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High-Head Bypass Fish Passage Investigations: Year One

Final Report

January 2021

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James S. Hughes

U.S. DEPARTMENT OF ENERGY

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Executive Summary

The High-Head Bypass Fish Passage Investigations at Green Peter: Year One study occurred at Green Peter Dam—a Willamette Valley Project (WVP) high-head dam—located on the Middle Santiam River, near Sweet Home, Oregon. The study had two objectives. The primary objective examined the effects (stress, injury, and survival) of downstream passage conveyances on healthy juvenile Chinook salmon (*Oncorhynchus tshawytscha*), and the secondary objective examined the effects of relocation on survival of copepod-infected juvenile Chinook salmon. To achieve the primary objective, healthy juvenile Chinook salmon surrogates were exposed to two downstream passage conveyance simulations that may be applicable for high-head dams in the WVP. The first simulated downstream fish passage at a collection facility with transport conveyance (transport simulation) and the second simulated downstream fish passage through a high-head bypass system (bypass pipe simulation). There were also control fish that did not undergo any downstream passage conveyance simulation. The transport simulation consisted of three treatments, representative of different durations fish may be held in a collection facility prior to transport to a downstream release site. Holding durations were 1 h, 12 h, and 24 h, with 24 h being the maximum amount of time fish would be held at a collection facility. The bypass pipe simulation had one treatment, as fish would not be held in a collection facility prior to downstream passage. Sub-samples of healthy fish from the four treatments and controls were analyzed for the amount of cortisol (a stress hormone) present in the blood plasma, presence of major injuries (lacerations, bulging eyes, torn operculum, etc.), and rate of survival. The goal of the primary objective was to understand if one of the two conveyances would best support achieving biological performance metrics and inform a preferred passage conveyance that could be implemented to reduce stress and mortality in healthy juvenile Chinook salmon. For the secondary objective, infected juvenile Chinook salmon surrogates were relocated from rearing facilities at Oregon State University (OSU) to Green Peter Dam. Infected fish were monitored for short-term (48 h) rate of survival post-relocation. The goal of the secondary objective was solely to understand the practicality of relocating infected fish for potential use in future studies (i.e., would they survive the stress of relocation).

Results from the primary objective indicated all healthy fish within the transport and bypass pipe treatments were stressed immediately post-treatment. Stress levels began to decrease by 3 h post-treatment and were nearing baseline stress levels by 24 h post-treatment. Fish in transport treatments simulating a longer holding duration in the collection facility (i.e., held for 12 or 24 h prior to downstream transport) were more stressed post-treatment (≤ 6 h) compared to fish in the transport treatment simulating a shorter holding duration (i.e., held for 1 h) and compared to fish in the bypass pipe treatment (regression tree analysis; $P < 0.05$). Nevertheless, stress levels of fish in all four treatments (bypass and transport) were no longer different and were nearing baseline levels by 24 h post-treatment (regression

tree analysis; $P > 0.05$), indicating fish were recovering from the stress caused by the treatments. Injury rates were greater for fish in the bypass pipe treatment compared to fish in the transport treatment simulating the 1 h holding duration (Fisher's exact test; $P < 0.012$) but were not different compared to fish in the transport treatments simulating the 12 h and 24 h holding durations. There was no difference in survival among the three transport treatments and one bypass pipe treatment (Fisher's exact test; $P = 0.18$).

Copepod-infected fish from the secondary objective experienced 100% short-term (48 h) survival post-relocation, indicating it would be practical to relocate infected fish from OSU to Green Peter Dam for future studies. As a result of the high survival rate, after the 48-h post relocation from OSU, infected fish underwent an ad hoc test for a transport treatment simulating the 1 h holding duration, a bypass pipe treatment, or were control fish. The purpose of this ad hoc test was to evaluate the effects of the treatments on infected fish survival, allowing for an additional understanding of the practicality of using infected fish for future studies. Post-treatment survival was high for infected fish in the transport and bypass pipe treatments (≥ 94%), further confirming the practicality of using infected fish for future studies.

Ultimately, results from both objectives in this study will be used to inform the U.S. Army Corps of Engineers Product Delivery Teams charged with designing alternatives for downstream juvenile fish passage at high-head dams in the WVP. The broader intent is to support management decisions on long-term measures and operations to rebuild populations of Upper Willamette River spring Chinook salmon listed as threatened under the Endangered Species Act.

Acronyms and Abbreviations

°C	degree(s) Celsius
ANOVA	analysis of variance
BiOp	Biological Opinion
BL	baseline
BP	bypass pipe
C	control
DO	dissolved oxygen
ESA	Endangered Species Act
fmsl	feet above mean sea level
FSS	floating screen structure
h	hour(s)
HHB	high-head bypass
L	liter
mg	milligram
mL	milliliter
MW	megawatt(s)
n	number
ng	nanogram
NMFS	National Marine Fisheries Service
OSU	Oregon State University
PDT	product delivery team
sec	second(s)
T	transport
USACE	U.S. Army Corps of Engineers
WVP	Willamette Valley Project

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1.0 Introduction

The Willamette River Basin, Oregon, encompasses more than 11,200 square miles (approximately 12% of the total area of the state) and holds most of the state's population, larger cities, and industries (USACE 2019). Congress authorized the U.S. Army Corps of Engineers (USACE) to construct and operate a series of 13 dams across the Willamette River Basin as part of the multiple Flood Control Acts. These 13 dams are collectively referred to as the Willamette Valley Project (WVP). The primary purpose of the WVP is to reduce the risks of flooding downstream communities during winter months although the dams provide additional benefits by storing and releasing water for hydropower generation, irrigation, water quality, and recreation (USACE 2019). However, the development and operation of the WVP has also adversely affected nationally and regionally important fish species, most notably, migratory salmonid populations (USACE 2019). The dams have blocked access to historical salmonid spawning habitat, altered river discharge patterns, affected water temperature and sediment supply, and caused mortality to migrating anadromous fish (Keefer and Caudill 2010).

Two migratory salmonid species that have been negatively affected by the WVP include the Upper Willamette River spring Chinook salmon (*Oncorhynchus tshawytscha*) and winter steelhead (*O. mykiss*). In 2005 and 2006, spring Chinook salmon and winter steelhead were listed as threatened in a final ruling under the Endangered Species Act (ESA), respectively (70 FR 37159, 71 FR 833). Subsequently, the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NMFS) issued a Willamette Biological Opinion (BiOp) regarding the operation of the WVP (NMFS 2008). The BiOp concluded spring Chinook salmon and winter steelhead populations would be jeopardized by continued operation and maintenance of the WVP. As such, a major requirement within the BiOp was to provide safe downstream fish passage alternatives for juvenile spring Chinook salmon and winter steelhead at dams in the WVP to avoid jeopardizing the populations of these two species. To achieve this goal, the USACE was required to initiate operational or structural modifications across the WVP (NMFS 2008). There was a specific focus on two high-head dams in the region, Cougar Dam and Detroit Dam, because of their connection to natural spawning habitats and inadequate safe downstream passage methods for juvenile spring Chinook salmon and winter steelhead.

The current methods for downstream juvenile fish passage at Cougar Dam are through the turbines and regulating outlets (ROs), and through the turbines, ROs, or the spillway at Detroit Dam. However, there is very little passage through these routes and when passage does occur, survival is poor (Beeman et al. 2014; Beeman and Adams 2015; Kock et al. 2015). For context, the turbines and ROs are

very deep in the water column and the spillway at Detroit Dam can only be operated during summer months when the reservoir is at full pool elevation.

To address the concern of poor survival and meet the BiOp requirements for improving downstream fish passage at Cougar and Detroit dams, specifically, the USACE is designing passage structures to implement fish collection in the reservoirs of the dams and vehicle conveyance to release the fish downstream of the dams (i.e., trap and haul). Traditionally, trap and haul methods required fish to be removed from the river, held (i.e., holding in a facility or collector) and handled (i.e., human contact or exposure to air), transported via truck, and released into the river downstream of a dam.

A Floating Screen Structure (FSS; i.e., a large floating fish collector) is being designed for placement at the upstream dam face, at the Water Temperature Control Tower, for Cougar Dam (USACE 2019). Queuing from attraction flows, fish in the reservoir would swim into the FSS when migrating downstream. Using the hydraulic flow configuration of the FSS, fish would be directed (funneled) to a chute within the FSS. Fish would then swim through a chute into a pod (i.e., a transportable holding tank). Depending on density of fish in the pod (with a maximum 0.25 cubic feet per pound; USACE 2019), the pod may be removed from the FSS and transported to the downstream release site several times daily, with a minimum of once every 24 h. A floating lid on the surface of the water in the pod will be used to minimize water loss and splashing, as well as minimize fish stress during movement and transport. For transport to the downstream release site, the pod would be hoisted out of its location in the back of the FSS and transported via a monorail to the front of FSS to be loaded onto a boat for transport to shore. A crane aboard the boat would lift the pod from the monorail onto the boat, and the boat would drive to the ramp at the shore. Again, the pod would be moved via crane from the boat to a transport truck and the truck would drive the fish to the release site downstream of the dam, where they would be released into the river via a pipe connected to the pod (USACE 2019). There will be a similar FSS design and transport for Detroit Dam. Each of the steps for the collection and transport methods at Cougar and Detroit dams are intended to minimize handling and stress for the fish.

Traditional trap and haul methods may be stressful for fish. Schreck et al. (2016) reported fish experience high levels of stress from crowding and handling in a trap and haul system, which could degrade fish health and cause high rates of mortality after release. Additionally, recent studies showed copepods (*Salmincola californiensis*)—a native freshwater parasite—have infected juvenile Chinook salmon at high rates in the Willamette River Basin and predominantly in Cougar reservoir (Monzyk et al. 2015; Herron et al. 2018). Copepods attach to salmon and can infest areas around the fins or in the branchial cavity on the gills (Monzyk et al. 2015). Branchial cavity attachments can produce respiratory problems for fish, particularly at times of oxygen or temperature stress (Kabata and Cousens 1973). A

laboratory study also indicated the presence of copepods reduced the swimming abilities of Chinook salmon; this reduction was influenced by copepod-induced gill damage (Herron et al. 2018). The percentage of surface area damage of the gills was highly correlated to the number of copepods present, and high rates of infection have been linked to lower water flow rates, as seen in hatcheries and reservoirs (Roberts et al. 2004; Monzyk et al. 2015; Herron et al. 2018). In the WVP, Chinook salmon in the reservoirs of dams, including Cougar and Detroit dams, were found to have higher infection rates than salmon rearing in streams upstream of dams. Fish in a reservoir had 52% more copepod attachments in their branchial cavities than fish in streams (Monzyk et al. 2015). Copepod infested juvenile fish were collected in an experimental floating fish collector at Cougar Dam and transported to Oregon State University (OSU); however, this effort led to an 85% mortality rate after transport (Herron et al. 2018). Copepod infections raise concerns for downstream passage survival as the increased stress from infection compounded with stress from trap and haul could lead to increased fish mortality.

Thus, alternative designs for downstream fish passage that minimize handling or transport, and therefore stress on fish, are preferred. To address the concern of stress and mortality to juvenile salmon in the collection (FSS) and transport conveyance, especially to fish infected with copepods, the USACE created a High-Head Bypass (HHB) Product Delivery Team (PDT) in 2014 to investigate operational or structural alternatives to improve downstream fish passage and survival at Cougar and Detroit dams. The HHB PDT determined the FSS would be required to attract and collect downstream migrating fish, and therefore the focus for safely passing fish at the dam after collection in the FSS would be the type of conveyance.

A potential alternative to a transport conveyance at Cougar and Detroit dams may be through bypass pipes constructed within and through the dam. Juvenile fish would be collected in the FSS, swim through a chute, and be immediately passed downstream of the dam through the bypass pipes. A bypass pipe conveyance has the potential to minimize delays in migration, eliminates the need to hold and handle fish for truck transport, and may reduce the overall stress on fish and the spread of copepods within the holding pods. Another high-head dam in the WVP, Green Peter Dam (hereafter: Green Peter), has an integrated bypass pipe system that was constructed with the dam to pass downstream migrating juvenile salmon and steelhead. Although the juvenile bypass system was successful initially at attracting and passing juvenile Chinook salmon during the first few years of operation, steelhead attraction and passage rates were very low. Anecdotal evidence suggested the system was not effective in the long term at attracting and passing juvenile salmon and steelhead in the reservoir. Additionally, the rates of adult salmon and steelhead returning to Green Peter were poor, prompting managers to decommission the bypass system. As such, the juvenile bypass system was decommissioned in 1988. However, the bypass

pipes remain intact and are available for research, making it an ideal site for the HHB PDT to perform studies to inform design of passage alternatives. Several studies have been conducted at Green Peter to evaluate direct injury and short-term (48 h) survival of juvenile salmonids after passage through the bypass pipes. For example, Normandeau Associates, Inc. (2015; 2016) conducted direct injury and short-term (48 h) survival studies of juvenile Chinook salmon and steelhead that passed through the juvenile fish bypass pipes. The authors concluded the current configuration of bypass pipes at Green Peter safely passed ≥ 96% of juvenile salmonids when the flow control valves were ≥ 50% open (Normandeau Associates, Inc. 2015; 2016). Based on the high passage survival and low injury rates, the HHB PDT determined it was beneficial to continue studies at the bypass system to investigate high-head passage alternatives and inform the design of downstream passage alternatives at Cougar and Detroit dams.

When comparing between downstream passage methods, one metric that can be used is a comparison of stress levels. Stress is a physiological response to a perceived or real threat (e.g., physical or chemical; Barton 2002; Schreck et al. 2016). When fish experience a stressor, a stress hormone—cortisol—is released into the blood as part of the initial stress response (i.e., fight-or-flight). Increased cortisol can lead to increased metabolic activity and act as an immunosuppressant (Sneddon et al. 2016). The fight-or-flight effects can be advantageous for fish trying to avoid natural stressors, such as predators; however, it can take 12 h or more for cortisol levels to return to pre-stressed levels (Strange et al. 1978). The amount of time and the magnitude of the stress response are dependent on the amount of time a stressor occurs and the severity of the stressor (Schreck et al. 2016). Traditional trap and haul methods have caused a significant increase in stress for juvenile Chinook salmon compared to control fish (Adams et al. 2018). It is likely that bypass pipe passage would also cause an increase in stress levels; however, this has not been studied. Quantifying and understanding the stress response of fish is an important consideration when comparing between downstream passage conveyances.

Evaluations of fish stress responses after exposure to downstream passage conveyances could provide a better understanding of how to best mitigate stress when finding alternative methods for downstream passage. The goal of this study was to understand if there is a preferred downstream passage conveyance that minimizes stress to healthy juvenile salmonids, and to evaluate the feasibility of relocating fish with copepod infestations. Two objectives were outlined: the primary objective evaluated healthy juvenile Chinook salmon stress, injury, and survival after exposure to one of two downstream passage conveyances, representative of transport conveyance (truck transport) or bypass pipe passage. Metrics to evaluate fish stress levels utilized cortisol as a proxy for stress. Injuries (lacerations, bulging eyes, torn operculum, etc.) and rate of survival were also recorded. The secondary objective evaluated the practicality of relocating copepod-infected juvenile Chinook salmon for future downstream passage

conveyance evaluations. For the secondary objective, the rate of survival was evaluated after relocation from the rearing facility at OSU to Green Peter, as a poor rate of survival would potentially bias results in a future study. This report will focus on the primary objective with healthy fish (stress, injury, and survival evaluations). The secondary objective (copepod-infected fish survival post-relocation) will be briefly reported and discussed after the healthy fish findings because it was a practicality objective to be used as a resource to inform potential future studies.

2.0 Methods

2.1 Study Site

Green Peter is located on the Middle Santiam River in the Willamette River Basin near Sweet Home, Oregon (Figure 2.1). Completed in 1968, it is a high-head dam that is 1020 feet above mean sea level (fmsl) with a hydraulic head of about 300 feet. The dam is equipped with two hydropower generating units with a total capacity of 80 MW. Construction of the dam included an intricate bypass system of four horizontal pipes (12-in. diameter) through the dam at varying elevations connected to a single vertical and horizontal 24-in. transportation pipe on the downstream face of the dam to pass downstream migrant juvenile salmonids to a juvenile fish evaluator (hereafter: juvenile fish collector; Figure 2.2) or directly into the river downstream of the dam. The juvenile fish collector is a facility to re-collect fish for examination before release into the river after passage through the bypass pipe. This bypass system was decommissioned in 1988 because of its poor performance of attracting and passing juvenile Chinook salmon and steelhead, and low return rates of adult fish to Green Peter, but remains mostly intact, including the juvenile fish collector. The bypass system is still suitable to serve as a research platform for investigations of passage at high-head dams that can support bioengineering design efforts at USACE projects in the WVP and elsewhere. For the purposes of this study, fish that were passed through the bypass pipe were released through the 935 fmsl elevation pipe (Figure 2.2).

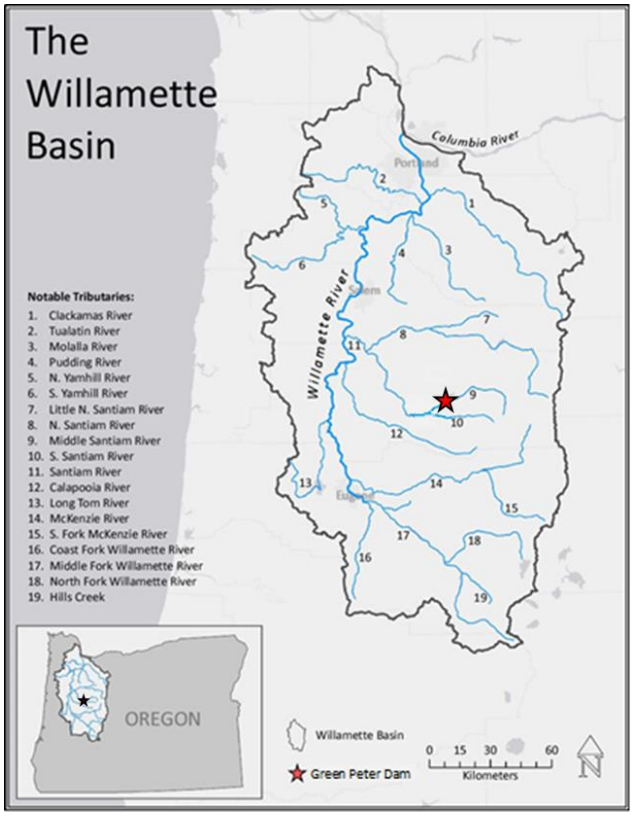


Figure 2.1. Map of the Willamette Valley Project. The red star depicts the approximate location of Green Peter Dam.

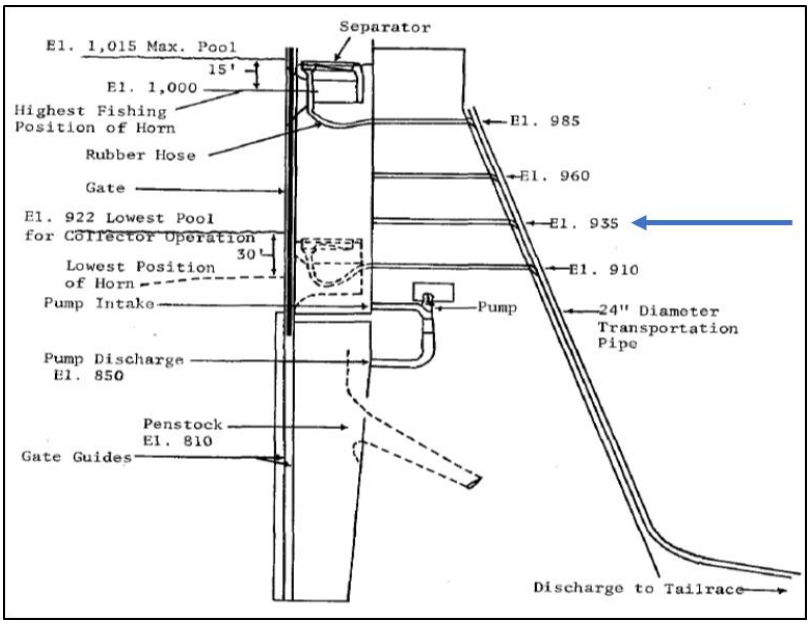


Figure 2.2. Sectional View of Green Peter Depicting the Piping for the Juvenile Bypass System. Four 12-in. horizonal bypass pipes are located at varying elevations (El.). The 24-in. transportation pipe runs vertically the length of the downstream face of the dam to the juvenile fish collector (not shown). The blue arrow points to the bypass pipe elevation used in this study (935 fmsl).

2.2 Fish Source and Holding Conditions

The OSU Wild Fish Surrogate Program provided healthy (*n* = 670) and separately copepod-infected (*n* = 200) juvenile Chinook salmon, reared to the approximate size of age-1 wild juveniles (mean: 140–160 mm in fork length). For the primary objective, two weeks prior to the start of the study healthy fish were relocated to Green Peter from the OSU facility to allow for acclimation and to recover from relocation stress. As soon as healthy fish arrived at Green Peter, they were randomly separated into 18 holding tanks of the same size and dimensions (110-gallon; semi-square with a conical bottom and a 4-in. diameter drain to facilitate fish release while minimizing injury and stress during simulations). Sixteen holding tanks held healthy fish that were in either a treatment or control group (*n* = 40 fish per tank), and two holding tanks held healthy fish used for post-relocation baseline sampling used to evaluate recovery from relocation stress (*n* = 15 fish per tank). Infected fish for the secondary objective were not relocated to Green Peter until after the healthy fish objective was completed.

Two downstream passage simulations using healthy fish were evaluated for this study: a truck transport simulation (hereafter: transport), representative of the Cougar Dam FSS transport conveyance described above, and a bypass pipe simulation. Fish within the transport simulation (treatment or control fish), and one of the holding tanks of post-relocation baseline fish, were located in the yard on the downstream side of the dam (*n* = 13 tanks; Figure 2.3). Fish for the bypass pipe simulation (as treatment or control fish), and the other holding tank of post-relocation baseline fish, were located at the top deck of Green Peter next to the upstream side of bypass system (*n* = 5 tanks; Figure 2.3). A third location of post-simulation tanks was at the juvenile fish collector approximately 0.12 km downstream of Green Peter. After transport and bypass pipe simulations, all treatment fish were recollected in one of two post-simulation tanks (hereafter: holding baskets) located in the juvenile fish collection facility. The holding baskets were 24-in. wide × 46-in. long × 24-in. tall. The lower half of the basket height consisted of continuous siding and the upper half of mesh grating allow excess water to spill over.

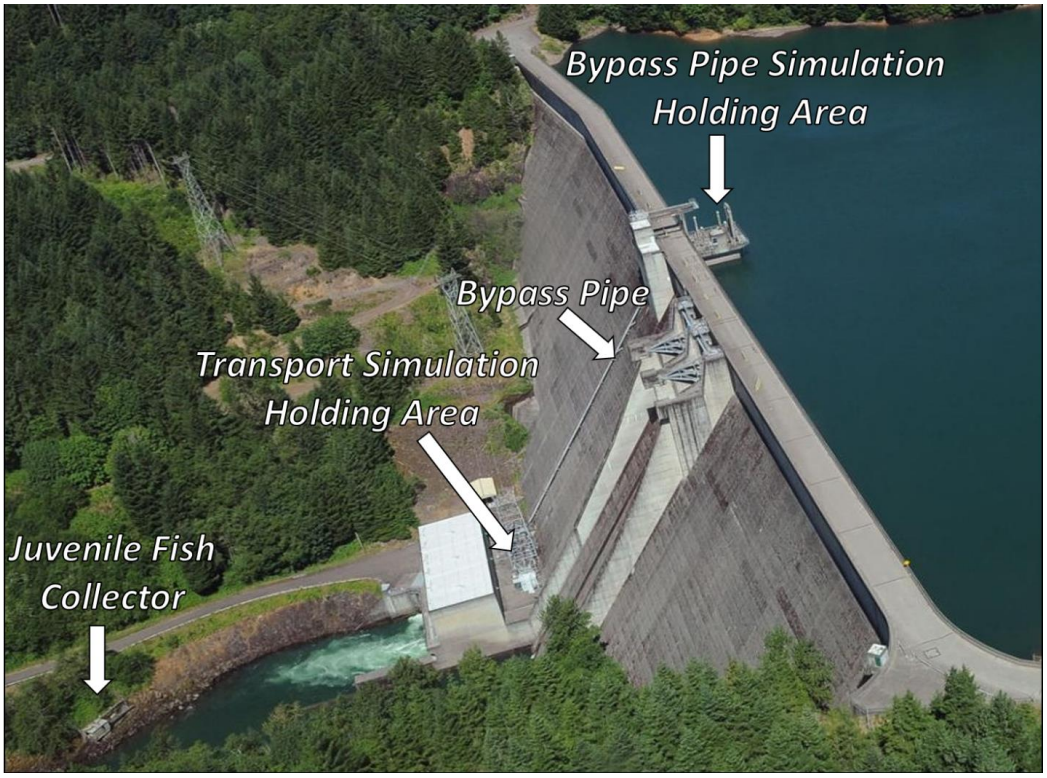


Figure 2.3. Transport Simulation Tanks were Held in the Area below Green Peter. Bypass pipe simulation tanks were held at the top deck of Green Peter for access to the bypass pipe. Fish from both simulations were recollected in the juvenile fish collector, located downstream of Green Peter. (Photo credit: Mark Ylen – Democrat Herald.)

There was no public access or road activity at the bottom of Green Peter (transport holding tanks) and tanks were exposed to more shade, as a natural artifact of location. At the top of Green Peter (bypass pipe holding tanks) a public road crosses the dam and the tanks were exposed to direct sunlight, also as a natural artifact of location. The juvenile fish collector (post-simulation holding baskets) was a covered, open air facility with no public activity and holding baskets were exposed to more shade. Transport and bypass pipe holding tanks were filled with 60 gallons of flow-through water and the holding baskets were filled with 52 gallons of flow-through water. Water for the fish tanks came from two sources, although both were from deep in the Green Peter reservoir. Transport holding tanks and holding baskets were provided with subsurface reservoir water supplied from a penstock (penstock intake elevation is 810 fmsl) and the bypass pipe holding tanks were provided with subsurface reservoir water on the upstream side of Green Peter at elevation 857 fmsl.

During the 2-week acclimation, fish were fed once daily, and the environmental conditions of the tanks were recorded (temperature and dissolved oxygen [DO]). A dip net was also introduced and gently

moved around inside the tank daily to acclimatize fish to the presence of a net and limit the potential stress response during post-treatment blood sampling.

2.3 Downstream Passage Simulations

Healthy fish were subjected to one of two downstream passage simulations (transport or bypass pipe) or a control, which did not undergo a simulation. Within the two simulations there were four treatments (Table 2.1; Figure 2.4). The transport simulation had three treatments, representative of different durations fish may be held in the pod prior to transport (simulating three different holding times in a pod on the FSS) and the bypass pipe simulation had one treatment (BP treatment) because there would be no holding (delay) of fish in a bypass system. Transport treatments included a 1-hour holding duration prior to the simulation (1 h treatment); a 12-hour holding duration (12 h treatment); and a 24-hour holding duration (24 h treatment). The amount of time fish may be held in a collection facility is variable and depends on the density of fish collected. For example, during peak migrations (high densities) fish may be held for a short duration (as short as a few minutes to a few hours) compared to the beginning or end of the migrations (low densities), when fish may be held for a long duration (maximum of 24 h). Our three holding durations were intended to represent a range of durations fish may be held in a pod before downstream transport. The holding density of fish in the pod for this study (e.g., 40 fish) represented 7.3% of the maximum density allotted in a pod in the FSS, according to the NMFS criterion of 0.25 cubic feet per pound (USACE 2019). All four treatments contained three replicates (Figure 2.4).

There were also four control groups (Figure 2.4) that did not undergo simulations. Control groups also had three replicates; each day a treatment replicate occurred, fish from an associated control replicate were also sampled for blood. A set of pre-relocation baseline blood samples (not depicted in Figure 2.4) occurred at OSU before fish were relocated to Green Peter, and a set of post-relocation baseline samples were taken at Green Peter after the 2-week acclimation. The pre- and post-relocation baseline samples provided a benchmark for comparison of fish stress levels at the OSU facility (pre-relocation stress) compared to fish stress levels after acclimation (post-relocation stress), to ensure the stress levels of relocated fish had returned to pre-relocation stress levels. Green Peter post-relocation baseline fish (n = 5) were sampled on the same day as the first simulation for each downstream passage scenario (i.e., one post-relocation baseline replicate for bypass pipe and one post-relocation baseline replicate for transport, representative of all three transport treatments).

Table 2.1. Replicates and Sample Sizes at each Post-Simulation Blood Sampling Time for the Transport and Bypass Pipe Simulations. Abbreviations are OSU = Oregon State University; BL = Baseline; BP = Bypass Pipe; BPC = Bypass Pipe Control; T = Transport; TC = Transport Control.

Group	Replicate Identification	Pre-Simulation	Sampling Times (h post-simulation; *n*)						Total
			0	0.5	1	3	6	24	
OSU Pre-Relocation Baseline (BL)	1-OSU_BL	5	—	—		—	—	—	5
	Total – OSU Baseline	5							5
		Bypass Pipe							
Bypass Pipe (BP) Post-Relocation Baseline (BL)	BP_BL	5	—	—	—	—	—	—	5
	Total – Bypass Pipe Baseline	5							5
Bypass Pipe Treatment	1-BP		8	5	5	6	6	5	35
	2-BP		6	5	5	5	5	5	31
	3-BP		5	6	5	5	5	5	31
	Total – Bypass Pipe		19	16	15	16	16	15	97
Bypass Pipe Controls	1-BPC	6	—	—	—	—	—	—	6
	2-BPC	5	—	—	—	—	—	—	5
	3-BPC	5	—	—	—	—	—	—	5
	Total – Bypass Pipe Controls	16							16
		Transport							
Transport (T) Post-Relocation Baseline	T_BL	5	—	—	—	—	—	—	5
	Total – Transport Baseline	5							5
1-hour hold Treatment	1-T_1 h		6	5	5	5	5	5	31
	2-T_1 h		5	5	5	5	5	6	31
	3-T_1 h		7	5	5	5	5	6	33
12-hour hold Treatment	4-T_12 h		6	5	6	5	5	5	32
	5-T_12 h		5	5	5	5	5	6	31
	6-T_12 h		6	7	6	5	5	6	35
24-hour hold Treatment	7-T_24 h		6	5	5	5	5	5	31
	8-T_24 h		7	7	5	5	5	5	34
	9-T_24 h		5	6	5	6	6	5	33
	Total – Transport Treatment		53	50	47	46	46	49	291
1-hour hold Controls	1-TC_1 h	5	—	—	—	—	—	—	5
	2-TC_1 h	5	—	—	—	—	—	—	5
	3-TC_1 h	5	—	—	—	—	—	—	5
12-hour hold Controls	4-TC_12 h	8	—	—	—	—	—	—	8
	5-TC_12 h	5	—	—	—	—	—	—	5
	6-TC_12 h	6	—	—	—	—	—	—	6
24-hour hold Controls	7-TC_24 h	5	—	—	—	—	—	—	5
	8-TC_24 h	5	—	—	—	—	—	—	5
	9-TC_24 h	5	—	—	—	—	—	—	5
	Total – Transport Controls	49							49
	Grand Total	**80**	**72**	**66**	**62**	**62**	**62**	**64**	**468**

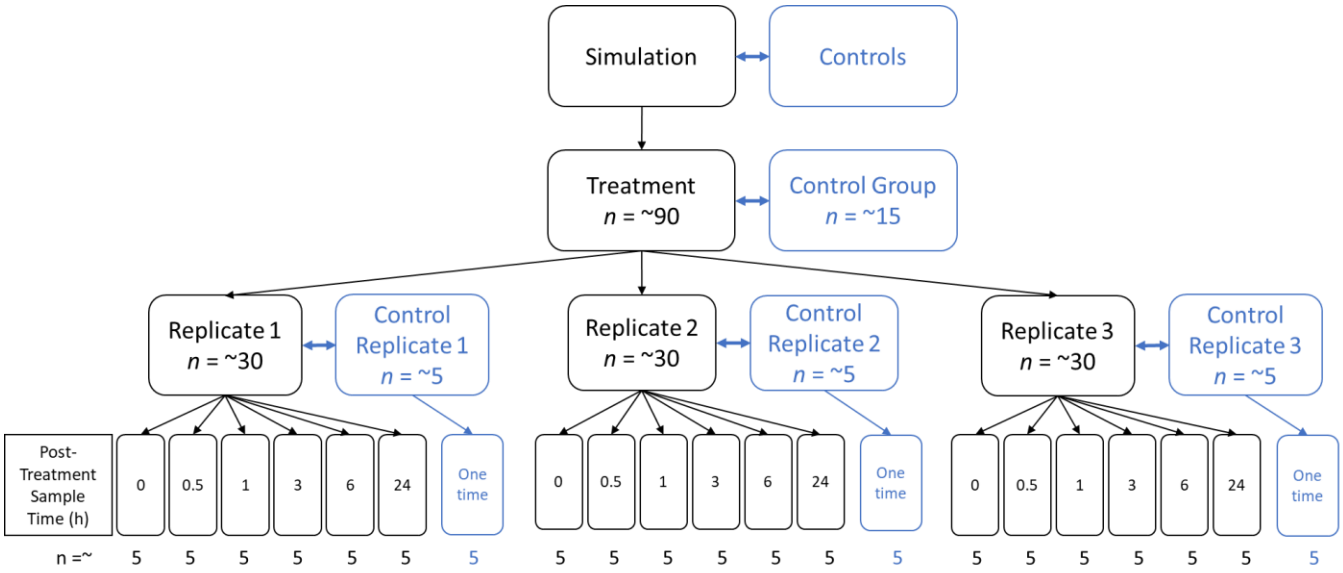


Figure 2.4. Study Design Example of One Treatment for Fish Undergoing a Transport or Bypass Pipe Simulation (black outline) and One Associated Control Group (blue outline). The study design was repeated for a total of four treatments (i.e., three for transport, representative of different holding periods [1 h, 12 h, and 24 h], and one for bypass pipe) and four associated control groups. Each treatment and its associated control group had three replicates. Within each treatment replicate, subsamples of fish (n ~ 5) were taken at six post-treatment sample times for blood collection. Subsamples from control fish replicates had only one sample time for blood collection, taken on the same day as the associated treatment replicate.

The two simulations were designed to induce alternative stressors that simulated real-world stressors experienced by juvenile salmonids. Real-world stressors were identified through collaboration with the USACE using planned passage methods for WVP high-head dams (e.g., Cougar and Detroit dams). Green Peter lacked the infrastructure to perform all the real-world stressors for transport and bypass pipe passage, resulting in the need for alternatives. As such, a collaboration with the USACE and OSU allowed for identification and minimization of stressors to the fish that would not be associated with the real-world or simulation stressors (i.e., human-induced stressors). Each real-world stressor corresponded with an alternative simulation stressor (Table 2.2 and Table 2.3 for transport and bypass pipe, respectively), except for the first two stressors for both simulations. Stressor 1, Relocation to Green Peter, would not be experienced by real-world populations as fish would be free-swimming in the river. Subsequently, the 2-week acclimation period (Stressor 2) was implemented to mitigate for the stress of relocation, so fish cortisol concentrations could return to pre-stress levels before undergoing simulations.

Table 2.2. Side-by-Side Comparison of Real World and Simulation Stressors for the Transport Simulation for Healthy Juvenile Chinook Salmon

Real World Stressors	Simulation Stressors
1. —	1. Relocate fish from OSU to Green Peter
2. —	2. Acclimate fish for 2 weeks
3. Fish enter floating screen structure (FSS)	3. Forklift lifts tank
4. Fish undergo chute passage from FSS into holding pod	4. Fish pass through flex pipe from holding tank into test pod
5. Fish held in pod for 0–24 h	5. Fish held in test pod for 1 h, 12 h, or 24 h
6. Floating lid applied to pod, monorail transports pod from FSS to boat (~15 min)	6. Floating lid applied to test pod, forklift drives pod for ~15 min
7. Crane lifts pod from monorail onto boat	7. Forklift lifts test pod onto truck
8. Boat drives pod to boat ramp (~13 min)	8. Truck drives test pod for ~13 min
9. Pod moved from boat to truck	9. Forklift removes/replaces test pod on truck
10. Truck drives pod to release site (~15 min)	10. Truck drives test pod for ~15 min
11. Pipe attached to pod; fish released into the river	11. Flex hose attached to test pod; fish released into juvenile fish collector; blood sampling occurs

Table 2.3. Side-by-Side Comparison of Real World and Simulation Stressors for the Bypass Pipe Simulation for Healthy Juvenile Chinook Salmon

Real World Stressors	Simulation Stressors
1. —	1. Relocate fish from OSU to Green Peter
2. —	2. Acclimate fish for 2 weeks
3. Fish enter floating screen structure (FSS)	3. Pull center standpipe in the holding tank
4. Fish undergo chute passage from FSS into bypass pipe	4. Fish pass through flex pipe from holding tank into bypass pipe
5. Bypass pipe passage	5. Bypass pipe passage
6. Fish released into the river	6. Fish released into the juvenile fish collector; blood sampling occurs

2.3.1 Transport Simulation

The transport simulation for this study imitated the collection (FSS) and truck transport design for Cougar Dam and was comprised of three treatments (1 h, 12 h, and 24 h) and each treatment consisted of three replicates paired with three control replicates (Table 2.1). There was one transport post-relocation baseline at Green Peter (T_BL) sample to represent all three transport treatments (Table 2.1).

Collaboration with the USACE and OSU identified eight potential real-world stressors that juvenile salmonids may experience during transport, eleven simulation stressors to mimic real-world stressors, and methods to minimize human-induced stress from the three additional stressors of the simulation (Table 2.2 and Figure 2.5). Transport treatment tanks were anchored to wooden pallets for ease of forklift access to conduct transport simulation stressors. Real-world fish transport would involve a transportable holding tank (i.e., pod) to hold the fish once collected in the reservoir of a high-head dam. In our study, a separate tank (same dimension as the transport and bypass pipe holding tanks) with a removable lid (i.e., floating lid) was used to simulate the pod (hereafter: test pod).

The first alternative simulation stressor that had a real-world stressor associated with it was Stressor 3 (Table 2.2 and Figure 2.5). Fish undergoing transport would be collected in an FSS followed by a chute passage (Stressors 3 and 4) that would direct fish into the pod. In our study, fish were held in the test pod for 1, 12, or 24 h depending on treatment (Stressor 5). After the holding duration, a floating lid was placed on top of the water in the test pod to reduce splashing and loss of water during transport and minimize stress to the fish. The test pod was driven on a forklift for approximately 15 minutes to simulate the monorail trip on the FSS to the transport boat next to the FSS(Stressor 6). The test pod was forklifted onto a truck bed (Stressor 7), where it was secured before driving for approximately 13 minutes to simulate the amount of time it would take the boat to reach the boat ramp upstream of the dam (Stressor 8), during which time the water temperature and DO in the pod were monitored. If necessary, supplemental oxygen was added to the test pod during transport to maintain starting DO levels. After the 13-minute drive time, the forklift removed the test pod and subsequently replaced it on the truck to simulate the transition (hoisting) of the test pod from the boat to a transport truck (Stressor 9). Fish were driven again, this time for approximately 15 minutes (Stressor 10), to simulate the truck driving time from the top of the dam to the release site below the dam. At the end of the drive time, the truck stopped on the road adjacent to and at a higher elevation than the juvenile fish collector. A 4-in. diameter flex pipe was connected to the test pod and ran from the truck to the release location approximately 100 ft downhill to the entrance of the juvenile fish collector flume. The flume was located where the bypass pipe emptied into the juvenile fish collector. The center standpipe of the pod was removed, and fish were released into the juvenile collector into holding baskets (Stressor 11), simulating the pipe release fish would experience from a truck into the river in the real world. Study fish remained in the holding baskets for 24 h for post-treatment blood sampling. The simulation, excluding the test pod holding (i.e., Stressor 5), took approximately 60 minutes. All transport driving times noted above were obtained from the Cougar Downstream Fish Passage Design Documentation Report (USACE 2019).

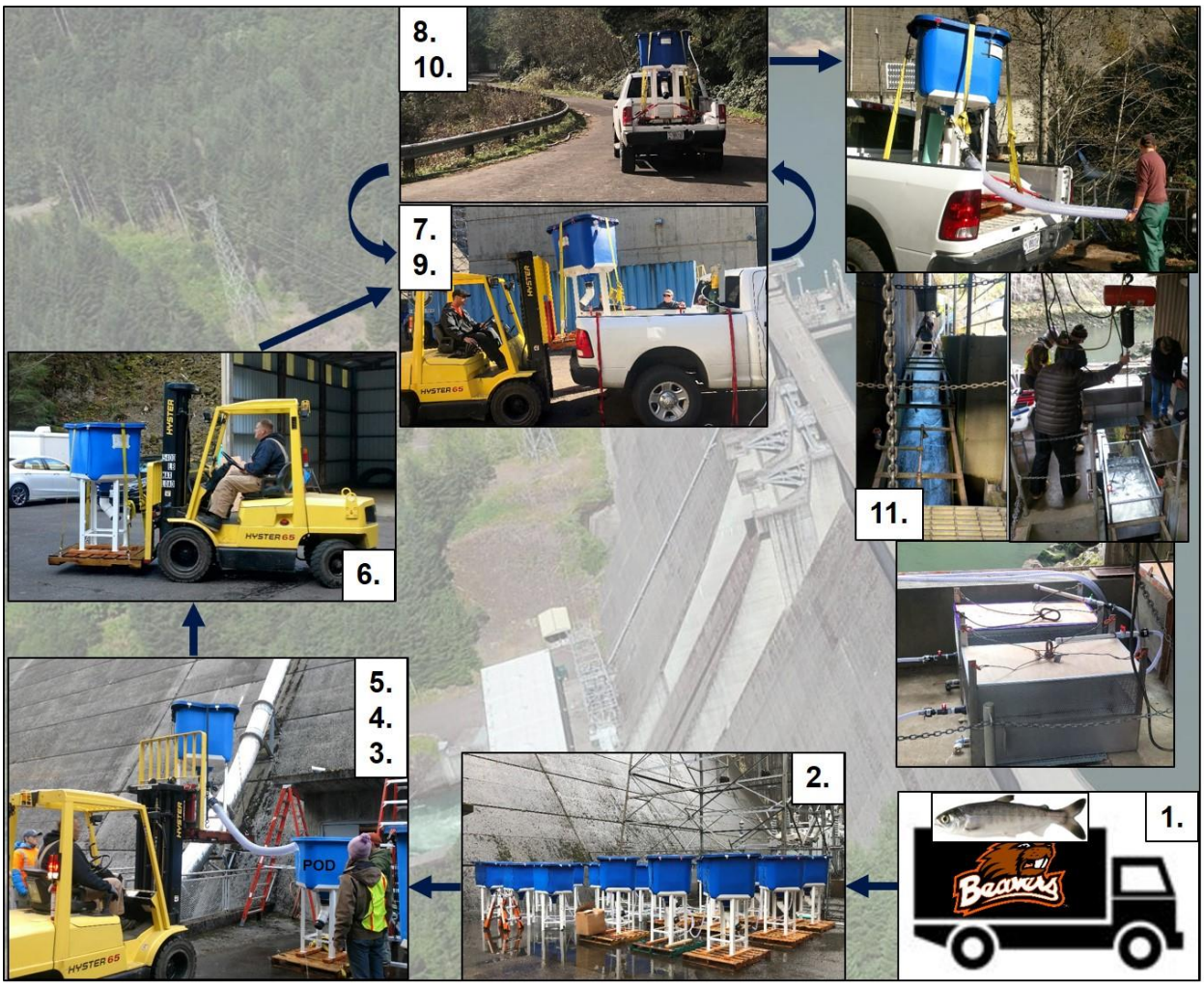


Figure 2.5. Photographs to Correspond with each of the Transport Simulation Steps Outlined in Table 2.2

2.3.2 Bypass Pipe Simulation

The bypass pipe simulation mimicked fish collected in the FSS and bypassed and had one treatment that consisted of three replicates paired with three control replicates and one bypass pipe post-relocation baseline sample at Green Peter (BP_BL; Table 2.1). Similar to the transport simulation, a collaboration with the USACE and OSU allowed for identification of potential real-world stressors that juvenile salmonids may experience during bypass pipe passage, simulation stressors that correspond to and mimic each real-world stressor, and methods to minimize human-induced stress. Four real-world stressors were identified, with six simulation stressors (Table 2.3 and Figure 2.6). Similar to the real-world transport method, the first alternative simulation stressor for bypass pipe that had a real-world stressor associated with it was Stressor 3 – entrance into the FSS. Removing the center standpipe from the tank created an abrupt water movement to induce the stress response, as a stress response would likely

occur when fish enter the FSS. Fish would then enter the chute and undergo chute passage in the FSS into the bypass pipe at the back (Stressor 4). Stressors 3 and 4 would be the same for transport and bypass pipe fish in the real-world, except bypass pipe fish would be directed from the chute into the bypass pipe, whereas transport fish would be directed from the chute into a pod. The simulation stressors were different; however, to allow for completion of the different subsequent steps required to complete the simulations while minimizing human-induced stressors. In our study, fish flowed through a y-shaped coupler and flexible tubing connected to the holding tank before traveling through the bypass pipe through the dam (Stressor 5 – bypass pipe passage). Finally, the fish were recollected at the juvenile fish collector in holding baskets (Stressor 6 – release into river) and remained in the holding baskets for up to 24 h for post-treatment blood sampling. The entire bypass pipe simulation (Stressors 3–6) took less than five minutes.

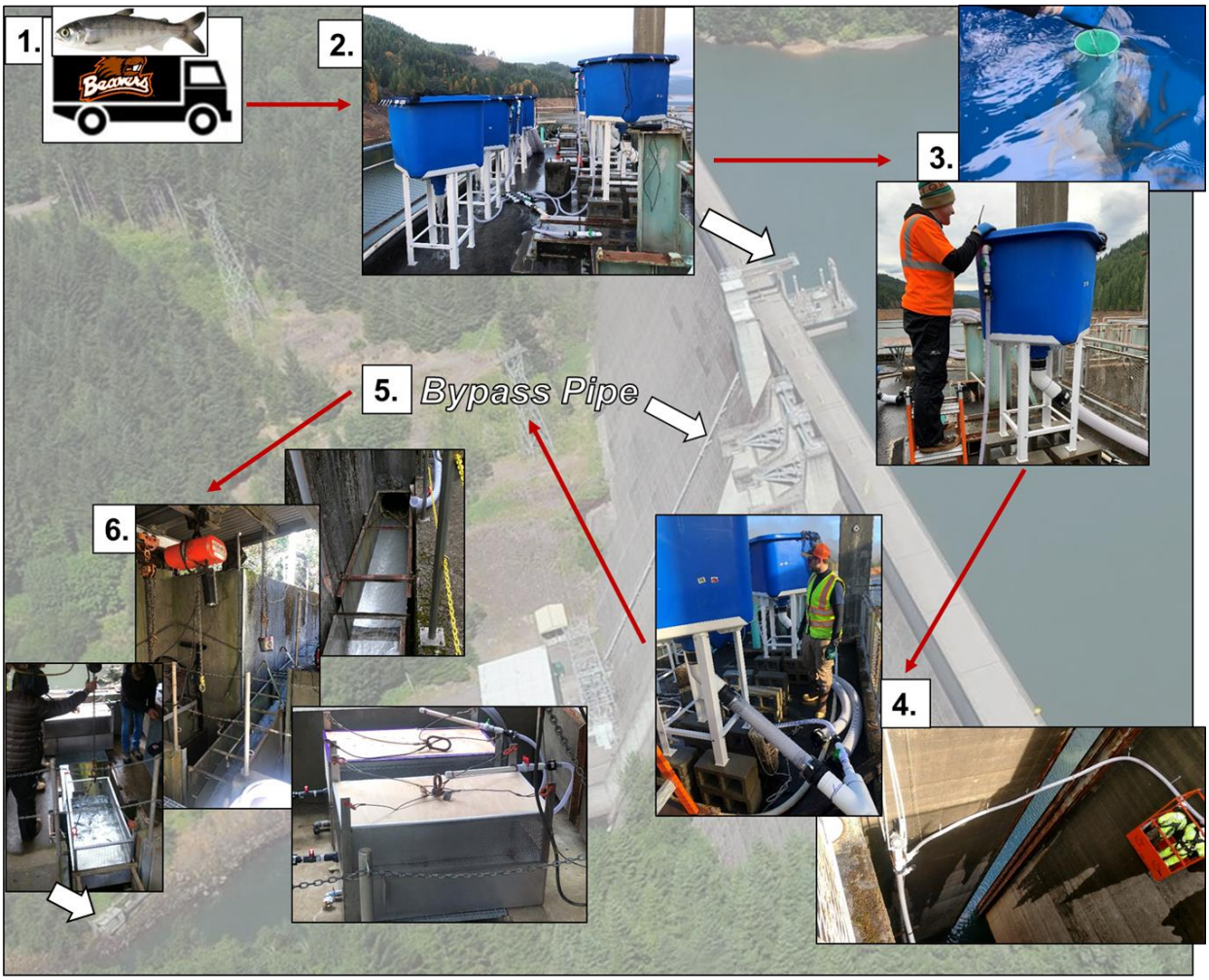


Figure 2.6. Photographs to correspond with each of the bypass pipe simulation steps outlined in Table 2.3

2.4 Blood Sampling

Treatment fish were recollected immediately post-treatment in one of two holding baskets at the juvenile fish collector. Fish remained in the holding basket for 24 h for blood sampling and injury and survival evaluations, as most mortalities were anticipated to occur within 24 h post-treatment. Blood samples were collected at six times post-treatment (i.e., at 0, 0.5, 1, 3, 6, and 24 h) and fish were concurrently monitored for survival.

Blood sampling protocols were the same for all fish (i.e., treatment, control, and pre- and post-relocation baseline fish). At each post-treatment sampling time (or for the one-time sampling time for control and both types of baseline fish), approximately 5 fish were randomly and gently netted from the holding basket (treatment fish) or tank (control and both types of baseline fish) for blood sampling. Fish were placed into 5 L of water treated with a lethal dose of anesthesia (250 mg/L MS-222) for 30 seconds; i.e., the amount of time it took for fish to lose equilibrium, become sedated, and slow or stop the increase in cortisol (i.e., the stress response). Syringes (1-mL) and 21-gauge needles (BD, Franklin Lakes, NJ) were pre-rinsed with a heparin sodium solution (1,000 units heparin to 1 mL water) to prevent blood from clotting (Houston 1990). Once fish were immobilized, 0.2–0.3 mL of whole blood was removed via caudal venipuncture (Figure 2.7A). Blood samples were collected in 2.6 min on average (SD: ± 1.3, range:0.8–9.3 min). Whole blood was placed into an individually labeled microcentrifuge tube and spun in a centrifuge (model HS120301; Heathrow Scientific, Vernon Hills, IL) at 6,000 rpm for 4 minutes to separate red blood cells from plasma (Figure 2.7B). Plasma was extracted using disposable pipettes and placed into duplicate labeled microcentrifuge tubes. The samples were immediately placed in a -20°C freezer until they could be transported and stored at OSU in a -80°C freezer. Samples remained in the -80°C freezer until laboratory analysis.

After blood sampling, fish were individually identified by microcentrifuge tube number and their replicated details were recorded (e.g., as a treatment, control, or pre- or post-relocation baseline fish). The post-treatment blood sampling time (i.e., 0, 0.5, 1, 3, 6, or 24 h post-treatment for treatment fish, or one-time sampling time for controls and both types of baseline fish); fish condition via visual inspection for injuries; and the amount of time it took for blood sample collection to occur were recorded. Sampled fish were euthanized in 250 mg/L of MS-222. After the 24-h post-treatment blood sampling occurred, all remaining unsampled fish were euthanized.

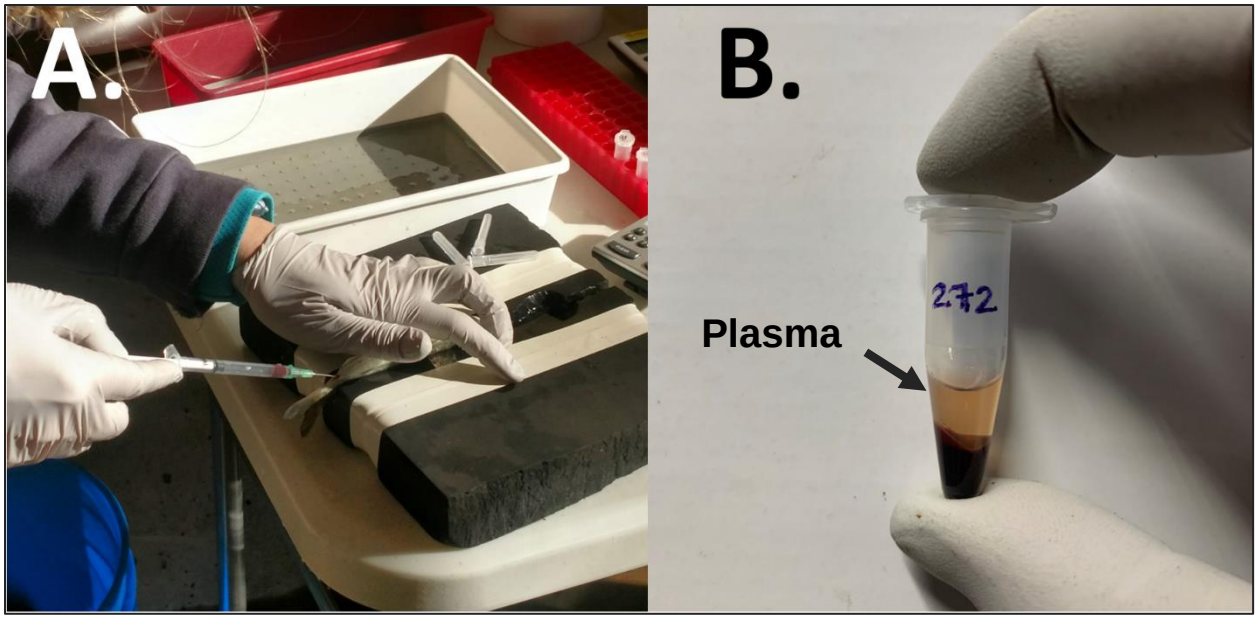


Figure 2.7. A) Blood Drawn via Caudal Venipuncture. B) Vial Depicting the Separation of Red Blood Cells (bottom) and Plasma (top) after being Centrifuged

2.5 Laboratory Analysis

Plasma samples were analyzed at OSU for cortisol concentrations (ng/mL). Analyses followed the methods of Redding et al. (1984) using a radioimmunoassay to quantify concentrations of cortisol in the blood plasma. All samples were run in duplicate. Only samples with < 5% variability across duplicates were used and samples with > 5% variability across duplicates were reanalyzed.

2.6 Copepod-Infected Fish

For the secondary objective, copepod-infected fish were a separate group of wild surrogate juvenile Chinook salmon (n = 200) that were infected with copepods at the OSU facility. Once the healthy fish study was complete, infected fish were relocated from OSU to Green Peter, to evaluate their short-term post-relocation survival and the practicality of using infected fish for future studies. At Green Peter, infected fish were randomly separated into 4 tanks (n = 50 fish per tank, same size and dimensions as the transport and bypass pipe holding tanks used for healthy fish). Two tanks were located at the bottom of the Green Peter (transport simulation location) and two located at the top deck of the Green Peter (bypass pipe simulation location). However, infected fish did not receive the same 2-week acclimation period as the healthy fish because the focus for this secondary objective was survival after relocation from OSU to Green Peter. Survival-only evaluations were short-term (48 h), as previous studies showed infected natural-origin fish that were heavily compromised (i.e., very infested with copepods) had high mortality rates within 48 h after relocation (Herron et al. 2018).

Fish were observed daily for mortalities for 48 h after relocation to Green Peter. Infected fish were postulated to have a lower rate of survival because they were predisposed to higher stress levels due to compromised immune systems; however, all infected fish survived. Higher survival rates of infected fish in this study compared to previous literature may have occurred because study fish were not as heavily compromised as the fish documented in Herron et al. (2018). As such, after the 48-h survival evaluation infected fish were put through ad hoc simulations or were control fish (i.e., infected fish did not undergo a simulation) to understand the effect of the simulations on infected fish survival. Infected fish were not evaluated for stress for these ad hoc simulations. They were not sampled for blood because they did not undergo the 2-week acclimation period required to recover from relocation stress. As such, infected fish that underwent a simulation were not compared to the healthy fish from the primary objective. Although stress levels were not evaluated, exposing infected fish to the simulations provided an additional metric to understand the practicality of using infected fish in a potential future study.

Two tanks of infected fish at the bottom of Green Peter were randomly assigned as transport treatment (1 h holding duration in the pod) or transport control and the two tanks of infected fish at the top of Green Peter to bypass pipe treatment or bypass pipe control. Infected fish were recollected at the juvenile fish collector and remained in the holding basket for 24 h post-treatment for evaluations of survival (similar to the methods performed for healthy treatment fish), as most mortalities were anticipated to occur within 24 h post-treatment. After 24-h post-treatment, infected fish were sampled for length, weight, and visual assessment of injuries (n = 5 fish per treatment and control group). Pictures of the gills were also taken where copepod infections occurred (Figure 2.8; Herron et al. 2018).

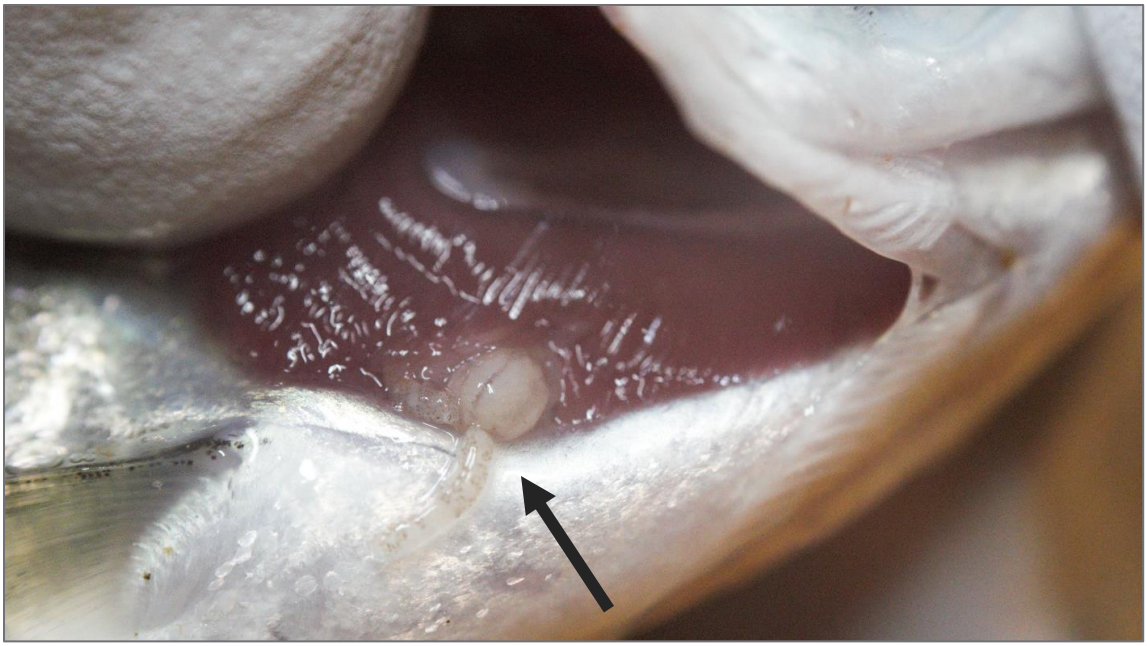


Figure 2.8. Adult Copepod Attached to the Gills of a Juvenile Chinook Salmon, Depicted by the Black Arrow

2.7 Statistical Analyses

Large sample sizes were not required to evaluate differences in cortisol. A small number of fish (e.g., $n = 5$ per sample) in a replicate is commonly used for stress effects studies to successfully identify if any variations in cortisol occur (Congleton 2006; Liebert and Schreck 2006; Cook et al. 2012; Stewart et al. 2016). Statistical analyses were conducted using $\alpha = 0.05$. Data were assessed for normality (Shapiro-Wilk test [Shapiro and Wilk 1965]) and unequal variances (for normally distributed data, Levene Test [Levene 1960]), and the appropriate one-way analysis of variance (ANOVA) was determined (Welch's test for unequal variance data [Welch 1951], standard ANOVA for normally distributed and equal variance data, or Kruskal-Wallis Test for non-normally distributed data [Kruskal and Wallis 1952]). If a significant difference was detected from the ANOVA, pairwise comparisons were made using either the Mann-Whitney (Mann and Whitney 1947), Tukey, or Dunnett's (Dunnett, 1955) test to identify the replicate(s) or treatment(s) that differed. Analyses of environmental and morphometric characteristics, injury, and survival were performed in R statistical software (R Development Core Team, 2018), while cortisol concentration analyses were performed in JMP statistical software (version 14.3; SAS Institute, Cary, NC).

2.7.1 Environmental and Morphometric Characteristics

Environmental characteristics measurements of tank temperature and DO taken during the 2-week acclimation period were compared to evaluate whether there was a difference based on tank location at Green Peter (e.g., comparing bypass pipe and transport tank locations). Morphometric characteristics of fish fork length and weight for each treatment (i.e., 1 h, 12 h, 24 h transport, and bypass pipe) were evaluated to understand if there was a size effect among treatments.

2.7.2 Cortisol Analyses

Control replicates of each treatment (i.e., 1 h, 12 h, 24 h transport, and bypass pipe) were compared to determine whether it was appropriate to combine (pool) them. Similarity among control replicates and control groups, either pooled or by replicate, were characterized by their median value. Cortisol concentrations of the bypass pipe control group and the three transport control groups showed consistent differences, indicating a pre-simulation holding tank location effect. The differences were confirmed by environmental characteristic analyses, which identified temperature and DO differences between the holding locations. Cortisol concentrations of bypass pipe controls were consistently higher than those of transport controls. To account for these differences between holding locations, the treatment:control cortisol ratio was used as the response metric in evaluations of treatment effects. The treatment:control cortisol ratios were calculated by dividing the cortisol values of each treatment fish at each post-simulation blood sample time (0, 0.5, 1, 3, 6, 24 h) by the median cortisol value of the corresponding control group.

For treatment fish, cortisol ratios were compared among the three replicates of a treatment to determine whether replicates could be pooled. Replicates within a treatment that differed were further evaluated by fitting cortisol ratios as a function of sample time using spline regression to identify those that deviated from the general trend displayed by the other replicates. Replicates that differed significantly and did not follow the general trend of other replicates were removed from further analysis.

Cortisol treatment:control ratios were plotted versus post-treatment sample time for each treatment. Because the relationship was nonlinear, decision tree analyses were used to identify differences among treatments across the range of sample times tested. Regression trees, such as those generated by decision tree analyses, are flexible and robust, able to deal with nonlinear relationships and high-order interactions yet are easy to understand and interpret (De'ath and Fabricius 2000). The 'Partition' platform within JMP was used to perform decision tree analyses. Within this platform, the data were partitioned at each split into two mutually exclusive groups, each of which were as homogeneous as possible with regard to response (treatment:control cortisol ratio) and predictor (treatment, post-treatment sample time)

values. The splitting procedure was then applied to each group separately. Partitioning was done according to a splitting "cut" value for the predictor variables. Splitting was based on maximizing the LogWorth significance value, which is the negative log of the adjusted *P* value, for each split candidate (Sall 2002). The adjusted *P* value was set to 0.05. For purposes of reporting, each split group was characterized by their median to avoid mischaracterization of non-normal data.

True cortisol concentrations (ng/mL) for each sampled fish were also plotted to promote ease of access and comparability with other studies. However, the statistical analyses were only conducted on the treatment:control cortisol ratios to account for holding location stress that was not associated with the bypass pipe or transport simulations.

2.7.3 Major Injuries and Rate of Survival

Major injuries documented during evaluations included > 20% descaling, eye damage, hemorrhaging, lacerations, or operculum damage. Some injuries were identified in control and treatment fish (e.g., missing eye, caudal fin or dorsal fin damage, and jaw damage). These injuries may have been a result of rearing or holding conditions as opposed to transport or bypass pipe simulations; therefore, they were excluded from injury analysis. The presence of major injuries was binomially quantified (e.g., 1 = injury present; 0 = no injury present) and analyzed using a Fisher's exact test with a Bonferroni correction for multiple comparisons (Fisher 1992; McDonald 2014). A difference in injury intensity (i.e., more than one injury present compared to one injury or no injuries) was also analyzed using Fisher's exact test with a Bonferroni correction. Rate of survival evaluations were short-term and lasted for 24 h post-treatment (i.e., until the 24 h post-treatment blood sample time). Survival rates were quantified per treatment by using proportions (e.g., number survived:number of fish that underwent the simulation) and a Fisher's exact test.

3.0 Results

3.1 Environmental Characteristics

Tank water temperatures and DOs differed significantly among location (Table 3.1; Kruskal-Wallis, $H = 131.2$ and 43.95, repetitively, $P \leq 0.001$). Tanks held at the top deck of Green Peter (bypass pipe) had significantly higher water temperatures than tanks located at the base of the dam (transport) and the juvenile fish collector (post-simulation holding tanks). There was no significant difference in temperature between transport and post-simulation holding tanks.

Table 3.1. Tank Water Temperatures and Dissolved Oxygen (DO) Levels by Location. Values represent the mean ± standard deviation, with ranges in parentheses. Tank locations were at the base of Green Peter (transport), top of Green Peter (bypass pipe), and the juvenile fish collector (post-simulation). Dissimilar superscripts within each column indicate a significant difference at the $\alpha = 0.05$ level.

Location	Temperature (°C)	DO (mg/L)
Transport	11.6 ± 0.7 (10–12.7) [b]	8.0 ± 0.4 (6.97–8.83) [b]
Bypass Pipe	13.8 ± 0.8 (11.5–14.9) [a]	8.4 ± 1.1 (6.72–9.69) [a]
Post-Simulation	12.0 ± 0.7 (11–14) [b]	8.6 ± 0.3 (7.91–8.97) [a]

3.2 Morphometric Characteristics

Blood samples were collected from an approximately equal number of fish from each treatment that were used for morphometric characteristics (Table 3.2). Fork lengths were not significantly different among treatments (Kruskal-Wallis; $H = 7.08$; $P = 0.07$). There was a significant difference in weight among treatments (ANOVA; $F = 5.43$, $P = 0.001$). However, it was not considered to be biologically significant because the difference in mean weights was very small (< 3.0 g) and could have been attributed to error during weight measurements (i.e., unexpected additional or reduced water in the weigh container during measurements).

Table 3.2. Sample Sizes and Measurements (Mean ± Standard Deviation, with Ranges in Parentheses) for Fish from each Treatment. Dissimilar superscripts within each column indicate a significant difference at the $\alpha = 0.05$ level and a lack of superscripts indicates no significant differences.

Treatment	*n*	Fork Length (mm)	Weight (g)
1 h transport	95	138 ± 10.8 (107–158)	25.5 ± 5.6 (12.5–43.5) [a]
12 h transport	99	139 ± 9.6 (111–161)	25.5 ± 5.2 (10.3–41.2) [a]
24 h transport	98	141 ± 9.1 (111–161)	27.6 ± 5.4 (12.8–41.0) [b]
Bypass pipe	97	139 ± 7.3 (120–157)	27.7 ± 4.8 (16.3–38.7) [b]
Total	389	139 ± 9.3 (107–161)	26.6 ± 5.4 (10.3–43.5)

3.3 Cortisol Concentrations and Treatment:Control Ratios

Control replicates of each treatment were similar enough to be pooled into groups (Kruskal-Wallis $H \leq 4.69$; $P \geq 0.09$). Once pooled, the bypass pipe control group had a median cortisol concentration of 10.6 ng/mL, and transport control groups had median cortisol concentrations of 7.2, 3.9, and 3.9 ng/mL for 1 h, 12 h, and 24 h treatments, respectively (Figure 3.1).

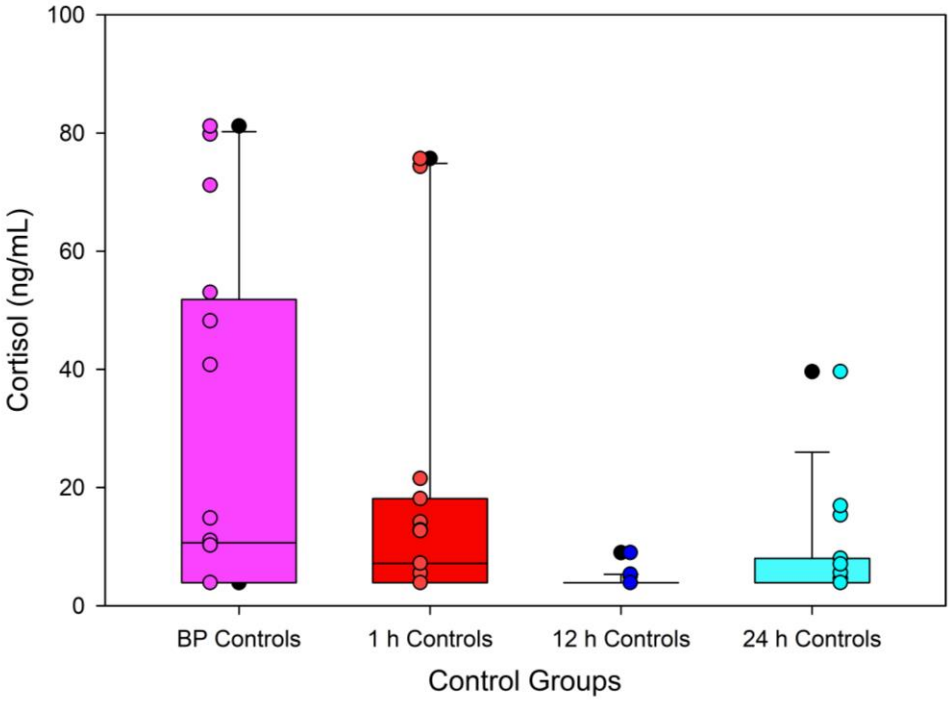
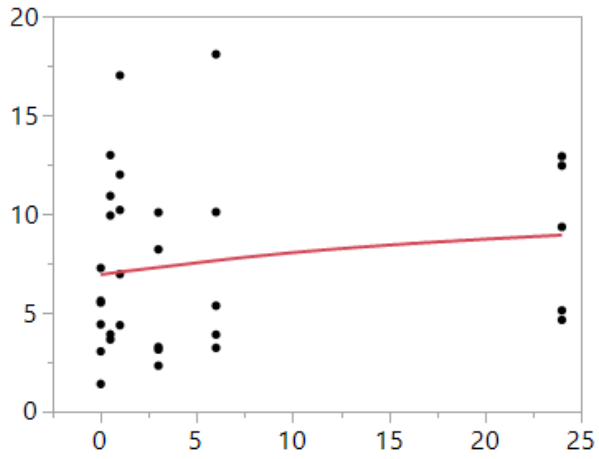


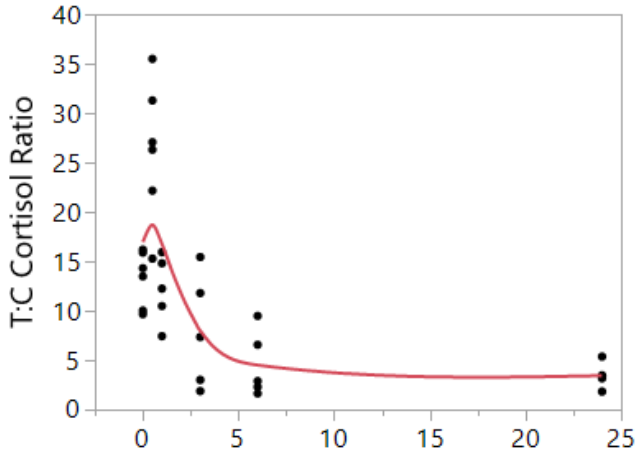
Figure 3.1. Scatter Plot Overlaying a Box Plot Displaying Cortisol Concentrations of Control Groups that Aligned with each Treatment (BP = bypass pipe [n = 16]; 1 h transport controls [n = 15]; 12 h transport control [n = 19]; and 24 h transport controls [n = 15]). Lower and upper bounds of each box represent the 25th and 75th percentiles, respectively. Lines within each box indicate the median. Whiskers represent 90th percentiles and black dots indicate 5th and 95th percentiles. The colored dots represent cortisol concentrations of individuals within each control group.

In general, treatment replicates were similar enough to pool with one exception. One of the 24 h transport treatment replicates (7-T_24 h; Figure 3.2) differed from the other two replicates at three post-treatment sample times: 0 h (Welch's; F = 7.9, P = 0.001), 0.5 h (ANOVA; F = 23.5, P < 0.001), and 24 h (Kruskal-Wallis; H = 8.5, P = 0.014). Fitting a spline regression to the cortisol ratio versus sample time data of each 24 h transport replicate also indicated replicate 7-T_24 h did not follow the same general trend of the other two replicates; therefore, the 7-T_24 h replicate was removed from further cortisol analyses. The other two replicates (8-T_24 h and 9-T_24 h) were combined to form the 24 h transport treatment.

a) 7-T_24 h



b) 8-T_24 h



c) 9-T_24 h

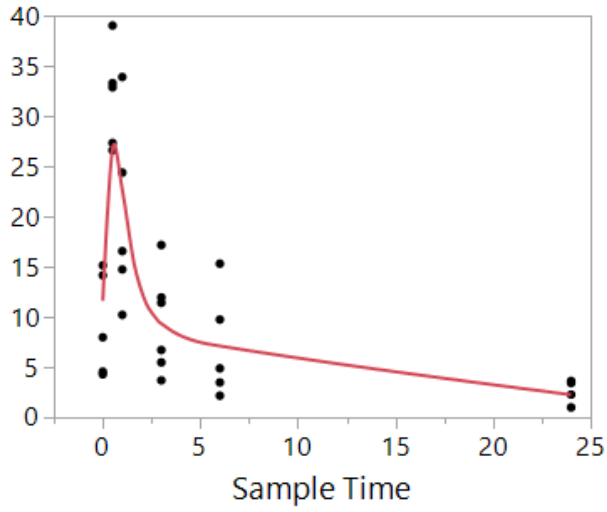


Figure 3.2. Spline Regression Models Fit to the Treatment:Control (T:C) Cortisol Ratios of each Replicate within the 24 h Transport Treatment. Replicate 7-T_24 h was removed from further analysis.

Results from the regression tree model indicated the bypass pipe and 1 h transport treatments had significantly lower treatment:control cortisol ratios than the 12 h and 24 h transport treatments at post-treatment sample times 0, 0.5, 1, 3, and 6 h post-treatment (Figure 3.3). The cortisol ratios of the bypass pipe and 1 h transport treatments were similar at all post-treatment sample times with one exception. Immediately following the simulation (post-treatment blood sample time = 0 h), the bypass pipe and 1 h transport treatments had cortisol concentrations that were 2.1 and 3.1 times higher, respectively, than the controls, suggesting the bypass pipe fish were slightly less stressed than 1 h transport fish. However, cortisol concentration ratios of both treatments increased to similar levels at 0.5 and 1 h post-treatment blood sample times before declining at similar rates at 3 and 6 h post-treatment blood sample times. By the 24 h post-treatment blood sample time, cortisol concentrations of both groups were becoming more similar to but were still slightly higher than the values of the controls (1.9 times higher than the controls).

The 12 h and 24 h transport treatments shared similar cortisol ratios at all post-treatment sample times except at the 0.5 h sampling time (Figure 3.3). Immediately following the simulation (post-treatment blood sample time = 0 h), 12 h and 24 h transport treatments had cortisol concentrations that were 13.2 times higher than the controls. At the 0.5 h post-treatment blood sample time, the 24 h transport treatment had a significantly higher cortisol ratio than the 12 h transport treatment, suggesting a slightly greater delayed stress response for 24 h transport fish. Thereafter, cortisol concentrations of both treatments declined at 1, 3, and 6 h post-treatment blood sampling times. By the 24 h post-treatment blood sample time, cortisol ratios of the 12 h and 24 h transport treatments declined to match those of the 1 h transport and bypass pipe treatments (i.e., 1.9 times higher than controls).

Concentrations of true cortisol (ng/mL) for each sampled fish were plotted (Figure 3.4), except for post-treatment sample times of fish from the 7-T_24 h replicate. The median values were also recorded (Table 3.3) for comparability with other studies.

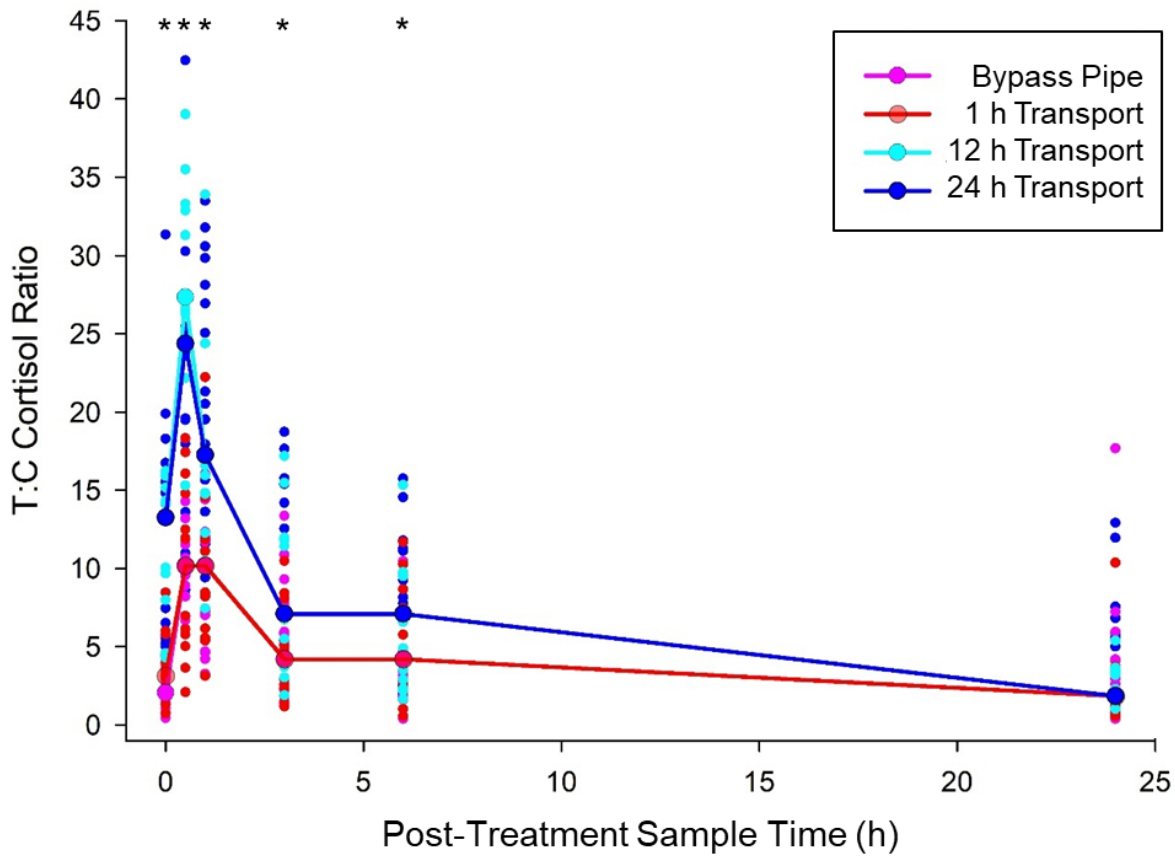


Figure 3.3. Scatter Plots of Treatment:Control (T:C) Cortisol Ratios of each Sampled Fish from each Treatment (Except for Ratios from Post-Treatment Sample Times of Fish in the 7-T_24 h Replicate; Small Dots), and the Results of a Regression Tree Model that Split the Data into Mutually Exclusive Groups (larger dots and line plots). An '*' above the post-treatment sample time indicates a significant difference ($P < 0.05$) between treatments.
Note: regression tree dots and lines overlap for treatments that form mutually exclusive groups. The bypass pipe and 1 h transport treatments formed mutually exclusive groups (i.e., are depicted by the same line [red]) at 0.5, 1, 3, and 6 h post-treatment sample times; the 12 h and 24 h transport treatments formed mutually exclusive groups (blue line) at 0, 1, 3, and 6 h post-treatment sample times; and all four treatments were grouped at the 24 h post-treatment sample time.

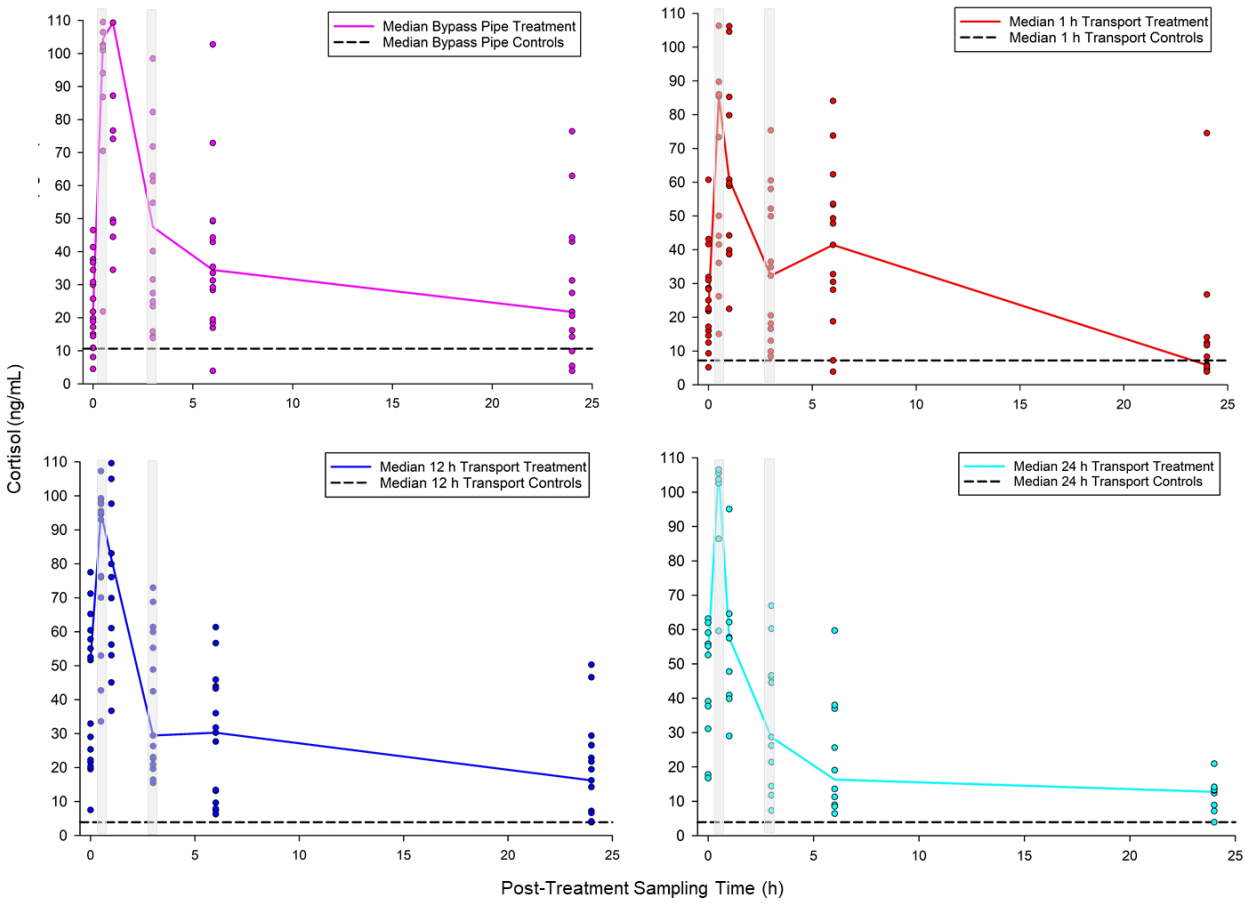


Figure 3.4. Scatter Plots of True Cortisol Concentrations of each Sampled Fish from each Treatment (Except for Cortisol Concentrations from Post-Treatment Sample Times of Fish in the 7-T_24 h Replicate). The line plots (solid colors) represent median cortisol concentrations for each treatment and the dashed line plots depict the median control group cortisol concentrations for each treatment. The gray bars on each plot are for ease of viewing data from the 0.5 h and 3 h post-treatment sampling times.

Table 3.3. True Cortisol Concentrations for Fish at each Post-Simulation Sampling Time for each Treatment (except for Cortisol Concentrations from Post-Treatment Sample Times of Fish in the 7-T_24 h Replicate) and the Associated Control Group. Values represent the median with ranges in parentheses.

Post-Simulation Sampling Time	Cortisol Concentrations (ng/mL)			
	Bypass Pipe	1 h Transport	12 h Transport	24 h Transport
0 h	21.8 (4.5–46.5)	22.5 (5.2–60.7)	51.6 (7.5–122.2)	52.6 (5.5–63.2)
0.5 h	104.5 (21.9–151.3)	85.4 (15.0–131.7)	95.0 (33.6–165.6)	106.6 (14.3–152.2)
1 h	109.3 (34.5–170.1)	60.8 (22.5–160.0)	81.5 (36.7–130.6)	57.6 (17.2–132.2)
3 h	47.5 (13.8–141.4)	32.3 (8.4–75.4)	29.4 (15.5–73.0)	28.7 (7.3–66.9)
6 h	34.4 (3.9–110.6)	41.4 (3.9–84.1)	30.3 (6.3–61.4)	16.3 (6.4–70.5)
24 h	21.8 (3.9–187.1)	5.9 (3.9–74.5)	16.2 (3.9–50.3)	12.7 (3.9–50.4)
Controls	10.6 (3.9–81.2)	7.2 (3.9–75.7)	3.9 (3.9–9.0)	3.9 (3.9–39.6)

3.4 Major Injuries and Rate of Survival

Injury classifications and the number of fish with observed injuries varied among treatments (Table 3.4). A significant difference was detected for total injuries by treatment (Fisher's exact test; $P < 0.012$), with pairwise comparison determining the only significant difference being between 1 h transport and bypass pipe treatments ($P = 0.015$). The bypass pipes are old and could be the cause for some of the observed injuries for the bypass pipe treatments, especially descaling and lacerations (Table 3.4). Additionally, hemorrhaging may have occurred from flows within the bypass pipe during treatments (Table 3.4).

Survival rates were similar among treatments (Table 3.5), and no significant differences were identified among treatments (Fisher's exact test; $P = 0.18$). All fish mortalities occurred within the three transport treatments; however, a comparison at a higher level between the two simulations (transport and bypass pipe) was also not significant (Fisher's exact test; $P = 0.20$).

Table 3.4. Injury Classifications by Treatment (1 h, 12 h, 24 h Transport, and Bypass Pipe [BP])

Treatment	Descaling	Hemorrhage	Laceration	Operculum Damage	Eye Damage	Total Injured	Total Healthy
1 h	0	0	0	0	1	1	94
12 h	4	2	0	0	1	7	92
24 h	4	2	0	1	1	8	90
BP	2	5	4	4	1	12 [(a)]	85

[(a)] Four fish from the bypass pipe treatment were found with multiple injuries but were not double counted in the total injured column.

Table 3.5. Rate of 24 h Post-Treatment Survival by Treatment

Treatment	Survived	Died	Survival Rate
1 h Transport	94	1	98.9%
12 h Transport	97	2	98.0%
24 h Transport	94	4	95.9%
Bypass Pipe	97	0	100%

3.5 Copepod-Infected Fish

All infected fish survived the initial relocation to Green Peter from the OSU facility, and the 48-h post-relocation evaluation once at Green Peter. To understand if the downstream passage conveyance simulations would have a negative effect on survival for the infected fish, infected fish were exposed to the 1 h transport and bypass pipe treatments. Infected fish from the 1 h transport treatment had an overall survival of 94% by 24-h post-treatment (n = 47 of 50). Two fish died immediately (0 h) post-treatment, and 1 fish died < 6 h post-treatment. There was 100% survival for the bypass pipe infected fish during the 24-h post-treatment holding period. Collectively, the relocation and post-treatment survival rates suggest it is practical to use infected fish for future studies.

4.0 Discussion

The results of this year one study demonstrated the feasibility of conducting a larger scale study to evaluate and compare fish stress and survival via truck transport and bypass conveyances. To our knowledge, this is the first study to evaluate fish stress using an intact bypass pipe to compare to a transport method intended to imitate a trap and transport method that may be used in real-world scenarios (e.g., imitating the potential stressors at Cougar and Detroit dams once the passage structures are built). Comparisons of cortisol ratios suggested that regardless of treatment, all fish were stressed as a result of the bypass pipe and transport simulations. Trends were similar among the four treatments: cortisol began to rise immediately post-treatment and peaked shortly thereafter; but returned to near-baseline levels by 24 h post-treatment. The stress response curve identified in our results was similar to previous studies evaluating the cortisol stress response in salmonids (Fast et al. 2008; Pankhurst 2011; Schreck et al. 2016; Cogliati et al. 2019). Additionally, the overall trend of treatment fish cortisol nearing baseline concentrations (i.e., similar to the concentrations found in control fish) by 24 h post-treatment is a positive one, indicating fish were recovering and the effects of the initial (fight-or-flight) stress response were temporary.

Although the overall trends were similar among the four treatments, a few differences between treatments at each post-treatment sampling time were identified. Cortisol ratio trends indicated the bypass pipe and 1 h transport treatments had comparable cortisol ratios for nearly all sampling times except 0.5 h post-treatment, although the difference was small, with bypass pipe fish having a lower cortisol ratio (2.1) than 1 h transport fish (3.1). The comparisons between bypass pipe and 1 h transport fish may indicate fish respond similarly when the duration of the stressor is short term. For example, the bypass pipe simulation took less than five minutes from start (pulling the center standpipe) to finish (fish recollected post-simulation). The timing would be likely similar for real world scenarios, excluding time spent in the FSS, once fish enter the chute that would lead fish to the bypass pipe at the rear of the FSS and passage though the bypass. The 1 h transport simulation took approximately 2 h from start (fish moved from acclimation tank to pod for 1 h hold) to finish (fish recollected post-simulation). Again, this timing would likely be similar for real world scenarios, excluding time spend in the FSS, once fish enter the chute that would lead fish to the pod and the 1 h holding period before transport.

Collectively, the bypass pipe and 1 h transport cortisol ratios were lower than the ratios for the 12 h and 24 h transport treatments at the majority of the post-treatment sampling times, except 24 h when all ratios were approximately the same and nearing baseline levels. The length of time a stress response lasts, and the extent of the response are dependent on the length of time a stressor occurs and the severity

of the stressor (Schreck et al. 2016). The total time from start (fish moved from acclimation tank to pod for 12 h or 24 h hold) to finish (fish recollected post-simulation) for the 12 h and 24 h treatments was approximately 13 h and 25 h, respectively. The comparison between the shorter-term stressors (bypass pipe and 1 h transport) and longer-term stressors (12 h and 24 h transport) may indicate the longer fish are held in the pod or collection facility (≥ 12 h), the more stressful the transportation event may be for fish because recovery took longer in the short-term (≤ 6 h) although recovery was not impaired by 24 h. Fish in the 12 h and 24 h treatments may have experienced a greater energetic cost associated with pod holding time (i.e., continued energy towards overcoming the stressor resulting in increased cortisol release). Cortisol levels for these fish may have increased with the longer holding duration in the pod and remained at a higher concentration as the transport simulation began. However, no blood samples were taken after the pod-holding time to avoid an unnecessary human-induced stressor that would not occur in a real-world transport scenario for the fish that would still undergo the transport simulation.

True cortisol concentrations provided ease of access and comparability with other studies. For example, previous studies have shown the resting (unstressed) cortisol levels of juvenile salmonids to be 0.0–25.0 ng/mL (Pickering and Pottinger 1989; Pankhurst 2011; Baker and Vynne 2014). The resting range aligns with the post-relocation baseline cortisol levels outlined in our study for the control fish from all four treatments (median cortisol concentrations of 10.6 ng/mL for bypass pipe, 7.2 ng/mL for the 1 h transport, and 3.9 ng/mL for 12 h and 24 h transport). However, our results indicated bypass pipe control fish had consistently higher true cortisol concentrations than transport control fish. Bypass pipe treatment fish also had consistently higher true cortisol concentrations at nearly all post-treatment sampling times than treatment fish from the three transport treatments. The higher true cortisol concentrations for the bypass pipe fish may have been attributed to the pre-simulation tank holding location, an unavoidable effect of study constraints. Bypass pipe tanks were located at the top deck of Green Peter, where there was substantial vehicle activity due to a road that crosses the dam. The tanks also had exposure to more direct sunlight throughout the day. Conversely, transport tanks were located at the base of Green Peter and had no road activity, little human access and more shade. As such, a treatment:control cortisol ratio was used for comparisons among treatments to account for the underlying stress that was not caused by the simulations but likely resulted from pre-simulation tank holding location. Subsequently, using cortisol ratios provided a more accurate comparison among treatments than the true cortisol concentrations.

The maximum or peak cortisol concentrations for teleosts may range from 30–300 ng/mL and are typically species-dependent (Pankhurst 2011). Peak cortisol for fish in our study fell within that range (median cortisol concentrations of 104.5 ng/mL [bypass pipe], 85.4 ng/mL [1 h transport], and 95.0 ng/mL [12 h transport] and 106.6 ng/mL [24 h transport]). The stress response curve for most fish

species shows peak cortisol concentrations occur within 0.5–1 h post-stressor (Barton 2002). For example, rainbow trout (*O. mykiss*) had an approximately 25-fold increase in cortisol 1 h after being subjected to a 30-sec exposure to air (Barton 2002). The greatest increase in cortisol levels for Atlantic salmon (*Salmo salar*) was at 1 h after being subjected to a one-time 15-sec exposure to air (Fast et al. 2008). For Chinook salmon exposed to a transportation stress test, mean cortisol peaked at 3 h, although no cortisol samples were taken at 0.5 or 1 h (Cogliati et al. 2019). The stress response curve trends in our study demonstrated the peak cortisol concentrations at 0.5 h post-simulation for all four treatments (10–26 times greater than controls), although cortisol at 1 h post-simulation was similar to the 0.5 h concentrations for fish in the bypass pipe, 1 h and 12 h treatments (i.e., remained high).

To our knowledge, this is a first of its kind study to evaluate fish stress response from bypass pipe conveyance compared to transport conveyance. Because the type of stressors in our study were different than the types of stressors found in other studies, direct one-to-one comparisons of true cortisol concentrations was difficult. However, the trends from our results to those of other stress response studies were compared, and the trends were similar. Collectively, comparisons among the four treatments within our study suggested the shorter the duration of the stressor (bypass pipe and 1 h transport treatments), the quicker the short-term recovery; although fish from all treatments were nearly recovered by 24 h post-stressor, suggesting minimal long-term initial cortisol response effects. Further studies are recommended for a more robust comparison across years, as there can be substantial variations in stress depending on the physiological condition of the fish at the time of sampling, as well as from differences in environmental factors (i.e., genetic differences, temperature, DO, human contact, etc.; Schreck et al. 2016).

5.0 Management Applications

A short-term recovery (< 6 h) may be advantageous compared to a longer-term recovery (24 h) for fish being released directly into a river post-simulation. The energetic cost of overcoming a stressor (e.g., resisting, recovering, coping) can affect the amount of energy available for other necessary physiological mechanisms that contribute to the overall fitness of the fish (i.e., development, growth, reproduction, etc.; Wendelaar Bonga 1997). One response to the energetic demand for increased cortisol is immunosuppression, which can affect fish health and resistance to disease, growth, predator avoidance, or reproduction (Schreck et al. 2016). However, even if cortisol returns to normal within a few hours or days after the stressor, the immune system may take longer to return to normal (Wendelaar Bonga 1997). A quicker recovery of the initial stress response (cortisol) may reduce the amount of energy needed to overcome the stressor, so the energy can instead be used towards repairing the immune system or other necessary physiological mechanisms that contribute to fish fitness.

Although trends indicated bypass pipe *or* a shorter holding time in the pod prior to transport may be less stressful for fish, the results are based on a first of its kind initial study and the environmental factors or fish responses could change from year to year. This year one study used appropriate sample sizes to determine these trends; however, a year two study with additional replicates is recommended. In year one, one of the replicates from analysis was removed (e.g., replicate 7-T_24 h from the 24 h transport treatment) as its trends were significantly different than the other two replicates from the 24 h transport treatment. Including additional replicates in year two will be beneficial for an increased sample size, as well as alleviate the concern if the need to remove a replicate occurs again. A year two study will allow for a more holistic dataset with two years of comprehensive data and provide a comparison to the trends identified from this year one study. Data from a year two study may also aid in accounting for variations in stress depending on the physiological condition of the fish at the time of sampling, as well as from differences in environmental factors.

Additionally, the stress evaluation component of the year one study used only healthy fish. However, copepods have been identified as an increasingly prevalent issue in the WVP, specifically at high-head dams such as Cougar, Detroit, and Lookout Point (Monzyk et al. 2015). Increased cortisol levels may affect fish resistance to disease (Schreck et al. 2016), potentially increasing their susceptibility to copepod infections. Previous laboratory studies have shown fish infected with copepods have a greater mortality rate than healthy fish (Herron et al. 2018). Evaluating the effects of bypass pipe and transport on the stress response of infected fish is critical for future management applications, to understand if copepod-infected fish in the field respond differently than healthy fish. The year one study demonstrated

the feasibility of relocating infected fish from OSU to Green Peter. Infected fish had high short-term survival post-relocation and high survival post-simulation (although they were not acclimated for two weeks at Green Peter prior to simulations). A year two study will allow for a full-scale evaluation of the stress response of infected fish (e.g., blood sampling, full sample sizes and treatments, and a two-week acclimation prior to simulations).

A year two study will also allow for improvements to the study design for healthy and infected fish, that were unforeseen in the year one study. Although the tank locations for the bypass pipe tanks (top deck of Green Peter) and the transport tanks (bottom of Green Peter) cannot be changed in a year two study (i.e., tank location is an unavoidable study constraint), measures to help reduce environmental effects could be taken. For example, at the top of Green Peter shade tarps over the tanks could be used to minimize exposure to direct sunlight. The tanks could also be wrapped in a thermal bubble roll to keep the water temperature in the tanks as cool as possible. The thermal bubble roll may also help provide noise protection from vehicles on the road deck.

Another advantage of a year two study includes the evaluation of additional stress physiology metrics. Cortisol is the one of the most commonly evaluated stress hormones, as it is released during the primary stress response (i.e., provides an immediate fight-or-flight response; Barton 2002; Schreck et al. 2016). However, other metrics such as glucose and lactate can provide supplementary information about fish stress (Barton 2002; Schreck 2016). Glucose and lactate are released during the secondary stress response and may provide information about the delayed stress response in fishes (Barton 2002). The two metrics are easy to evaluate *in situ* with hand-held devices and have been commonly used in fisheries research (Brown et al. 2008; Stoot et al. 2014; Wells and Pankhurst 1999). Glucose and lactate, used in conjunction with cortisol, may provide a more well-rounded understanding of the fish stress response to the bypass pipe and transport simulations for both healthy and infected fish.

The benefits of a year two study will allow for a more detailed response to management implications. Year one was a successful study and provided data with interesting trends relating to the healthy fish stress response. Both simulations (bypass pipe and transport) may have been equally as severe in magnitude for stressing the fish, but the quicker recovery may have been driven by the shorter duration of the stressor, leading to quicker short-term recovery. When combined, year two will allow for greater interpretation of the trends from the healthy fish stress response findings from year one and will identify trends for the infected fish stress response.

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