled into designed location.

Ejection of Tags and Final Adjustments

Integrated into the design of the molds was a precisely placed ejection pin. After 12 h of curing, the external tags were easily removed by tapping on the pins with a hammer. The tags were then filed around the edges for final adjustments to ensure neutral buoyancy (Figure 1.5). Overall, one round of fabrication took approximately 24 h. For this study, the mold for each tag design had 12 slots, leading to 12 neutrally buoyant external tags per design a day. More molds can be made if a large number of tags are needed.

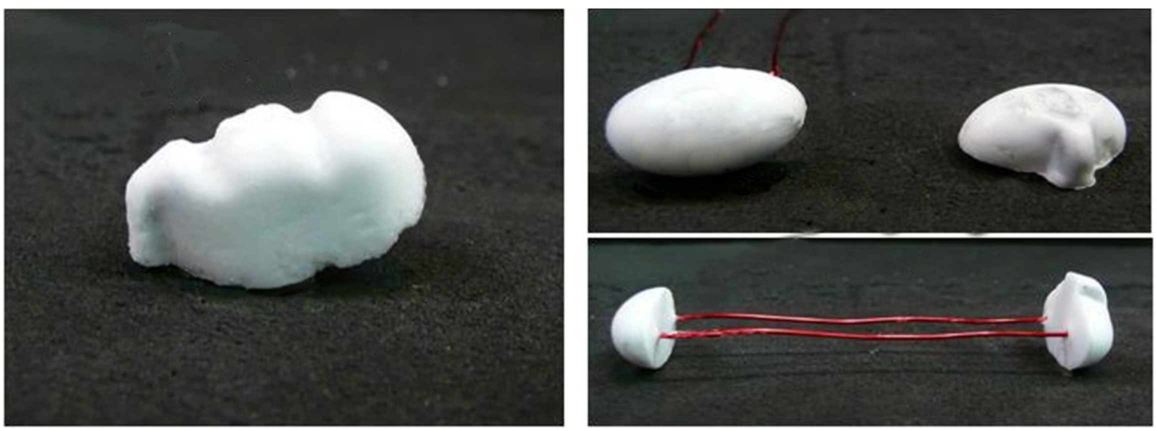

Figure 1.5. Prototype external transmitters. Left: Type A tag; right: Type B tag.

Performance Check

An existing transmitter from the Juvenile Salmon Acoustic Telemetry System (JSATS, 2008 model; Advanced Telemetry Systems, Isanti, Minnesota) was used to evaluate the impact of the manufacturing process on acoustic properties of the transmitter. The original source level was 153.4 dB re 1µPa at 1 m. After undergoing the manufacturing process for the Type A design to make it neutrally buoyant, the transmitter had a source level of 153.6 dB re 1µPa at 1 m, which was within the uncertainties of the measurement system.

Experimental Methods

Study Animals

Juvenile fall Chinook salmon were originally obtained as eyed eggs from the Washington Department of Fish and Wildlife Priest Rapids Hatchery in December 2009. Fish were reared at the Aquatic Research Laboratory (ARL) at the Pacific Northwest National Laboratory in Richland, Washington. During the study period, the test population was held inside the ARL in a 650-L circular tank. The holding tank was supplied with 16.8–17.8°C well water. Fish within the rearing and test population were fed Bio Vita Starter (Bio-Oregon, Longview, Washington) ad libitum. Test fish (subyearling Chinook salmon) had a mean fork length (± SD) of 122 mm ± 7 (range 95–139 mm) and mean weight (± SD) of 20.0 g ± 4.0 (range 8.4–29.9 g).

Tag Attachment

Both the external and internal surgeries were performed by one surgeon to eliminate surgeon bias (Deters et al. 2010). The order in which surgeries were performed was randomized. Fish were anesthetized with a solution of 80 mg tricaine methanesulfonate (MS-222) /L of water buffered with an 80-mg/L sodium bicarbonate solution until reaching stage 4 anesthesia (as described by Summerfelt and Smith 1990). All fish were marked for identification by clipping the caudal fin in a unique pattern, and fork length (FL, in millimeters) and mass (grams) for all treatment groups (including controls) were measured while fish were anesthetized. Fish were placed dorsal side up on a foam rubber pad for external attachment, and ventral side up for internal implantation. A small tube was inserted in the fish's mouth during surgery to provide a constant maintenance flow of 40-mg/L MS-222 buffered with a 40-mg/L solution of sodium bicarbonate.

Type A tags were externally attached anterior to the dorsal fin with two sutures (see Figure 1.6), each secured by a 2 × 2 × 2 × 2 knot as described by Deters et al. (in press). The suture rested in grooves formed in the tag to improve retention. Two different types of sutures were used for attachment when mortality and growth were examined, and only one type of suture was used when the effects of shear were examined. For the examination of mortality and growth, fish were tagged with Type A tags using either Ethicon Monocryl 5–0 absorbable monofilament sutures or Ethicon Vicryl Rapide absorbable 4–0 sutures (Deters et al., in press). Vicryl Rapide sutures are made of a material designed to absorb at a faster rate than typical absorbable monofilament sutures when used on humans. Due to the nature of many turbine survival studies (duration is less than one week), they may be an effective alternative, allowing the tag to be released sooner. Both types of sutures had a precision point-reverse cutting needle.

Type B tags (Figure 1.7) were attached using two 25-7/8-gauge needles (Becton, Dickinson and Company, Franklin Lakes, New Jersey) mounted on 3-mL syringes (Becton, Dickinson Medical). The needles were used to pass the 25-gauge enamel coated magnet wires (attached to the battery side) through the dorsal musculature. On the opposite side, the needles were removed, and the wires were threaded through the transducer side and the excess wire trimmed.

Internally implanted fish were surgically implanted with a JSATS acoustic transmitter (with an expired battery) and a passive integrated transponder (PIT) tag (Destron Technologies, St. Paul, Minnesota). The PIT tags were 12.5 mm (length) by 2.1 mm (width) and weighed 0.10 g in air (0.06 g in water, 0.04 cm^3 volume). Acoustic transmitters were 12.0 × 5.2 × 3.8 mm; they weighed 0.43 g in air (0.30 g in water; 0.14 mL volume). Tags (acoustic and PIT) were surgically implanted using methods similar to Panther et al. (in press). This included making a 6- to 7-mm-long incision on the linea alba and closing the incision with two simple interrupted sutures using a 1 × 1 × 1 × 1 knot (detailed in Deters et al., in press). Following surgery (or handling for controls), all fish were placed in a 5-gal bucket containing oxygenated water to recover. Food was withheld for 24 h prior to either external attachment or internal implantation of tags.

Figure 1.6. Type A tag attached to a fish.

Figure 1.7. Type B tag attached to a fish.

Biological Response

To determine any influence the tags had on growth, tag retention, and tissue response over 14 d, we included five groups of fish in the study, each group with a different tag treatment:

- nontagged (controls)
- Type A tag attached with Monocryl sutures
- Type A tag attached with Vicryl Rapide sutures
- Type B tag
- surgically implanted tag.

After recovery from surgery, fish were transferred to a 650-L circular tank inside the ARL with a maintained temperature between 16.8 and 17.8°C for the duration of the 14-d holding period. Lights inside the ARL were automatically controlled to follow the natural photoperiod, and fish were fed Bio Vita Starter (Bio-Oregon, Longview, Washington) ad libitum. The tank was checked daily for mortalities and tag loss.

At the end of the 14-d holding period, all fish were euthanized with a lethal dose of MS-222 (250-mg/L) and identified by their fin clip. Fork length (millimeters) and mass (grams) were measured for all treatment groups (including controls). Growth (percentage increase in length or mass) was calculated for each fish that survived to the end of the 14-d study period by subtracting the initial length or mass from the final length or mass.

Tissue response to the attachment and bearing of external transmitters was examined in only the treatment fish with Type A and Type B tags. The amount of tissue tearing (millimeters) was determined by measuring the longest tear resulting from the suture tearing the tissue. Discoloration beneath the tag was classified as either not present, greater than 50%, or less than 50% of the surface area of the tag. Indentation from the tag was defined as none, mild, or severe; and tissue laceration, caused by the tag rubbing against the tissue, was classified as either not present, greater than 50% of the tag outline, or less than 50% of the tag outline.

Shear Exposure

For testing the efficacy of the different tag designs under shear exposure, only three treatment groups were used—the untagged control fish, fish with Type A tags attached with Monocryl sutures and, fish with Type B tags.

Test Facility

A round water jet (6.35 cm in diameter) submerged in a rectangular fiberglass flume (9 m long, 1.2 m wide, and 1.2 m deep) was used to create a quantifiable shear environment consistent with conditions expected within a hydroelectric turbine (Figure 1.8). Flow was generated using a centrifugal pump with a programmable electronic speed controller that could produce jet velocities in excess of 20 m/s. Jet velocities were measured with a two-dimensional laser Doppler velocimeter (Deng et al. 2010).

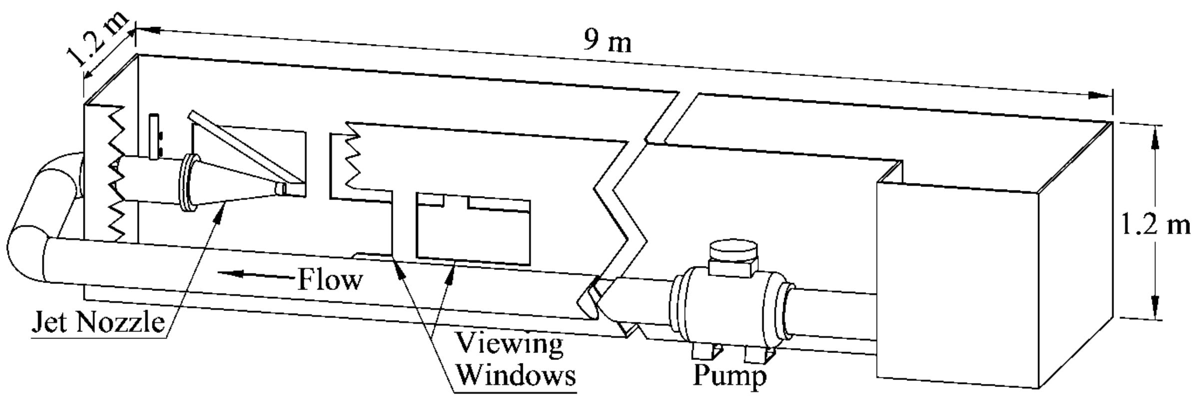

Figure 1.8. Flume used to create shear environment consistent with conditions expected within a hydroelectric turbine.

Test fish were actively introduced from standing water into the round water jet through an introduction tube (Figure 1.9). The terminus of the introduction tube was positioned above and in front of the terminus of the nozzle, with only a 1-mm vertical gap to ensure that test fish were entrained into the jet. This exposure mechanism, termed *the slow-fish–to–fast-water scenario*, is typical of conditions within the turbine environment, where turbine-passing fish go from a relatively slow region at approach to the turbine wicket gates before rapid acceleration to high velocity during runner passage the entrance to the highly turbulent region downstream of the turbine runner.

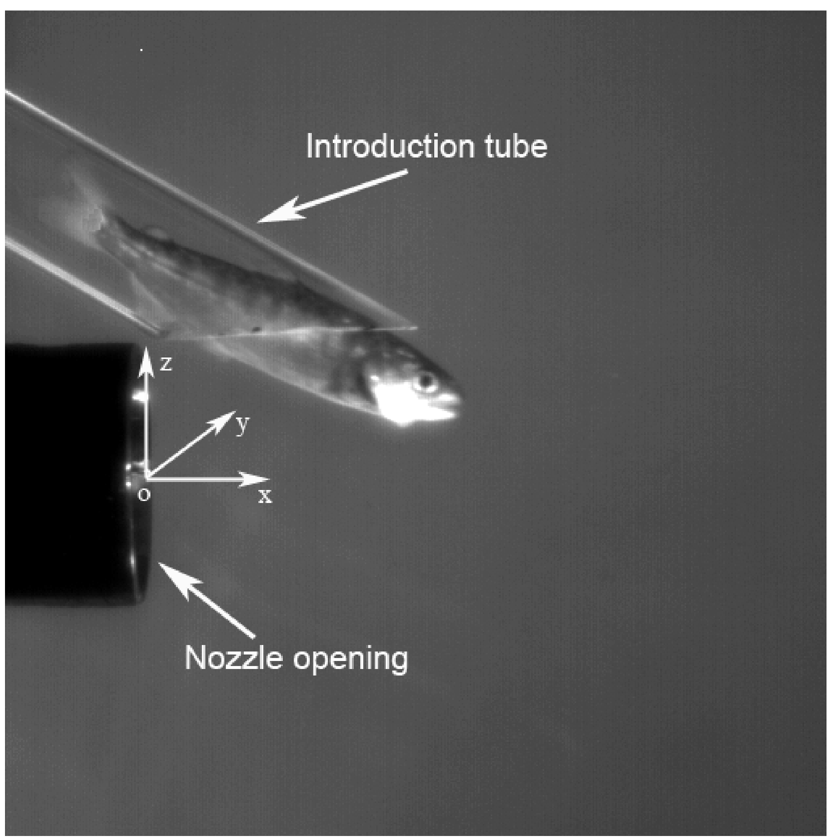

Figure 1.9. The coordinate system, nozzle dimensions, and fish release mechanisms.

Fish Handling and Shear Injury Characterization

For each test, a fish was randomly captured from the holding tank and placed in a section of clear tubing (cartridge) containing a small volume of water. The fish was then identified based on tag type, tag number, and fin clip. Photographs of both the left and right sides of the fish were taken to compare to post-shear exposure photographs. Each fish was then transferred to the introduction tube until the jet stabilized and then introduced into the flow field of the jet. The duration of injection was about 1 s, and the entire deployment and exposure process took approximately 30 s. Within about 10 s following each individual exposure, the pump was turned off and the fish were captured from the flume with dip nets. Swimming impairments, such as loss of equilibrium, lethargy, and disorientation, as well as immediate mortality were evaluated during recapture. After recapture, each fish was examined externally to assess the type and severity of injuries (i.e., biological responses) sustained. Photographs of both the left and right sides of the fish, as well as close-up images of any injuries seen, were also taken to document injuries. Injury categories included eye damage, descaling, gill/operculum damage, and bruising/ discoloration. Injuries were scored as present/absent; when an injury was observed, the side of the body and location were recorded, where applicable. Injury levels were calculated using methods outlined in Deng et al. (2005, 2010). Following injury evaluation, fish were placed in 1000-L holding tanks for 4 d to monitor delayed mortality and other biological responses indicative of stress or injury.

Video Recording and Processing

Two high-speed digital cameras (Photron PCI FastCAM 1280; Photron USA, Inc., San Diego, California) simultaneously recorded the exposure process of all test fish at 2000 frames per second through clear viewing windows in the side and bottom of the tank. Halogen lamps provided the desired illumination, and a gray back panel provided optimal contrast. The trajectories of three separate points on each fish (head, centroid, and tail) were tracked manually frame by frame in a motion-tracking software package (Visual Fusion 4.2; Boeing-SVS Inc., Albuquerque, New Mexico). The side- and bottom-view tracks were then combined to form 3D trajectories. Time series of velocity and acceleration were computed from the 3D trajectories using a five-point-stencil scheme (Abramowitz and Stegun 1970) and smoothed using a zero-phase forward and reverse digital filtering technique based on a running average filter (Mitra 2001, Sections 4.4.2 and 8.2.5; Gustafsson 1996). Finally, the peak values of each variable were computed for each time series and used in the statistical analysis.

Necropsy

After the 4-d holding period, fish were removed from the holding tank and euthanized with a lethal dose of MS-222 (250 mg/L). A full necropsy was conducted on each fish to evaluate the tag attachment site for tissue trauma and to identify areas of external or internal injuries resulting from exposure to shear forces. Each fish was also photographed on both the left and right sides for comparison of injuries throughout the study.

Data Analysis

The proportion increase in length and weight, and the amount of tissue tearing over the 14-d holding period was compared among groups using an analysis of variance (ANOVA). If differences were present, pairwise comparisons were made among test groups. A Šidák correction was used to adjust the alpha for pairwise comparisons. The family-wise rejection region for proportion increase in length and proportion increase in weight, using a Šidák correction, based on 10 tests between five tag types was

$$\alpha_{\text{family}} = 1 - (1 - 0.05)^{1/10} = 0.0051$$

Pairwise chi-square tests of independence were used to compare tissue tearing, discoloration, indentation, and laceration among groups externally attached with transmitters. The family-wise rejection region for tissue tearing, discoloration, indentation, and laceration, using a Šidák correction, based on three tests between three tag types was

$$\alpha_{\text{family}} = 1 - (1 - 0.05)^{1/3} = 0.0170$$

Shear injury and tissue damage at the tag attachment location were the two biological response variables evaluated. A shear injury was a binary variable indicating damage or no damage to the eyes, operculum, or skin (bruising). It was considered a binary variable because there were no cases in which a fish received multiple injuries (more than one minor or major injury). Tissue damage was measured as a continuous variable indicating either none = 0, mild = 1, or severe = 2. Tissue damage was considered a continuous variable because the metric was based on an underlying continuous scale (Snedecor and Cochran 1989). When the tissue damage data were analyzed, control fish were removed from tag type because only tagged fish could develop tissue damage. An analysis of deviance (ANODEV) based on a binomial error structure and log-link and scatter plots were employed to investigate the differences in shear injuries or tissue damage for fish exposed to different flow speeds and containing different tag types.

Results

Biological Response

Mortality and Tag Loss

Throughout the 14-d study, no mortalities were observed, and tag loss was limited. None of the Type B tagged fish or internally implanted fish lost any tags. One (4.8%) Type A tag attached with Monocryl sutures and one (4.8%) attached with Vicryl Rapide sutures were lost on Day 13 of the study. In addition, two other Vicryl Rapide sutures were lost during the study; however, the fish still retained their tags.

Changes in Length and Weight

Although there were no significant ($P < 0.05$) differences in initial length and weight among test groups, there were changes in both length ($P < 0.001$; Table 1.1) and weight ($P < 0.001$; Table 1.2) among the groups after 14 d (Table 1.3). The percentage increase in length was significantly lower for tag Type B than for all of the other tag treatments or the control (Tables 1.1 and 1.4; Figure 1.10). There were no significant differences in the percentage increase in length among the control group, fish tagged with tag Type A (using either suture type), or fish internally implanted with a transmitter and PIT tag. The percentage increase in weight was significantly lower for tag Type B than for all of the other tag treatments or the control (Tables 1.1 and 1.5; Figure 1.10). The control and internally implanted fish had significantly higher increases in weight than fish tagged with Type A transmitters using Vicryl Rapide sutures.

Table 1.1. Mean initial, final, and percentage differences in mean lengths and mass (± SD) of test fish by tag type.

		Initial		Final		Percentage Difference	
Treatment	*n*	Mean length (mm) ± SD	Mean mass (g) ± SD	Mean length (mm) ± SD	Mean mass (g) ± SD	Mean length (%) ± SD	Mean mass (%) ± SD
Control	21	120 ± 7	21.0 ± 3.9	129 ± 8	28.8 ± 5.3	7.0 ± 2.5	37.5 ± 10.2
Type A (Monocryl)	21	121 ± 7	21.2 ± 3.4	129 ± 7	28.2 ± 4.0	6.4 ± 2.3	34.3 ± 10.2
Type A (Vicryl Rapide)	21	122 ± 7	21.8 ± 3.6	129 ± 8	27.8 ± 5.5	5.2 ± 1.7	27.5 ± 9.2
Type B	21	124 ± 7	23.1 ± 4.5	127 ± 9	26.9 ± 6.1	2.8 ± 1.6	15.9 ± 7.6
Internal surgery	21	122 ± 8	21.5 ± 4.3	130 ± 9	30.0 ± 5.9	6.7 ± 1.5	37.2 ± 6.9

Table 1.2. Analysis of variance of proportion increase in length with a covariate of tag type.

Source	df	Sum square	Mean square	*F*	*P*
Tag type	4	0.024954	0.00062385	16.08	<0.0001
Residuals	95	0.036858	0.000388		

Table 1.3. Analysis of variance of proportion increase in weight with a covariate of tag type.

Source	df	Sum square	Mean square	*F*	*P*
Tag type	4	0.67517	0.168792	21.136	<0.0001
Residuals	95	0.75868	0.007986		

Table 1.4. Pairwise comparison of proportion increase in length. Differences were considered significant at an alpha of 0.0051.

	Control	Surgery	Type A (Monocryl)	Type A (Rapide)
Surgery	0.66755	--	--	--
Type A (Monocryl)	0.31102	0.55463	--	--
Type A (Rapide)	0.00552	0.01687	0.0683	--
Type B	<0.0001	<0.0001	<0.0001	0.00034

$\alpha_{family} = 1 - (1 - 0.05)^{1/10} = 0.0051$

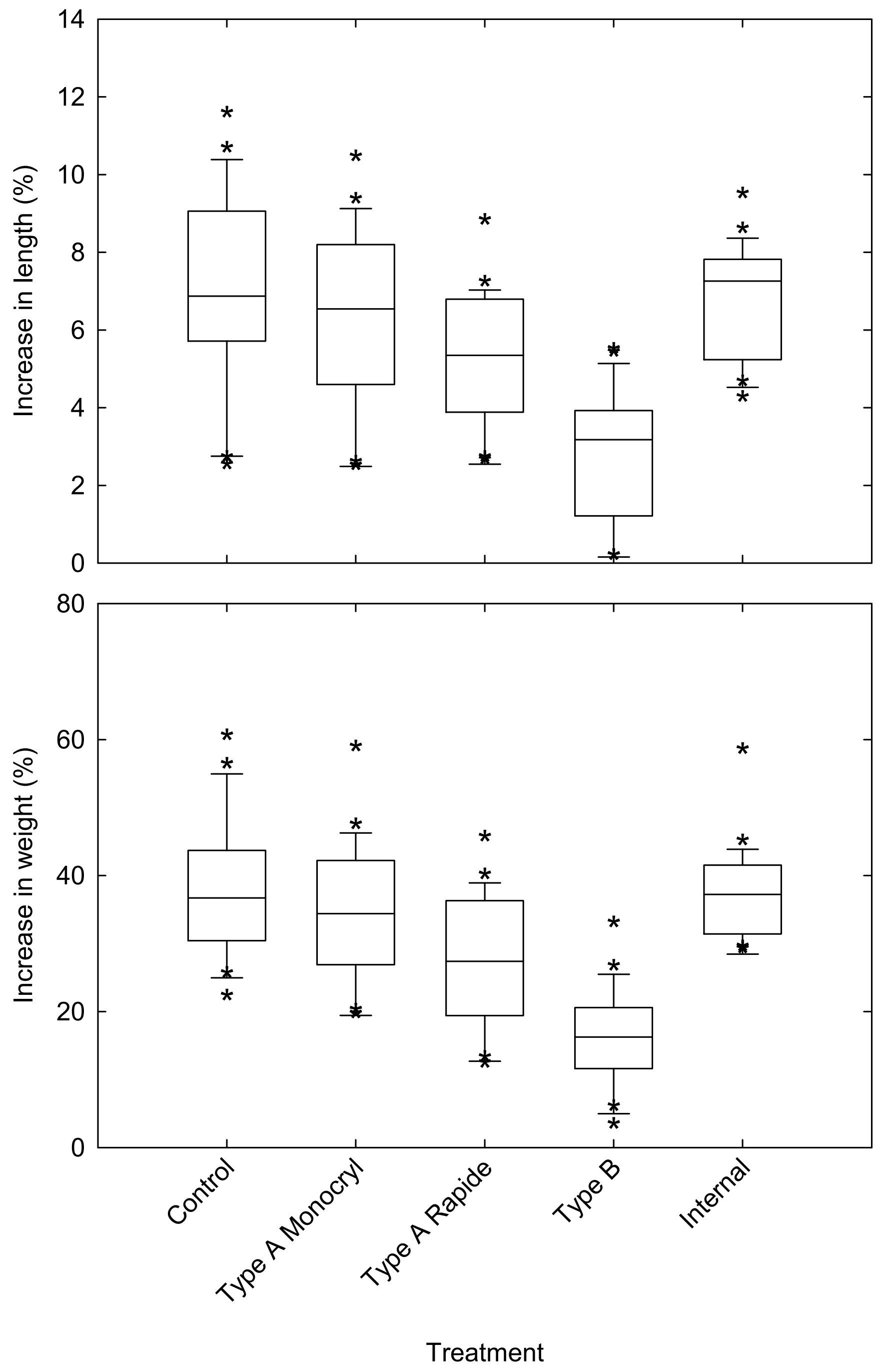

Figure 1.10. Box plots of difference in length and weight (%) for each treatment 14-d post-surgery. The top and bottom edges of the boxes indicate the 25th and 75th percentile of data, the line within each box indicates the median of the data. Whiskers indicate 1.5 × the interquartile range beyond the box, and asterisks indicate outliers.

Table 1.5. Pairwise comparison of proportion increase in weight. Differences were considered significant at an alpha of 0.0051.

	Control	Surgery	Type A (Monocryl)	Type A (Rapide)
Surgery	0.9124	--	--	--
Type A (Monocryl)	0.2651	0.3141	--	--
Type A (Rapide)	0.0033	0.0044	0.0574	--
Type B	<0.0001	<0.0001	<0.0001	<0.0001

$\alpha_{\text{family}} = 1 - (1 - 0.05)^{1/10} = 0.0051$

Tissue Reaction

Differences were found in tissue reaction to external attachment of transmitters. Type B tags generally caused more negative tissue reaction than Type A tags (Figure 1.11). Tissue tearing was significantly ($P < 0.01$) higher among fish tagged with Type B than fish tagged with Type A tags attached with either suture type (Table 1.6). However, there was no significant ($P = 0.19$) difference in tissue tearing between Type A tags attached with Monocryl or Vicryl Rapide. A similar pattern was seen for discoloration and indentation; fish tagged with Type B transmitters had significantly more discoloration ($P < 0.01$) and indentation ($P < 0.01$) than fish with Type A transmitters attached with either suture type (Figure 1.12). However, there were no differences in discoloration between Type A tags attached using Monocryl and Vicryl Rapide. The lack of indentation among fish implanted with Type A transmitters (observed in only one fish) precluded analysis between fish tagged using the two different types of suture. There was no significant ($P = 0.07$) difference in lacerations among the different tag type attachments; minor lacerations generally were found in all external tag types.

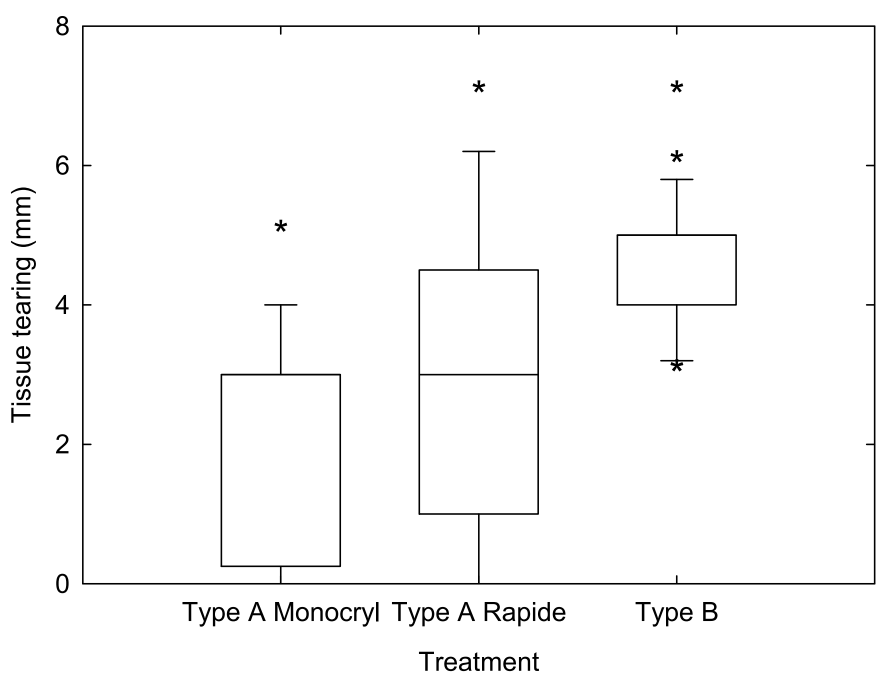

Figure 1.11. Longest measured tissue tear per fish observed for each tag treatment group after 14-d holding period.

Table 1.6. Analysis of variance of tissue tearing with a covariate of tag type.

Source	df	Sum square	Mean square	*F*	*P*
Tag type	2	60.77	30.3851	12.141	<0.0001
Residuals	55	137.64	2.5026		

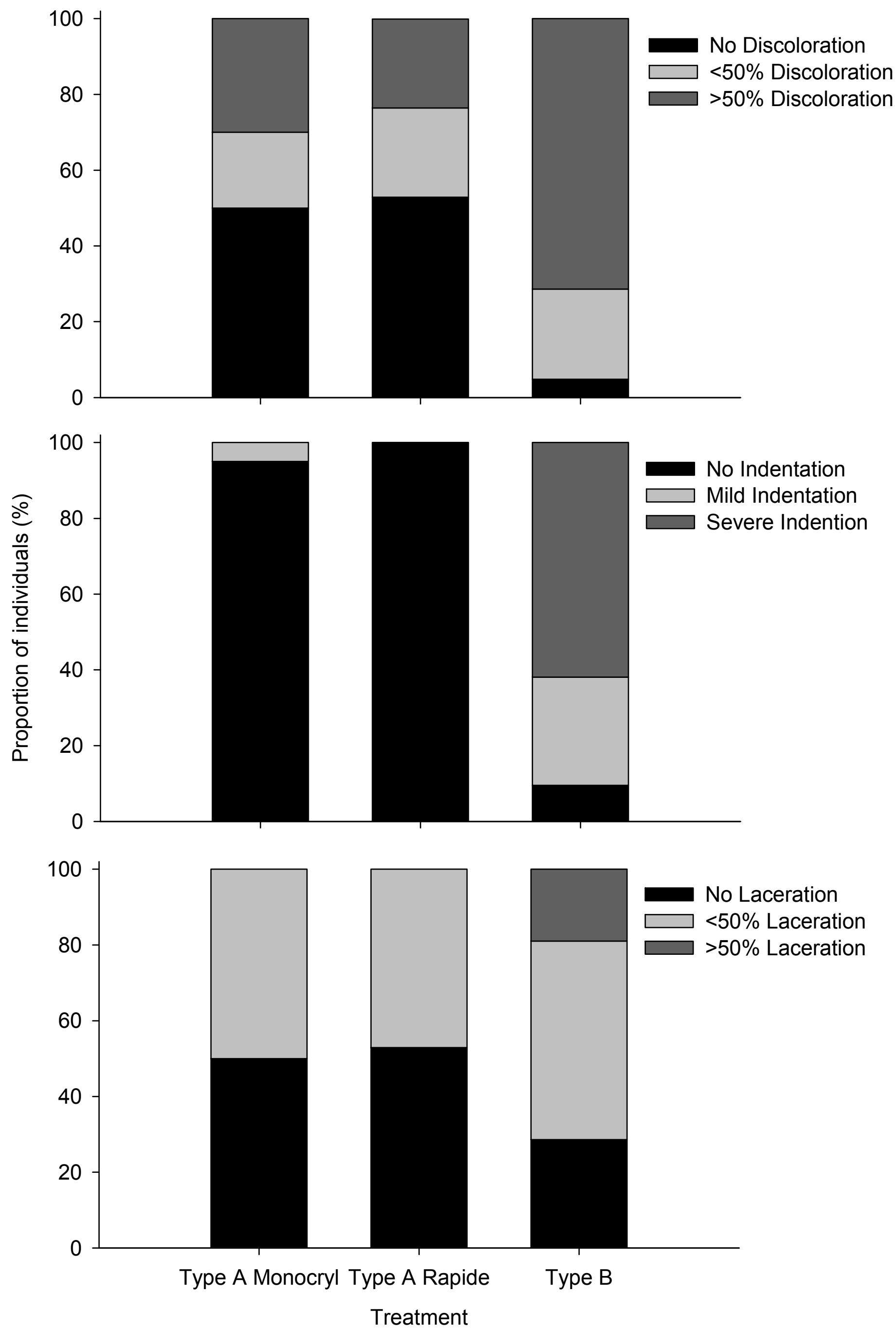

Figure 1.12. Proportion of individuals with discoloration, indentation (mild or severe), and laceration (%) at the end of the 14-d holding period for juvenile fall Chinook salmon with externally attached neutrally buoyant transmitters.

Shear Exposure

A total of 151 fish were exposed to shear forces at three nozzle velocities: 3.0 m/s, 9.1 m/s, and 12.2 m/s. Basic characteristics of the fish used are summarized in Table 1.7. Fish exposed to the 3.0-m/s nozzle velocity had significantly greater lengths and mass when compared to fish exposed to the 12.2-m/s velocity. In addition, the fork length of fish exposed to the 3.0-m/s velocity was significantly greater than fish exposed to the 9.1-m/s velocity.

Table 1.7. Basic characteristics of the fish exposed to shear flows.

Tag type	Nozzle velocity (m/s)	*n*	Mean length ± SD (mm)	Mean mass ± SD (g)
Control (nontagged)	3	11	127 ± 7	22.2 ± 4.4
	9.1	19	122 ± 8	18.2 ± 3.6
	12.2	20	120 ± 9	18.5 ± 3.9
Type A	3	12	125 ± 7	21.5 ± 4.2
	9.1	19	123 ± 8	20.0 ± 4.3
	12.2	20	123 ± 6	19.9 ± 3.4
Type B	3	9	123 ± 4	19.8 ± 1.8
	9.1	21	121 ± 6	18.6 ± 2.6
	12.2	20	119 ± 5	17.0 ± 3.0

Shear Injuries

Overall, no mortalities were observed throughout the study. Of the 151 test fish, 6.0% ($n = 9$) had injuries that were observed immediately after shear exposure, all of which were classified as minor (Table 1.8). Of these injuries, 77.8% ($n = 7$) were classified as bruising, 11.1% ($n = 1$) were classified as opercular damage, and 11.1% ($n = 1$) were classified as eye damage (i.e., exophthalmia). Shear injuries were observed among fish exposed to nozzle velocities of only 9.1 m/s and greater.

Table 1.8. Summary of shear injuries observed immediately following exposure to shear flows.

Tag type	Nozzle velocity (m/s)	*n*	Bruising	Opercular damage	Eye	Total
Control (nontagged)	3	11	0	0	0	0
	9.1	19	0	0	0	0
	12.2	20	2 (10.0%)	0	0	2 (10.0%)
Type A	3	12	0	0	0	0
	9.1	19	1 (5.3%)	0	0	1 (5.3%)
	12.2	20	0	1 (5.0%)	0	1 (5.0%)
Type B	3	9	0	0	0	0
	9.1	21	0	0	0	0
	12.2	20	4 (20.0%)	0	1 (5.0%)	5 (25.0%)

A correlation matrix showed a strong positive relationship between all kinematic parameters computed from the fish tracks (Table 1.9). The occurrence of shear injuries (Table 1.10) was significantly ($P < 0.001$) associated with head velocity; however, tag type was not a significant ($P = 0.4093$) predictor of injury. In addition, head velocity was the most predictive variable among the six kinematic parameters evaluated.

Table 1.9. Correlation matrix of kinematic parameters.

	Head velocity	Head acceleration	Middle velocity	Middle acceleration	Tail velocity	Tail acceleration
Head velocity	1	0.80	0.86	0.76	0.76	0.59
Head acceleration		1	0.83	0.79	0.80	0.72
Middle velocity			1	0.87	0.88	0.71
Middle acceleration				1	0.79	0.68
Tail velocity					1	0.86
Tail acceleration						1

Table 1.10. Analysis of deviance of the response variable shear injuries.

	df	Deviance	Residual df	Residual deviance	*F*	*Pr*(>*F*)
Null			150	68.214		
Head velocity	1	14.6227	149	53.591	41.3756	<0.0001
Tag type	2	0.6354	147	52.956	0.8989	0.4093
Interaction	2	1.7102	145	51.245	2.4195	0.0925

Tissue Damage

At the time of necropsy, 64.7% of fish tagged with Type A tags compared to 100% of fish tagged with Type B exhibited mild or severe tissue damage in the area around the tag (Table 1.11). A chi-square test for independence demonstrated that tag type and tissue damage were dependent (chi-square = 27.3367; P-value < 0.0001). Preliminary analysis examining the effect of tail velocity and tag type on the rate and severity of tissue damage showed that there was a significant ($P = 0.0051$) interaction between the two variables, even though tag type was significant for tissue damage (Table 1.12).

Table 1.11. Tissue damage in the area around the tag.

	Nozzle velocity					
	3 m/s	9.1 m/s	12.12 m/s	3 m/s	9.1 m/s	12.12 m/s
Tag type	Minor tissue damage			Major tissue damage		
Control (nontagged)	0	0	0	0	0	0
Type A	2	9	6	0	12	6
Type B	3	2	5	6	19	15

Table 1.12. Analysis of deviance of the response variable tissue damage.

	df	Deviance	Residual df	Residual deviance	*F*	*Pr*(>*F*)
Null			100	60.158		
Tail velocity	1	4.9022	99	55.256	13.3227	0.0004
Tag type	1	16.541	98	38.715	44.9534	<0.0001
Interaction	1	3.0228	97	35.692	8.2151	0.0051

When tail velocity was separated into two categories (0.0–6.0 m/s and 6.1–14.0 m/s), it was shown that there is a significant difference in the rate of tissue damage between fish tagged with Type A and Type B tags (Table 1.13). In all cases, fish tagged with Type A tags had significantly ($P < 0.001$ and $P = 0.0001$ respectively) lower levels of tissue damage at necropsy, when compared with fish tagged with Type B tags, while tail velocity and tail velocity by tag type interaction were not significant. It should be noted that as tail velocity increased, the difference in tissue damage between the two treatment groups decreased (Figure 1.13). Similarly, when the occurrence of tissue damage was evaluated within nozzle velocity groups, tail velocity and the tail velocity by tag type interaction were no longer significant ($P > 0.05$) and were removed from the model. For all three nozzle velocity groups, there were significantly ($P < 0.04$) lower levels of injury for fish tagged with Type A tags when compared with those tagged with Type B (Table 1.14).

Table 1.13. Analysis of variance comparing tail velocity and tag type for tissue damage occurrence, with tail velocity separated into two categories (0.0–6.0 m/s and 6.1–14.0 m/s).

Tail velocity (m/s)	Source	df	Sum square	Mean square	*F*	*Pr*(>*F*)
0.0–6.0	Tail velocity	1	2.558	2.558	13.917	0.0013
	Tag type	1	11.965	11.965	65.093	<0.0001
	Interaction	1	0.759	0.759	4.129	0.0556
	Residuals	20	3.676	0.184		
6.1–14.0	Tail velocity	1	0.218	0.218	0.558	0.4576
	Tag type	1	6.349	6.349	16.211	0.0001
	Interaction	1	0.012	0.012	0.03	0.864
	Residuals	73	28.59	0.392		

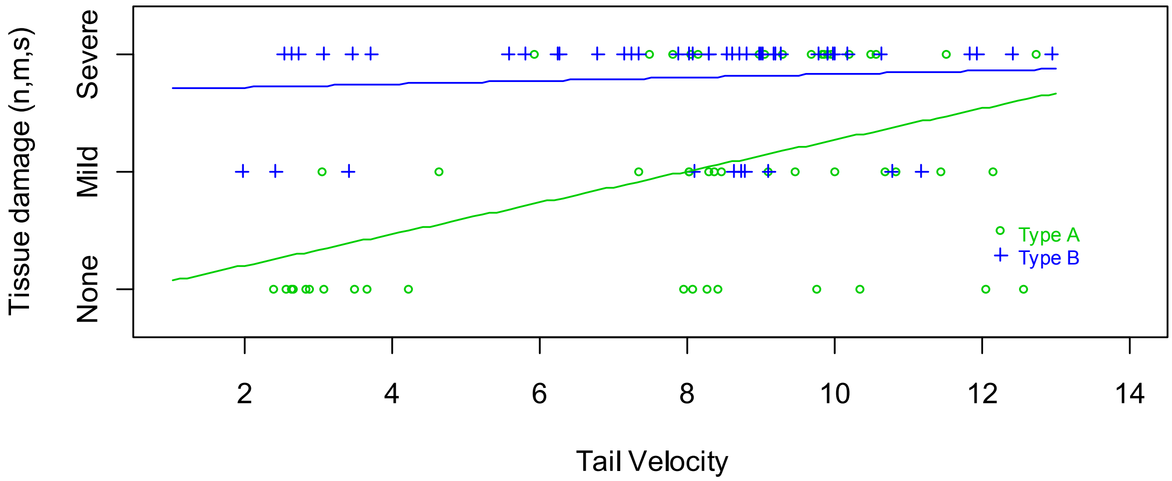

Figure 1.13. Scatter plot of tail velocity versus tissue damage.

Table 1.14. Analysis of variance comparing tail velocity and tag type for tissue damage occurrence within nozzle velocity groups.

Nozzle velocity (m/s)	Source	df	Sum square	Mean square	*F*	*Pr*(>*F*)
3.0	Tag type	1	11.571	11.571	59.961	<0.0001
	Residuals	19	3.667	0.193		
9.1	Tag type	1	0.744	0.744	4.54	0.0396
	Residuals	38	6.231	0.164		
12.2	Tag type	1	7.225	7.225	15.644	0.0003
	Residuals	38	17.55	0.462		

Discussion and Conclusions

The biological response noted in this study varied with tag type and tagging technique (internal vs. external). However, no mortality occurred in any of the tagged fish. Overall, the attachment and bearing of Type A transmitters led to a less negative influence on growth and tissue response than Type B transmitters. It did not appear that either Type A transmitters attached using Monocryl sutures or surgically implanted internal transmitters negatively influenced fish growth over the 14-d holding period.

Tag retention was high (no transmitter loss until Day 13) over the 14-d holding period but did vary by tag type and tagging technique. Tags were designed for use on short-term studies (3 d to 1 week) of survival of fish through hydroelectric turbines. There were no losses of Type B transmitters or internally implanted transmitters during the 14-d holding period. Vicryl Rapide sutures are designed to dissolve faster in humans than other types of sutures (Deters et al. 2010). However, there was no difference in tag loss over the 14-d period between the tags attached with either Monocryl or Vicryl Rapide sutures. Although retention of Type B transmitters was slightly higher than that of Type A transmitters, their use resulted in lower growth and more negative tissue response.

This research indicated much less damage to fish than previous research examining the use of external transmitters (Table 1.15). For example, Greenstreet and Morgan (1989) found higher mortality for externally tagged than untagged Atlantic salmon (*Salmo salar*). In their study, mortality was associated with size; smaller fish suffered higher mortality. They attached a two-part tag on the back of the fish beneath the dorsal fin. Other researchers (Makiguchi and Ueda 2009) also have found that mortality was higher for externally tagged fish (masu salmon, *Oncorhynchus masou*) than for PIT-tagged or surgically implanted fish.

Table 1.15. Details of studies conducted to determine the effects of externally attaching transmitter on salmonids.

Reference	Species[(a)]	*N*	Study period	Tag type	Method of attachment[(b)]	Range (mean) in length (mm)	Range (mean) in weight (g)	Tag weight in air (g)	Tag weight in water (g)	Tag burden (%)
Greenstreet and Morgan (1989)	AS	150	15 d	Acoustic	EX	101–200	--	2.7	--	--
Makiguchi and Ueda (2009)	MS	86	68 d	Radio	EX, SI	138–143	27.2–31.6	0.8	--	2.5–2.8
Mellas and Haynes (1985)	RT	80	45 d	Acoustic	EX, SI, GI	245–305	168–372	3.0	--	0.8–1.8
Thorstad et al. (2000)	AS	168	8 d	Radio	EX, SI	450–590	1021–2338	14.9–25.2	6.8–10.9	1.1–1.5
This study	CS	105	14 d	Acoustic	EX, SI	(122) 95–139	(21.7) 11.3–29.9	0.53	0.0,[(c)] 0.36	0.0,[(c)] 1.8–4.7

(a) AS = Atlantic salmon (*Salmo salar*), MS = masu salmon (*Oncorhynchus masou*), RT = rainbow trout (*O. mykiss*), CS = Chinook salmon (*O. tshawytscha*).
(b) EX = external attachment, SI = surgical implantation, GI = gastric implantation.
(c) External transmitter neutrally buoyant in water.

Our research also developed an external transmitter that did not negatively influence the growth of juvenile Chinook salmon (Table 1.15). Other research examining juvenile salmonids (Atlantic salmon) found that bearing an external transmitter resulted in reduced growth (Greenstreet and Morgan 1989). However, Makiguchi and Ueda (2009) found no difference in growth over 68 d among PIT-tagged, internally implanted, and externally tagged juvenile masu salmon. They used plastic wire to attach external transmitters through the dorsal musculature anterior to the dorsal fin.

Similar to this research, others have found that externally tagging fish can lead to some negative tissue response. Makiguchi and Ueda (2009) noted wounds and inflammation in the vicinity of the attachment wire in externally tagged masu salmon. However, we found that attachment of Type A transmitters led to less damage than the typical two-part mount attached below the dorsal fin. However, the influence of an external transmitter may be associated with fish size. Thorstad et al. (2000) found that Atlantic salmon bearing larger external transmitters had more signs of wounds associated with the external transmitter than fish bearing smaller external transmitters. Thus, use of a Type A external transmitter may have even less tissue response when attached to larger juvenile salmon.

Fish externally implanted with Type A tags sustained slightly lower injuries than fish externally implanted with Type B tags. However, there was no significant difference in shear injury rates between externally implanted and untagged fish. When tissue damage was evaluated within nozzle velocity groups or tail velocity, there were significantly lower levels of injury for fish tagged with Type A tags when compared to those tagged with Type B tags. Therefore, Type A tagged fish sustained significantly lower risk of tissue damage than Type B tagged fish for all groups combined. However, as flow speed or tail velocity increased; the difference in tissue damage between tag types greatly decreased.

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Chapter 2

The Effects of Neutrally Buoyant Externally Attached Transmitters on Predator Avoidance and Swimming Performance of Juvenile Chinook Salmon

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Introduction

Biotelemetry is commonly used to study the survival and migratory behavior of juvenile salmonids in the Federal Columbia River Power System (FCRPS), particularly in the Columbia and Snake rivers (Skalski et al. 1998, 2001; Hockersmith et al. 2003; Plumb et al. 2006; McMichael et al. 2010). For the resulting data to be used to make decisions about entire populations, the assumption is made that the fish's behavior, movement, and survival are unaffected by the presence of the transmitter or the tagging process (Peven et al. 2005). Swimming performance and the ability to avoid predators are two elements of fish behavior frequently used by researchers to determine if the presence of a tag or the tagging process has a negative influence (Peake et al. 1997; Anglea et al. 2004; Brown et al. 2006).

Several researchers have examined the effects of surgical implantation of transmitters on the swimming performance of juvenile salmonids (Table 2.1). Adams et al. (1998) found critical swimming speeds for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) surgically implanted with radio transmitters lower than the speeds of controls 1 and 21 days after tagging. However, swimming performance in that study may have been negatively influenced by drag forces associated with the presence of a trailing external antenna (31 cm in length). In a study by Brown et al. (1999), external antennas were trimmed to 2.5 cm, and no differences in critical swimming speeds were detected between tagged juvenile rainbow trout (*O. mykiss*) and controls, suggesting that the length of the antenna may influence the fish's ability to swim. One of the benefits of acoustic transmitters compared to radio transmitters is the absence of an external antenna. Neither Anglea et al. (2004) nor Brown et al. (2006) found a difference in critical swimming speed between juvenile Chinook salmon or sockeye salmon (*O. nerka*) surgically implanted with an acoustic transmitter compared to controls.

Although many studies have compared swimming performance of fish surgically implanted with transmitters to the performance of nontagged control fish, only a few examined the influence of externally attached transmitters. Thorstad et al. (2000) found no differences in swimming performance among groups of adult Atlantic salmon (*Salmo salar*) externally attached with radio transmitters, surgically implanted with transmitters, or implanted with no transmitters (controls). Peake et al. (1997) compared swimming performance of externally, internally, and gastrically implanted radio-tagged wild and hatchery-reared Atlantic salmon smolts. They found no differences between fish externally or internally tagged. However, swimming performance was lower for externally and internally tagged fish compared to the performance of nontagged controls.

In addition to swimming performance, the effects of surgical implantation of transmitters on juvenile salmonids have been studied by examining growth, survival, simulated turbine passage (barotrauma), and predator avoidance (Table 2.2; Zale et al. 2005; Brown et al. 2009, 2010; Carlson et al. 2010). Increased rates of predation on tagged fish may be attributed to trauma from the tagging procedure, visibility of the tag to predators, and impaired swimming performance due to drag associated with the transmitter or the antenna (Ross and McCormick 1981). Some studies of tagging effects on predator avoidance of juvenile salmonids (Anglea et al. 2004; Jepsen et al. 1998; Table 2.2) have found no difference in predation rates between tagged and nontagged fish. However, Adams et al. (1998) reported increased rates of predation for juvenile Chinook salmon surgically and gastrically implanted with radio transmitters compared to nontagged controls.

Table 2.1. Summary of studies examining effects of transmitters on swimming performance of salmonids. Tag burden is the weight of the transmitter in air divided by the weight of the fish in air. Externally attached transmitters used in this study were neutrally buoyant, thus having no tag burden when fish were in water.

Reference	Species[a]	*N*	Tag type	Method of attachment[b]	Mean (range) length (mm)	Mean (range) mass (g)	Tag mass in air (g)	Tag burden (%)
Adams et al. (1998)	CS	128	Radio	GI, SI	95–160	--	1.0	2.2–10.4
Brown et al. (1999)	RT	38	Radio	SI	84.9–91.9	5.0–10.0	0.6	6.0–12.0
Anglea et al. (2004)	CS	156	Acoustic	SI	139–143	34.0–37.0	1.5	1.4–6.7
Brown et al. (2006)	CS	150	Acoustic	SI	104–105	12.0–12.4	0.7	4.3–9.7
Brown et al. (2006)	SS	150	Acoustic	SI	113–114	12.1–12.3	0.7	4.6–7.2
Thorstad et al. (2000)	AS	168	Radio	EX, SI	450–590	1021–2338	15.1–25.0	<1.0 (in water)
Peake et al. (1997)	AS	126	Radio	SI, GI, EX	185–218	54.0–112.5	2.6	1.9–6.0
Robertson et al. (2003)	AS	80	Radio	SI	143–144	29.2–31.9	0.75	2.4–2.5
This study	CS	101	Acoustic	EX, SI	98–135	21.2–23.1	0.53–0.85	2.3–3.7

(a) CS = Chinook salmon (*Oncorhynchus tshawytscha*), RT = rainbow trout (*O. mykiss*), SS = sockeye salmon (*O. nerka*), AS = Atlantic salmon (*Salmo salar*).
(b) GI = gastric implantation, SI = surgical implantation, EX = external attachment.

Table 2.2. Summary of studies examining predator avoidance of salmonids. Tag burden is the weight of the transmitter in air divided by the weight of the fish in air. Externally attached transmitters used in this study were neutrally buoyant, thus having no tag burden when fish were in water.

Reference	Species[a]	*N*	Tag type	Method of attachment[b]	Mean (range) length (mm)	Mean (range) mass (g)	Tag mass in air (g)	Tag burden (%)
Adams et al. (1998)	CS	696	Radio	GI, SI	95–160	--	1.0	2.2–10.4
Anglea et al. (2004)	CS	40	Acoustic	SI	139–143	34.0–37.0	1.5	1.4–6.7
Jepsen et al. (1998)	AS, BT	50, 24	Radio	SI	160–240	--	1.4–1.7	--
Mesa (1994)	CS	541	--	--	--	10.8	--	--
This study	CS	113	Acoustic	EX, SI	98–135	27.7–28.6	0.53	1.9

(a) CS = Chinook salmon (*Oncorhynchus tshawytscha*), AS = Atlantic salmon (*Salmo salar*), BT = brown trout (*S. trutta*).
(b) GI = gastric implantation, SI = surgical implantation, EX = external attachment.

Juvenile salmonids migrating through hydropower facilities often encounter multiple stressors, including handling and passage through traveling screens, fish sorters, spillways, and turbines. Mesa (1994) found increased predation rates of juvenile Chinook salmon (age-0, mean mass 10.7 g) by northern pike minnow (*Ptychocheilus oregonensis*, fork length > 275 mm) shortly after being subjected to multiple stressors (multiple handlings and agitations) characteristic of some conditions encountered by fish during dam passage. Juvenile salmonids passing through the turbines are of particular concern for managers and researchers (Mathur et al. 1996; Čada et al. 2006). Carlson et al. (2010) surgically implanted juvenile Chinook salmon with acoustic transmitters (tag burden range 0.0% to 6.6%) and subjected them to simulated turbine passage. Fish were exposed to rapid decompression similar to pressures experienced by fish during passage through turbines in the FCRPS. The rate of mortality and injury in fish increased with tag burden, suggesting that the additional mass of the transmitter and/or the volume of the transmitter inside the body cavity increased the likelihood of mortal injury during rapid decompression. Carlson et al. (2010) suggest that this tagging bias likely leads to inaccuracies in survival studies of fish passing through turbines. These results led to this investigation of whether a neutrally buoyant externally attached transmitter could provide more accurate estimates of survival during turbine passage.

Although previous studies have found that external attachment of transmitters can alter the swimming performance and behavior of fish, there is a paucity of research on the effects of externally attached acoustic transmitters on juvenile salmonids. Recent technological advances have led to the reduction in size of acoustic transmitters, making it possible to study smaller fish. With the decrease in transmitter size resulting in lower tag burdens, external attachment of acoustic transmitters to juvenile salmonids has become a more plausible option for biotelemetry studies. The objective of this research is to determine if the swimming performance and predator avoidance of juvenile Chinook salmon will be compromised by the external attachment of a neutrally buoyant acoustic transmitter developed for tracking juvenile salmonids.

Experimental Methods

Fish Acquisition, Holding, and Surgical Procedures

Juvenile fall Chinook salmon were originally obtained as eyed eggs from the Washington Department of Fish and Wildlife Priest Rapids Hatchery in December 2009. Fish were reared at the Aquatic Research Laboratory (ARL) at the Pacific Northwest National Laboratory in Richland, Washington. During the study period, all test fish were held inside the ARL in 650-L circular tanks. All holding and test tanks were supplied with 15.0–17.8°C well water. Fish within the rearing and test population were fed Bio Vita Starter (Bio-Oregon, Longview, Washington) ad libitum. Fish selected for testing were not fed for 24 h prior to surgery or testing. Fish in both test groups (swimming performance and predator avoidance) ranged from 98 to 152 mm long (fork length, FL) and 9.0–39.7 g in weight (Table 2.3, Table 2.4).

Adult rainbow trout were used as predators and were obtained from Trout Lodge Hatchery (Soap Lake, Washington) in November 2010. All predators were held outside the ARL in two 2000-L circular tanks prior to the study period. Holding tanks were supplied with 15–16°C well water. Predator fish ranged in length from 300 to 460 mm (FL) and in weight from 400 to 1200 ± 200 g.

Externally tagged fish were designated with one of two tag designs for swimming performance—the tag anterior to the dorsal fin (Type A) or the two-part tag beneath the dorsal fin (Type B) (Table 2.3). Only transmitter Type A was used for predation trials. Information on the size and characteristics of the external transmitters is detailed in Chapter 1.

Table 2.3. Mean fork length (FL) and weight ± SD (range) of swimming performance test fish by treatment.

		FL (mm)		Mass (g)	
Treatment	*n*	Mean ± SD	Range	Mean ± SD	Range
External transmitter					
Type A (anterior to dorsal fin)	30	123 ± 6.4	111–135	21.2 ± 4.2	14.2–30.4
Type B (two-part beneath dorsal fin)	31	126 ± 7.3	102–135	22.6 ± 4.5	11.9–30.3
Internal transmitter					
JSATS + PIT	10	125 ± 4.4	119–132	23.1 ± 2.4	20.3–27.7
Control (nontagged)	31	124 ± 8.6	98–135	21.8 ± 5.5	9–30.7
Overall	101	124 ± 7.2	98–135	22.0 ± 4.6	9–30.7

Table 2.4. Mean fork length (FL) and weight ± SD (range) of predator avoidance test fish for each trial.

	Tagged			Nontagged		
Trial	*n*	Mean ± SD (range) FL (mm)	Mean ± SD (range) Mass (g)	*n*	Mean ± SD (range) FL (mm)	Mean ± SD (range) Mass (g)
1	7	136 ± 8 (117–145)	29.8 ± 5.0 (18.9–35.9)	10	137 ± 12 (106–152)	31.0 ± 6.9 (13.2–39.7)
2	7	140 ± 6 (127–149)	30.5 ± 4.2 (22.2–38.8)	10	143 ± 7 (128–155)	33.3 ± 4.4 (25.2–40.4)
3	10	125 ± 7 (113–135)	24.0 ± 4.2 (16.1–31.2)	10	129 ± 6 (114–135)	25.1 ± 3.8 (18.5–32.4)
4	10	129 ± 5 (120–134)	25.9 ± 3.1 (19.1–29.4)	9	128 ± 6 (120–135)	25.1 ± 4.7 (14.8–31.4)
5	10	130 ± 5 (115–135)	28.9 ± 3.3 (19.4–32.1)	10	130 ± 4 (125–135)	28.6 ± 2.6 (23.5–31.9)
6	10	128 ± 8 (105–135)	28.4 ± 5.5 (14.1–35.0)	10	130 ± 5 (118–134)	29.7 ± 3.4 (22.6–34.4)
Overall	54	131 ± 8 (105–149)	27.7 ± 4.7 (14.1–38.8)	59	133 ± 9 (106–155)	28.6 ± 5.2 (13.2–40.4)

All surgeries were performed by one surgeon to eliminate surgeon bias (Deters et al. 2010). The daily order in which surgeries were performed (i.e., Type A tag or Type B tag) was randomized. Fish were anesthetized with a solution of 80 mg tricaine methanesulfonate (MS-222)/L of water buffered with an 80-mg/L sodium bicarbonate solution until they reached stage 4 anesthesia (as described by Summerfelt and Smith 1990). Fork lengths (in millimeters) and mass (in grams) for all fish were measured while they were anesthetized. Fish were placed dorsal side up on a foam rubber pad for external attachment, and ventral side up for internal implantation. A small tube was inserted in the fish's mouth during surgery to provide a constant maintenance flow of 40-mg/L MS-222 buffered with a 40-mg/L solution of sodium bicarbonate.

For predation trials, Type A transmitters were air-brushed with a mixture of green, black, white, and blue paint (CS Coatings, Wausau, Wisconsin) before attachment. The paint camouflaged the tag by mimicking the coloring of Chinook salmon (Figure 2.1). External tag attachment and surgical implantation was performed as described in Chapter 1 (similar to Deters et al., in press; Panther et al., in press). Following all surgeries (or handling for controls), fish were placed in a 20-L bucket containing oxygenated water to recover. After recovery, fish were placed in a floating, perforated 20-L bucket to allow flow-through water and then placed in a 650-L circular tank inside the ARL. Lights inside the ARL were controlled automatically to follow the natural photoperiod.

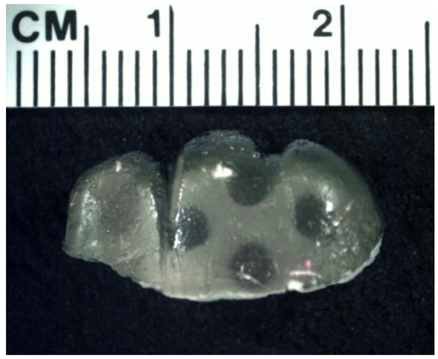

Figure 2.1. Type A tag used for predation trials, painted with a green base coat and dark green spots.

Swimming Performance Tests

A Blazka-type respirometer (Figure 2.2) was used to conduct swimming performance tests. The relationship between water velocity in the swim chamber and motor speed was calibrated using a Type "S" pitot tube (United Sensor Corporation, Amherst, New Hampshire). Flow straighteners at the upstream end of the tube were used to achieve uniform water velocity within the swim chamber. The swim chamber had an electrified grid at the downstream end. A black shade was placed at the upstream end of the swim chamber during testing to provide shelter and orientation. Flow-through well water (16.8–17.8°C) was supplied to the swim chamber during testing.

Figure 2.2. The Blazka-type respirometer used for testing swimming performance.

Swimming performance tests were conducted from November 8 through December 17, 2010. For each trial, one fish was selected at random and placed inside the swim chamber. Fish were given a 30-min acclimation period with the respirometer velocity set at 1 FL s^{-1}. Thereafter, the velocity was increased by 0.5 FL s^{-1} every 15 min. When a fish stopped swimming and fell back to the downstream end of the swim chamber, the shocking grid was activated to emit a 6- to 12-V shock. The fish received a

1-s shock if it came in contact with the grid. If the fish did not swim away from the grid, the fish was shocked consecutively at 1-s intervals for 10 s. If the fish remained on the grid at the end of 10 s, the motor was stopped to allow the fish to swim away from the grid. The velocity was set back to the acclimation speed and increased gradually to the last velocity setting. If the fish did not swim away from the grid, the fish was considered to be fatigued and received no further shocks. If the fish continued to swim, the procedure would be repeated until the fish was fatigued. At the end of the test, the fish was removed from the swim chamber and euthanized with MS-222 (250 mg/L). Critical swimming speed was calculated based on the formula of Brett (1964):

$$U_{crit} = u_1 + (t_i / t_{ii} \cdot u_{ii}) \tag{2.1}$$

where u_1 = the highest velocity maintained for the prescribed period (cm s^{-1})
u_{ii} = the velocity increment (cm s^{-1})
t_i = time (min) fish swam at the ''fatigue'' velocity
t_{ii} = prescribed period of swimming (min).

Predator Avoidance Tests

Juvenile fall Chinook salmon were randomly designated as treatment (tagged; Type A external transmitter) or control (nontagged) fish for the predation trials. Sample size for both groups combined was between 17 and 20 fish for each trial. Some trials had fewer fish as a result of fish jumping out of the tank during testing.

Rainbow trout were chosen as predators because of their performance as test predators in previous studies and ease of acclimation to the test environment (Neitzel et al. 2000; Anglea et al. 2004). Ten rainbow trout were held in the 2000-L circular test tank for an acclimation period of 8 weeks prior to the start of the predation trials. During the acclimation period, predators were conditioned to feed on live fish (as described by Anglea et al. 2004) by presenting them with 10 juvenile Chinook salmon (FL ~ 130 mm, 30 g) daily.

Predation trials were conducted in a 2000-L circular tank (15–16°C). Predators were not fed between trials. To begin the trial, 10 tagged and 10 nontagged fish were transferred in 20-L buckets and introduced into the predation tank by emptying the buckets directly into the tank.

Video cameras were set up away from the tank to monitor the rates of predation and minimize outside disturbances. Observations were made at 15-min intervals from the live video feed and every hour at the tank; the goal was to stop each trial when 50% of the prey were consumed. If injuries from predation attempts were serious (e.g., fish lying on the tank bottom), fish were categorized as "consumed" based on the assumption that those fish would not survive the trial. Trials ended when 50% of prey were consumed or after 8- or 24-h periods if less than 50% of prey were consumed. Fish with predation marks were identified, and injuries were examined.

One additional predator avoidance trial was conducted on a pilot scale in a rectangular fiberglass flume (9.14 m long, 1.22 m wide) with a water depth of 0.76 m (8,475 L). A submerged water jet (as described by Deng et al. 2005) was used to mimic conditions experienced by migrating juvenile salmonids in the tailrace of hydroelectric facilities. Flow velocity of 20 m s^{-1} was generated by using a centrifugal pump. The tank was partitioned with netting attached to a polyvinyl chloride frame to confine the test fish to the area (3.58 m long, 1.22 m wide) of the flume in front of the water jet. Ten rainbow trout were transferred to the tank and allowed to acclimate for 7 days prior to testing. Testing and monitoring procedures were carried out in a manner similar to that of the other predation trials.

Statistical Analysis

Differences in critical swimming speed among tag treatment groups were tested using analysis of variance (ANOVA). The first analysis included three groups (Type A, Type B, and control). The analysis was done again with the addition of internally implanted fish (Surgery) as a fourth treatment group. In addition to tag type, the influence of fish length on critical swimming speed was examined. ANOVA was used also to compare all combinations of two tag types. To control for the increased probability of a Type I error, a Šidák correction was used to adjust the rejection region, depending on the number of pairwise tests.

$$\begin{aligned} &\alpha_{family} = 1-(1-\alpha_{comparison})^{1/t} \\ &t = \text{\# of pairwise t-tests} \\ &\alpha_{comparison} = 0.05 \\ &\alpha_{family} = \text{ new familywise error rate} \end{aligned} \tag{2.2}$$

For swimming performance, power curves were constructed to show the sample size needed for comparing any combination of two tag types. Assuming homogeneous variances, the mean squared error (MSE) from the overall ANOVA test can be used as an estimate of variance in making calculations involving power. Assuming the MSE and sample mean difference between two tag types do not change with increased sample size, the estimated power and percentage detectable difference can be calculated for different levels of *n*. This was done for the observed sample mean differences.

For predator avoidance, analysis of variance was used to test for differences between the proportion of survival between tagged and nontagged fish. All assumptions of parametric tests were met (i.e., independence, normality, homogeneity of variance). A significance level of 0.05 was used.

Results

Swimming Performance

Comparison of External Transmitters to Controls

Mean critical swimming speed (U_{crit}) for juvenile Chinook salmon ranged from 36.7 to 46.7 cm s^{-1} (Table 2.5 and Figure 2.3). Critical swimming speed varied significantly with both fish size ($P < 0.0001$; decreasing with increasing fish size) and tag type ($P < 0.0001$; Table 2.6 and Figure 2.4). Control fish had significantly higher critical swimming speeds than fish with either Type A ($P = 0.0087$) or Type B ($P < 0.0001$) external transmitters (Table 2.7). No significant ($P = 0.038$) difference in critical swimming speed was observed for fish tagged with Type A transmitters compared to fish with Type B transmitters.

Table 2.5. Mean ± SD relative critical swimming speed (U_{crit}) expressed in cm s^{-1} and FL s^{-1} for each treatment.

Treatment	*n*	Mean U_{crit} (cm s^{-1})	Mean U_{crit} (FL s^{-1})
External transmitter			
Type A	30	41.2 ± 7.97	3.36 ± 0.70
Type B	31	36.7 ± 7.27	2.93 ± 0.61
Internal transmitter			
JSAT + PIT	10	42.9 ± 7.66	3.44 ± 0.64
Control (nontagged)	31	46.7 ± 9.37	3.78 ± 0.84

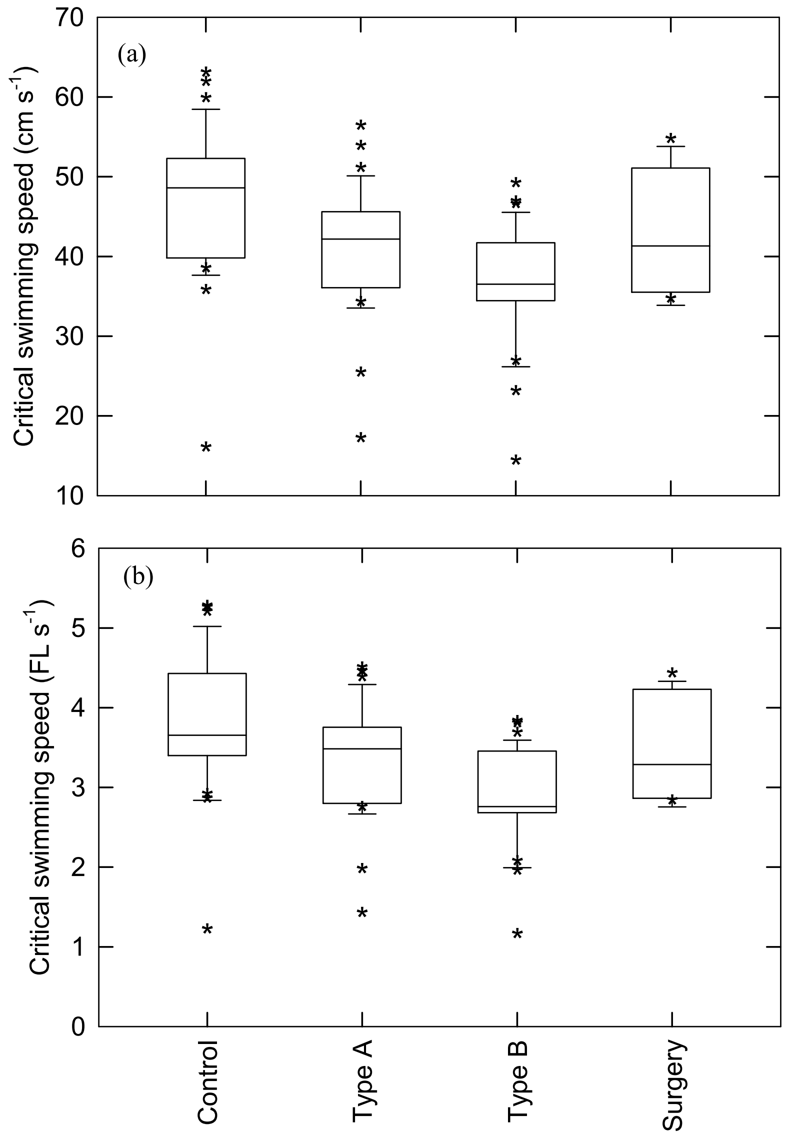

Figure 2.3. Box plots of critical swimming speed in (a) cm s^{-1} and (b) FL s^{-1} for each treatment. The top and bottom edges of the boxes indicate the 25th and 75th percentile of data; the line within each box indicates the median of the data. Whiskers indicate 1.5 × interquartile range beyond the box, and an asterisk indicates outliers.

Table 2.6. Analysis of variance of U_{crit} scores with covariates length and tag type.

Source	df	Sum square	Mean square	*F*	*P*
Length	1	10.09	10.09	23.55	<0.0001
Tag type	2	9.816	4.9078	11.455	<0.0001
Residuals	88	37.704	0.4285		

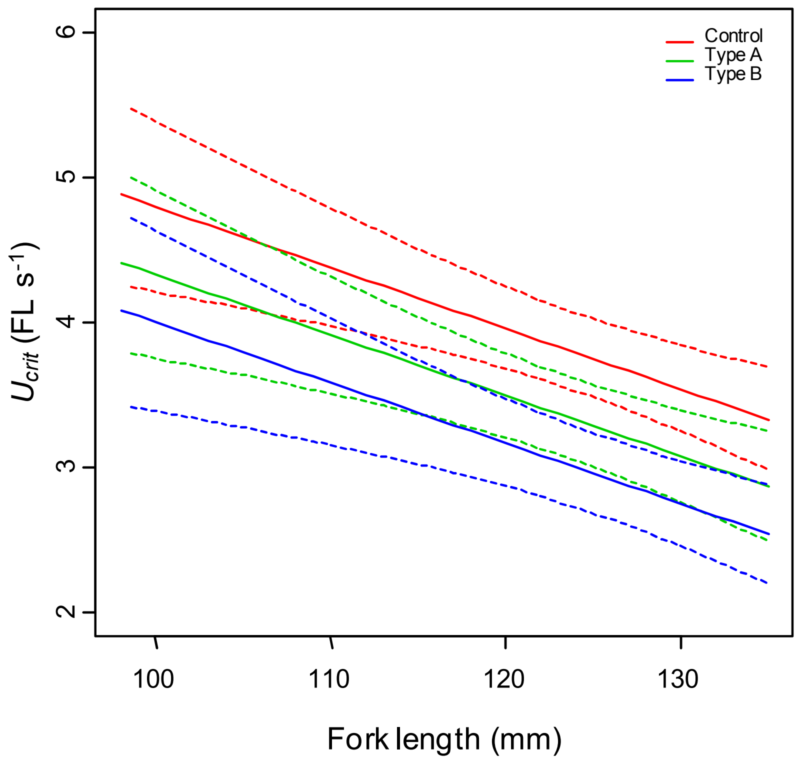

Figure 2.4. Predicted U_{crit} values for test and control fish with differing lengths and tag types. Dotted lines show 95% confidence intervals of the regression lines.

The sample sizes from these experiments provided high overall power to determine differences among treatment groups. The sample data from experiments showed a maximum difference in sample means between treatment groups of 0.07 FL/s (the maximum difference between Type B tag and control fish). The data obtained were sufficient to detect a difference of 10% with a power of 75%, a power of 97% to find a 15% difference, and a power approaching 100% to find a 20% difference. The mean critical swimming speed for control fish was 11.3% higher than the mean for Type A tagged fish (mean U_{crit}). Data obtained from these experiments were sufficient to detect this difference with a power of 84%. The mean U_{crit} for Type B tagged fish was 22.5% lower than controls, with a power of 99.99% to detect this difference. The mean U_{crit} for Type A tagged fish was 12.6% higher than Type B tagged fish, with a power of 91% to detect this difference.

Table 2.7. Analysis of variance of U_{crit} scores with covariates length and tag type for comparing between each pair of test groups. Significant *P*-values (*P* < 0.017 after Šidák correction, Equation 2.2) are in italics.

Source	df	Sum square	Mean square	*F*	*P*
Type A vs. Type B					
Length	1	4.4522	4.4522	11.8299	*0.0011*
Tag type	1	1.6902	1.6902	4.4909	0.0384
Residuals	58	21.8284	0.3764		
Control vs. Type B					
Length	1	7.7723	7.7723	17.568	*0.0001*
Tag type	1	9.7116	9.7116	21.952	*<0.0001*
Residuals	59	26.1017	0.4424		
Control vs. Type A					
Length	1	7.2809	7.2809	15.5248	*0.0002*
Tag type	1	3.4605	3.4605	7.3788	*0.0087*
Residuals	58	27.201	0.469		

$\alpha_{family} = 1 - (1 - \alpha_{comparison})^{1/t} = 1 - (1 - 0.05)^{1/3} = 0.017$

Comparison of External Transmitters to Controls and Internally Implanted Fish

When internally implanted fish were added as a pilot-scale comparison (Figure 2.5), there was also a significant difference in swimming perfomance related to fish length (decreasing with increasing fish length; *P* < 0.001) and tag type (*P* = 0.001; Table 2.8). Control fish still had signficantly higher critical swimming speeds than fish externally implanted with Type A (*P* = 0.0087; Table 2.9) or Type B external transmitters (*P* < 0.0001). However, there was no significant difference between internally implanted fish and either control fish (*P* = 0.2245) or fish externally implanted with Tag Type A (*P* = 0.512) or Type B external transmitters (*P* = 0.0317).

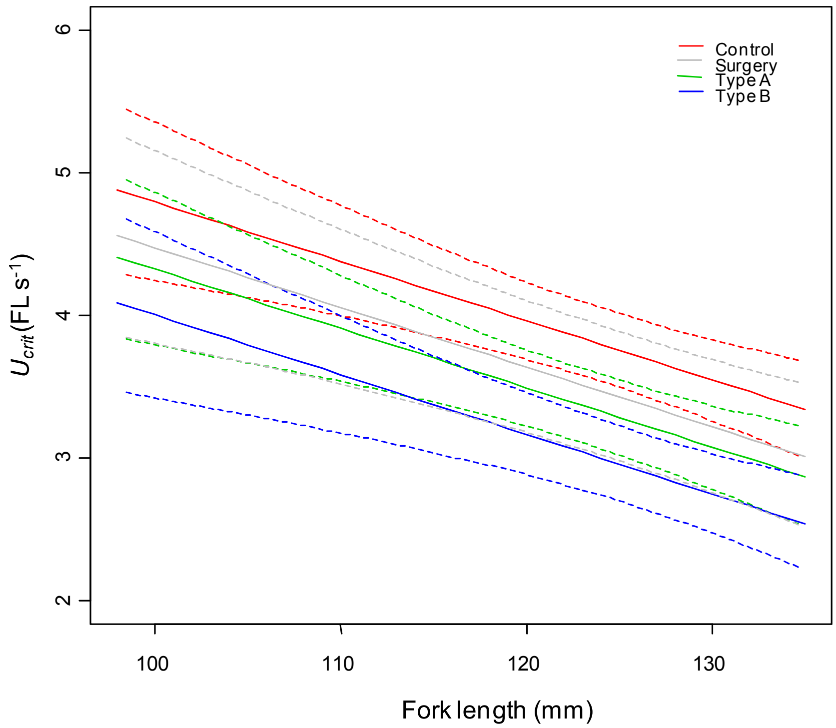

Figure 2.5. Predicted U_{crit} values for control, externally tagged, and surgically implanted fish with differing lengths and tag types. Internally implanted fish have been added as the grey line. Dotted lines show 95% confidence intervals of the regression lines.

Table 2.8. Analysis of variance of U_{crit} scores with covariates length and tag type.

Source	df	Sum square	Mean square	*F*	*P*
Length	1	10.393	10.3933	24.5263	<0.0001
Tag type	3	9.906	3.3021	7.7923	0.0001
Residuals	97	41.105	0.4238		

The data obtained with the addition of internally implanted fish were sufficient to detect a difference of 10%, with a power of 31%, a power of 58% to find a 15% difference and a power approaching 100% to find a difference of ≥ 20%. The mean critical swimming speed for internally implanted fish was 9.0% lower than control fish, with 88% power to detect this difference. The mean U_{crit} for internally implanted fish was 2.5% higher than for Type A tagged fish, with a power of 15% to detect this difference. The U_{crit} of internally implanted fish was 15% higher than the U_{crit} for Type B tagged fish, with a power of 99.9% to detect this difference.

Predator Avoidance

There was no significant difference ($P = 0.2622$) between tagged and nontagged fish in the percentage of fish consumed (Table 2.10). No significant difference ($P = 0.8263$) was detected in the percentage consumed among the six predation trials conducted. The mean percentage of nontagged fish consumed was 38.9% compared to 47.6% for tagged fish (Figure 2.6). The estimated difference in survival was 8.7%.

For the pilot-scale predator avoidance trial conducted in the flume (n = 9 tagged, 10 nontagged), 44.4% of tagged fish (n = 4) and 40.0% of nontagged controls (n = 4) were consumed after 24 h. Two surviving nontagged fish were found to have evidence of predation attempts. This trial was not included in the statistical analysis because it was done on a pilot scale and involved additional variables (e.g., different tank, turbulent flow) not included in the other trials.

Table 2.9. Analysis of variance of U_{crit} scores with covariates length and tag type for comparing between each groups. Significant P-values ($P < 0.009$ after Šidák correction, Equation 2.2) are in italics.

Source	df	Sum square	Mean square	F	P
Type A vs. Type B					
Length	1	4.4522	4.4522	11.8299	*0.0011*
Tag type	1	1.6902	1.6902	4.4909	0.0384
Residuals	58	21.8284	0.3764		
Control vs. Type B					
Length	1	7.7723	7.7723	17.568	*0.0001*
Tag type	1	9.7116	9.7116	21.952	*<0.0001*
Residuals	59	26.1017	0.4424		
Control vs. Type A					
Length	1	7.2809	7.2809	15.5248	*0.0002*
Tag type	1	3.4605	3.4605	7.3788	*0.0087*
Residuals	58	27.201	0.469		
Control vs. Surgery					
Length	1	5.9544	5.9544	11.8974	*0.0014*
Tag type	1	0.763	0.763	1.5246	0.2245
Residuals	38	19.0182	0.5005		
Type A vs. Surgery					
Length	1	2.6528	2.6528	6.5527	0.0147
Tag type	1	0.1775	0.1775	0.4384	0.512
Residuals	37	14.979	0.4048		
Type B vs. Surgery					
Length	1	1.6785	1.6785	4.7378	0.0358
Tag type	1	1.7626	1.7626	4.9751	0.0317
Residuals	38	13.4625	0.3543		

$\alpha_{family} = 1 - (1 - \alpha_{comparison})^{1/t} = 1 - (1 - 0.05)^{1/6} = 0.009$

Table 2.10. Analysis of variance of six predation trials between juvenile Chinook salmon that were nontagged or tagged with a neutrally buoyant external transmitter (Tag type).

Source	df	Sum square	Mean square	*F*	*P*
Trial	5	0.2654	0.0531	0.408	0.8263
Tag type	1	0.2077	0.2077	1.596	0.2622
Error	5	0.6506	0.1301		

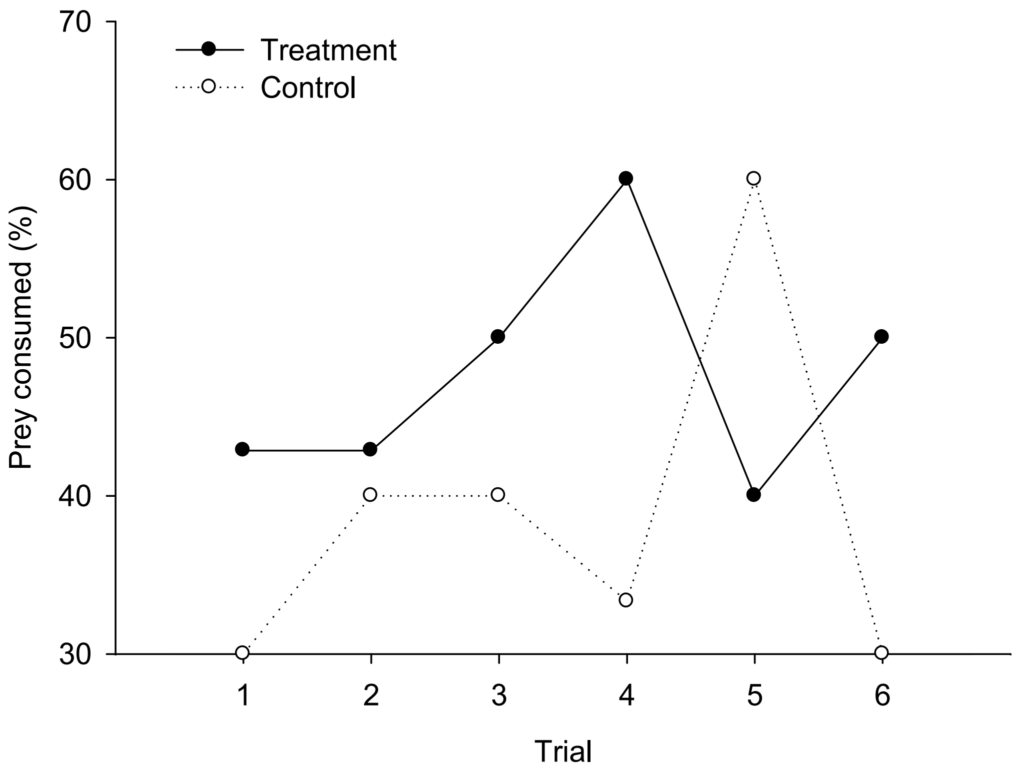

Figure 2.6. Percentages of juvenile Chinook salmon consumed by rainbow trout during each of six predation trials. Control fish were nontagged; treatment fish were tagged with a neutrally buoyant external transmitter.

Discussion

Swimming Performance

Compared to nontagged juvenile Chinook salmon, both external transmitter Types A and B negatively influenced the swimming performance of juvenile Chinook salmon (98–135 mm). Similar results have been reported by researchers examining the effects of external transmitters on the swimming performance of Atlantic salmon smolts (175–221 mm; Peake et al 1997; see Table 2.1 for size and tag burden details for this and other studies). However, Thorstad et al. (2000) found no negative effect on the swimming performance of adult Atlantic salmon (450–590 mm) from the presence of an externally attached radio transmitter.

Swimming performance of fish internally implanted with acoustic transmitters was similar to that of the controls. Other researchers found similar results for swimming performance of juvenile Chinook salmon surgically implanted with acoustic transmitters (124–154 mm, Anglea et al. 2004; 94–125 mm, Brown et al. 2006). However, Brown et al. (2006) found that juvenile sockeye salmon (101–133 mm) surgically implanted with acoustic transmitters had poorer swimming performance than their nontagged counterparts.

Swimming performance of internally implanted juvenile Chinook salmon was also similar to that of fish externally tagged with Type A and Type B transmitters. Although the 10 internally implanted fish were initially added on a pilot scale, difference in critical swimming speed was detected with high statistical power for all tag types combined (power approaching 100% to detect a 20% difference), as well as for the comparison of internally implanted transmitters to Type B transmitters (99.99% power to detect a difference of 15%) and controls (88% power to detect a difference of 9.0%). However, there was much lower statistical power to detect a difference, if one existed, between Type A transmitters and internally implanted transmitters (15% power to detect a difference of 2.5%). When the swimming performance was compared between the two external transmitter types, this research demonstrated that there was no difference in swimming performance for fish carrying Type A external transmitters compared to Type B transmitters. No difference between Type A or Type B transmitters can be concluded.

Swimming performance also decreased with increasing fish length. This trend was also noted in critical swimming speeds among control fish tested by Adams et al. (1998). In addition, Brett (1964) stated that the swimming ability of fish decreases as size increases. However, Peake et al. (1997) found no correlation between critical swimming speed and fish length for radio-tagged Atlantic salmon smolts (175–221 mm). Those results mirror those of Brown et al. (2006) for acoustic-tagged juvenile Chinook salmon.

Predation

Predation research was carried out after the swimming performance (outlined in this chapter) and the holding and shear work (outlined in Chapter 1) were conducted. Fish tagged with Type A transmitters attached using Monocryl sutures had superior growth compared to those with Type B transmitters or Type A transmitters attached with Vicryl Rapide. The differences in growth due to suture type were likely due to the difference in the suture material (Vicryl Rapide is a braided suture while Monocryl is a monofilament suture). Because of these differences, only Type A transmitters attached with Monocryl were compared to nontagged fish during predation trails.

In this study, no detectable difference among predation rates of tagged and nontagged fish were found. Of the few studies that have examined the effects of transmitters on predator avoidance of juvenile salmonids, results similar to this study were reported by Anglea et al. (2004) for juvenile Chinook salmon. However, acoustic transmitters in that study were internally implanted. In contrast, Adams et al. (1998) reported that juvenile Chinook salmon tagged with radio transmitters (external antenna length 31 cm) were consumed in higher proportions than control fish. In that study, swimming performance of tagged fish was found to be lower than that of nontagged fish. Many factors are involved in a fish's ability to avoid predation. Swimming performance, prey conspicuousness, and ability to detect predators may lead to differential predation (Bams 1967; Mesa 1994). The presence of an external transmitter has the potential to impair some of these avoidance abilities by possibly creating drag as well as visible differences among prey. Multiple stressors associated with the tagging process itself may also lead to increased risk of predation by eliciting physiological and behavioral stress responses potentially resulting in substandard condition of the prey at the time of the predatory interaction (Schreck 1990; Temple 1987).

The additional mass of a transmitter can result in an increase in fish density, which potentially leads to increased energy expenditure (Lefrancois et al. 2001). This potential increase in energy expenditure could be a factor in both swimming performance and the ability to avoid predation. Although the attachment of an external transmitter adds more surface area to the fish and thus potentially leads to drag forces, the transmitter used in this study was neutrally buoyant in water. Thus, for this research, there was no tag burden (the ratio of transmitter mass to fish mass). In addition, predation of tagged fish may not have been higher than that of nontagged fish, contrary to the results of Adams et al. (1998), due to the lack of an antenna on the external acoustic transmitter. The external tags we used were also painted to minimize their visibility. This could have played in the role in the lack of a difference in predation.

For the pilot predation trial conducted in the flume, four (44.0%) of the nontagged fish were consumed compared to four (40.0%) of the tagged fish. Despite the presence of more turbulent conditions in the flume, there was no difference in the number of tagged and nontagged fish eaten. These results are similar to those found in the circular tank where turbulent conditions did not exist. Although turbulent flow in the tailrace is one type of condition confronting juvenile salmonids during dam passage, other factors such as rapid decompression during turbine passage, handling at fish-sorting facilities, and damage while going though bypass facilities may also influence their ability to avoid predators. These cumulative factors and their effect on predation rates in the tailrace should be examined further.

Conclusion

Although this research indicates that the swimming performance of externally tagged juvenile Chinook salmon was lower than that of nontagged fish, there was no difference in swimming performance between fish externally implanted with Type A or Type B transmitters and internally implanted fish. In addition, no difference in predation rates between externally implanted and nontagged fish was detected. These are good indications that an externally attached neutrally buoyant transmitter may be a viable option for studies to estimate survival of juvenile salmonids passing through turbines. However, as suggested by Zale et al. (2005), Thorstad et al. (2009), and Brown et al. (2010), conclusive evidence of transmitter effects as well as whether bias will be present from the use of these transmitters will require comparative laboratory (as detailed in other chapters of this report) and field studies that involve tagging a wide size range of juvenile salmonids with transmitters and measuring their rates of migration, growth, predation, and survival. We also suggest that research be conducted to examine the differences in predation between internally implanted and externally tagged (with a neutrally buoyant transmitter) juvenile Chinook salmon after exposure to simulated turbine passage. In addition, results from the other chapters of this report provide insight into the use of neutrally buoyant transmitters as a tool for estimating survival of juvenile Chinook salmon passing through turbines.

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Chapter 3

The Effect of an Externally Attached Neutrally Buoyant Transmitter on Mortal Injury during Simulated Turbine Passage

Richard S. Brown, Brett D. Pflugrath, Thomas J. Carlson, Z. Daniel Deng

Introduction

Survival of juvenile salmonids passing through turbines can vary depending on several factors. The environment experienced by fish when passing through turbines can vary depending on the type of turbine present, the ways in which turbines are operated, the route the fish take when passing through the turbine, the discharge rate, and the head differential (forebay and tailrace elevation differential; Čada 1990; Carlson et al. 2008; Deng et al. 2010). Researchers typically use telemetry tags (acoustic or radio) as a tool to evaluate how these factors influence survival of turbine-passed fish. However, recent research indicates that the presence of a telemetry tag (acoustic, radio, inductive) implanted inside the coelum of a juvenile salmon increases the likelihood that the fish will be injured or die during turbine passage (Carlson et al. 2010). Carlson et al. (2010) found that the variability in injury and mortality sustained due to simulated turbine passage (due to changes in pressure, i.e., barotrauma) varied with the fish's tag burden and the amount by which pressures changed. Thus, previous research conducted using telemetry tags implanted into the coelom of fish may have been inaccurate.

Accurate and precise assessments of turbine survival are critical for evaluating turbine operations and for assessment of turbines prior to and after their replacement, to determine if different operations or turbine designs improve survival. This is especially important because a large proportion of the existing turbines in North America are nearing the end of their functional lifespan and need replacement. Thus, a new technique is needed to provide unbiased estimates of survival through turbines.

The research presented in this chapter provides an evaluation of the effectiveness of a neutrally buoyant externally attached acoustic transmitter. We hypothesized that a neutrally buoyant externally attached acoustic transmitter would reduce bias in survival studies because it will not add excess mass (the weight in water of an object) to the fish or take up space within the coelum. Previous research has indicated that fish bearing acoustic transmitters that have an excess mass, leading fish to increase the volume of the swim bladder; that is, they increase their displacement to balance the increased excess mass (Gallepp and Magnuson 1972; Perry et al. 2001). This increased volume of gas in the swim bladder leads to a higher likelihood that fish will be injured during the rapid decompression associated with turbine passage (Stephenson et al. 2010). In addition, the volume of the transmitter present in the coelom would also likely lead to a higher incidence of barotrauma when fish are exposed to rapid decompression. The swim bladder may be more likely to rupture, and there may be a higher likelihood of compression-related injuries.

We hypothesized that juvenile Chinook salmon tagged with a neutrally buoyant externally attached acoustic transmitter would not experience a higher degree of barotrauma than their nontagged counterparts. To test this hypothesis, both nontagged fish and fish tagged with a neutrally buoyant external transmitter were exposed to a range of rapid decompressions simulating turbine passage.

Methods

Hatchery-reared juvenile Chinook salmon *Oncorhynchus tshawytscha* (*n* = 368; mean length = 123.4 mm, range 95 to mm 137; mean weight = 21.2 g, range 7.9 to 33.7 g) were exposed to simulated turbine passage (STP) treatments between January 8 and 27, 2011 (Table 3.1). The fish were either acquired as fry or hatched and reared from eggs at the PNNL Aquatic Research Laboratory (ARL).

Table 3.1. Sample sizes and mean length and weight of juvenile Chinook salmon examined for each treatment.

		Fork length (mm)	Mass (g)
Transmitter treatment	*n*	Mean ± SD (range)	Mean ± SD (range)
Nontagged	184	124 ± 8 (97–137)	21.2 ± 4.8 (9.6–32.1)
Externally tagged	184	123 ± 8 (95–137)	21.1 ± 4.9 (7.9–33.7)

Testing of juvenile salmon was conducted in the hyper/hypobaric chambers described in Stephenson et al. (2010). During testing, ambient well water (median temperature = 16.9°C; range 16.6 to 17.4°C) was pumped to the chambers. Total dissolved gas levels were a median of 102.4% (range 101.8 to 102.9). Total dissolved gas (TDG) was monitored with sensors installed within each chamber (Model T507, In-Situ Inc., Fort Collins, Colorado; ±1.5 mmHg accuracy). Levels of TDG were recorded on a data logger (Campbell Scientific, Logan, Utah) controlled by a program written in CRBasic and implemented via LoggerNet. Water was supplied to all chambers at a continuous rate of 7.6 L/min with a flow control accuracy of ±0.95 L/min (see Stephenson et al. 2010 for a description of water treatment).

Acclimation Prior to Pressure Exposure and Simulated Turbine Passage

Juvenile Chinook salmon were marked and loaded into chambers as described in Stephenson et al. (2010). Acclimated pressures were equivalent to the absolute pressures that would exist at depths of 15 ft (21.2 psia) in fresh water. Fish were held at acclimation pressure for 16–24 h prior to testing to allow ample time to attain neutral buoyancy and equilibration of gas tensions in bodily fluids and tissues. The determination of buoyancy, exposure to STP, and necropsy procedures were conducted using observations and video equipment described in Stephenson et al. (2010). Although we tested 368 fish, a small proportion (7.3%) of fish were negatively buoyant (15 nontagged fish and 12 externally tagged fish; none of the fish were positively buoyant) and never gained neutral buoyancy following 16 h of acclimation. Given the results from Stephenson et al. (2010), and the assumption that in-river fish are neutrally buoyant when approaching hydroelectric facilities (due to energy conservation in wild systems), we included only neutrally buoyant fish in statistical analyses.

Exposure Pressures and Rate of Pressure Change

Exposure pressures (i.e., the nadir) during STP ranged from 1.6 to 11.6 psia, with a median of 4.6 psia. The rate of pressure change (i.e., rate of decompression) during STP ranged from 129 to 385 psia/s (median = 239 psia/s). The pressure exposure profiles used represent passage through Kaplan turbine units typical of the hydropower projects on the lower Snake and Columbia rivers (Figure 3.1). The exposure profile simulated the pressure of flow passing through the turbines, which may increase to nearly 400 kPa over approximately a 20-s period, as fish enter the turbine intake and approach the turbine runner. As fish pass between the turbine runner blades, they are exposed to a sudden pressure decrease (<1 s) before returning to near surface pressure as they enter the downstream channel (~20 s). The magnitude of the pressure drop during turbine passage is dependent upon the turbine runner design, the operation of the turbine, the rate of flow through the turbine, the submergence of the turbine runner (i.e., elevation of the turbine runner relative to the downstream water surface elevation), the total project head, and the flow path (Čada 1990; Carlson et al. 2008; Deng et al. 2010). The overall pressure change will increase with increased project head.[1] Pressures are higher on the upstream side of the turbine blades (pressure side) and lower on the downstream side (suction side). Locally higher pressures occur near the leading edges of the blades on the upstream side, and lower pressures occur near the blade tips on the downstream side. However, all fish that pass through a turbine runner experience decompression (ENSR 2008). A turbine with a deep submergence will generally have lower nadir pressures than a turbine with a shallow submergence; further, for any given turbine, the nadir pressure will decrease with increased turbine flow. The lowest pressure a fish may experience during turbine passage can vary from approximately 200 to –2 kPa (Carlson et al. 2008), dependent on turbine design, operation, head, and passage route.

Mortal Injury

After they were exposed to STP, many fish died within a few minutes or received pressure-related injuries (barotrauma) sufficient to cause eventual mortality. It is not always feasible to hold fish following rapid decompression testing, and the conditions in which fish could be held could be highly variable (pressure, temperature, total dissolved gas, or other conditions may vary). Although we can accurately simulate fish passage through the turbine, we cannot simulate with any accuracy the conditions they experience following passage as they pass downstream from the dam. Subsequently, a metric that predicted mortal injury, derived by McKinstry et al. (2007), was used as the response variable in this study instead of mortality and a multitude of different injury types. The mortal injury metric was derived by analysis of a large data set of juvenile Chinook salmon exposed to rapid decompression. The metric associated fish that died within minutes of rapid decompression with the injuries that were observed during necropsy. The injuries seen most often in fish that died (determined using odds ratios, Fisher's exact tests, and stepwise logistic regression modeling using the Akaike information criterion [AIC; McKinstry et al. 2007]) were included in the metric. These injuries included exophthalmia (eye-pop); hemorrhaging in the pericardium, liver, or kidney; ruptured swim bladder; blood secretions from the vent; and emboli in the gills or pelvic fins. Fish that died were also considered to be mortally injured. Although emboli in the pelvic fins appears to be an injury that would not be associated with mortality, among the fish that had this malady (n = 284), 187 or 65.8% died during or within a few minutes of exposure to rapid decompression. Thus, the injury, emboli in the pelvic fins, acts as an externally

[1] The total project head (difference between the upstream and downstream water surface elevations) is between approximately 17 and 30 m at projects on the lower Snake and Columbia rivers.

observable predictor of mortality. Therefore, mortal injury served as the endpoint and response variable for these analyses. Fish with any one of these eight injuries present, or fish that were dead shortly following testing (within ~10 min), were classified as mortally injured. Although other injuries were noted that could lead to delayed mortality or increased chance of predation, they were not highly associated with mortality shortly after STP and therefore were not included in this analysis.

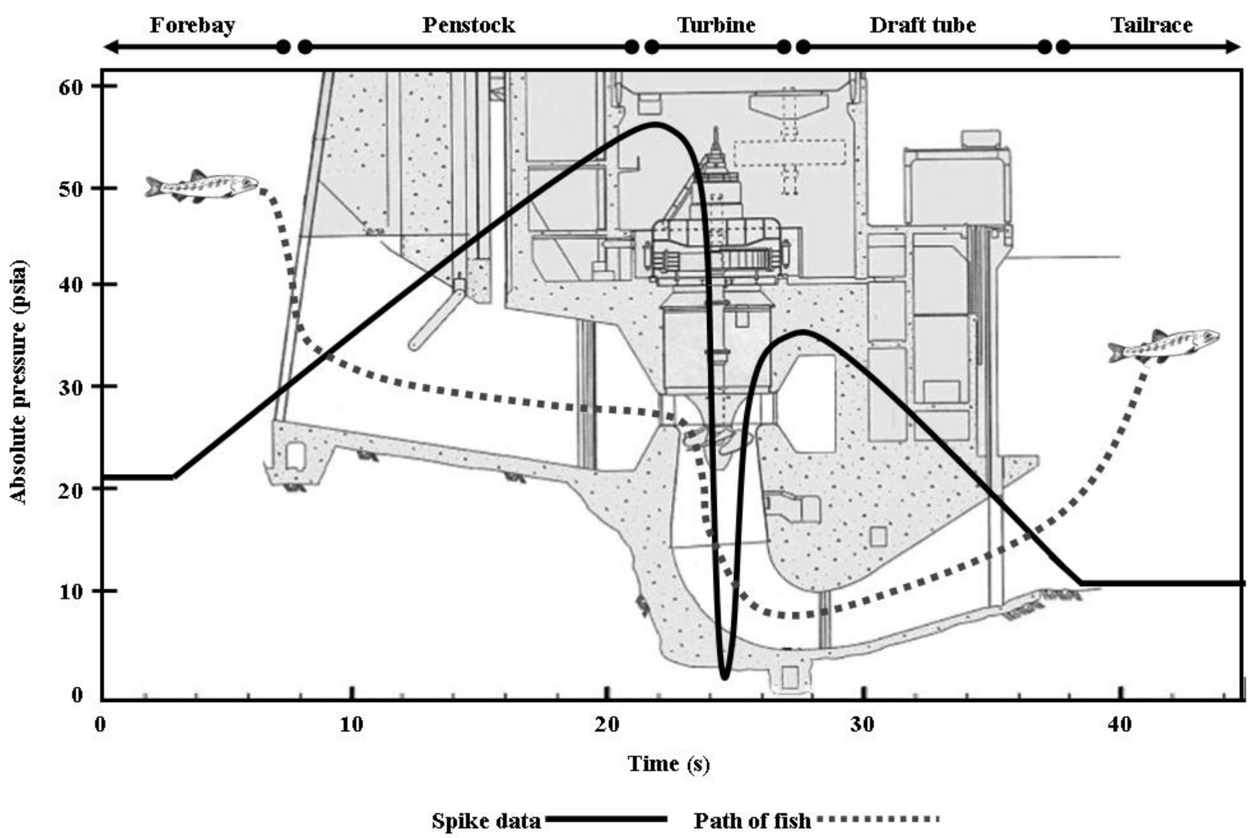

Figure 3.1. Example of trajectory through a Kaplan turbine used to compute simulated turbine passage. In this 15-ft depth-acclimation trajectory (dashed line), fish are assumed to move through the turbine runner while being passively transported by water flow in approximately 45 to 90 s, depending upon depth of entry to the turbine intake (acclimation depth). After their passage through the turbine runner, turbine-passed fish exit through the turbine draft tube, enter the tailrace, and go up to the water surface. The solid line shows the pressure changes the fish undergo during turbine passage. The rapid decrease in pressure and the nadir in the simulated turbine passage occur during passage through the turbine runner (blade assembly).

Statistical Models

An analysis of deviance table was constructed to examine the differences in mortal injury between tagged and nontagged fish. Analysis of deviance based on a binomial error structure and log-link was used in modeling the data and testing the hypotheses. The independent variables included nadir as a continuous variable and tag type as a categorical variable (tagged or nontagged). A confidence interval was also constructed on the proportion difference in mortality from nontagged to externally tagged and fitted regression lines using a log-link. A plot of nadir versus proportion of mortal injury shows that fish with a nadir higher than 7.4 psia did not experience mortal injury. These fish were removed from the analysis because they may obscure an effect of tag type.

Results

The relationship between the probability of mortal injury and nadir was not significantly ($P = 0.3804$) different between externally tagged and nontagged juvenile Chinook salmon (Table 3.2). An overall significant ($P < 0.001$) decrease in mortal injury was seen as the nadir to which fish were exposed decreased (Table 3.3; Figure 3.2). The equation for predicting mortal injury at a given logged ratio of pressure change (the acclimation pressure divided by the exposure pressure; logged using natural log) for nontagged fish is

$$\text{Probability of mortal injury} = e^{-2.963+1.064*LRP} \quad (3.1)$$

The equation for predicting mortal injury at a given logged ratio of pressure change (logged using natural log) for fish tagged with an externally attached neutrally buoyant transmitter is

$$\text{Probability of mortal injury} = e^{-2.837+1.061*LRP} \quad (3.2)$$

Because there was no significant difference in mortal injury between nontagged and externally tagged fish, the data for these two groups were combined to provide the following equation for predicting mortal injury at a given ratio of pressure change:

$$\text{Probability of mortal injury} = e^{-2.922+1.075*LRP} \quad (3.3)$$

Table 3.2. Analysis of deviance of the factors associated with the mortal injury of juvenile Chinook salmon with respect to simulated turbine passage.

Source	df	Deviance	Mean deviance	*F*	*P*
NULL	61	115.36			
Nadir	1	47.988	47.988	42.581	<0.0001
Tag type	1	0.88	0.88	0.781	0.3804
Error	59	66.492	1.127		

Table 3.3. Coefficients of the model describing the relationship between mortal injury and nadir. Tag type refers to fish being either nontagged or externally tagged with a neutrally buoyant acoustic transmitter.

Source	Estimate	SE	*T*	*P*
Intercept	0.336	0.197	1.701	0.089
Nadir	–0.382	0.064	–5.961	<0.001
Tag type	0.134	0.13	1.031	0.303

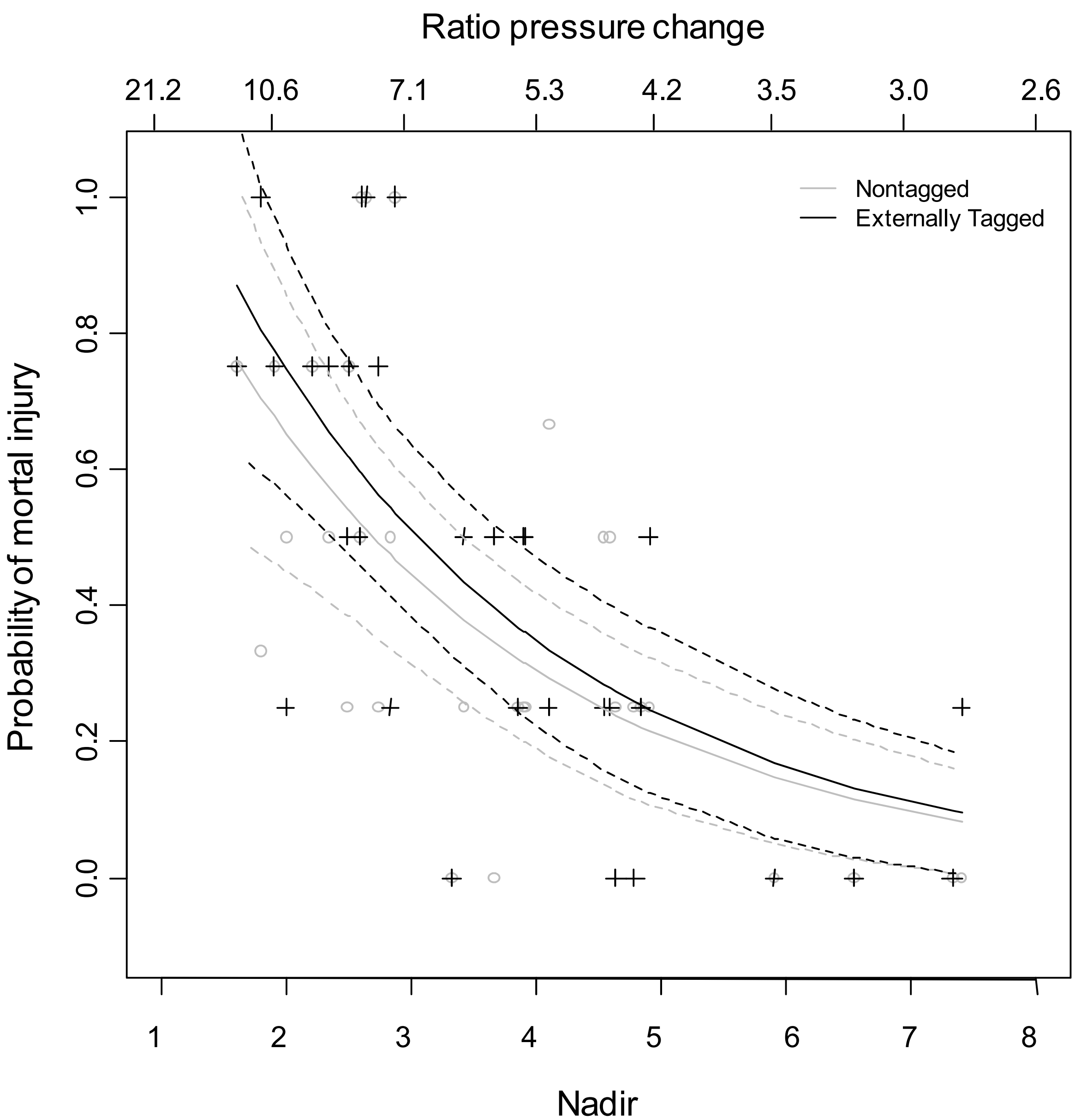

Figure 3.2. Probability of mortal injury along a range of nadir for juvenile Chinook salmon. The 95% confidence interval is shown on either side of the regression line (solid center line). Each dot on the graph indicates an individual fish exposed to simulated turbine passage.

Discussion

We hypothesized that juvenile Chinook salmon tagged with a neutrally buoyant externally attached acoustic transmitter would not receive a higher degree of barotrauma than their nontagged counterparts. Our hypothesis was confirmed because there was no difference in mortal injury between these two groups. Other research has indicated that having a negatively buoyant tag either implanted gastrically or inserted into the coelom of a fish by injection or via surgery can increase the likelihood that fish will be injured or killed during turbine passage (Brown et al. 2009; Carlson et al. 2010). However, the use of this neutrally buoyant externally attached transmitter did not cause increased injury or mortality. This is likely due to the lack of a implantation of negatively buoyant tag into the coelom.

Fish implanted with telemetry tags can compensate for the additional excess mass of the tag by increasing their displacement via increased swim bladder volume (Gallepp and Magnuson 1972; Perry et al. 2001). The presence of this additional gas in the swim bladder has been associated with higher mortality and swim bladder ruptures when fish are exposed to rapid decompression. Stephenson et al. (2010) noted these higher injuries and mortality among fish that were neutrally buoyant (thus having more gas in the swim bladder) than fish that were negatively buoyant when exposed to rapid decompression.

The volume occupied by the tag may also be an important factor influencing barotrauma. During rapid decompression, the expansion of gases in the swim bladder and tissues may reduce the available volume of space within the coelom where the tag rests, which is finite. The presence of a tag may limit the volume to which the gases can expand before barotraumas such as compression-related injuries occur.

Bearing a tag that increases the mass of a fish could also influence the behavior of juvenile salmonids by changing the maximum depth at which the fish can become neutrally buoyant. As mentioned above, fish can compensate for the additional excess mass of a transmitter by increasing displacement by adding more gas to the swim bladder (Gallepp and Magnuson 1972; Perry et al. 2001). This compensation, however, could influence the behavior of the fish. Salmon are physostomous and are therefore required to gulp air at the water surface to fill the swim bladder. To become neutrally buoyant at depth, the fish has to fill its swim bladder at the surface and then dive to a depth where it becomes neutrally buoyant. A fish bearing a transmitter would not have the ability to attain neutral buoyancy at a depth as deep as that of a nontagged fish. If a fish compensates for the excess mass of the tag by filling its swim bladder to the capacity limited by available coelom volume, it will have less swim bladder capacity available to attain neutral buoyancy at a greater depth. Also, the space taken up in the coelom by the transmitter could limit the inflation of the swim bladder and thus the depth at which the fish can attain neutral buoyancy.

Reduction of the maximum depth at which a fish can attain neutral buoyancy due to the presence of a tag with excess mass may bias studies examining survival and behavior. This bias may be due to several possible outcomes. The fish will have two options—remain neutrally buoyant at a shallower depth or become negatively buoyant at deeper depths. If a tagged fish remains neutrally buoyant at a shallower depth than its nontagged counterpart, it may be exposed to less than ideal surroundings, for a number of reasons. Fish that migrate at shallower depths are likely more prone to bird and other predation. In addition, in rivers where increased TDG levels are an issue, fish with transmitters may not be able to protect themselves from gas bubble disease by hydrostatic compensation (Beeman and Maule 2006). Conversely, if tagged fish occupy deeper depths, they would be negatively buoyant, likely leading to greater energy expenditures.

Reducing the maximum depth where a fish can become neutrally buoyant may also bias studies designed to determine the routes that fish use to pass hydroelectric facilities. Surface-oriented fish may be more likely to pass through the spillway than passage routes like turbines or juvenile bypass systems. Survival of juvenile salmonids that pass through hydroelectric dams is generally greatest through the spillway (Muir et al. 2001). A change in behavior due to carrying a transmitter may skew the results of a study. A neutrally buoyant transmitter alleviates the need for the fish to compensate for the excess mass through inflation of the swim bladder. Therefore, a fish with a neutrally buoyant tag can become neutrally buoyant at the same depth as a nontagged fish with the same amount of gas in the swim bladder, reducing behavioral biases.

This research, combined with results from Chapters 1 and 2, indicates that this neutrally buoyant, externally attached transmitter could be a useful tool for assessing survival of juvenile salmonids passing through turbines or for survival studies in general. The use of this transmitter eliminated the bias observed among juvenile Chinook salmon tagged with negatively buoyant transmitters and exposed to simulated turbine passage (Carlson et al. 2010). We suggest that future research include field-based comparisons of survival and behavior among fish tagged with a neutrally buoyant external transmitter and those implanted internally with JSATS transmitters.

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