

NOTE

Efficacy of Single-Suture Incision Closures in Tagged Juvenile Chinook Salmon Exposed to Simulated Turbine Passage

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Abstract

Reductions in the size of acoustic transmitters implanted in migrating juvenile salmonids have resulted in the use of a shorter incision—one that may warrant only a single suture for closure. However, it is not known whether a single suture will sufficiently hold the incision closed when fish are decompressed and when outward pressure is placed on the surgical site during turbine passage through hydroelectric dams. The objective of this study was to evaluate the effectiveness of single-suture incision closures on five response variables in juvenile Chinook salmon *Oncorhynchus tshawytscha* that were subjected to simulated turbine passage. An acoustic transmitter (0.43 g in air) and a passive integrated transponder tag (0.10 g in air) were implanted in each fish; the 6-mm incisions were closed with either one suture or two sutures. After exposure to simulated turbine passage, none of the fish exhibited expulsion of transmitters. In addition, the percentage of fish with suture tearing, incision tearing, or mortal injury did not differ between treatments. Expulsion of viscera through the incision was higher among fish that received one suture (12%) than among fish that received two sutures (1%). The higher incidence of visceral expulsion through single-suture incisions warrants concern. Consequently, for cases in which tagged juvenile salmonids may be exposed to turbine passage, we do not recommend the use of one suture to close 6-mm incisions associated with acoustic transmitter implantation.

Migrating juvenile salmonids can experience rapid pressure changes associated with passage at hydroelectric dams, particularly passage through turbines (Cramer and Oligher 1964; Cada 2001; Carlson et al. 2008). Pressure can vary widely along the span of a turbine passage route owing to factors such as turbine relative efficiency and proximity to the turbine blade tip or hub (Carlson et al. 2008). At some Columbia River basin

dams, nadir pressures (the lowest pressure to which fish may be exposed during turbine passage) between 200 and -2 kPa have been recorded with pressure-sensitive equipment (e.g., Sensor Fish; see Deng et al. 2007). Most ($\geq 93\%$) of these trials recorded nadir pressures of 55.2 kPa or greater, which probably represent conditions that a majority of migrating juvenile salmonids would encounter during turbine passage.

Pressure increases as fish move downward in the water column toward a turbine intake (Figure 1). This increase in pressure compresses air-filled structures (e.g., swim bladder) and gases within the fish. As fish pass by the turbine blades, rapid decompression can occur, causing gases within the fish to expand in proportion to the rapid reduction in pressure. This rapid expansion of gases within the fish can cause a suite of barotraumas and mortality (Cramer and Oligher 1964; Cada 2001; Rummer and Bennett 2005; Brown et al. 2009). As fish exit the draft tube and enter the tailrace, the pressure changes to near-atmospheric levels (Figure 1). Given the large number of possible trajectories through a turbine passage route, each fish probably experiences a unique pressure profile.

Surgically implanted acoustic transmitters are increasingly being used in the Columbia River basin and elsewhere to assess the survival of migratory salmonids after passage at dams, including hydroturbine passage. A fish that receives an implanted transmitter must offset the additional mass of the transmitter by increasing the volume of air in the swim bladder. Inflation of the swim bladder during the rapid decompression associated with turbine passage can exert pressure on the transmitter within the fish. Implanted transmitters generally lie between the swim bladder and the surgical incision. Thus, one possible effect of

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Received November 8, 2010; accepted February 28, 2011

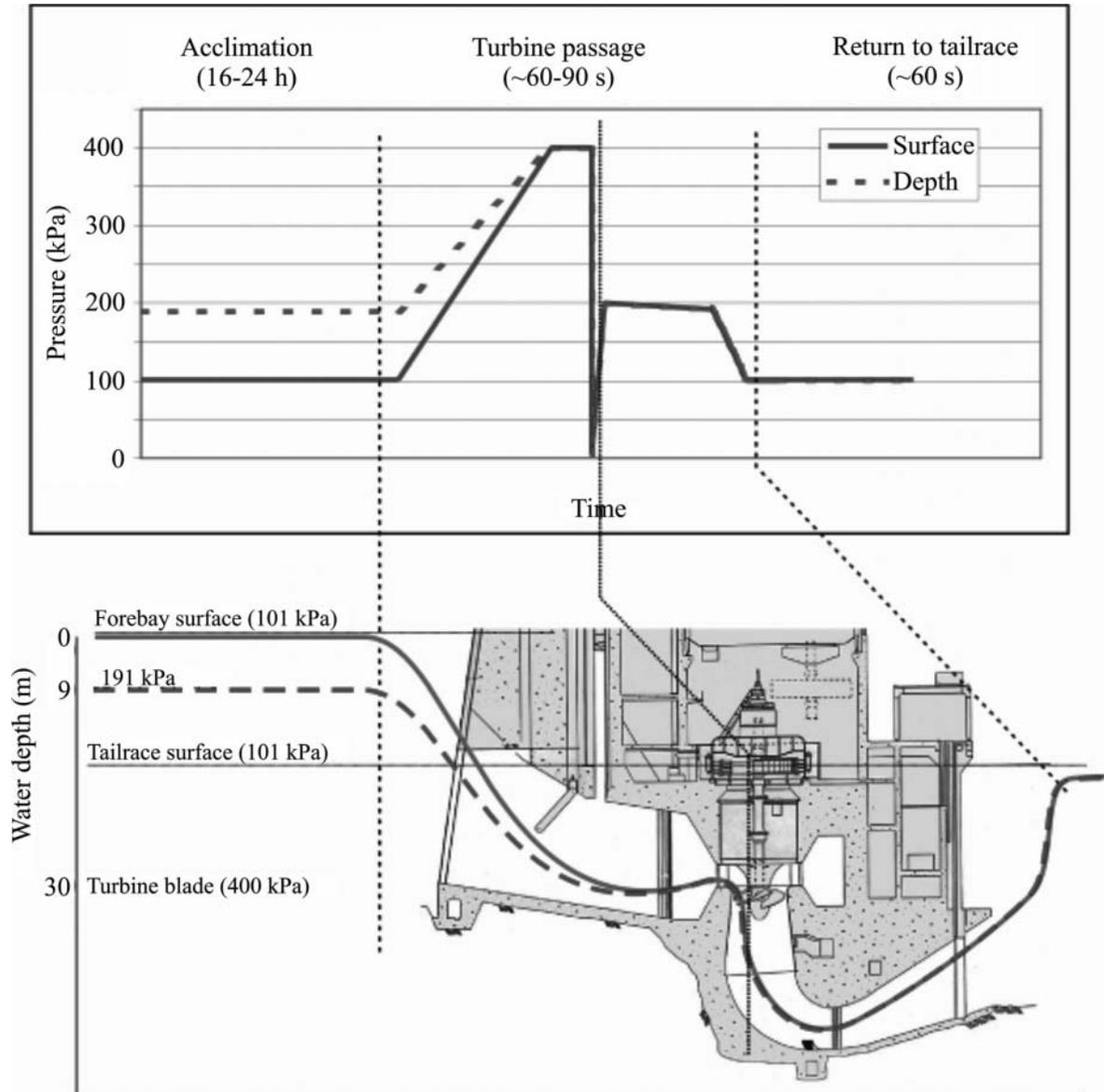


FIGURE 1. Hypothetical pressure profile (upper panel) experienced by a migrating juvenile salmon in relation to dam structures (lower panel) during turbine passage through a hydroelectric dam.

this extra swim bladder inflation may be the expulsion of the transmitter through the incision. In survival studies, transmitter loss biases the survival estimates because fish that lose transmitters cannot be detected and are functionally “dead.” For this reason, the minimization or elimination of transmitter expulsion is needed to ensure accurate interpretation of survival study results.

Recently, the miniaturization of acoustic transmitters has led to a reduction in the incision length that is required to implant a transmitter. In past years, two sutures were used to close incisions after the implantation of acoustic transmitters that were 0.5–0.8 g in weight, 16–19 mm in length, and approximately 6 mm wide (for examples, see Chittenden et al. 2009; Deters et al. 2010). However, smaller transmitters are now available

(0.43 g, 12 mm long, ~5 mm wide), so it seems likely that a shorter incision can be used for implanting these smaller transmitters. A shorter incision may increase the likelihood that one suture will adequately close the incision. Given the reduced suturing time and tissue trauma (i.e., less suture material in contact with fish tissue) that would result from one suture instead of two sutures, potential benefits to fish include reductions in the handling time, duration of anesthesia, and overall stress associated with surgery. However, the effects of a dynamic river environment, including rapid decompression associated with hydroturbine or spillway passage, on the performance of one suture for closing surgical incisions and retaining acoustic transmitters are unknown.

There has been little comparative research on the number of sutures required to close surgical incisions and ensure proper apposition to expedite healing. In addition, precise incision length is seldom reported by researchers. In general, Swaim (1980) suggested that sutures should be spaced the same distance apart as each suture is wide; however, such a recommendation appears to have no scientific basis for use with fish, and fish that are exposed to rapid decompression may need more sutures to ensure that the transmitters are not expelled. Thus, the objectives of this study were to compare tag retention, suture tearing, incision tearing, mortal injury, and expulsion of viscera after simulated turbine passage (STP) for juvenile Chinook salmon *Oncorhynchus tshawytscha* that received surgical incisions (controlled length: 6 mm) closed by one suture or two sutures. Exposure of tagged fish to a range of pressure changes that are likely to be encountered by the majority of turbine-passed juvenile Chinook salmon in the Columbia River basin should provide insight into the performance of single sutures for closing surgical incisions. This study furthers the understanding of turbine passage effects on fish that are subjected to surgical implantation of an acoustic transmitter and a passive integrated transponder (PIT) tag; additionally, this study has implications for future surgical protocols used in the Columbia River basin and other areas where hydroturbine passage-related research is of interest.

METHODS

Experimental fish and surgery.—Hatchery-reared juvenile fall Chinook salmon ($n = 206$) with fork lengths of 95–135 mm (mean = 125 mm) and weights of 9.5–32.8 g (mean = 22.4 g) were used in this study. Procedures for anesthesia and surgical setup followed those described by Deters et al. (2010) except for some modifications to the surgery and wound closure. Surgeries were performed over a 9-d period. With the fish facing ventral side up, a 6-mm incision (measured by use of calipers) was made on the linea alba anterior to the pelvic girdle. An acoustic transmitter and a PIT tag were implanted, and the incision was closed with either one suture (one-suture treatment, $n = 91$ fish) or two sutures (two-suture treatment, $n = 99$ fish; 5-0 Monocryl absorbable sutures; Ethicon, Somerville, New Jersey). Each fish was double tagged (acoustic and PIT tags) to simulate field studies on the Snake and Columbia rivers, where the presence of a PIT tag prevents fish from being sorted into transport barges or trucks at juvenile bypass facilities. Currently, two sutures are typically used to close surgical incisions associated with acoustic transmitter and PIT tag implantation in Columbia River basin juvenile salmonids. A simple interrupted suturing pattern was used for both treatments, and all sutures were tied with reinforced surgeon's knots (Wagner et al. 2011). Acoustic transmitters were $12.0 \times 5.2 \times 3.8$ mm, and they weighed 0.43 g in air (0.30 g in water; 0.14-mL volume). The PIT tags were 12.5×2.1 mm and weighed 0.10 g in air (0.06 g in water; 0.04-mL volume). Mean tag burden (2.6%; range = 1.6–5.7%) was identical between treatments. Tag burden was

equal to the combined weight of the acoustic transmitter and PIT tag, expressed as a percentage of fish body weight.

All fish that were randomly assigned to a given pressure chamber (Stephenson et al. 2010) received a unique clip on the distal portion of a fin for purposes of individual identification. After surgery, fish were placed in a 20-L recovery bucket (marked with the chamber identification) containing aerated water until they regained equilibrium; the fish were then transferred to one of four perforated, 20-L buckets that were designed to float within a circular tank. Water temperature in the tank was 17°C. Each bucket housed eight fish (four fish from the one-suture treatment and four from the two-suture treatment). Fish remained in the perforated buckets within the circular tank for approximately 24 h before being transferred to their respective pressure testing chambers; thus, eight fish (one bucket) were placed in a given pressure chamber. Sixteen fish (two buckets) were withheld from further testing at this point to examine changes in incision length. Incisions were measured to the nearest 0.5 mm. These measurements were used to control for the effects of acclimation and STP testing on incision tearing.

Acclimation pressure chamber.—After water-to-water transfer of fish to the pressure chambers, the pressure within each chamber was immediately set to 146 kPa (the pressure equivalent to the absolute pressure at 4.6-m depth in freshwater given standard atmospheric pressure) by using methods similar to those described by Stephenson et al. (2010). The physoclistous test fish were given an air pocket during the 16–18-h acclimation period—ample time to achieve neutral buoyancy—and freshwater (at 17°C) flowed continuously. To determine fish buoyancy (as described by Harvey 1963) before STP exposure, fish behavior was remotely observed through a video imaging system. All nonneutrally buoyant fish and mortalities were identified based on their unique fin clips and were removed from further analysis.

Simulated turbine passage exposure.—Exposure tests were conducted with four hyperbaric–hypobaric chamber systems that were designed and built by Reimers Engineering (Springfield, Virginia), Pacific Northwest National Laboratory (Richland, Washington), and Tice Engineering (Broadway, Virginia). Stephenson et al. (2010) provide a detailed description of the chambers and testing system. At the conclusion of the acclimation period, STP exposure was initiated by means of the virtual interface, which read a programmable decompression profile that was set to achieve nadir pressures (the lowest pressures present during STP) between 55.2 and 82.7 kPa, thereby increasing the volume of the swim bladder within the fish by 1.8–2.6 times. During this period, the automated air bubble was slowly removed from the chamber without altering the pressure in the chamber. The STP profile simulated (1) the passage of fish through a hydroelectric turbine intake, (2) their approach to the turbine runner, and (3) their movement out of the turbine draft tube, into the tailrace, and up to the water surface, where pressure was returned to atmospheric pressure (~101 kPa; Stephenson et al. 2010).

TABLE 1. Response variable outcomes (percentage of fish) after simulated turbine passage for Chinook salmon that each received an acoustic transmitter and a passive integrated transponder tag through a 6-mm incision that was closed with either one suture or two sutures. Asterisks denote a significant difference ($P < 0.05$) between treatments.

Treatment	<i>n</i>	≥1 mortal injury (%)	Expulsion of viscera (%)	Incision tearing (%)	Suture tearing (%)	Tag retention (%)
One suture	91	15	12*	94	9	100
Two suture	99	19	1*	95	8	100

Postexposure holding.—After STP exposure, all fish were held in their respective chambers for a 10-min observation period during which mortality and tag expulsion were recorded. After 10 min, chambers were drained to a depth of 15 cm, and all fish from one of the four pressure chambers were lightly anesthetized (tricaine methanesulfonate [MS-222] at 10–40 mg/L) and transferred (water to water) to an observation room. Anesthetization of fish within the remaining pressure chambers was staggered by approximately every 15 min to ensure that anesthesia times were similar among chambers. Next, fish were individually placed in a bucket containing MS-222 at 80 mg/L until they experienced a loss of equilibrium. At this point, a photograph was taken of the incision and sutures, incision lengths were measured with calipers, and the incidence of visceral expulsion was noted. Fish exposure groups were allowed to recover in oxygenated water within 20-L buckets before being transferred into one of four circular (890-L) tanks supplied with flow-through water at 17°C. A postexposure holding time of 8 h in the circular tanks allowed for assessment of delayed mortality as well as delayed loss of transmitters, PIT tags, and sutures. Holding for 8 h postexposure is within the range of time taken by acoustic-tagged Chinook salmon to reach the first downstream acoustic array (i.e., for estimating survival) below dams in Columbia River basin field studies. After the 8-h holding period, all fish were euthanized with a 250-mg/L dose of MS-222. All mortalities were removed from circular tanks when they were discovered.

Necropsy.—Necropsies were performed on all euthanized fish to identify any mortal injuries that were incurred during STP. Euthanization of fish within postexposure holding tanks was staggered approximately every 15 min to ensure that the necropsies were initiated immediately after fish death. A mortal injury index (determined from fish that either died or received injuries sufficient to cause eventual mortality) constructed for a previous study was used to classify mortal injury in this study (see McKinstry et al. 2007). Suture tearing and suture retention were also noted during internal and external necropsy. Two researchers that were experienced in necropsy procedures and techniques performed all of the necropsies.

Data analysis.—Initially, pressure chambers were treated as experimental units; however, scores for most response variables were zero, which precluded meaningful analysis and interpretation of data. Therefore, for each individual fish, the response variables—tag retention, incision tearing, suture tearing, mortal injury, and visceral expulsion—were expressed as Bernoulli

random variables and modeled by means of generalized linear models based on a logistic link function and Bernoulli error structure. Predictor variables used in the generalized linear models included nadir pressure and fish length. Analysis of deviance was used in modeling the data and testing hypotheses. Incision tearing was a discrete numeric variable (0.5-mm intervals); treatment and STP effects were analyzed with a Mann–Whitney *U*-test because the data were not normally distributed. Alpha level was 0.05 for all analyses.

RESULTS

Tag expulsion and mortal injury were not influenced by the number of sutures present on incisions. No acoustic transmitters, PIT tags, or sutures were expelled across the observed nadir pressure range of 44–101 kPa (mean = 75 kPa; Table 1). In addition, mortal injury did not differ significantly ($F = 1.29$, $df = 1$, $P = 0.26$) between treatments, as 15% of fish in the one-suture treatment and 19% of fish in the two-suture treatment incurred at least one mortal injury during STP (Table 1). Nadir pressure ($F = 97.44$, $df = 1$, $P < 0.01$) and fish length ($F = 12.22$, $df = 1$, $P < 0.01$) were significant predictors of mortal injury. Lower nadir pressures caused a greater incidence of mortal injury, and smaller fish were more prone to mortal injury than were larger fish.

Incidence of suture tearing was low (<10%) for both treatments (Table 1) and did not differ significantly ($F = 0.08$, $df = 1$, $P = 0.78$) between treatments. However, fish length ($F = 133.57$, $df = 1$, $P < 0.01$) was a significant predictor of suture tearing. Suture tearing occurred more frequently in smaller fish.

Incidence of incision tearing was similar between treatments (Table 1). Amount of incision tearing did not differ significantly (Mann–Whitney $U = 3,500.5$, $P = 0.09$) between the one-suture treatment group (1.8 mm) and the two-suture treatment group (1.4 mm). Incision tearing did not differ between STP-exposed fish and unexposed fish in the one-suture treatment (Mann–Whitney $U = 339.5$, $P = 0.86$) or the two-suture treatment (Mann–Whitney $U = 285.0$, $P = 0.99$).

Incidence of visceral expulsion differed significantly ($F = 37.01$, $df = 1$, $P < 0.01$) between treatments. Expulsion of viscera (Figure 2) occurred in 12% of fish in the one-suture treatment but only 1% of fish in the two-suture treatment (Table 1). Fish length ($F = 27.06$, $df = 1$, $P < 0.01$) and nadir pressure ($F = 11.55$, $df = 1$, $P < 0.01$) were significant predictors of visceral expulsion. Incidence of visceral expulsion was

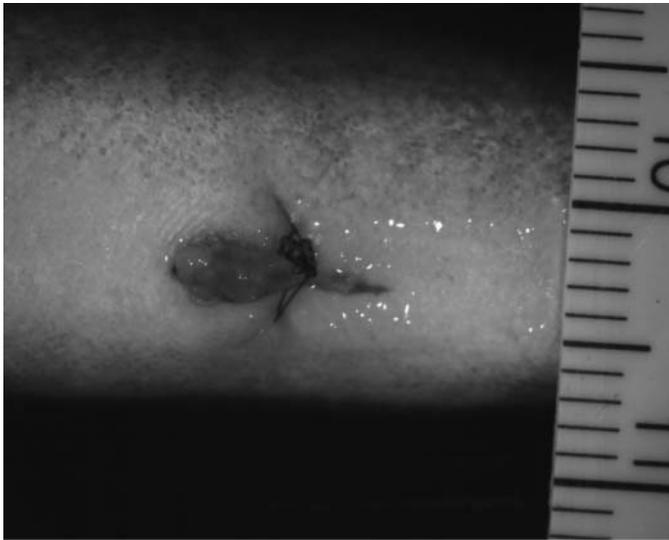


FIGURE 2. Expulsion of viscera through an incision closed by a single suture in a tagged Chinook salmon that was examined after simulated turbine passage.

higher for small fish than for large fish, and visceral expulsion occurred more frequently at relatively low nadir pressures than at high nadir pressures.

DISCUSSION

Despite the lack of acoustic transmitter or PIT tag expulsion among fish in the one-suture treatment, the greater incidence of visceral expulsion in one-suture fish than in two-suture fish suggests that one suture does not provide adequate incision closure (for a 6-mm incision) to retain viscera during STP. Incision openness caused by expulsion of viscera could create a pathway for pathogens (e.g., *Saprolegnia* spp.) to enter or colonize on the body and could compromise fish health in the long term. In a natural riverine environment, protruding viscera could abrade against substrate, causing further physiological complications, infection, or death. In addition, it is unlikely that fish with protruding viscera would behave in a manner similar to that of untagged fish. Although no fish with expelled viscera died during the 8-h postexposure holding period, the effect of visceral expulsion on longer-term survival remains unknown. Thus, we do not recommend the use of one suture to close 6-mm or longer surgical incisions on the linea alba in juvenile Chinook salmon that may experience rapid decompression.

Probability of visceral expulsion appeared to be size-mediated in this study, suggesting that some characteristics of smaller fish increase the likelihood of this outcome. Brown et al. (2009) reported similar results in which 28% of small (subyearling) Chinook salmon and 9% of large (yearling) juveniles exhibited visceral protrusions through the incision after STP exposure to nadir pressures between 8.3 and 19.3 kPa. One possible explanation for these observations is that smaller fish have thinner, less-rigid skin than do larger fish, which can make

proper apposition of the wound margins difficult. In addition, the less-rigid skin of smaller fish would provide less support against the outward pressure exerted by an implanted transmitter. Available volume within the body cavity may be another factor influencing visceral expulsion in smaller fish. Acoustic transmitters occupy a significant volume within the bodies of small fish. Given that there is less available volume in smaller fish, the expansion of the swim bladder and the resulting pressure exerted on surrounding viscera at a constant nadir pressure are probably greater than those in larger fish. Viscera (e.g., the pyloric caeca and spleen) that lie between the swim bladder and the incision are probably forced out of the incision through a path of least resistance.

Mortal injury and visceral expulsion occurred more frequently at lower nadir pressures in this study—a result that was probably due to changes in the volume of gas within the body of fish during rapid decompression. Gas bubbles in the blood or other bodily fluids increase in size as pressure decreases. This expansion of existing gas bubbles or the formation of new bubbles released from solution can increase blood volume and rupture vasculature, which in turn can cause internal hemorrhaging and other injuries (Cramer and Oligher 1964; Rummer and Bennett 2005; Brown et al. 2009). Greater expansion of the swim bladder occurs at progressively higher pressure changes and exerts more pressure on the surrounding viscera, possibly causing expulsion through an unhealed incision.

No acoustic transmitter expulsion occurred in either treatment in this study (~48 h elapsed between implantation and the end of postexposure holding). Similarly, no radio transmitters were expelled from juvenile Chinook salmon that had two sutures and were exposed to low nadir pressures (8.27–19.3 kPa) during STP (Brown et al. 2009). Tag or transmitter expulsion has been observed across a wide range of postsurgery durations in some biotelemetry studies (Moore et al. 1990; Bunnell and Isely 1999; Jepsen et al. 2008; Brown et al. 2010; Deters et al. 2010); however, the temporal component of tag retention in juvenile Chinook salmon that receive one suture for closure of incisions was beyond the scope of this study and remains unknown.

Interestingly, incision tearing occurred before acclimation and rapid decompression from STP, as incision lengths did not differ between control fish and fish that experienced acclimation, STP exposure, and post-STP holding. One plausible explanation for this result may be related to the orientation of the incision relative to surrounding muscle tissue. Both humans and fish have abdominal muscles that are joined on the ventral midline (i.e., linea alba). In humans, contraction of abdominal muscles results in the lateral retraction of midline incision edges (Burger et al. 2002). If the same is true for fish, contractions of the abdominal muscles used for swimming may pull laterally on the incision, possibly causing it to tear. In this study, fish swam within recovery buckets after surgery and often burst-swam when bucket lids were removed after the recovery period. Thus, it is conceivable that this swimming activity accounted for the incision tearing observed during this study.

The length of incision should dictate the number of sutures that are needed to ensure proper apposition of incision edges and optimal wound healing. Too few sutures may cause dehiscence of a wound (Swaim 1980), whereas too many sutures can increase tissue trauma from additional suture entry and exit holes and the increased surface area of suture-to-skin contact. Suture spacing (inferred from reported incision lengths and number of sutures) varied from approximately 2.5 to 6.0 mm during several studies in which biotelemetry devices were implanted into fish (Hart and Summerfelt 1975; Harvey et al. 1984; Wagner and Stevens 2000; Anglea et al. 2004; Brown et al. 2006; Caputo et al. 2009; Deters et al. 2010). In the present study, mean incision length was 7.8 mm (after incision tearing) for fish with one suture, resulting in a gap of 3.9 mm between the suture and the incision end. Although this gap lies in the midrange of suture spacing reported from similar studies, one suture was not adequate to prevent visceral expulsion during STP. Comparatively, suture spacing for fish in the two-suture treatment was 2.5 mm and resulted in only one occurrence of visceral expulsion.

Most recorded nadir pressures at Columbia River basin dams are between 34.5 and 200 kPa and probably give a realistic representation of nadir pressures experienced by the majority of turbine-passed fish (Carlson et al. 2008). By exposing treatment fish in this study to nadir pressures within that range (e.g., 44–101 kPa), we hoped to provide a realistic performance assessment of single sutures for closing surgical incisions in juvenile Chinook salmon. Despite a lack of acoustic transmitter, PIT tag, or suture loss in the STP-exposed fish, the potential effects of visceral expulsion on the long-term health, behavior, and survival of fish certainly warrant concern. Thus, for studies in which tagged fish may be exposed to rapid decompression associated with turbine or spillway passage, we do not recommend the use of a single suture to close 6–8-mm incisions on the linea alba. More research is needed to determine the incision length at which one suture would be adequate for maintaining proper apposition and visceral retention in fish that are exposed to STP.

ACKNOWLEDGMENTS

Funding was provided by the U.S. Army Corps of Engineers, Portland District. We especially thank John Stephenson, Brett Pflugrath, Piper Benjamin, Andrew Gingerich, Ricardo Walker, Kasey Knox, Marybeth Gay, Andy LeBarge, and Bob Mueller (Pacific Northwest National Laboratory) for their valuable assistance. We are grateful to John Skalski and Adam Seaburg (University of Washington) for scientific and statistical advice. Animal facilities were certified by 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 were approved by the Institutional Animal Care and Use Committee of Battelle–Pacific Northwest Division. Reference to trade names

does not imply endorsement by Battelle, the Pacific Northwest National Laboratory, or the U.S. Government.

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