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Development of external and neutrally buoyant acoustic transmitters for juvenile salmon turbine passage evaluation

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ABSTRACT

Fish can sustain injury or mortality when they pass through hydroelectric facilities. To develop a method to monitor the passage and survival of juvenile salmonids without bias through turbines within the Federal Columbia River Power System, we developed and fabricated two designs of neutrally buoyant transmitters: 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). To determine the efficacy of the two designs under non-turbine passage-related conditions, fish had one of the tags attached and were held for 14 days to determine any potential effects of the tags 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. When compared with non-tagged individuals, fish tagged with Type A tags did not differ significantly with respect to growth or mortality over a 14-d holding period. However, fish tagged with Type B transmitters had lower growth rates than the nontagged controls or other tag treatments. The efficacy of two designs was also compared to nontagged individuals under shear exposure. Fish were exposed to a submerged, 6.35-cm-diameter water jet at velocities ranging from 3.0 to 12.2 m/s in a water flume to simulate turbine conditions within the Columbia River basin. 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 untagged fish and fish tagged with Type A or Type B tags. 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. Overall, Type A tags may be a viable tag design for juvenile Chinook salmon passing through hydropower facilities.

1. Introduction

Biotelemetry is commonly used to monitor the passage and survival of juvenile salmonids at hydroelectric facilities throughout the Columbia River basin (CRB) (Steig, 1999; Matter and Sandford, 2003; McMichael et al., 2010; Weiland et al., 2011; Deng et al., 2011). 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 does 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 CRB 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 (e.g., shear force, blade strike) that can lead to injury (Cada, 2001; Deng et al., 2005, 2007, 2010a). However, all fish are exposed to rapid decompression as they pass by the turbine blade even though the levels of exposure vary. This rapid decompression causes gases in the swim bladder and tissues to expand, which can

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result in numerous barotraumas, including ruptured swim bladder, exophthalmia, and internal hemorrhaging (Brown et al., 2009, in press).

For a fish, the regulation of gas in the swim bladder allows it to maintain neutral buoyancy in the water column. Salmonids are physostomes and can regulate the amount of gas in the swim bladder through the pneumatic duct. To maintain neutral buoyancy below the water surface, salmonids increase the volume of gas in their swim bladder by gulping air at the water surface. In addition, they can expel gas from the swim bladder through their mouth to maintain buoyancy when moving up in the water column. As they move up in the water column, the swim bladder expands as pressure decreases, leading to gas expulsion. When juvenile salmonids pass through hydroturbine, free gas in the swim bladder expands as they are exposed to rapid decompression. However, the change in pressure can be so rapid that fish may not be able to expel gas from the swim bladder, potentially leading to a ruptured swim bladder (Cramer and Oligher, 1964; Feathers and Knable, 1983; Rummer and Bennett, 2005).

In a recent laboratory study, Carlson et al. (2010) demonstrated that the presence of a negatively buoyant telemetry tag inside the body of a juvenile Chinook salmon increased the likelihood of barotrauma, notably the rupture of swim bladders. The presence of a tag inside the body of a fish can increase the likelihood of damage due to hydroturbine passage through several pathways. The volume occupied by the tag may 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. In addition, fish implanted with telemetry tags can compensate for the additional excess mass of a negatively buoyant 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).

Bearing a tag that has excess mass (is negatively buoyant) 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 adding more gas to the swim bladder (Gallepp and Magnuson, 1972; Perry et al., 2001). A fish bearing a transmitter would not have the ability to attain neutral buoyancy at a similar depth as an untagged fish. If a fish compensates for the excess mass of the tag by filling its swim bladder, 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 negatively buoyant tag violates the assumption that tagged fish behave similarly to untagged fish, potentially biasing studies examining survival and behavior. 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.

Carlson et al. (2010) found that tag burden was a predictor of mortal injury (mortality or injury leading to mortality) among juvenile Chinook salmon exposed to rapid decompression. As tag burden increased, so too did the rates of mortal injury (Carlson et al., 2010). By reducing the excess mass of the tag, it is suggested that the bias associated with mortal 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 and the issues with available space in the coelom addressed above, external attachment would be favored over internal implantation.

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,b; 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 (Lewis and Muntz, 1984; Thorstad et al., 2001). Due to these concerns, novel tag designs need to be evaluated to determine any potential bias they may create in telemetry studies.

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, we designed an externally attached transmitter that would be hydraulically streamlined and have limited negative influence to fish (survival, growth, tissue damage). This paper describes the design and construction of this transmitter and evaluation of the tagging effects and tag retention with juvenile Chinook salmon.

2. 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, mock external transmitters were constructed from clay. The mock transmitters were attached using Ethicon Monocryl 5-0 absorbable monofilament sutures (Ethicon, Inc., New Brunswick, NJ) with and without moleskin (Dr. Scholl's Super Moleskin Plus; Schering-Plough Healthcare Products, Inc., Berkeley Heights, NJ) under the mock transmitter. The mock transmitters were also glued to the fish using Tissumend and Vetbond glue, but neither 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. Both tag types were designed to be streamlined with the fish to minimize any drag created by the presence of the tag. To manufacture the external transmitters, molds were created for each design. For

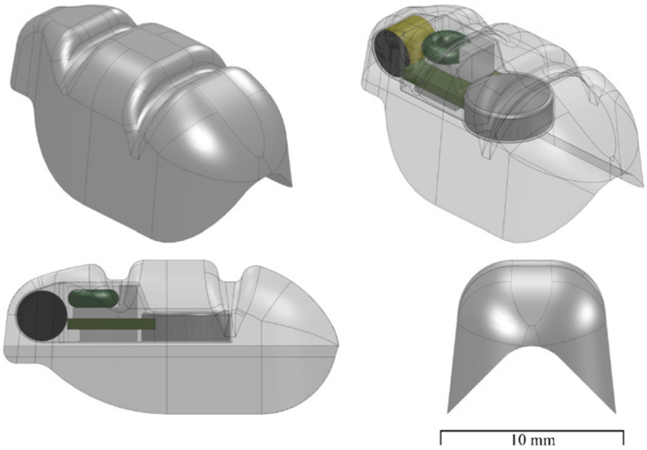

Fig. 1. Computer aided design (CAD) model drawings of Type A tag outlining the placement of the negatively buoyant JSATS tag within the positively buoyant resin and glass bubble mixture, creating a neutrally buoyant tag.

the initial prototype molds, a three-dimensional printer (Stratasys FDM Vantage rapid prototyper, Stratasys Inc., Eden Prairie, MN) was used with the fine (T10) tip. This tip is capable of depositing 0.13-mm-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.

2.1. 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° with a 2.1-mm-radius fillet (Fig. 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. Since both Type A and B transmitters were neutrally buoyant they, unlike conventional transmitters, did not represent any tag burden to the fish. For the acoustic portion of the Type A tag, an acoustic transmitter with one size 337 silver oxide button cell battery is used.

To manufacture the Type A tags, a two-part mold was machined from aluminum bar stock using a CNC mill. 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.

2.2. Type B tag design

The Type B tag is a two-part design; each half of the transmitter is attached beneath the dorsal fin (Fig. 2). 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 (ks964-49MG, Advanced Interconnections, West Warwick, RI) 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

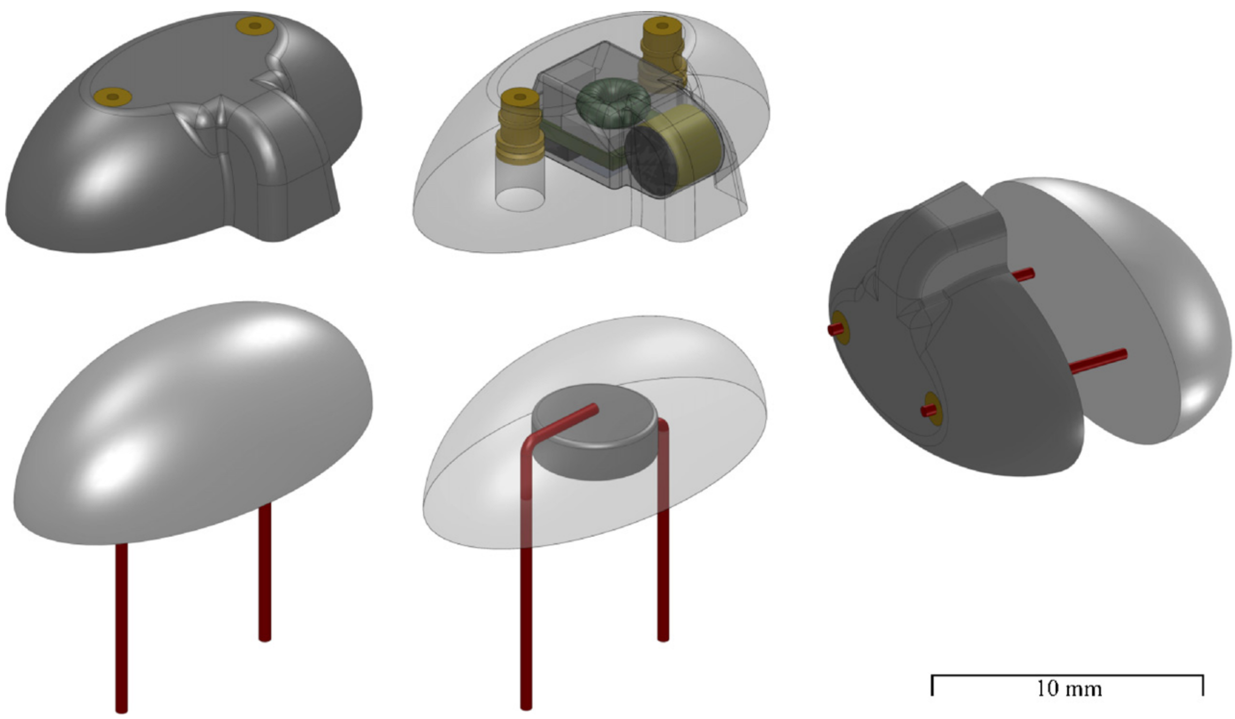

Fig. 2. Computer aided design (CAD) model drawings of Type B tag outlining the placement of the negatively buoyant JSATS tag within the positively buoyant resin and glass bubble mixture, creating a neutrally buoyant tag.

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. 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.

2.3. 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, MN). 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 μm and a typical density of 0.16 g/cm^3.

By varying the proportions of the resin and glass bubbles, the density of the resulting mixture could be altered 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, CA) was combined with 30-min epoxy.

2.4. Performance check

An existing transmitter from the Juvenile Salmon Acoustic Telemetry System (JSATS, 2008 model; Advanced Telemetry Systems, Isanti, MN) 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 1 μPa 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 1 μPa at 1 m, which was within the uncertainties of the measurement system.

3. Experimental methods

3.1. 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, WA. 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, WA) ad libitum. Test fish (subyearling Chinook salmon) were randomly assigned to treatment groups and 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).

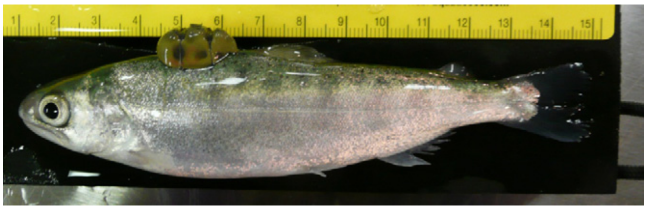

Fig. 3. A photograph outlining the placement and attachment of a Type A tag on a juvenile Chinook salmon anterior to the dorsal fin. The tag was painted to mimic coloration of fish. The scale in the photo indicates centimeters.

3.2. 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 threaded through the dorsal musculature of the fish (Fig. 3), 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., 2010). 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 1 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 (Fig. 4) were attached using two 25-7/8-gauge needles (Becton, Dickinson and Company, Franklin Lakes, NJ) 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. Due to the two part design of the Type B tag, a conductive material was required to complete the circuit between the battery and

Fig. 4. A photograph outlining the placement and attachment of a Type B tag on a juvenile Chinook salmon through the dorsal musculature.

transducer. Therefore, a variety of different attachment materials could not be tested as with Type A tags.

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, MN). 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 mm × 5.2 mm × 3.8 mm; they weighed 0.43 g in air (0.30 g in water; 0.14 mL volume). These transmitters comprised a median tag burden of 1.8% (range 1.3–3.5). 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 22-L bucket containing oxygenated water to recover. Food was withheld for 24 h prior to either external attachment or internal implantation of tags.

The amount of time it takes to attach internal and external transmitters was also evaluated. Fifteen fish were surgically implanted with an acoustic transmitter (described above). In addition, fifteen fish were each tagged with external tag Type A and Type B. Each of these tagging procedures were timed for comparison. Timing began after fish were anesthetized and reached the surgery table. Timing ended when fish were removed from the surgery table for recovery.

3.3. 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, WA) 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). Since other researchers have noted differences in growth between externally tagged and untagged fish over a relatively short term (15 d; Greenstreet and Morgan, 1989), 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.

3.4. 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.

3.4.1. 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 (see Deng et al., 2010b for more information about the shear system). 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., 2010b).

Test fish were actively introduced from standing water into the round water jet through an introduction tube (Deng et al., 2005). 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).

3.4.2. 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. Care was taken to minimize disturbance to other fish in the holding tank. 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., shear injuries) 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, 2010b). Following injury evaluation, fish were placed in 1000-L holding tanks for 4 d (96 h) to monitor delayed mortality and tag loss.

3.4.3. Video recording and processing

Two high-speed digital cameras (Photron PCI FastCAM 1280; Photron USA, Inc., San Diego, CA) 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, NM). 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 et al., 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.

3.4.4. 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.

3.5. 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 ANOVA. If differences were present, pairwise comparisons were made among test groups. A Sidak correction was used to adjust the alpha for pair-wise comparisons. The family-wise rejection region for proportion increase in length and proportion increase in weight, using a Sidak correction, based on 10 tests between five tag types was

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

The time required for each tag attachment type (Type A, Type B and internal surgery) was compared among groups using an ANOVA on ranks, with a post hoc Dunn's test.

Pair-wise 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 Sidak correction, based on 3 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 are the two biological response variables that were 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, <50% = 1, or >50% = 2. Tissue damage was considered a continuous variable because the metric was based on an underlying continuous scale (Snedecor and Cochran, 1989; similar to Panther et al., in press). When the tissue damage data were analyzed, control fish were removed from tag type because only tagged fish could develop tissue damage due to the presence of the tag. 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.

4. Results

4.1. Biological response

4.1.1. 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 anterior sutures were lost during the study; however, the two fish still retained their tags.

4.1.2. 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 ($P < 0.001$) and weight ($P < 0.001$) among the groups after 14 d (Table 1). The percentage increase in length was significantly lower for tag Type B than for all of the other tag treatments or the control (Table 1; Fig. 5). 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 (Table 1; Fig. 5). The control and internally implanted fish had significantly higher increases in weight than fish tagged with Type A transmitters using Vicryl Rapide sutures.

4.1.3. 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 (Fig. 6). 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. 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 (Fig. 7). However, there were no differences in discoloration between Type A tags attached using Monocryl and Vicryl Rapide. The lack of indentation among fish tagged 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.

4.1.4. Tagging time

External transmitters took less time to attach to the fish than it took to surgically implant a transmitter ($P < 0.001$). It took a median time of 42 s (range 35–51) to attach external tag Type A and 44 s (range 34–75) to attach external tag Type B. The median time it took to surgically implant a transmitter was 57 s (range 43–70).

Table 1
Mean initial, final, and percentage differences in mean lengths and mass (±SD) of test fish by tag type. Dissimilar letters indicate significant ($P < 0.05$) differences in length and weight change. There were no significant ($P > 0.05$) differences in initial weight or length.

Treatment	*n*	Initial		Final		Percentage difference	
		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[a]	37.5 ± 10.2[a]
Type A (Monocryl)	21	121 ± 7	21.2 ± 3.4	129 ± 7	28.2 ± 4.0	6.4 ± 2.3[a]	34.3 ± 10.2[b]
Type A (Vicryl Rapide)	21	122 ± 7	21.8 ± 3.6	129 ± 8	27.8 ± 5.5	5.2 ± 1.7[a]	27.5 ± 9.2[b]
Type B	21	124 ± 7	23.1 ± 4.5	127 ± 9	26.9 ± 6.1	2.8 ± 1.6[b]	15.9 ± 7.6[c]
Internal surgery	21	122 ± 8	21.5 ± 4.3	130 ± 9	30.0 ± 5.9	6.7 ± 1.5[a]	37.2 ± 6.9[a]

4.2. 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 2. 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 that of fish exposed to the 9.1-m/s velocity.

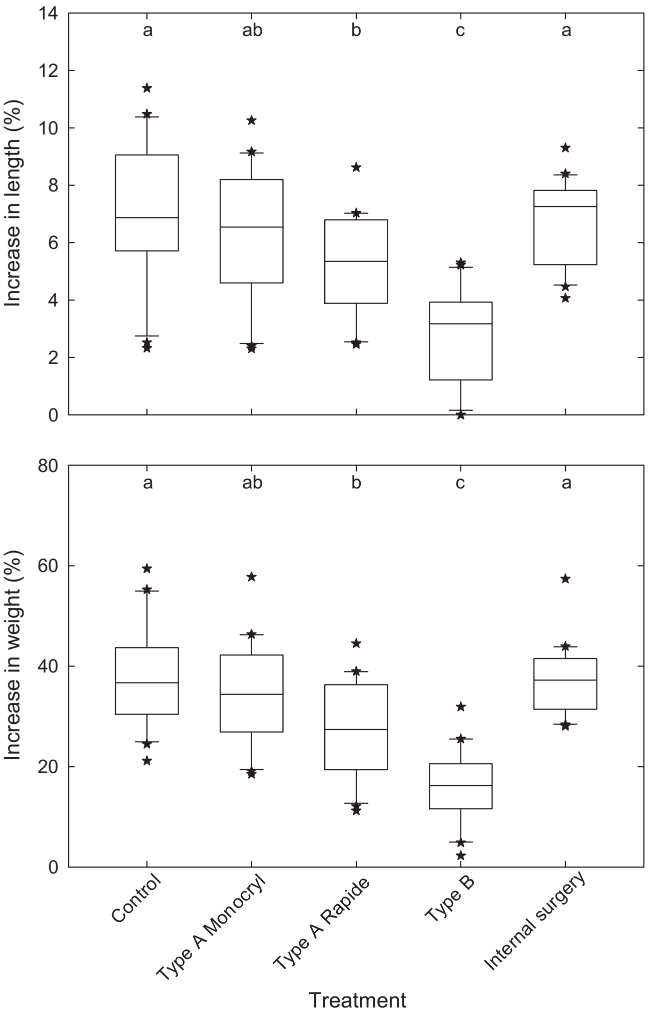

Fig. 5. Box plots of difference in length and weight (%) for each treatment 14-d post-surgery. The top and bottom edges of the boxes indicate the 25th and 75th percentile of data, the line within each box indicates the median of the data. Whiskers indicate 1.5× the interquartile range beyond the box, and asterisks indicate outliers. (Difference in length: $F_{4,95} = 16.08$, $P < 0.0001$. Difference in weight: $F_{4,95} = 21.14$, $P < 0.0001$.) Differences were considered significant at an alpha of 0.0051 and are indicated by dissimilar letters.

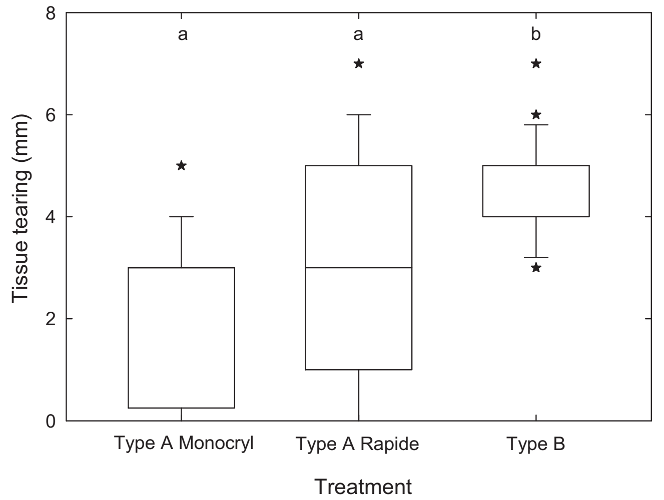

Fig. 6. Box plots of the longest measured tissue tear per fish observed for each tag treatment group 14-d post-surgery. The top and bottom edges of the boxes indicate the 25th and 75th percentile of data, the line within each box indicates the median of the data. Whiskers indicate 1.5× the interquartile range beyond the box, and asterisks indicate outliers. (One-way ANOVA: $F_{2,55} = 12.14$, $P < 0.0001$.) Dissimilar letters above boxes indicate significant differences.

4.2.1. 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 3). 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.

A correlation matrix showed a strong positive relationship between all kinematic parameters computed from the fish tracks (Table 4). The occurrence of shear injuries (Table 5) was

Table 2
Basic characteristics of the fish for fish exposed to shear flows. Dissimilar letters indicate significant differences.

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

Table 3
Summary of shear injuries observed immediately following exposure to shear flows.

Tag type	Nozzle velocity (m/s)	*n*	Bruising	Opercular damage	Eye	Total
Control	3	11	0	0	0	0
(nontagged)	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%)

Table 4
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

significantly ($P<0.001$) associated with head velocity; however, tag type (Type A, B, or untagged control) 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.

4.2.2. *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 6). There was no injury among untagged control fish at the time of necropsy. A chi-square test for independence demonstrated a dependent relationship between tag type (Type A, B, or untagged control) and tissue damage (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 7).

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 was a significant difference in the rate of tissue damage between fish tagged with Type A and Type B tags (Table 8). 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 (Fig. 8). 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 9).

5. Discussion and conclusions

One of the primary concerns of telemetry studies is the influence of the tag on survival and behavior of the tagged individual (Nielsen, 1992; Baras and Lagardère, 1995; Bégout Anras et al., 1998). The information collected from telemetry studies is also dependent on tag retention. Differences in behavior and survival, or the premature loss of a tag may lead to biases in estimates of survival or understanding of behavior. In the current study, there were no mortalities observed and tag retention was high for all tag types tested. Tags were designed for use on short-term survival studies (3 d to 1 week) for fish passing through hydroelectric turbines. While one of the Type A tags attached with Vicryl Rapide and one of the Type A tags attached with Monocryl sutures were lost, this was on Day 13 of the study, and would not influence the results of a short term hydropower survival study. 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 tags (no tag loss over 14 d), their negative influence on growth and tissue response should also be considered.

Our research shows that the Type A tag design and surgically implanted tags did not influence the growth rate of juvenile Chinook salmon over a 14-d holding period, while Type B tag design did influence growth when compared to untagged individuals (Table 1). There is a lack of consensus in previous research examining the influence of external tags on juvenile salmonids growth rates (studies outlined in Table 10). Greenstreet and Morgan (1989)

Table 5
Analysis of deviance of the response variable shear injuries. Tag type included controls (non-tagged), Type A and Type B.

	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

Table 6
Number of individuals (%) with tissue damage in the area around the tag.

Tag type	Nozzle velocity					
	Minor tissue damage			Major tissue damage		
	3 m/s	9.1 m/s	12.12 m/s	3 m/s	9.1 m/s	12.12 m/s
Control (nontagged)	0 (0.0%)	0 (0.0%)0	0 (0.0%)0	0 (0.0%)0	0 (0.0%)0	0 (0.0%)0
Type A	2 (16.7%)	7 (36.8%)	6 (30.0%)	0 (0.0%)	12 (63.2%)	6 (30.0%)
Type B	3 (33.3%)	2 (9.5%)	5 (25.0%)	6 (66.7%)	19 (90.5%)	15 (75.0%)

Table 7
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

Table 8
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		

Table 9
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		

found that bearing an external transmitter resulted in reduced growth for Atlantic salmon (*Salmo salar*) over a 15-d holding period. These results are contrasted by Makiguchi and Ueda (2009), who found no difference in growth over 68 d among PIT-tagged, internally implanted, and externally tagged juvenile masu salmon (*Onchorynchus masou*). Tag effects are dependent on a number of factors including tag size, fish species, and method of tag attachment (Nielsen, 1992). Our research would suggest that the use of suture material, in lieu of wires, may be a better option for juvenile Chinook salmon, however, further experimentation would be needed to confirm this. Differences in growth rate between tagged and untagged fish suggest the presence of the Type B tag

Table 10
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 were neutrally buoyant in water.

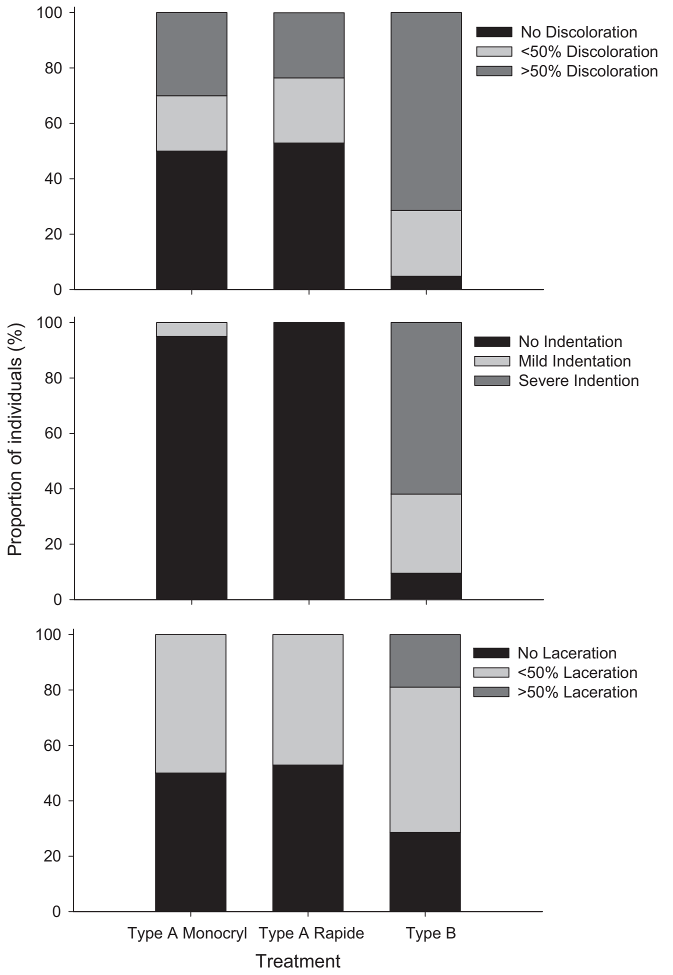

Fig. 7. The proportion of individuals with discoloration, indentation (mild or severe), and laceration (%) at the end of the 14-d holding period for juvenile Chinook salmon with externally attached neutrally buoyant transmitters.

may compromise foraging efficiency (Serafy et al., 1995) and energy expenditure, which can have direct impacts on the survival of the fish.

Negative tissue response is a commonly noted issue with external tags (Herke and Moring, 1999; Crook, 2004). In our comparison of externally attached tags, Type B tags resulted in more severe tissue damage and irritation than Type A tags. Tissue tearing, tissue indentation and discoloration were all significantly higher for fish tagged with Type B tags, when compared to those tagged with Type A tags (Fig. 7). Makiguchi and Ueda (2009) also found that external tags attached with wire resulted in wounds and inflammation for masu salmon. Research comparing suture material for fish surgeries has suggested that monofilament sutures elicit less tissue reaction

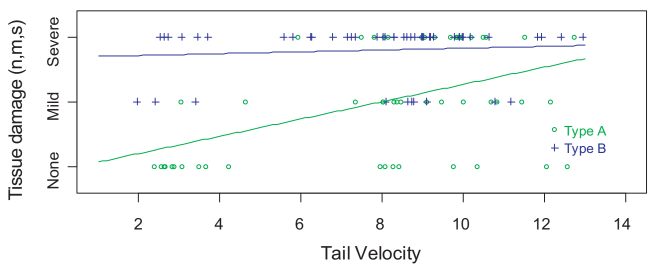

Fig. 8. A scatter plot of tail velocity versus tissue damage for juvenile Chinook salmon exposed to shear conditions.

than other materials (Kaseloo et al., 1992; Gilliland, 1994; Wagner et al., 2000; Hurty et al., 2002; Mulcahy, 2003; Harms, 2005; Deters et al., 2010). Increased tissue damage and infection can negatively influence growth and survival, due to allocation of energy to tissue repair and healing.

There were no tags lost during exposures to shear forces in the current study. Tags used were designed to be streamlined when attached to juvenile Chinook salmon. Type A tags had a curved contact surface to fit closely to the curved dorsal surface (Figs. 1 and 3). Type B tags had a flat contact surface, which allowed the tags to fit along the flat lateral surfaces to which they were attached (Figs. 2 and 4). Sutton and Benson (2003) have shown that tag shape and size influence retention rates for juvenile lake sturgeon (*Acipenser fulvescens*), and proposed that this was based on the unique morphology of sturgeon. In their study, cylindrical tags (length: 21–40 mm, diameter: 10–11 mm) had higher retention rates than compressed tags (length: 24–34 mm, width: 11–16 mm, height: 6–8 mm) due to the limited number of sites available to tightly attach compressed tags. Our research also demonstrates the importance of tag shape based on the morphology of the fish as both Type A and Type B were able to withstand shear forces up to 12.2 m/s. These shear forces are similar to what is observed in hydro turbines in the CRB and demonstrates the efficacy of these externally attached tags for use in hydroturbine passage survival studies.

There were no mortalities observed in the current study within <96 hours of shear force exposure (3.0–12.2 m/s). Previous research reported immediate mortality (<1 h after exposure) when fish were exposed to minimum shear forces of 16.8 and 22.9 m/s (Deng et al., 2005, 2010b; respectively). Deng et al. (2005, 2010b) also found that delayed mortalities (<96 h) occurred for fish exposed to a minimum of 16.8 and 15.2 m/s (Deng et al., 2005, 2010b; respectively).

Overall, rates of injury for fish exposed to shear forces in the current study were low (6.0%; 9 of 151 fish; Table 3). Injuries were observed for fish exposed to 9.1 and 12.2 m/s shear forces and the frequency of occurrence was not associated with tag type (Table 5). No major injuries were observed in the current study under the flow velocities tested, and this is similar to results of Deng et al. (2005) where approximately 5% of fish exposed to 12.2 m/s shear force exhibited major injuries. Similar to fish held for 14 d, tissue damage was significantly higher for fish tagged with Type B tags when compared to those tagged with Type A tags that were exposed to shear forces and held for 4 d post-exposure.

The results of the current study suggest that Type A tag design is a viable option for hydroturbine survival studies. Fish tagged with Type A tags did not differ in survival or growth when compared to untagged fish or those surgically implanted with transmitters, and tag retention did not differ among the applicable treatment groups. In addition, exposure to shear forces did not produce differences in immediate injury between untagged fish or those with Type A tags. Previous studies testing the efficacy of external transmitters included a variety of different metrics not investigated in the current study. Future studies should focus on the influence of these novel neutrally buoyant externally attached tag designs on swimming performance, predator avoidance and the rate of barotraumas associated with rapid decompression for juvenile Chinook salmon.

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