Articles

Physical, Physiological, and Reproductive Effects on Adult Fall Chinook Salmon Due to Passage Through a Novel Fish Transport System

***David R. Geist, Alison H. Colotelo, Timothy J. Linley, Katie A. Wagner, Ann L. Miracle**

D.R. Geist, A.H. Colotelo, T.J. Linley, K.A. Wagner, A.L. Miracle

Earth Systems Science Division, Pacific Northwest National Laboratory, Richland, Washington, 99352

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. A total of $N = 147$ adult fall Chinook salmon (*Oncorhynchus tshawytscha*) nearing maturation were apportioned 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 their injury, stress, and immune system responses and reproductive function were compared 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 post-treatment 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

Received: November 5, 2015; Accepted: July 13, 2016; Published Online Early: July 2016;
Published: xxx

Citation: Geist DR, Colotelo AH, Linley TJ, Wagner KA, Miracle AL. 2016. Physical, physiological, and reproductive effects on adult fall Chinook salmon due to passage through a novel fish transport system. Journal of Fish and Wildlife Management 7(2):xx-xx; e1944-687X. doi: 10.3996/102015-JFWM-108

*Corresponding author: David.Geist@pnnl.gov

Running Title: Cheat Lake Channel Catfish Age, Growth and Diet

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 pre-spawning mortality rates in adult spring Chinook Salmon (*O. tshawytscha)* out-planted above barrier dams. Further, trap and haul is not typically operated continuously which can substantially delay upstream migration. Sockeye salmon (*O. nerka*) were delayed a median of 0.4 to 8.7 days 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

77 instream barriers. The system is highly portable and consists of a flexible transport conduit
78 (hereafter tube) that utilizes differential internal air pressure to move fish. Widespread adoption
79 of this technology, however, has been hindered because of limited evaluations on how the WFTS
80 could impact fish health, particularly for populations of conservation interest. A major concern
81 for application of the WFTS for migrating adult salmon is that fish are de-watered during
82 transport through the system, which can potentially cause epithelial injury, increase stress and
83 impair behavioral and endocrine functions that are important for reproductive success (Goetz
84 1983; Schreck et al. 2001). Mesa et al. (2013) evaluated a prototype of the WFTS to assess the
85 physiological responses in hatchery adult rainbow trout (*O. mykiss*) and showed that mean
86 plasma cortisol, glucose, and lactate did not differ between controls and fish transported 15 m
87 through the WFTS. In addition, none of the fish showed signs of injuries such as descaling,
88 abrasions or loss of mucous (Mesa et al. 2013). The authors noted, however, that important
89 questions for widespread acceptance of the device would be to explore the transport
90 effectiveness was well as the short term (injury) and long term (reproductive viability of
91 gametes) effects on fish moved over longer distances of tens to hundreds of meters (Mesa et al.
92 2013).

93 Improvements in the design of the WFTS since the original study (e.g., optimizing
94 internal pressure configuration) have allowed for transport of fish over longer distances (~ 80 m),
95 but the effects on physiological responses and injury at these distances have not been examined.
96 In addition, the WFTS has not been compared to traditional active transport techniques, such as
97 trap and haul. In this study, we compared the effects of transport distance within the WFTS
98 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

Male and female adult fall Chinook salmon in a pre-spawning condition were collected 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 (WDFW). The hatchery is located in eastern Washington State at approximately river kilometer (rkm) 639 on the Columbia River. Fish collected in the trap are predominantly of hatchery origin and only hatchery origin fish were used in this study. After capture at the trap, the salmon were loaded into fish transport trucks using an Archimedes pump, driven approximately 0.8 km, and transferred by pipe into a concrete raceway measuring 30 m long, 3 m wide, and 1 m deep. Fish were prevented 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 hour.

Prior to treatment, the salmon were crowded into a confined area and individual fish were netted from the raceway into a buffered anesthetic solution (100 mg/L MS-222) where they were held until they reached stage 4 anesthesia (loss of equilibrium; Summerfelt and Smith 1990). Once fish were anesthetized, their fork length (FL), wet weight, and maximum circumference were measured and recorded. A preliminary assessment of the external condition of the fish was

121 also conducted to document eye or nose damage, and any abrasions, lacerations, cuts, or scars on
122 the body. Fish of the appropriate size and condition were tagged with a passive integrated
123 transponder (PIT) tag injected into the dorsal sinus to identify individual fish throughout the
124 study. Following tagging, fish were returned to the raceway to recover from anesthesia.

125 **Experimental treatment**

126 The movement of fish was conducted November 4-6, 2014. Adult fall Chinook salmon
127 were randomly assigned to one of four groups which consisted of a control and three treatments
128 (Table 1). Control fish ($N = 36$) were netted from the holding raceway into anesthesia (35 ppm
129 Aqui-S 20E, Aqui-S New Zealand LTD, New Zealand). Once fish reached stage 2 anesthesia
130 (Summerfelt and Smith 1990) they were either examined for macroscopic injuries using
131 fluorescein ($N = 12$) or placed in the holding raceway to recover ($N = 24$). Rubber mesh carry
132 totes were used to transfer fish among the anesthesia and fluorescein dye stations and the
133 raceway.

134 Fish used in the WFTS treatment groups were randomly lifted by hand from the raceway,
135 scanned for a PIT tag and placed into the WFTS one at a time, in groups of 3 to 5; fish were not
136 anesthetized when they transported through the tube. The WFTS consisted of an accelerator
137 (pump-house), pump, generator, hanger system, and two lengths of tubing (12 m, hereafter
138 WFTS-12, or 77 m, hereafter WFTS-77) that could be interchanged with the accelerator
139 depending on the treatment (Figure 1A). The diameters of both the WFTS-12 and WFTS-77
140 tubes were sized for salmon approximately 7-14 kg in mass (maximum circumference = 48-60
141 cm); proper tube diameter relative to the fish circumference ensures optimal system pressures are
142 created to move fish through the tube. The accelerator of the WFTS was placed in the end of the
143 raceway where the fish were crowded. The opening to the accelerator was positioned so that fish

144 could be moved directly from the water by hand into the accelerator where they were transported
145 through the WFTS-12 or WFTS-77 tube into a separate raceway filled to a water depth of
146 approximately 1 m. While the slope for both tubes was essentially zero (i.e., flat with no
147 elevation gain), the layout of the two tubes was different. The 12-m tube went straight from the
148 fish holding raceway to the fish exit raceway while the 77-m tube followed a racetrack
149 configuration with four corners (Figure 1B). After exiting the WFTS, the salmon were crowded,
150 netted, and placed into an anesthetic bath (35 ppm Aqui-S 20E) where they were either examined
151 for macroscopic injuries using fluorescein or returned to the holding raceway. A total of $N = 36$
152 and $N = 39$ adult salmon were sent through the WFTS-12 and WFTS-77, respectively (Table 1).

153 Salmon used for the trap and haul treatment ($N = 36$; Table 1) were crowded in the
154 raceway and individually placed into a tote (volume = 850 L) using a polypropylene transfer
155 sleeve; fish were not anesthetized when they were placed into the tote. The tote with fish ($N = 6$-
156 11) was then lifted with a fork lift and poured into the holding tank of the fish transport truck.
157 Fish were held in the truck for 30 to 45 min after which they were transferred by pipe into a
158 raceway, crowded, and then netted into an anesthetic bath (35 ppm Aqui-S 20E). Once fish
159 reached stage 2 anesthesia (Summerfelt and Smith 1990) they were either examined for
160 macroscopic injuries using fluorescein ($N = 10$) or placed in the holding raceway to recover ($N =$
161 26).

162 **Epithelial damage detection**

163 Fluorescein dye (Fluorescein, Disodium Salt; Aldon Corp., Avon, New York) was used
164 on a subsample of fish to assess latent injuries to epithelial tissue (Table 1). Fluorescein binds to
165 hemoglobin where epithelial damage has occurred and can be detected with ultraviolet (UV)
166 light to quantify the amount of injury (Colotelo and Cooke 2011). Following treatment,

anesthetized fish (35 ppm Aqui-S 20E) were placed in a 0.2 mg/mL solution of aerated fluorescein for 6 min. They were then transferred to an aerated anesthetic rinse bath (35 ppm Aqui-S 20E) for 6 min. After the rinse bath, fish were photographed on both sides under 254 nm UV light using a digital SLR Nikon D200 camera (Nikon Inc., Millville, New York). Fresh water was flushed over the gills during photography.

Photographs were evaluated using Image-J software (http://rsb.info.nih.gov/ij//, January 2015; National Institute of Health, Bethesda, Maryland), which was 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. The damaged tissue pixel classification algorithm was implemented 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 median Otsu threshold calculated from the extensive damage image set. The proportion of epithelial damage on each side of the fish was calculated by dividing the total number of fluorescein-stained pixels by the total number of pixels encompassed by the fish. The proportion of epithelial damage for the left and right sides were then summed and used for statistical analysis (Colotelo and Cooke 2011).

Stress, reproductive readiness and immune response

Cortisol and vitellogenin (Vtg) were measured in plasma and immunoglobulin M (*IgM*) and interleukin-1 beta (*IL1-β*) gene expression were measured in spleen tissue collected from adult female fall Chinook salmon at the time of spawning (November 7-8, 2014). Blood samples were collected 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, the fish were euthanized to collect spleen tissue samples. Blood samples were placed on ice and spleen samples in RNA later® (Ambion Inc., Foster City, California) for transport to the lab. Upon arrival, the blood samples were centrifuged at 3,000 rpm for 15 min to separate the plasma and stored at -80°C until analysis.

Plasma cortisol concentrations were measured from un-extracted female plasma using competitive plasma cortisol expression enzyme immunoassay (EIA) following manufacturer's protocol (Cayman Chemical Company, Ann Arbor, Michigan). Plasma Vtg was measured using an EIA following manufacturer's protocol (Biosense Laboratories, Bergen, Norway). Differential expression of *IgM* and *IL1- β* in spleen ribonucleic acid (RNA) was determined using semi-quantitative polymerase chain reaction (qPCR; Freeman et al. 1999). Total RNA was isolated from spleen tissue (Ambion TriReagent, Austin, Texas), and relative concentrations were determined by ultraviolet spectrophotometry (GENE SYS 10). Complementary DNA (cDNA) to the spleen RNA template was prepared using a High Capacity cDNA Reverse Transcription Kit and a GeneAMP® PCR system 9700 (Applied Biosystems, Foster City, California). Primers for amplification were designed using PrimerQuest (IDT, Coralville, Iowa) (*IL1-β*: F 5' AGCAGGGTTCAGCAGTACATCACA 3', R 5' ATCAGGACCCAGCACTTGTTCTCA 3'; *IgM heavy chain*: F 5'

GTGACCCTGACTTGCTACGTCAAA 3', R 5' GCTCATCGTTAACAAGCCAAGCCA 3') using Chinook salmon sequence. Briefly, 1 µg of total spleen RNA was reverse transcribed with 50 µM random hexamers. One tenth of the cDNA was used 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. Cycling was carried out 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. The cDNA expression levels for all samples were normalized to expression levels for 18S, using Ambion's Quantum RNA™ primers. DNA targets (*IL1-β* and *IgM*) were previously confirmed by comparing DNA sequences (sequencing performed by Agencourt, Danvers, Massachusetts) with known fish sequences for identity using the BLAST algorithm (NCBI, NIH). The resulting DNA sequence information was compared with known sequences for identity using BLAST (basic local alignment search tool) algorithm (http://blast.ncbi.nlm.nih.gov/, October 2012) and both target sequences were confirmed with >98% nucleotide match to other salmonid species.

Gamete viability

Males and females that were sexually mature at the time of post-treatment blood sampling were euthanized and stripped of eggs and sperm, which were placed in coolers on ice and returned to the Aquatic Research Laboratory (ARL) at the Pacific Northwest National Laboratory (PNNL), Richland, Washington. At the ARL, eggs and sperm from individual fish were mixed 1:1 within treatment groups to form a total of $N = 37$ full sib families. The fertilized eggs were divided into three sub-samples per family (approximately 100 eggs per sub-sample) and randomly assigned to a stack, tray and cell in vertical flow incubators. The incubation trays were modified with perforated PVC sheet to create 12 individual cells per tray and $N = 144$ total

cells ($N = 2$ stacks with $N = 5$ or 7 trays per stack and $N = 12$ cells per tray). Fertilized eggs were held in incubation trays at ~10°C until they reached the eyed egg stage (December 17, 2014, or approximately 40 days post-fertilization), at which time the number of live and dead eggs were counted to determine survival.

Data analysis

Differences among groups in post-treatment adult survival to spawning were determined by Chi-square analysis (Zar 1984). The average embryo survival to the eyed stage (subsamples were averaged per family) and proportion of adult salmon that experienced epithelial injury were both assessed by one-way analysis of variance (ANOVA) after arcsine transformations to meet the assumptions for normality and equal variances. Plasma cortisol and Vtg were also analyzed 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 were tested by Kruskal-Wallis analysis of variance by ranks. All analyses used $P = 0.05$ as a level of significance.

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 proportion that survived to spawning for the WFTS-12 and WFTS-77 was 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 was variable. The mean (± standard deviation) proportion 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 (± standard deviation) 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) 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 (± standard deviation) 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 (± standard deviation) 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 (± standard deviation) 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, $P = 0.24$).

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 (± standard deviation) values ranged from 0.28 ± 0.16 for the control fish to 0.77 ± 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 pre-spawning 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 are from a single population sampled over a few days when they were reproductively mature and nearing a state of post-spawning 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, e.g., 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 pre-spawning 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 post-treatment 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 (*IgM*) is an antibody produced by B cells in response to antigen exposure and general activation of the adaptive immune system. Interleukin-1 beta (*IL1-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 (≤ 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 two to three days post-treatment and the upregulation of antibodies in fish, such as *IgM*, has been demonstrated to occur more than three days post exposure to antigens as a result of infections or inflammation (Magnadottir 2010).

Plasma cortisol, a steroid hormone released into the blood stream in response to stress (Barton 2002), was measured 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 hours post-treatment for rainbow trout and roughly 10 times higher than those reported for migrating sockeye and pink (*O. 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 post-spawning 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 non-anesthetized 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. The highest survival was observed 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 is 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 (± standard deviation) concentrations occurred in the control fish (3,555 ± 1,469 ng/ml), while the lowest concentrations were found in the WFTS-12 group (2,451 ± 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, both 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 (*O. 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 (± standard deviation) plasma Vtg concentrations in the control fish (0.63 ± 0.34 mg/ml) were less than half of those found in the other treatment groups (≤ 1.21 ± 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, i.e., the salmon were not captured until the river water fell below 15 °C to reduce the likelihood of pre-spawning 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

436 additional physiological stress and injury occurs to fish if they are transported in the WFTS over
437 elevations and inclines that are necessary to pass fish upstream over moderate to high head dams.

438 The transmission of disease as fish pass through the WFTS has been raised as a potential
439 issue by management agencies (Dale Bambrick, National Marine Fisheries Service, personal
440 communication with DRG, May 14, 2014) but has not been studied to date. Run-of-river fish
441 may carry pathogens and deposit them in the tube due to the direct contact during passage. Fish
442 with compromised immune systems or damaged epithelial layers, whether due to transport or
443 previous injuries, may be more prone to infections which can have sub-lethal and lethal
444 consequences (Cooke and Sneddon 2007). For example, senescent adult salmon may be more
445 susceptible to disease transfer as they depress natural immune defense mechanisms as energy
446 stores are catabolized to fuel reproduction (Mommsen et al. 1999; Carruth et al. 2000). Future
447 research should consider rates of disease transmission and options for cleaning or disinfecting
448 the conduit to minimize disease transmission.

449 Volitional entry and the ability of the WFTS to pass multiple species of varied sizes may
450 enable the benefits of the WFTS to provide a lower cost alternative to traditional fish ladders
451 while reducing the labor associated with trap and haul efforts. A volitional entry system has
452 been incorporated into a WFTS used at Roza Dam on the Yakima River, Washington, and at
453 Buckley Dam on the White River, Washington. Although Whooshh Innovations has indicated
454 they are working on a system to identify, sort and pass a variety of fish sizes and species
455 (Vincent Bryan, CEO, Whooshh Innovations, personal communication with DRG, April 27,
456 2015), to our knowledge such as system has not been tested. This will be important for
457 widespread adoption because passage systems deployed in natural river settings may be required
458 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 (*O. 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° (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.

Archived Material

Please note: The Journal of Fish and Wildlife Management is not responsible for the content or functionality of any archived material. Queries should be directed to the corresponding author for the article.

To cite this archived material, please cite both the journal article (formatting found in the Abstract section of this article) and the following recommended format for the archived material.

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. Each adult salmon was given a Passive Integrated Transponder (PIT) tag with a unique identification code (column: PIT tag number). Their gender was determined (column:

Sex with M = male and F = female). Fork length was measured in cm (column: Length), body circumference was measured in cm (column: Circumference), and body weight taken in kg (column: Weight). 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. Treatment dates (column: Treatment date) ranged from November 4-6, 2014 and mortality dates (column: Mortality date) ranged from November 4-11, 2014. The following information was collected 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: Percent 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

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 sub-samples per family (approximately 100 eggs per sub-sample) 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

504 reached the eyed egg stage (December 17, 2014, or approximately 40 days post-fertilization), at
505 which time the number of live and dead eggs were counted to determine gamete survival by cell
506 (columns labeled 1st cell survival, 2nd cell survival, and 3rd cell survival, all percentages). Data
507 analysis was done on the average embryo survival to the eyed stage (subsamples were averaged
508 per sib family) (column: Average survival %). Available from the Dryad Digital Repository:
509 http://dx.doi.org/10.5061/dryad.4g1n8

510 Reference A1: Chokhachy R, Sorel M, Beauchamp D, Clark C, Lowery E. 2014. Development
511 of new information to inform fish passage decisions at the Yale and Merwin Hydro projects on
512 the Lewis River: a review of existing data to anadromous fish reintroduction, collection and
513 transport of anadromous fish above hydropower/dam facilities. Annual Report Prepared for
514 PacifiCorp, Portland, OR. Available from the Dryad Digital Repository:
515 http://dx.doi.org/10.5061/dryad.4g1n8

516

517 **Acknowledgements**

518 Funding for the research described in this article was provided by the U.S. Department of
519 Energy's (DOE) Wind and Water Power Technologies Office (WWPTO). None of the authors
520 have any financial interest in the WFTS. The authors thank Hoyt Battey and Jocelyn Brown-
521 Saracino of the WWPTO for their commitment and oversight of the project; neither Mr. Battey
522 nor Ms. Brown-Saracino participated in the research or reporting of its findings. We would also
523 like to thank Whooshh Innovations, LLC including CEO Vince Bryan III and staff Jim Otten,
524 Pete Kunzler, and Ryan Johnson for their assistance in setting up and operating the Whooshh
525 Fish Transport System. The tote dumper and forklift came from Cave B Estate Winery; it was

delivered to the hatchery by Bob Olson, a retired equipment operator for Cave B. The authors acknowledge Mike Lewis and Glenn Pearson of the Washington Department of Fish and Wildlife for providing fish for this study and Grant County Public Utility District for use of their facility. Finally, this research required the assistance of many Pacific Northwest National Laboratory staff members. The authors thank Erika Cutsforth, Jill Janak, Stephanie Liss, Sean Porse, Sadie Montgomery, Bob Mueller, Megan Nims, and Vanessa Paurus of PNNL. The Pacific Northwest National Laboratory is accredited 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 for our study were approved by the Institutional Animal Care and Use Committee. The Pacific Northwest National Laboratory is operated by Battelle for the U.S. DOE under contract DE-AC05-76RL1830.

538 **Tables**

539 Table 1. The sample sizes from a study on the effects of passage and handling conducted in
540 November, 2014, with adult fall chinook salmon (*Oncorhynchus tshawytscha*) that were captured
541 at Priest Rapids Salmon Hatchery, Mattawa, Washington. The sample sizes of adult fall
542 Chinook salmon are shown for the four groups (control, 12 m transport tube [WFTS-12], 77 m
543 transport tube [WFTS-77], and trap and haul) that were assessed for epithelial damage using
544 fluorescein dye, cortisol, vitellogenin (Vtg), immunoglobin M (IgM), interleukin-1 beta (IL1-β),
545 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

546

547

Figure Captions

Figure 1. 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. Photo of the entrance to the Whooshh Fish Transport System. The accelerator (pump-house) is suspended on supports placed within the raceway where the fish were held. The two interchangeable transport tubes measured 12 m and 77 m.

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).

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 middle of each box represents the median; the lower and upper boundaries of the box are the 25^{th} and 75^{th} percentiles; the whiskers represent the 10^{th} and 90^{th} percentiles; and the circles represent the 5^{th} and 95^{th} percentile.

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 the middle of 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 percentile.

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 the middle of 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 percentile.

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 mean (± standard deviation) relative gene expression for *IL1-β* and *IgM* 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).

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 the middle of 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 percentile.

References

Al-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, OR. (Reference S1)

Baker MR, Vynne CH. 2014. Cortisol profiles in sockeye salmon: sample bias and baseline values at migration, maturation, spawning and senescence. Fisheries Research 154:38-43.

Barry TP, Unwin MJ, Malison JA, Quinn TP. 2001. Free and total cortisol levels in semelparous and iteroparous Chinook salmon. Journal of Fish Biology 59:1673-1676.

Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Berg H, Modig C, Olsson PE. 2004. 17beta-estradiol induced vitellogenesis is inhibited by cortisol at the post-transcriptional level in Arctic char (*Salvelinus alpinus*). Reproductive Biology and Endocrinology vol 2, 10 pages.

Campbell PM, Pottinger TG, Sumpter JP. 1992. Stress reduces the quality of gametes produced by rainbow trout. Biology of Reproduction 47:1140-1150.

Campbell PM, Pottinger TG, Sumpter JP. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120:151-169.

616 Carruth LL, Dores RM, Maldonado TA, Norris DO, Ruth T, Jones RE. 2000. Elevation of
617 plasma cortisol during the spawning migration of landlocked kokanee salmon
618 (*Oncorhynchus nerka kennerlyi*). Comparative Biochemistry and Physiology C 127:123-
619 131.

620 Colotelo AH, Cooke SJ. 2011. Evaluation of common angling-induced sources of epithelial
621 damage for popular freshwater sport fish using fluorescein. Fisheries Research 107:217-
622 224.

623 Cook KV, McConnachie SH, Gilmour KM, Hinch SG, Cooke SJ. 2011. Fitness and behavioral
624 correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon
625 (*Oncorhynchus gorbuscha*) upon arrival at spawning grounds. Hormones and Behavior
626 60:489-497.

627 Cooke SJ, Sneddon LU. 2007. Animal welfare perspectives on recreational angling. Applied
628 Animal Behavior Science 104:176-198.

629 Ding JL, Hee EL, Lam TJ. 1993. Two forms of vitellogenin in the plasma and gonads of male
630 tilapia (*Oreochromis aureus*). Comparative Biochemistry and Physiology 93:363-70.

631 Ding JL, Lim EH, Lam TJ. 1994. Cortisol-induced hepatic vitellogenin mRNA in *Oreochromis*
632 *aureus* (Steindachner). General Comparative Endocrinology 96:276-287.

633 Donaldson EM, Fagerlund UH. 1968. Changes in the cortisol dynamics of sockeye salmon
634 (*Oncorhynchus nerka*) resulting from sexual maturation. General Comparative
635 Endocrinology 11:552-561.

636 Eriksen MS, Bakken M, Espmark Å, Braastad BO, Salte R. 2006. Prespawning stress in farmed
637 Atlantic salmon *Salmo salar*: maternal cortisol exposure and hyperthermia during

638 embryonic development affect offspring survival, growth and incidence of
639 malformations. Journal of Fish Biology 69:114-129.

640 Flores AM, Shrimpton JM, Patterson DA, Hills JA, Cooke SJ, Yada T, Moriyama S, Hinch SG,
641 Farrell AP. 2012. Physiological and molecular endocrine changes in maturing wild
642 sockeye salmon, *Onchorhynchus nerka*, during ocean and river migration. Journal of
643 Comparative Physiology B – Biochemical Systematic and Environmental Physiology
644 182:77-90.

645 Freeman WM, Walker SJ, Vrana KE. 1999. Quantitative RT-PCR: pitfalls and potential.
646 BioTechniques 26:112-125.

647 Goetz FW. 1983. Hormonal control of oocyte final maturation and ovulation. Pages 117-170 in
648 Hoar WS, Randall DJ, Donaldson EM, editors. Fish physiology. Volume 9, part B.
649 Orlando, Florida: Academic Press.

650 Jefferies KM, Hinch SG, Donaldson MR, Gale MK, Burt JM, Thompson LA, Farrell AP,
651 Patterson DA, Miller KM. 2011. Temporal changes in blood variables during final
652 maturation and senescence in male sockeye salmon *Oncorhynchus nerka*: reduced
653 osmoregulatory ability can predict mortality. Journal of Fish Biology 79:449-465.

654 Keefer ML, Taylor GA, Garletts DF, Gauthier GA, Pierce TM, Caudill CC. 2010. Prespawn
655 mortality in adult spring Chinook salmon outplanted above barrier dams. Ecology of
656 Freshwater Fish 19:361-372.

657 Kubokawa K, Watanabe T, Yoshioka M, Iwata M. 1999. Effects of acute stress on plasma
658 cortisol, sex steroid hormone and glucose levels in male and female sockeye salmon
659 during the breeding season. Aquaculture 172:335-349.

660 Lethimonier C, Flouriot G, Valotaire Y, Kah O, Ducouret B. 2000. Transcriptional interference
661 between glucocorticoid receptor and estradiol receptor mediates the inhibitory effect of
662 cortisol on fish vitellogenesis. Biology of Reproduction 62:1763-1771.

663 Magnadottir B. 2010. Immunological control of fish diseases. Marine Biotechnology 12:361-
664 379.

665 Mesa MG, Gee LP, Weiland LK, Christiansen H.E. 2013. Physiological responses of adult
666 rainbow trout experimentally released through a unique fish conveyance device. North
667 American Journal of Fisheries Management 33:1179-1183.

668 Mommsen TP, Vijayan MM, Moon TW. 1999. Cortisol in teleosts: dynamics, mechanisms of
669 action, and metabolic regulation. Reviews in Fish Biology and Fishes 9:211-268.

670 Murauskas JG, Fryer JK, Nordlund B, Miller JL. 2014. Trapping effects and fisheries research:
671 a case study of sockeye salmon in the Wenatchee River, USA. Fisheries 39:408-414.

672 Otsu N. 1975. A threshold selection method from gray-level histograms. Automatica 11:23-27.

673 Pottinger TG, Pickering AD. 1990. The effect of cortisol administration on hepatic and plasma
674 estradiol-binding capacity in immature female rainbow trout (*Oncorhynchus mykiss*).
675 General and Comparative Endocrinology 80:264-273.

676 Schreck CB, Contreras-Sanchez W, Fitzpatrick MS. 2001. Effects of stress on fish reproduction,
677 gamete quality, and progeny. Aquaculture 197:3-24.

678 Schwindt AR, Feist GW, Schreck CB. 2007. Stress does not inhibit induced vitellogenesis in
679 juvenile rainbow trout. Environmental Biology of Fishes 80:453-463.

680 Sheppard KL. 1994. Functions for fish mucus. Reviews in Fish Biology and Fishes 4:401-429.

Stratholt ML, Donaldson EM, Liley NR. 1997. Stress induced elevation of plasma cortisol in adult female coho salmon (*Oncorhynchus kisutch*), is reflected in egg cortisol content, but does not appear to affect early development. Aquaculture 158:141-153.

Summerfelt RC, Smith LS. 1990. Anesthesia, surgery, and related techniques. Pages 213-272 in Schreck CW, Moyle PB, editors. Methods for fish biology. Bethesda, Maryland: American Fisheries Society.

Svendsen YS, Bøgwald J. 1997. Influence of artificial wound and non-intact mucus layer on mortality of Atlantic salmon (*Salmo salar* L.) following a bath challenge with *Vibrio anguillarum* and *Aeromonas salmonicida*. Fish and Shellfish Immunology 7:317-325.

Tort L. 2011. Stress and immune modulation in fish. Developmental and Comparative Immunology 35:1366-1375.

Ueda H, Hiroi O, Hara A, Yamauchi K, Nagahma Y. 1984. Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. General and Comparative Endocrinology 53:2013-211.

Van West P. 2006. *Saprolegnia parasitica*, an oomycete pathogen with a fishy appetite: new challenges for an old problem. Mycologist 20:99-104.

Ventura MT, Grizzle JM. 1987. Evaluation of portals of entry of *Aeromonas hydrophila* in channel catfish. Aquaculture 65:205-214.

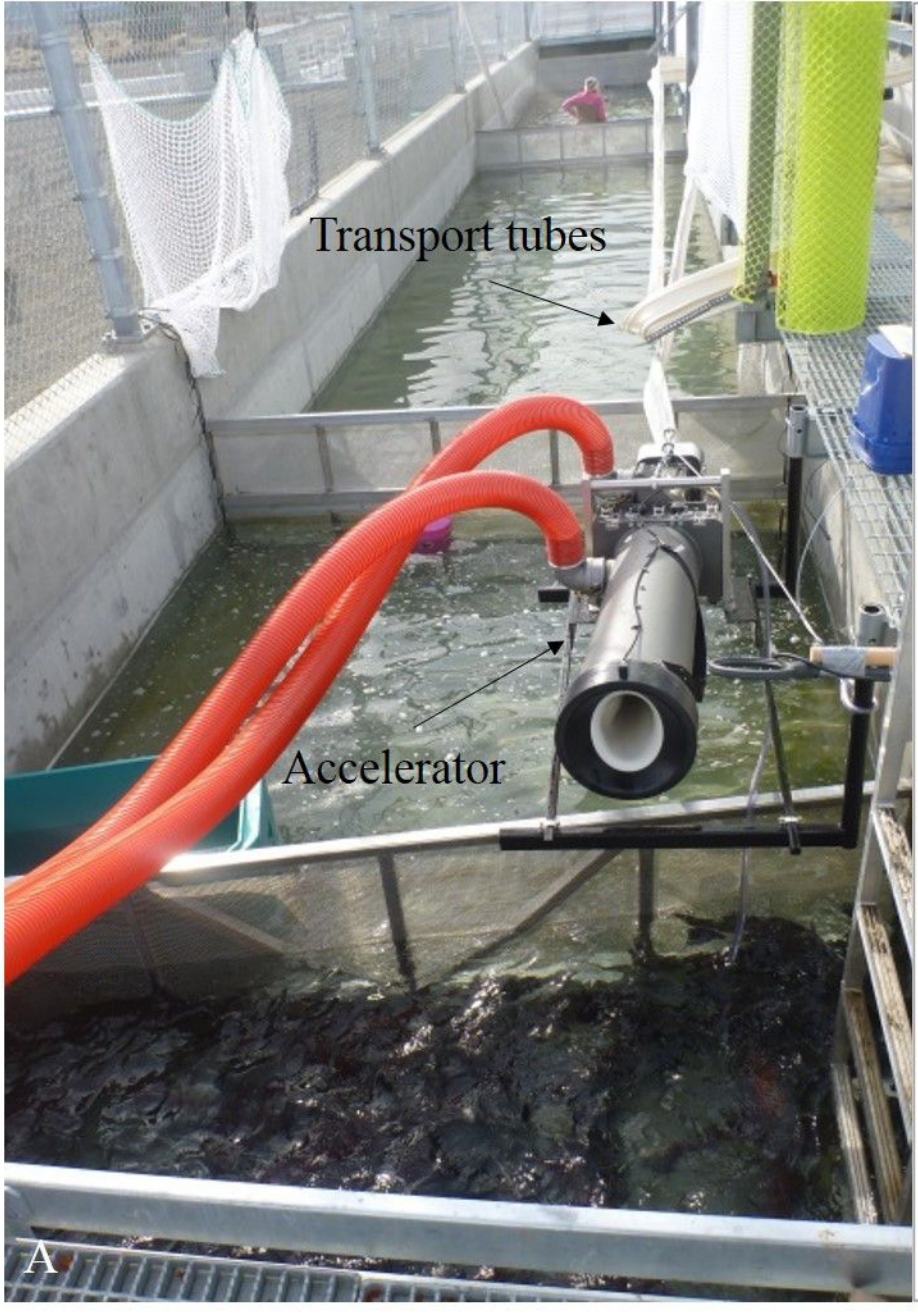
Wendelaar Bonga SE. 1997. The stress response in fish. Physiological Reviews 77:591-625.

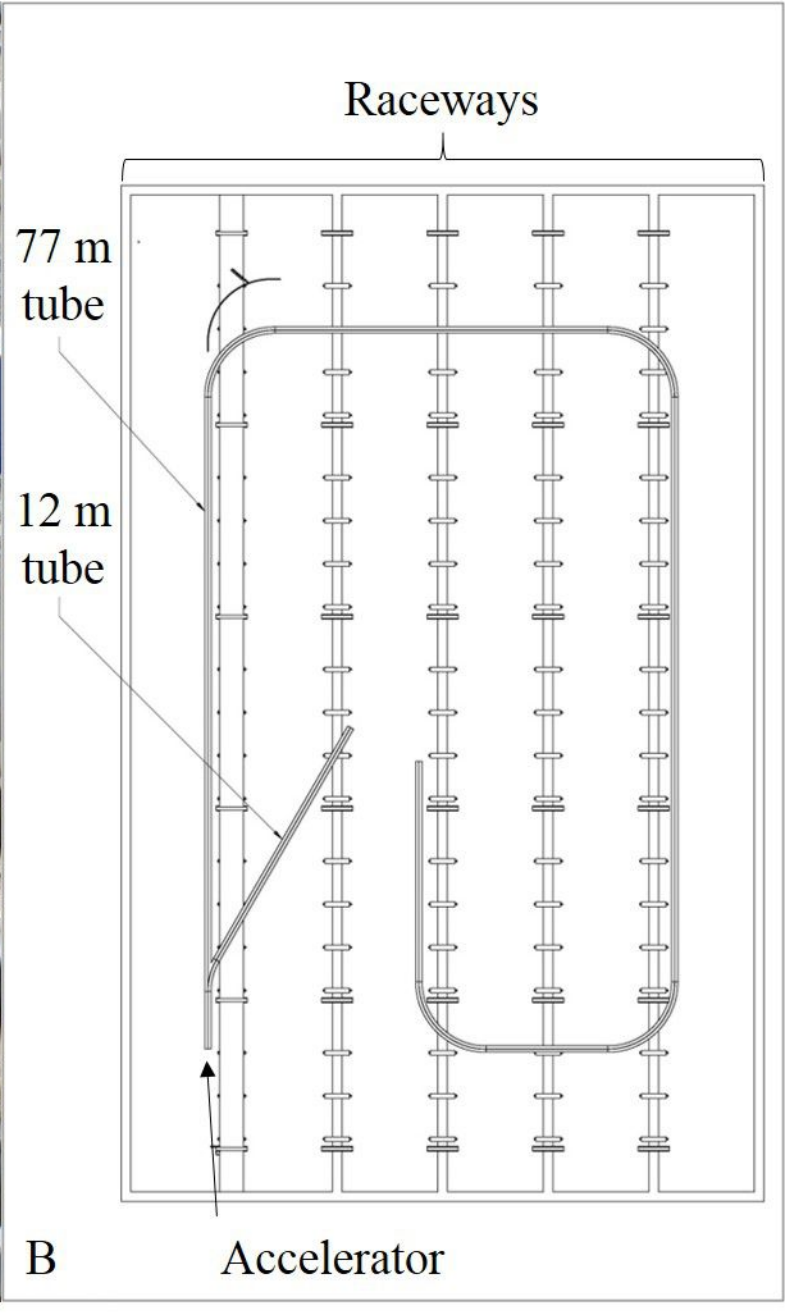
Whyte SK. 2007. The innate immune response of finfish – a review of current knowledge. Fish and Shellfish Immunology 23:1127-1151

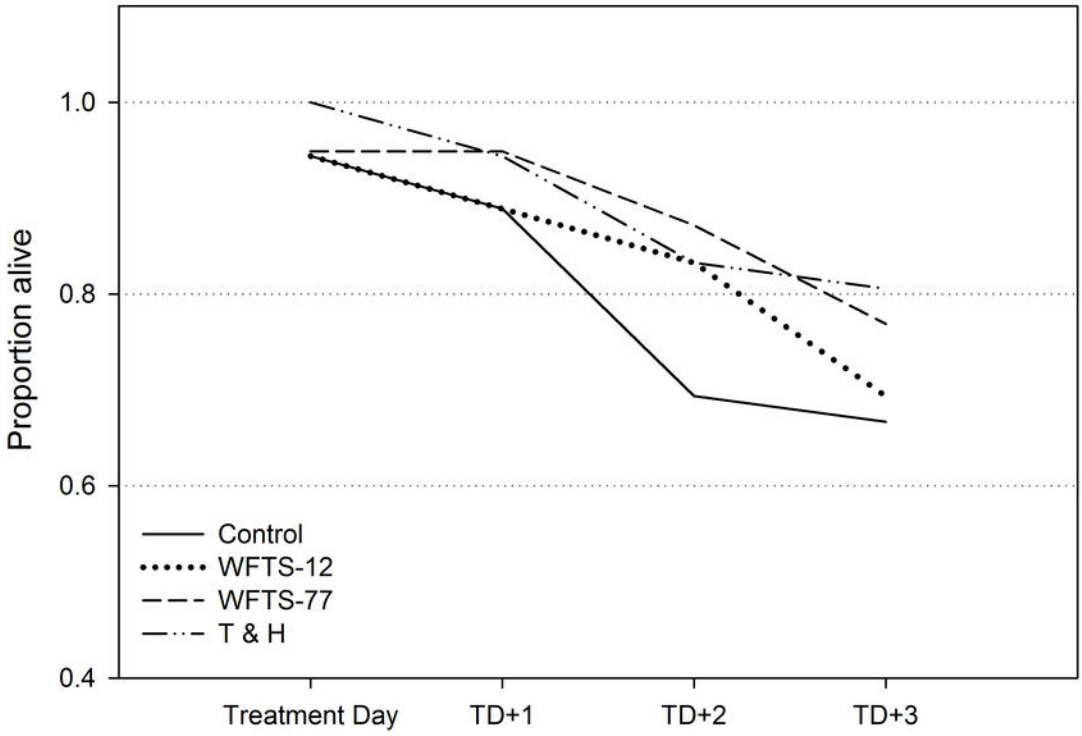
Wiegand MD. 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. Reviews in Fish Biology and Fisheries 6:259-286.

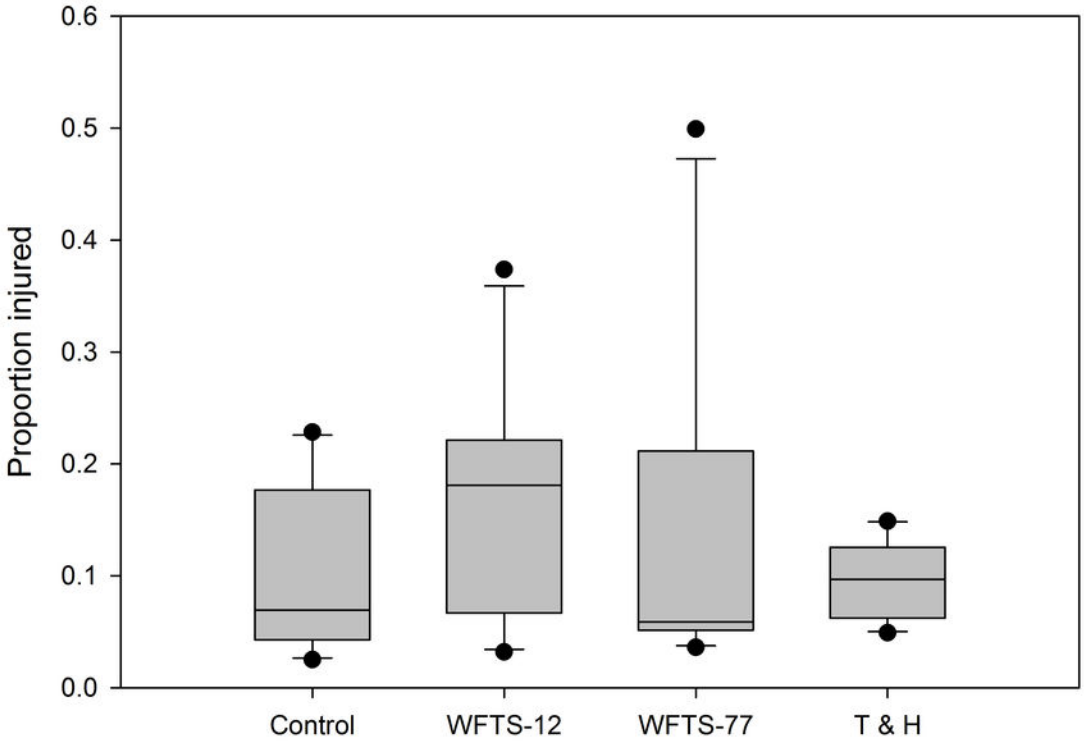
Zar JH. 1984. Biostatistical analysis. Englewood Cliffs, New Jersey: Prentice Hall.

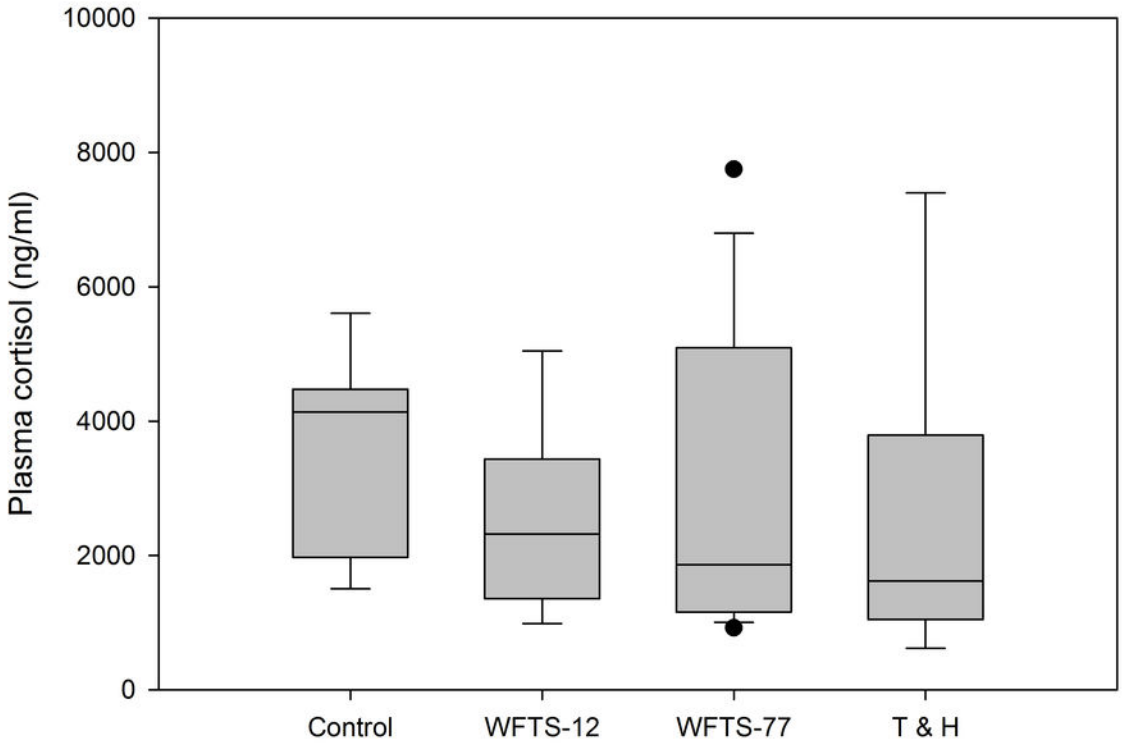
Zhu L, Nie L, Zhy G, Xiang L, Shao J. 2013. Advances in research for fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. Developmental and Comparative Immunology 39:39-62.

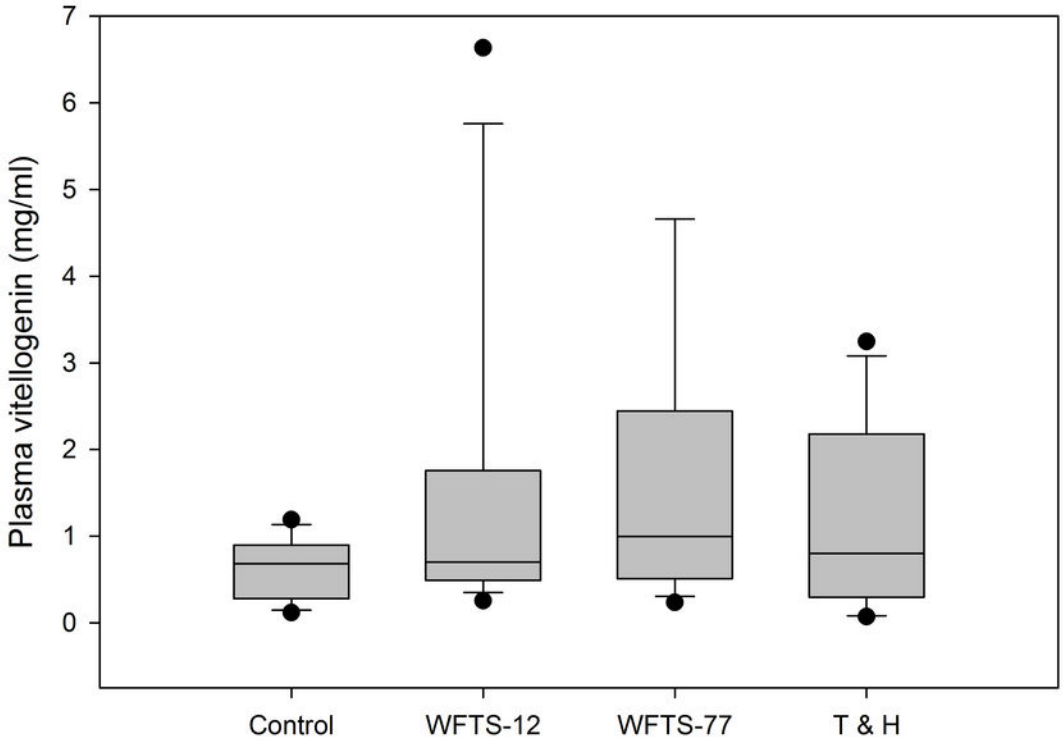

Transport tubes
Accelerator
A

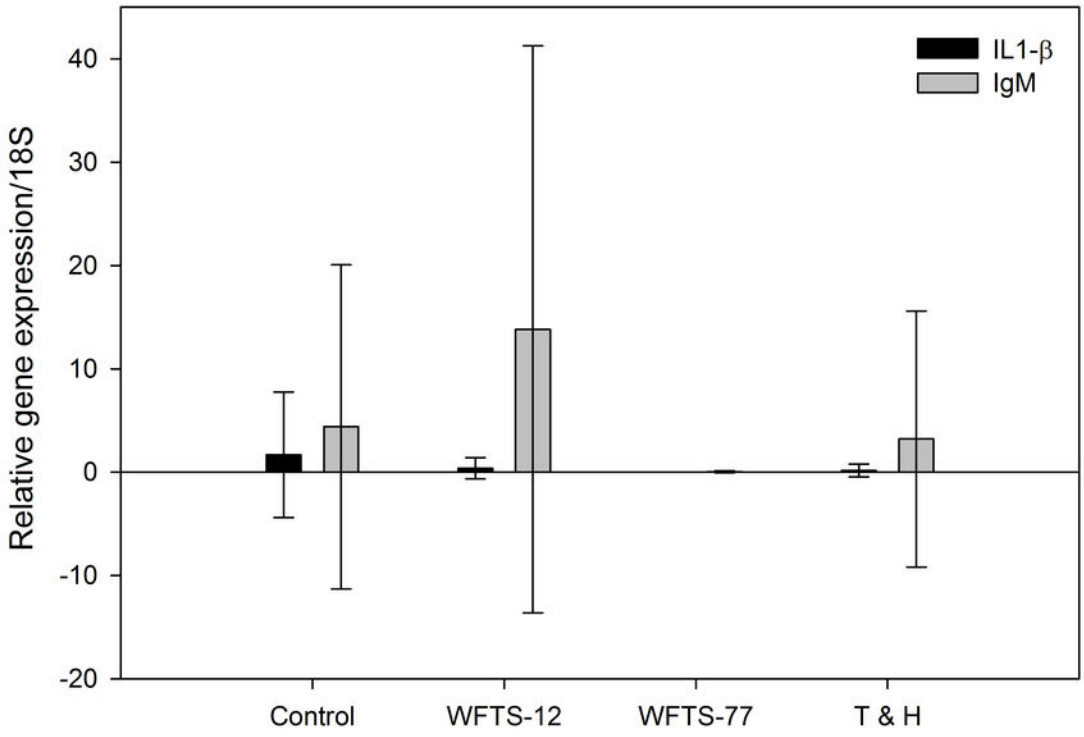

Raceways
77 m tube
12 m tube
B
Accelerator

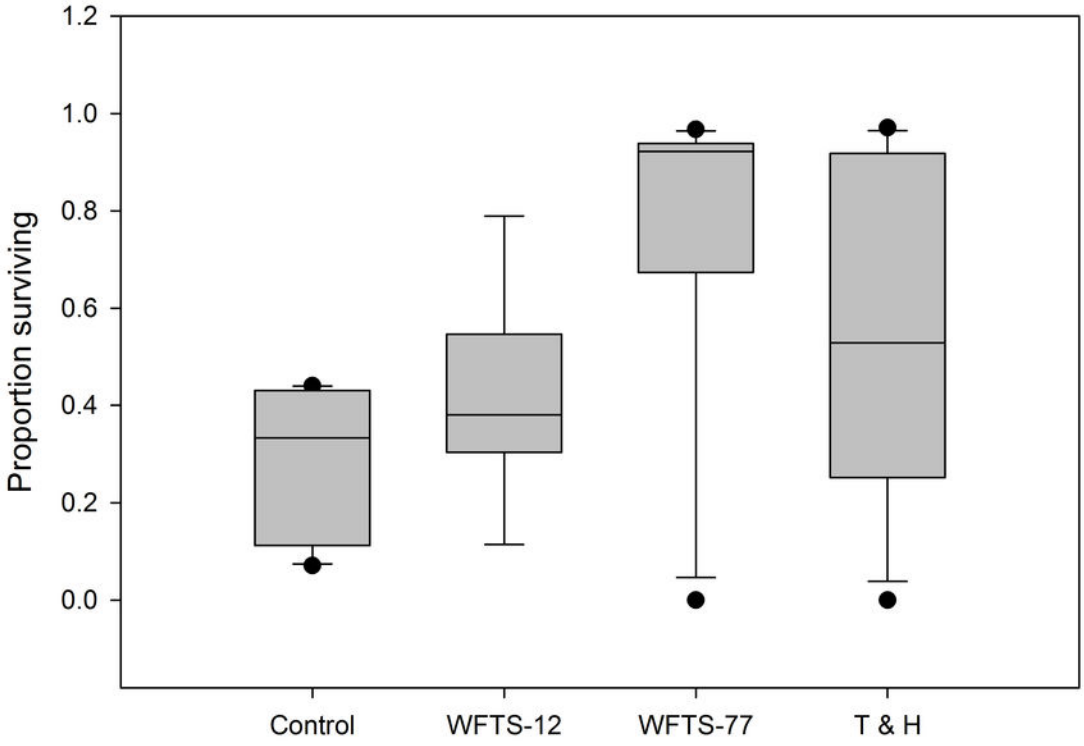

1.0
0.8
0.6
0.4
Proportion alive
Control
WFTS-12
WFTS-77
T & H
Treatment Day
TD+1
TD+2
TD+3


Proportion injured
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
WFTS-12
WFTS-77
T & H


10000
8000
6000
4000
2000
0
Plasma cortisol (ng/ml)
Control
WFTS-12
WFTS-77
T & H


Plasma vitellogenin (mg/ml)
7
6
5
4
3
2
1
0
Control
WFTS-12
WFTS-77
T & H


IL1-β
IgM
Relative gene expression/18S
40
30
20
10
0
-10
-20
Control
WFTS-12
WFTS-77
T & H


1.2
1.0
0.8
0.6
0.4
0.2
0.0
Proportion surviving
Control
WFTS-12
WFTS-77
T & H