Articles

Effects of a Novel Fish Transport System on the Health of Adult Fall Chinook Salmon

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Abstract

Movement past hydroelectric dams and related in-river structures has important implications for habitat connectivity and population persistence in migratory fish. A major problem is that many of these structures lack effective fish passage facilities, which can fragment spawning and rearing areas and negatively impact recruitment. While traditional fish passage facilities (e.g., ladders, trap and haul) can effectively enable fish to pass over barriers, their capital or operational costs can be significant. We evaluated the utility of a novel transport device that utilizes a flexible tube with differential internal air pressure to pass fish around in-river barriers. We apportioned a total of 147 adult fall Chinook salmon (*Oncorhynchus tshawytscha*) nearing maturation to three treatments and a control group. In two of the treatments, adult fall Chinook salmon were transported through the device via two lengths of tube (12 or 77 m) and we compared their injury, stress, and immune system responses and reproductive function to a third treatment where fish were moved by a standard trap-and-haul method and also to a control group. We observed no significant differences among the treatment or control groups in posttreatment adult survival, injury, or stress. Indicators of immune system response and reproductive readiness were also not significantly different among the four groups. Egg survival was significantly different among the groups, with the highest survival in the eggs from females transported 77 m and lowest in the control group; the differences were highly variable within groups and not consistent with the duration of treatment or degree of handling. Taken together, the results suggest the device did not injure or alter normal physiological functioning of adult fall Chinook salmon nearing maturation and may provide an effective method for transporting such fish around in-river barriers during their spawning migration.

Keywords: Whooshh; transport; in-stream barriers; hydropower

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Introduction

Adult Pacific salmon (*Oncorhynchus* spp.) are often assisted in their upstream migration over instream barriers through the use of active transport methods such as trap-and-haul techniques. Trap and haul involves capturing fish in a device such as a fish trap and then moving them by hand or other means to a tank on a vehicle which is then used to haul the tank and fish around the barrier where the fish are released to continue their upstream migration. While commonly used by fisheries management agencies, trap-and-haul techniques can have significant impacts to individual fish due to handling and confinement. For example, Keefer et al. (2010) noted that among other factors, handling stress associated with a trap-and-haul facility on the Willamette River, Oregon, may have contributed to high prespawning mortality rates in adult spring Chinook salmon (*Oncorhynchus tshawytscha)* out-planted above barrier dams. Further, trap and haul is not typically operated continuously, which can substantially delay upstream migration. Sockeye salmon (*Oncorhynchus nerka*) were

delayed a median of 0.4 to 8.7 d at a trapping facility on the Wenatchee River, Washington, and the facility may have precluded 8 to 38% of the return (2,387 to 21,090 adults) from reaching upstream spawning areas (Murauskas et al. 2014). The development of a cost-effective and adaptable system as an alternative to trap and haul for moving fish could have substantial benefits for affected fish populations. Ideally, such a system should minimize stress and injury and have no long-term impact on survival, growth, or reproduction.

The Whooshh fish transport system (WFTS) has been developed by Whooshh Innovations, LLC, Seattle, Washington, as an alternative to trapping and hauling fish around instream barriers. The system is highly portable and consists of a flexible transport conduit (hereafter tube) that utilizes differential internal air pressure to move fish. Widespread adoption of this technology, however, has been hindered because of limited evaluations on how the WFTS could impact fish health, particularly for populations of conservation interest. A major concern for application of the WFTS for migrating adult salmon is that fish are dewatered during transport through the system, which can potentially cause epithelial injury, increase stress, and impair behavioral and endocrine functions that are important for reproductive success (Goetz 1983; Schreck et al. 2001). Mesa et al. (2013) evaluated a prototype of the WFTS to assess the physiological responses in hatchery adult rainbow trout (*Oncorhynchus mykiss*) and showed that mean plasma cortisol, glucose, and lactate did not differ between controls and fish transported 15 m through the WFTS. In addition, none of the fish showed signs of injuries such as descaling, abrasions, or loss of mucous (Mesa et al. 2013). The authors noted, however, that important questions for widespread acceptance of the device would be to explore the transport effectiveness as well as the short-term (injury) and long-term (reproductive viability of gametes) effects on fish moved over longer distances of tens to hundreds of meters (Mesa et al. 2013).

Improvements in the design of the WFTS since the original study (e.g., optimizing internal pressure configuration) have allowed for transport of fish over longer distances (~80 m), but the effects on physiological responses and injury at these distances have not been examined. In addition, the WFTS has not been compared to traditional active transport techniques, such as trap and haul. In this study, we compared the effects of transport distance within the WFTS against standard trap-and-haul methods on survival to spawning, injury to epithelial tissue, general stress, immune response, reproductive readiness, and gamete survival in adult hatchery fall Chinook salmon.

Methods

Fish collection and sorting

We collected male and female adult fall Chinook salmon in a prespawning condition from the volunteer trap at Priest Rapids Hatchery on October 27–31, 2014. Priest Rapids Hatchery is owned by the Public Utility District of Grant County and operated by the Washington Department of Fish and Wildlife. The hatchery is located in eastern Washington State at approximately river kilometer 639 on the Columbia River. Fish collected in the trap are predominantly of hatchery origin and we used only hatchery-origin fish in this study. After capture at the trap, we loaded the salmon into fish transport trucks using an Archimedes pump, drove them approximately 0.8 km, and transferred them by pipe into a concrete raceway measuring 30 m long, 3 m wide, and 1 m deep. We prevented fish from jumping out of the raceway by a combination of cyclone fencing and plastic netting that extended vertically along both the sides and ends of the raceway. The raceway was supplied with water from the Columbia River that ranged between 14 and 15.5°C during the study, with a turnover rate of approximately once every 1 h.

Prior to treatment, we crowded the salmon into a confined area and netted individual fish from the raceway into a buffered anesthetic solution (100 mg/L MS-222) where we held them until they reached stage 4 anesthesia (loss of equilibrium; Summerfelt and Smith 1990). Once fish were anesthetized, we measured and recorded their fork length, wet weight, and maximum circumference. We also conducted a preliminary assessment of the external condition of the fish to document eye or nose damage, and any abrasions, lacerations, cuts, or scars on the body. We tagged fish of the appropriate size and condition with a passive integrated transponder tag injected into the dorsal sinus to identify individual fish throughout the study. Following tagging, we returned fish to the raceway to recover from anesthesia.

Experimental treatment

We moved fish November 4–6, 2014. We randomly assigned adult fall Chinook salmon to one of four groups which consisted of a control and three treatments (Table 1). We netted control fish ($n = 36$) from the holding raceway into anesthesia (35 ppm Aqui-S 20E, Aqui-S New Zealand Ltd., New Zealand). Once fish reached stage 2 anesthesia (Summerfelt and Smith 1990) we either examined them for macroscopic injuries using fluorescein ($n = 12$) or placed them in the holding raceway to recover ($n = 24$). We used rubber mesh carry totes to transfer fish among the anesthesia and fluorescein dye stations and the raceway.

We randomly lifted fish used in the WFTS treatment groups by hand from the raceway, scanned them for a passive integrated transponder tag, and placed them into the WFTS one at a time, in groups of 3 to 5; fish were not anesthetized when they transported through the tube. The WFTS consisted of an accelerator (pumphouse), pump, generator, hanger system, and two lengths of tubing (12 m, hereafter WFTS-12, or 77 m, hereafter WFTS-77) that could be interchanged with the accelerator depending on the treatment (Figure 1A). The diameters of both the WFTS-12 and WFTS-77 tubes were sized for salmon approximately 7–14 kg in mass

Table 1. The sample sizes from a study on the effects of passage and handling conducted in November, 2014, with adult fall Chinook salmon (*Oncorhynchus tshawytscha*) that were captured at Priest Rapids Salmon Hatchery, Mattawa, Washington. The sample sizes of adult fall Chinook salmon are shown for the four groups (control, 12-m transport tube [WFTS-12], 77-m transport tube [WFTS-77], and trap and haul) that were assessed for epithelial damage using fluorescein dye, cortisol, vitellogenin (Vtg), immunoglobin M (*IgM*), interleukin-1 beta (*IL1*-β), and gamete viability through the eyed egg stage.

Treatment	Total	Epithelial damage	Cortisol	Vtg	*IgM*	*IL1*-β	Gamete viability
Control							
Males	13	5	—	—	3	3	10
Females	23	7	8	11	10	10	10
Total	36	12	8	11	13	13	10 pairs
WFTS-12							
Males	12	5	—	—	4	4	6
Females	24	5	9	15	13	13	6
Total	36	10	9	15	17	17	6 pairs
WFTS-77							
Males	14	5	—	—	3	3	10
Females	25	5	15	19	17	17	10
Total	39	10	15	19	20	20	10 pairs
Trap and haul							
Males	15	5	—	—	3	3	11
Females	21	5	9	18	12	12	11
Total	36	10	9	18	15	15	11 pairs

(maximum circumference = 48–60 cm); proper tube diameter relative to the fish circumference ensures optimal system pressures are created to move fish through the tube. The accelerator of the WFTS was placed in the end of the raceway where the fish were crowded. We positioned the opening to the accelerator so that fish could be moved directly from the water by hand into the accelerator where they were transported through the WFTS-12 or WFTS-77 tube into a separate raceway filled to a water depth of approximately 1 m. While the slope for both tubes was essentially zero (i.e., flat with no elevation gain), the layout of the two tubes was different. The 12-m tube went straight from the fish-holding raceway to the fish-exit raceway whereas the 77-m tube followed a racetrack configuration with four corners (Figure 1B). After exiting the WFTS, we crowded the salmon, netted them, and placed them into an anesthetic bath (35 ppm Aqui-S 20E) where we either examined them for macroscopic injuries using fluorescein or returned them to the holding raceway. A total of 36 and 39 adult salmon were sent through the WFTS-12 and WFTS-77, respectively (Table 1).

We crowded salmon used for the trap-and-haul treatment (n = 36; Table 1) in the raceway and individually placed them into a tote (volume = 850 L) using a polypropylene transfer sleeve; fish were not anesthetized when we placed them into the tote. We then lifted the tote with fish (n = 6–11) with a forklift and poured them into the holding tank of the fish-transport truck. We held fish in the truck for 30 to 45 min, after which we transferred them by pipe into a raceway, crowded them, and then netted them into an anesthetic bath (35 ppm Aqui-S 20E). Once fish reached stage 2 anesthesia (Summerfelt and Smith 1990) we either examined them for macroscopic injuries using fluorescein (n = 10) or placed them in the holding raceway to recover (n = 26).

Epithelial damage detection

We used fluorescein dye (fluorescein, disodium salt; Aldon Corp., Avon, NY) on a subsample of fish to assess latent injuries to epithelial tissue (Table 1). Fluorescein binds to hemoglobin where epithelial damage has occurred and can be detected with ultraviolet light to quantify the amount of injury (Colotelo and Cooke 2011). Following treatment, we placed anesthetized fish (35 ppm Aqui-S 20E) in a 0.2-mg/mL solution of aerated fluorescein for 6 min. We then transferred them to an aerated anesthetic rinse bath (35 ppm Aqui-S 20E) for 6 min. After the rinse bath, we photographed fish on both sides under 254-nm ultraviolet light using a digital single-lens reflex Nikon D200 camera (Nikon Inc., Millville, NY). We flushed fresh water over the gills during photography.

We evaluated photographs using Image-J software (National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij), which we used to preview the images, perform contrast adjustments, and determine the number of pixels that represented the entire area of the fish. To provide a systematic and repeatable means of measuring injured regions, we developed an automated image classification algorithm to isolate pixels exhibiting a fluorescein signature. We implemented the damaged-tissue pixel classification algorithm using Python open-source scripting language. The algorithm proceeded in a step-wise fashion to 1) calculate summary pixel statistics of the approximate amount of damaged tissue (e.g., minor or major), 2) select a threshold based on this determination, 3) isolate and refine the damage pixels, and 4) compute the proportion of damage for each image. Images from fish that experienced extensive damage exhibited a bimodal pixel distribution and were ideally suited for classification via the Otsu threshold (Otsu 1975). Images of fish that experienced minor damage were not bimodal and were classified using the

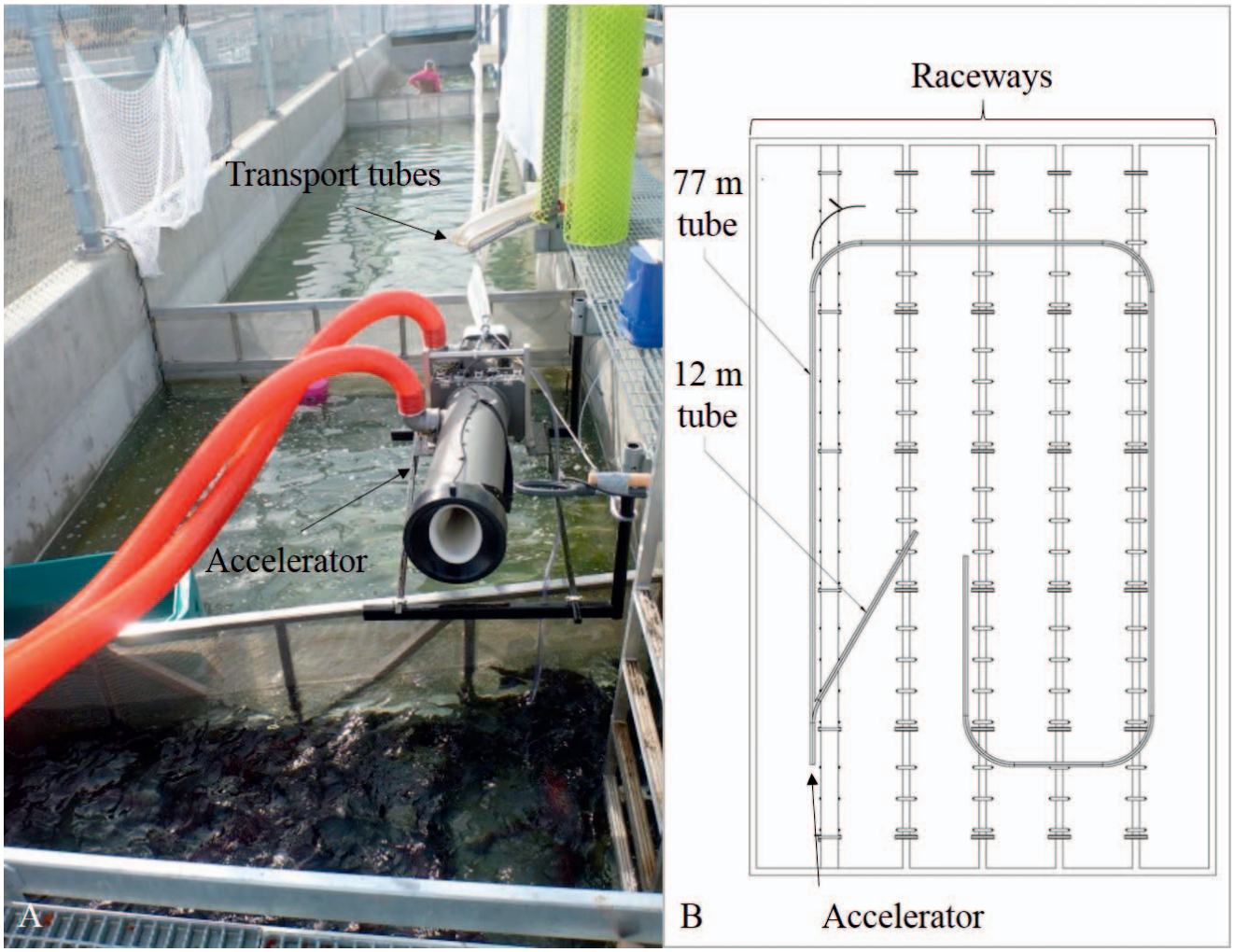

Figure 1. (A) Photo of the entrance to the Whooshh fish transport system used in the fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. The accelerator (pump house) is suspended on supports placed within the raceway where the fish were held. The two interchangeable transport tubes measured 12 and 77 m. (B) Configuration of raceways used with 12- and 77-m transport tubes.

median Otsu threshold calculated from the extensive damage image set. We calculated the proportion of epithelial damage on each side of the fish by dividing the total number of fluorescein-stained pixels by the total number of pixels encompassed by the fish. We then summed the proportion of epithelial damage for the left and right sides and used these for statistical analysis (Colotelo and Cooke 2011).

Stress, reproductive readiness, and immune response

We measured cortisol and vitellogenin (Vtg) in plasma and immunoglobulin M (*IgM*) and interleukin-1 beta (*IL1*-β) gene expression in spleen tissue collected from adult female fall Chinook salmon at the time of spawning (November 7–8, 2014). We collected blood samples from the caudal vasculature of anesthetized salmon (35 ppm Aqui-S 20E) using a 4-mL vacutainer containing sodium heparin and a 21-gauge needle. Following blood sampling, we euthanized the fish to collect spleen tissue samples. We placed blood samples on ice and spleen samples in RNA later® (Ambion Inc., Foster City, CA) for transport to the lab. Upon arrival, we centrifuged the blood samples at 2095 x *g* for 15 min to separate the plasma and stored them at –80°C until analysis.

We measured plasma cortisol concentrations from unextracted female plasma using competitive plasma cortisol expression enzyme immunoassay following manufacturer's protocol (Cayman Chemical Company, Ann Arbor, MI). We measured plasma Vtg using an enzyme immunoassay following manufacturer's protocol (Biosense Laboratories, Bergen, Norway). We determined differential expression of *IgM* and *IL1*- β in spleen ribonucleic acid (RNA) using semiquantitative polymerase chain reaction (Freeman et al. 1999). We isolated total RNA from spleen tissue (Ambion TriReagent, Austin, TX), and determined relative concentrations by ultraviolet spectrophotometry (GENE SYS 10). We prepared

complementary DNA (cDNA) to the spleen RNA template using a high-capacity cDNA reverse transcription kit and a GeneAMP® PCR system 9700 (Applied Biosystems, Foster City, CA). We designed primers for amplification using PrimerQuest (IDT, Coralville, IA) (*IL1*-β: forward 5′ AGCAGGGTTCAGCAGTACATCACA 3′, reverse 5′ ATCAGGACCCAGCACTTGTTCTCA 3′; *IgM heavy chain*: forward 5′ GTGACCCTGACTTGCTACGTCAAA 3′, reverse 5′ GCTCATCGTTAACAAGCCAAGCCA 3′) using a Chinook salmon sequence. Briefly, we reverse transcribed 1 μg of total spleen RNA with 50 μM random hexamers. We used one-tenth of the cDNA for each PCR reaction along with a SYBR® Green master mix and 2 pmol of primers. Reagents and protocols for primer construction were from Applied Biosystems. We carried out cycling with 40 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 10 s. Each gene assay included a standard curve of gel-purified, template-specific cDNA in serial dilutions for setting the cycle threshold. We normalized the cDNA expression levels for all samples to expression levels for 18S, using Ambion's Quantum RNA™ primers. We previously confirmed DNA targets (*IL1*-β and *IgM*) by comparing DNA sequences (sequencing performed by Agencourt, Danvers, MA) with known fish sequences for identity using the basic local alignment search tool (BLAST) algorithm (National Center for Biotechnology Information, National Institutes for Health, Bethesda, MD; available at http://blast.ncbi.nlm.nih.gov). We compared the resulting DNA sequence information with known sequences for identity using BLAST and confirmed both target sequences with >98% nucleotide match to other salmonid species.

Gamete viability

We euthanized males and females that were sexually mature at the time of posttreatment blood sampling and stripped them of eggs and sperm, which we placed in coolers on ice and returned to the Aquatic Research Laboratory at the Pacific Northwest National Laboratory, Richland, Washington. At the Aquatic Research Laboratory, we mixed eggs and sperm from individual fish 1:1 within treatment groups to form a total of 37 full-sib families. We divided the fertilized eggs into three subsamples per family (approximately 100 eggs per subsample) and randomly assigned them to a stack, tray, and cell in vertical-flow incubators. We modified the incubation trays with perforated polyvinyl chloride sheets to create 12 individual cells per tray and 144 total cells (two stacks with five or seven trays per stack and 12 cells per tray). We held fertilized eggs in incubation trays at ~10°C until they reached the eyed egg stage (December 17, 2014, or approximately 40 d postfertilization), at which time we counted the number of live and dead eggs to determine survival.

Data analysis

We determined differences among groups in post-treatment adult survival to spawning by χ^2 analysis (Zar 1984). We assessed the average embryo survival to the eyed stage (subsamples were averaged per family) and the proportion of adult salmon that experienced epithelial injury by 1-way ANOVA after arcsine transformations to meet the assumptions for normality and equal variances. We also analyzed plasma cortisol and Vtg by ANOVA after $\log_{10}$ transformation to meet these assumptions. *IL1*-β and *IgM* values did not meet the assumptions for normality and equal variances after $\log_{10}$ transformation and we tested these by Kruskal-Wallis ANOVA by ranks. All analyses used $P = 0.05$ as a level of significance.

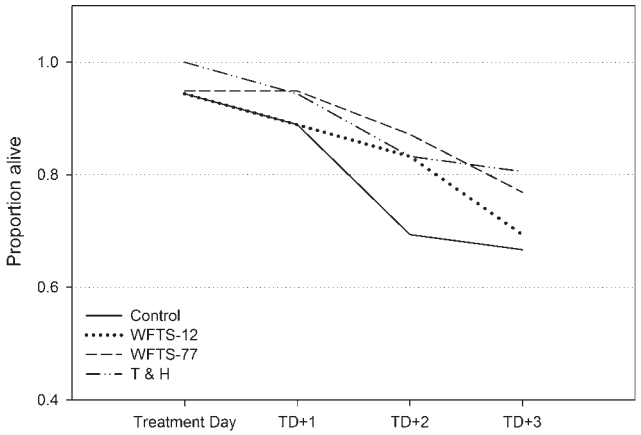

Figure 2. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. The cumulative adult fall Chinook salmon survival measured from the day of treatment (TD) until spawning (TD+3). Study groups were control, 12-m transport tube (WFTS-12), 77- m transport tube (WFTS-77), and trap and haul (T & H).

Results

Adult survival

The proportion of fish alive at the time of spawning ranged from 0.67 for the control group to 0.81 for trap and haul (Figure 2). The proportions that survived to spawning for the WFTS-12 and WFTS-77 were 0.69 and 0.77, respectively. On the day of treatment, two fish in each of the control, WFTS-12, and WFTS-77 groups died. Throughout the remainder of the study, mortality rates ranged from 0 to 7 individuals per treatment group per day. There was no significant difference in the survival of adults among treatment groups ($\chi^2 = 2.5$, $df = 3$, $P = 0.48$). Overall, travel time through the 12-m transport tube was 3 to 4 s, whereas transport through the 77 m transport tube occurred in a mean time of 13 s (range: 10–17 s).

Epithelial damage detection

In general, the proportion of fish from all groups that suffered damage to epithelial tissue was low but variable. The mean ± standard deviation (SD) proportion

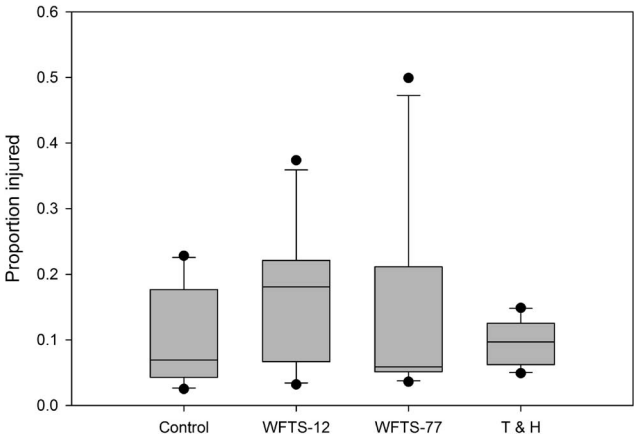

Figure 3. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. Comparison of the proportion of epithelial damage measured for each adult fall Chinook salmon among the four groups including control, 12-m transport tube (WFTS-12), 77-m transport tube (WFTS-77), and trap and haul (T & H). The horizontal line through the each box represents the median; the lower and upper boundaries of the box are the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the circles represent the 5th and 95th percentiles.

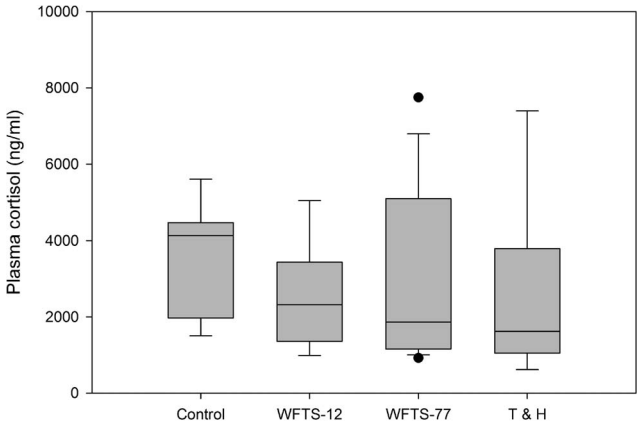

Figure 4. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. Comparison of the plasma cortisol concentration (ng/mL) among the four groups of adult fall Chinook salmon including control, 12-m transport tube (WFTS-12), 77-m transport tube (WFTS-77), and trap and haul (T & H). The horizontal line through each box represents the median; the lower and upper boundaries of the box are the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the circles represent the 5th and 95th percentiles.

of injury from the WFTS-12 (0.18 ± 0.11) and WFTS-77 (0.14 ± 0.17) groups was nearly twice as high as the mean values measured in the control (0.08 ± 0.08) and trap-and-haul (0.10 ± 0.04) groups. However, there was considerable variation within the two groups sent through the WFTS (Figure 3). As such, there was no significant difference in the measured proportions of epithelial damage among groups ($F = 2.14$; $df = 3,38$; $P = 0.11$).

Stress, reproductive readiness and immune response

The mean ± SD plasma cortisol concentration in the control group (3,554.9 ± 1,468.7 ng/mL) was 20 to 30% higher than the trap-and-haul (2,542.4 ± 2,264.2 ng/mL), WFTS-12 (2,451.0 ± 1,339.2 ng/mL), and WFTS-77 (2,827.8 ± 2,239.7 ng/mL) groups, but varied widely within all groups (Figure 4) and there were no significant differences among the groups ($F = 1.05$; $df = 3,37$; $P = 0.38$). The mean ± SD plasma Vtg concentrations in the WFTS-12 (1.6 ± 1.9 mg/mL), WFTS-77 (1.5 ± 1.3 mg/mL), and trap-and-haul (1.2 ± 1.1 mg/mL) groups were more than double that of the control group (0.6 ± 0.3 mg/mL); however, there were no significant differences ($F = 1.62$; $df = 3,59$; $P = 0.19$) among the groups, in part because of the large amount of variability observed across the groups (Figure 5). There was also considerable variability in values for *IL1*-β and *IgM* (Figure 6). The mean ± SD relative gene expression for *IL1*-β ranged from 0.003 ± 0.01 for WFTS-77 to 1.7 ± 6.1 for control fish and did not differ among the experimental groups ($\chi^2 = 3.20$, $df = 3$, $P = 0.36$). The mean ± SD values for *IgM* ranged from 0.03 ± 0.09 for the WFTS-77 to 13.8 ± 27.4 for the WFTS-12 and also did not differ among treatments ($\chi^2 = 4.15$, $df = 3$, $P = 0.24$).

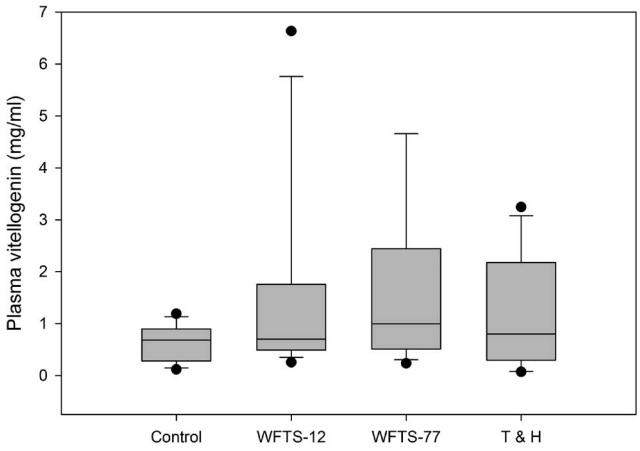

Figure 5. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. Plasma vitellogenin concentration (mg/mL) for the each of the groups of adult fall Chinook salmon including control, 12-m transport tube (WFTS-12), 77-m transport tube (WFTS-77), and trap and haul (T & H). The horizontal line through each box represents the median; the lower and upper boundaries of the box are the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the circles represent the 5th and 95th percentiles.

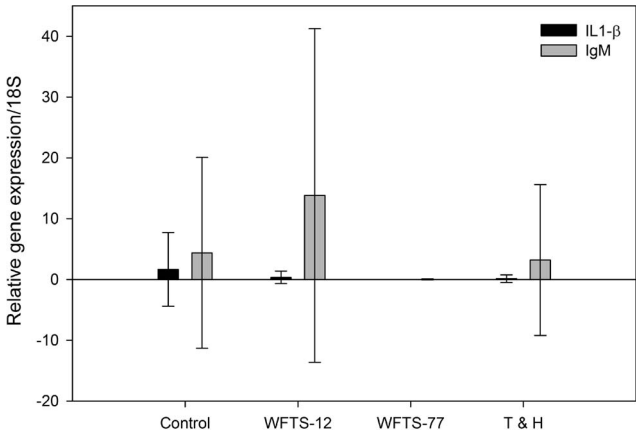

Figure 6. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. The relative gene expression for *IL1*-β and *IgM* is normalized with 18S for each of the groups of adult fall Chinook salmon including control, 12-m transport tube (WFTS-12), 77 m-transport tube (WFTS-77), and trap and haul (T & H). The bars represent the means and the whiskers represent the standard deviation of the mean.

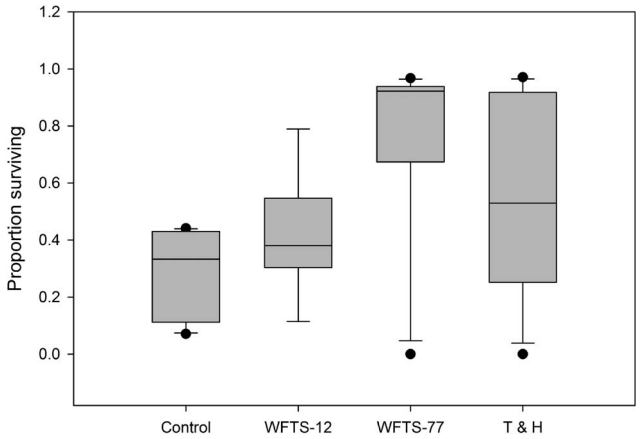

Figure 7. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. Proportion of fall Chinook salmon eggs surviving to the eyed stage for each group including control, 12-m transport tube (WFTS-12), 77-m transport tube (WFTS-77), and trap and haul (T & H). Values were based on the average survival of the three replicates for each mating pair. The horizontal line through each box represents the median; the lower and upper boundaries of the box are the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the circles represent the 5th and 95th percentiles.

Gamete viability

The mean proportion of eggs surviving to the eyed stage within the four groups were significantly different from one another ($F = 4.09$; $df = 3,33$; $P = 0.01$). The mean $\pm$ SD values ranged from 0.28 $\pm$ 0.16 for the control fish to 0.77 $\pm$ 0.31 for the WFTS-77. The variation within all the groups was high and in some cases ($n = 2$) survival for individual families was equal to zero (Figure 7).

Discussion

Taken together, our evaluation indicates that the WFTS moved prespawning adult fall Chinook salmon quickly and effectively, regardless of the transport distances included in this study (12 or 77 m). The measured physical and physiological effects of transport through the WFTS were not significantly different than those measured in fish exposed to minimal handling (control) or to standard trap-and-haul procedures. Egg survival was significantly different among the groups, with the highest survival in the eggs from females transported 77 m and lowest in the control group. These survival rates, however, were not consistent with the duration of treatment or degree of handling and did not reveal latent effects on offspring survival after transport through the WFTS. Some of our results, however, were highly variable, which may relate to the fact that the fish used in this study were from a single population sampled over a few days when they were reproductively mature and nearing a state of postspawning death.

Adult survival

Overall, the proportion of adult fall Chinook salmon alive at the end of the study that passed through the WFTS-12 (0.69) and WFTS-77 (0.77) were comparable to that of the control (0.67) and trap-and-haul (0.81) treatment groups. These survival rates are lower than previous research on the WFTS; Mesa et al. (2013) reported no injuries or mortalities of adult rainbow trout that traversed 15 m through an earlier version of the WFTS. Differences in the sexual maturity of fish in this study may account for the lower survival rates compared to previous tests with the WFTS. The adult fall Chinook salmon used in the current study were sexually mature at the time of testing. In comparison, the rainbow trout used by Mesa et al. (2013) were not sexually mature at the time of testing and did not have the added stress associated with semelparity, which may have contributed to the higher survival rates (Donaldson and Fagerlund 1968).

Overall, the survival rates of adult fall Chinook salmon observed in the current study are comparable to previous investigations into the effects of trap and haul. For example, Keefer et al. (2010) found that total prespawning mortality for adult spring Chinook salmon that were captured, transported, and out-planted above barrier dams in the Willamette River, Oregon, was 48%, ranging from 0 to 93% for individual release groups with mortality rates strongly correlated with fish condition and water temperature. Comparatively, Al-Chokhachy et al. (2014) reported survival rates of 98.3 to 100% for a variety of anadromous salmonid species; however, specific information on the individual fish characteristics and environmental conditions encompassed in this analysis is unknown.

Epithelial damage detection

Adult fall Chinook salmon that were transported through the WFTS in this study did not have significantly different proportions of epithelial damage when compared with fish that were handled using standard trap-and-haul procedures, or those that served as controls. The epithelial layer of fish serves as an important barrier to pathogens, ultraviolet light, and desiccation (Sheppard 1994). Damage to this layer may weaken the barrier, increasing the susceptibility of fish to infections (Ventura and Grizzle 1987; Svendsen and Bøgwald 1997; Van West 2006). For example, *Saprolegnia* spp., one of the most notable pathogens for adult salmon, infects its fish hosts via damage to the epithelial layer, beginning around the head or fins. These infections can then spread quickly over the entire body of the fish, ultimately resulting in mortality (Van West 2006). Due to the advanced state of maturation of fish included in this study and *Saprolegnia* spp. infections on some individuals prior to testing, it is unknown how the epithelial damage detected posttreatment may have influenced the infection and mortality rates. However, similar rates in mortality and epithelial damage among treatments suggest that transport through the WFTS did not increase the susceptibility of infection for adult fall Chinook salmon.

Stress, reproduction readiness, and immune response

We evaluated the expression of two genes that represent the potential for innate and adaptive immune responses and could be compared between treatments for differential expression. Immunoglobulin M is an antibody produced by B cells in response to antigen exposure and general activation of the adaptive immune system. Interleukin-1 beta β is a cytokine protein involved in rapid response to inflammation that could result from injury (Zhu et al. 2013). Tissue immune gene expression may provide information regarding physiological response to injury that requires tissue repair (Whyte 2007), such as abrasions or cuts that might occur during transport through the WFTS or by netting and handling during the trap-and-haul procedure, and response to secondary infections. We found no significant differences in immune gene expression among the treatment and control groups, which is consistent with there being no significant difference in the average proportion of epithelial damage among treatment groups in this study. In general, the proportions of injured fish were low ($\leq$0.16), which is surprising given their advanced state of maturation where even minor injuries can rapidly lead to widespread fungal (e.g. *Saprolegnia*) infestation and tissue necrosis (Van West 2006). The lack of an immune system response may indicate a lack of injuries sufficient to stimulate an inflammatory response (*IL1-* β), or may be a result of the high levels of cortisol suppressing the immune response due to senescence (Wendelaar Bonga 1997; Tort 2011). It is also important to note that the adaptive immune response marker was measured 2 to 3 d posttreatment and the upregulation of antibodies in fish, such as *IgM*, has been demonstrated to occur more than 3 d postexposure to antigens as a result of infections or inflammation (Magnadottir 2010).

We measured plasma cortisol, a steroid hormone released into the blood stream in response to stress (Barton 2002), in females as an indicator of the stress associated with the WFTS and trap-and-haul treatments. Similar to results found by Mesa et al. (2013) for rainbow trout, there were no significant differences seen in plasma cortisol among treatments. However, plasma cortisol levels measured in this study were approximately 100 times higher than those reported by Mesa et al. (2013) 0 to 24 h posttreatment for rainbow trout and roughly 10 times higher than those reported for migrating sockeye and pink (*Oncorhynchus gorbuscha*) salmon (Cook et al. 2011; Flores et al. 2012). It is possible that these elevated cortisol levels may be a result of Chinook salmon approaching senescence because cortisol is known to increase during the period of sexual maturation, spawning, and death in salmonids (Donaldson and Fagerlund 1968; Kubokawa et al. 1999; Jefferies et al. 2011; Baker and Vynne 2014). In addition, cortisol has been shown to be negatively correlated with fitness for pink salmon when measured on the spawning grounds (Cook et al. 2011). Further, Mesa et al. (2013) examined the effects of the WFTS on rainbow trout, an iteroparous species, whereas semelparous fish, such as Chinook salmon like those in this study, have been found to have higher cortisol concentrations, which is thought to facilitate postspawning death (Barry et al. 2001). Finally, the fish in this study were not anesthetized prior to transport treatment, which also may have contributed to the elevated levels of cortisol. In contrast, Mesa et al. (2013) anesthetized the adult rainbow trout before transport. They suggested that because anesthesia is a common practice at many fish-sorting facilities, this method would provide an effective test of the device. This is probably not the case at facilities that handle or pass large numbers of fish in a short time period and we were interested in determining how nonanesthetized fish would respond to WFTS transport in such applications.

Plasma Vtg is a yolk precursor lipoprotein that is synthesized in the liver and transported by the blood to developing eggs where it is cleaved into phosvitin and lipovittelin (high in omega-3 phospholipids) that serve as food for developing embryos (Wiegand 1996). Plasma Vtg levels decline as the fish approaches reproductive maturity and the eggs are fully developed and ready for fertilization (Ueda et al. 1984). Stress during certain stages of maturation has been found to delay or accelerate the maturation process (as reviewed by Schreck et al. 2001). Similar plasma Vtg concentrations among the groups studied here may be an indication that all fish were at a similar maturation level and that stress encountered from handling and/or treatment did

not have an effect on accelerating or delaying the maturation process.

Gamete viability

Gamete survival differed significantly among treatment and control fish, but it is not clear if these differences were biologically meaningful. We observed the highest survival in the WFTS-77 and trap-and-haul treatments, which seems counterintuitive because these two treatments involved the greatest amount of handling or time that the salmon were dewatered. One explanation for the counterintuitive result is that we collected gametes from only 6–11 pairs of spawners from each group. Clearly, larger sample sizes would have added additional statistical power to our study in order to detect meaningful differences had they existed. Another explanation is related to the excessive amount of handling of the fish that was required to complete the study. Additional handling or dewatering time could potentially elevate stress levels for these treatment groups, and although there were no significant differences in cortisol levels among the treatment groups, the highest mean $\pm$ SD concentrations occurred in the control fish (3,555 $\pm$ 1,469 ng/ml), while the lowest concentrations were found in the WFTS-12 group (2,451 $\pm$ 1,339 ng/ml). Increased maternal stress prior to spawning can lead to higher levels of cortisol in freshly ovulated eggs, but the effects on embryo viability are equivocal (Campbell et al. 1992; Stratholt et al. 1997; Eriksen et al. 2006). For example, Campbell et al. (1992) and Eriksen et al. (2006) reported lower survival rates for progeny from stressed rainbow trout and Atlantic salmon (*Salmo salar*), respectively, compared to unstressed adult fish, whereas Stratholt et al. (1997) found no difference in embryo survival for coho salmon (*Oncorhynchus kisutch*) from stressed and unstressed adults despite the fact that cortisol concentrations were significantly higher in the eggs from stressed females.

Increased levels of cortisol can also affect gamete quality by mediating other endocrine pathways such as estradiol (Pottinger and Pickering 1990) and Vtg (Ding et al. 1993; Campbell et al. 1994). We did not measure estradiol in this study, and although the differences were not significant, the mean $\pm$ SD plasma Vtg concentrations in the control fish (0.63 $\pm$ 0.34 mg/mL) were less than half of those found in the other treatment groups ($\leq$1.21 $\pm$ 1.07 mg/mL). Whether this reflects stress related suppression of Vtg or the fact that this group of fish was at a more advanced state of maturation is unclear because the effects of stress on synthesis of Vtg are highly variable (Ding et al. 1993, 1994; Campbell et al. 1994; Lethimonier et al. 2000; Berg et al. 2004; Schwindt et al. 2007). However, if these lower levels of Vtg in the control fish were indicative of more advanced maturation, it could partly explain the lower egg survival in this group because the dates the fish were spawned did not necessarily coincide with the time of optimum ripeness and free release of gametes, but were also influenced by the timing of study, which was water temperature dependent: the salmon were not captured until the river water fell below 15°C to reduce the likelihood of prespawning mortality.

Future research

Currently the WFTS is being proposed as an alternative to traditional fish passage routes over hydropower dams (e.g., fish ladders, elevators, or trap and haul). The development of a cost-effective and adaptable system to facilitate upstream passage at hydropower structures could have substantial benefits for affected fish populations and potentially expand opportunities for new hydropower systems globally. Ideally, such a system should minimize stress and injury for fish and have no long-term impact on survival, growth, or reproduction. The results of this study, combined with the earlier work of Mesa et al. (2013), suggest the WFTS is a promising fish passage alternative and the two studies provide a baseline upon which to conduct future research. Additional topics that deserve attention include disease transmission, the practicality of the WFTS in reducing the amount and duration of handling, and a determination of whether additional physiological stress and injury occurs to fish if they are transported in the WFTS over elevations and inclines that are necessary to pass fish upstream over moderate- to high-head dams.

The transmission of disease as fish pass through the WFTS has been raised as a potential issue by management agencies (D. Bambrick, National Marine Fisheries Service, personal communication) but has not been studied to date. Run-of-river fish may carry pathogens and deposit them in the tube due to the direct contact during passage. Fish with compromised immune systems or damaged epithelial layers, whether due to transport or previous injuries, may be more prone to infections, which can have sublethal and lethal consequences (Cooke and Sneddon 2007). For example, senescent adult salmon may be more susceptible to disease transfer as they depress natural immune defense mechanisms as energy stores are catabolized to fuel reproduction (Mommsen et al. 1999; Carruth et al. 2000). Future research should consider rates of disease transmission and options for cleaning or disinfecting the conduit to minimize disease transmission.

Volitional entry and the ability of the WFTS to pass multiple species of varied sizes may enable the benefits of the WFTS to provide a lower-cost alternative to traditional fish ladders while reducing the labor associated with trap-and-haul efforts. A volitional entry system has been incorporated into a WFTS used at Roza Dam on the Yakima River, Washington, and at Buckley Dam on the White River, Washington. Although Whooshh Innovations has indicated they are working on a system to identify, sort, and pass a variety of fish sizes and species (V. Bryan, Whooshh Innovations, personal communication), to our knowledge such as system has not been tested. This will be important for widespread adoption

because passage systems deployed in natural river settings may be required to handle a variety of sizes of fish (both within and among species). For example, in the Columbia River basin, adult Chinook salmon, sockeye salmon, and steelhead (*Oncorhynchus mykiss*) may all be migrating upstream at the same time and range widely in size and body shape.

Although our evaluation was conducted on a WFTS with no elevation gain (i.e., 0° angle), the system has successfully demonstrated the ability to move fish over a change in elevation of approximately 100 m and a maximum incline of around 40° (Whoosh Innovations 2016 http://www.whooshh.com/specifications1.html, May 2016). Rainbow trout were safely transported through a WFTS set at a 45° angle over about 3 m (Mesa et al. 2013). To our knowledge, however, no other studies have assessed physiological response of fish to passing vertical changes in elevation of 100 m or inclines of 40 to 45°. Further study on the physiological effects on fish transported over elevations differences approaching 100 m may be warranted if there will be an application to passing fish over hydropower dams.

Supplemental Material

Reference S1. Chokhachy R, Sorel M, Beauchamp D, Clark C, Lowery E. 2014. Development of new information to inform fish passage decisions at the Yale and Merwin hydro projects on the Lewis River: a review of existing data to anadromous fish reintroduction, collection and transport of anadromous fish above hydropower/dam facilities. Annual report prepared for PacifiCorp, Portland, Oregon. Archived in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4g1n8

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Table A1. The results of a fall Chinook salmon (*Oncorhynchus tshawytscha*) passage and handling study conducted in November, 2014, at the Priest Rapids Salmon Hatchery, Mattawa, Washington. We gave each adult salmon a passive integrated transponder (PIT) tag with a unique identification code (column: PIT tag number). We determined their gender (column: Sex with M = male and F = female). We measured fork length in centimeters (column: Length), body circumference measured in centimeters (column: Circumference), and body weight in kilograms (column: Weight). We divided fish into one of four treatment groups (column: Treatment): Whoosh Fish Transport System (WFTS 77-m tube (WFTS-77), WFTS 12-m tube (WFTS-12), trap and haul (T&H), or control. Treatment dates (column: Treatment date) ranged from November 4 to 6, 2014, and mortality dates (column: Mortality date) ranged from November 4 to 11, 2014. We collected the following information on a subset of fish—column: spleen *IL1*-β normalized with 18s ($n = 65$); column: Spleen *IgM* normalized with 18s ($n = 65$); column: Cortisol concentration (ng/mL) ($n = 41$); column: Day 3 vitellogenin concentration (mg/mL) ($n = 63$); and column: Percentage of epithelial injury (%) ($n = 42$). In all columns, NR represents no data were recorded. Available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4g1n8

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Table A2. The gamete survival results from a study on the effects of passage and handling conducted in 2014 with adult fall Chinook salmon (*Oncorhynchus tshawytscha*) that were captured at Priest Rapids Salmon Hatchery, Mattawa, Washington. Each fish was given a Passive Integrated Transponder (PIT) tag with a unique identification code (column: PIT tag number). Fish were divided into one of four treatment groups (column: Treatment) – Whoosh Fish Transport System (WFTS) 77-m tube (WFTS-77), WFTS 12-m tube (WFTS-12), trap and haul (T&H), or control. Their gender was determined (column: Sex with M = male and F = female). One female's eggs were fertilized with sperm from one male (column: Pair number) for a total of 37 pairings (i.e., sib family). The fertilized eggs were divided into three subsamples per family (approximately 100 eggs per subsample) and randomly assigned to a stack, tray, and cell in vertical flow incubators. Fertilized eggs were held in incubation trays at ~10°C until they reached the eyed egg stage (December 17, 2014, or approximately 40 d postfertilization), at which time the number of live and dead eggs were counted to determine gamete survival by cell (columns labeled 1st cell survival, 2nd cell survival, and 3rd cell survival, all percentages). Data analysis was done on the average embryo survival to the eyed stage (subsamples were averaged per sib family) (column: Average survival %). Available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4g1n8

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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