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ARTICLE

A Fish Out of Basin: Increased Stress Physiology and Reduced Performance of Salmon River Hatchery Chinook Salmon

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Abstract

Variation in rearing conditions across hatcheries and basins can affect the performance of hatchery salmonids in the wild. In 2008, the Shoshone-Bannock Tribes began planning an out-of-basin hatchery facility on the eastern Snake River Plain for rearing threatened Chinook Salmon *Oncorhynchus tshawytscha* for release in tributaries of the upper Salmon River, Idaho, USA. To help determine the viability of the planned out-of-basin hatchery, we reared 100,000 juvenile Chinook Salmon from the same genetic stock at one in-basin (Sawtooth Fish Hatchery on the Salmon River) and one out-of-basin (Springfield Fish Hatchery on the Snake River Plain) site in Idaho that are characterized by significant differences in water hardness and temperature regime. In October 2018 and April 2019, we tested whether fish condition, stress physiology, acute mortality, and downstream survival differed between the two groups at the parr and smolt life stages upon release in the Yankee Fork Salmon River, which is characterized by low water hardness. For both release groups, parr experienced low acute mortality during the 48 h after release; however, the out-of-basin group had a downstream survival rate through an unimpounded portion of the migration corridor that was an order of magnitude lower than that for the in-basin group. During the smolt release, the out-of-basin group showed signs of extreme physiological stress, acute mortality rates of 40–80%, and low survival in the unimpounded portion of the migratory corridor. The in-basin group recovered from the stress of transport and release, had no acute mortality, and survived through the unimpounded migratory corridor at a rate comparable to that of previous years' releases. Based on the results of this comparative study, comanagers are evaluating alternatives to the proposed out-of-basin

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hatchery program. This case study highlights the effects of differences between rearing and release conditions on salmon physiology and survival as well as the value of conducting preliminary evaluations prior to implementing large-scale hatchery supplementation programs.

There is immense variation in hatchery rearing and release conditions for anadromous and resident fishes. Because increasing fish abundance is a major goal of any conservation or harvest-focused hatchery program, managers should optimize environmental conditions to maximize juvenile-to-adult survival. Loading, transport, and release are all established stressors that are common to hatchery programs (Barton 2000) and can affect postrelease performance. Postrelease performance may also be affected by differences between rearing and release conditions, such as water temperature and water hardness (Vargas-Chacoff et al. 2018; Trushenski et al. 2019). Hatchery protocols often include guidance regarding temperature differences between transport water and release water. Yet, guidelines regarding water hardness gradients are less common, despite their potential to affect postrelease performance (McDonald and Robinson 1993).

The interaction between rearing conditions and juvenile-to-adult survival should be considered when designing, constructing, and implementing new hatchery programs (HSRG 2009). For example, significantly different smolt-to-adult return rates and adult age structure were reported for groups of the same genetic stock of Chinook Salmon *Oncorhynchus tshawytscha* that were reared at different hatchery facilities and released at the same location (Spangenberg et al. 2014; Beckman et al. 2017) or reared at the same facility and released at different locations (Harstad et al. 2018). In both of these cases, differences between rearing and release conditions may have contributed to stocking success.

In 2008, the Shoshone-Bannock Tribes began planning for the construction of the out-of-basin Crystal Springs Hatchery on the eastern Snake River Plain in eastern Idaho to supplement the Chinook Salmon populations in the Salmon River basin (Shoshone-Bannock Tribes 2011). The proposed Crystal Springs Hatchery site is adjacent to the Idaho Department of Fish and Game's (IDFG) Springfield Fish Hatchery and would draw from the same ground water source. Springfield Fish Hatchery serves as IDFG's primary hatchery for rearing endangered Snake River Sockeye Salmon *O. nerka* smolts. In 2017, IDFG determined that the difference in hardness between rearing and release water caused the low survival (mean = 16–31% to Lower Granite Dam) and high acute mortality of Springfield-reared Sockeye Salmon in the first two release years (Trushenski et al. 2019). Indeed, differences in hardness between rearing and release water is a known contributor to stress in fish (McDonald and Robinson 1993; McDonald and Milligan 1997); however, prior to the work of Trushenski et al. (2019), high levels of acute mortality had not been documented. To our knowledge, few investigations have identified strong interspecific differences in the ability to tolerate rapid changes in water hardness, particularly at the scale of a hatchery program. The negative response of Sockeye Salmon in the Snake River to the difference between rearing and release water hardness raised questions about whether Chinook Salmon would experience similarly poor performance and cause reduced success of the proposed Crystal Springs Hatchery.

We initiated a multipart study in autumn 2017 to evaluate whether fish condition, stress physiology, acute mortality, and downstream migration survival differed between the same genetic stock of Chinook Salmon that are reared at the out-of-basin Springfield Fish Hatchery versus the in-basin Sawtooth Fish Hatchery (Figure 1). Mirroring the study by Trushenski et al. (2019), we measured a suite of stress-related physiological parameters including plasma cortisol, plasma glucose, blood lactate, hematocrit, and ion balance via blood sodium (Na^+) and chloride (Cl^-). We performed paired releases of both hatchery groups at two life stages (fall parr and spring smolts) to further assess how smoltification may potentially compound the stress of abrupt changes in water temperature and chemistry. Finally, we used observations in a controlled environment and mark–recapture techniques to assess performance metrics that are related to survival. The overall objective of this case study was to help determine whether out-of-basin rearing at a site with different water hardness than that at the target release site would be effective in achieving long-term success in population recovery efforts for threatened Chinook Salmon in the upper Salmon River basin.

METHODS

Study Area

Hatchery rearing occurred at two facilities in Idaho, USA (Figure 1): an out-of-basin site at Springfield Fish Hatchery on the Snake River Plain near the town of Springfield, Idaho, and an in-basin site at Sawtooth Fish Hatchery on the upper Salmon River near the town of Stanley, Idaho. Both hatcheries are operated by the IDFG.

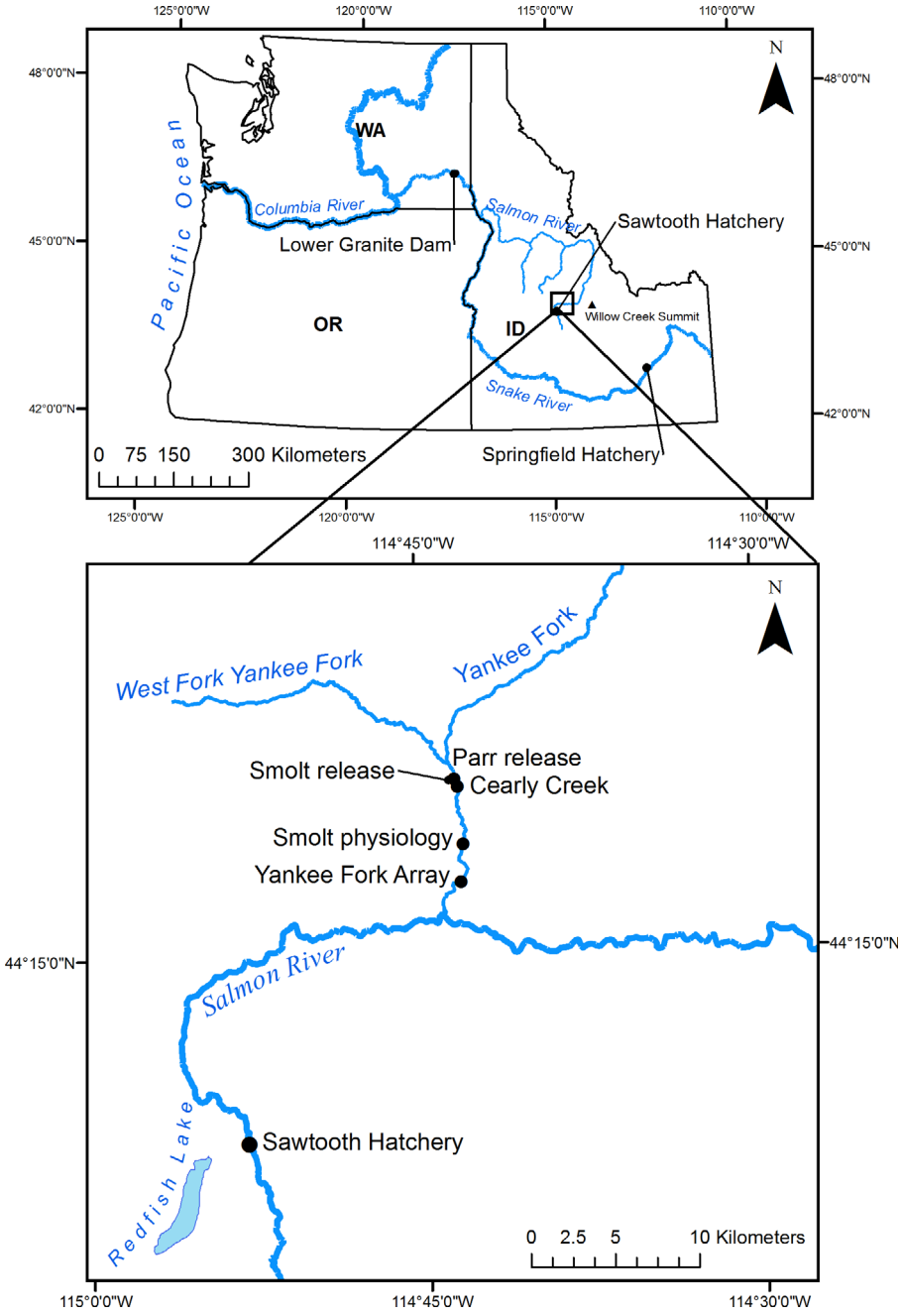

FIGURE 1. Map of locations referenced in this study. The top panel shows the location of the evaluations in southeast and central Idaho, including the rearing location of the Springfield group at Springfield Fish Hatchery (adjacent to the proposed Crystal Springs Hatchery site) relative to the Sawtooth group at Sawtooth Fish Hatchery. The bottom panel shows the locations in the upper Salmon River basin where we conducted the parr and smolt release evaluations.

The fish that were reared at the two hatcheries were released into the Yankee Fork Salmon River (henceforth, "Yankee Fork") located within the Salmon–Challis National Forest (Figure 1). The river begins at 2,500 m above sea level and flows from north to south for 42 river kilometers (rkm) to its confluence with the Salmon River, approximately 32 rkm downstream from Sawtooth Fish Hatchery at an elevation of 1,880 m above sea level. Smolts released in the upper Salmon River migrate through approximately 640 rkm of unimpounded river to Lower Granite Dam and another 800 rkm through eight hydropower dams to the Pacific Ocean.

Stock Characteristics

The Sawtooth Fish Hatchery broodstock originated from brood collections from 1981 to 1984 on the upper Salmon River near the present site of Sawtooth Fish Hatchery (Figure 1). Sawtooth Fish Hatchery rears and releases up to 2.4 million Chinook Salmon smolts annually. Most smolts are released at the hatchery, but up to 300,000 smolts are also released in the Yankee Fork as part of the Shoshone-Bannock Tribes' Chinook Salmon Supplementation Program. To facilitate a test of the effect of rearing conditions on juvenile Chinook Salmon performance, comanagers agreed to rear 100,000 individuals at the out-of-basin Springfield Fish Hatchery (for this test in addition to their standard practice of rearing Redfish Lake Sockeye Salmon) and 100,000 individuals at in-basin Sawtooth Fish Hatchery from brood year 2017 for release into the Yankee Fork in 2019 (IDFG et al. 2019a).

Rearing Conditions

Rearing conditions at the two hatcheries differed with respect to their water chemistry, temperature regime, feeding regime, and solar exposure. These differences form the basis of our case study. Water hardness at Springfield Hatchery was over fourfold higher than at Sawtooth Hatchery (Table 1). Water temperature was recorded twice daily at each hatchery and varied little across the rearing period at Springfield Hatchery (monthly mean temperature range of 9–10°C; Figure 2 top panel) relative to the Sawtooth Hatchery (monthly mean temperature range of 2–14°C; Figure 2 top panel). We endeavored to rear fish to a similar size at release of approximately 18 fish per pound (i.e., individual fish weight of approximately 25 g). Because of the difference in temperature regimes, the feeding regime (Figure 2 middle panel) and rearing density (Figure 2 bottom panel) necessarily varied seasonally between the two groups. Fish that were reared at Springfield Hatchery were reared in concrete raceways under permanent shade, whereas the concrete raceways at Sawtooth Hatchery were partially shaded from July to September and exposed to full sunlight or covered by ice for the remainder of the year. Standard operating procedures for both hatcheries are described in IDFG et al. (2019b).

Tagging

We marked approximately 5,000 parr from each group with passive integrated transponder (PIT) tags (Table 2). Tagging of the parr was contracted through Biomark, Inc. (Boise, Idaho) and occurred on September 6, 2018, and September 12, 2018, at Sawtooth and Springfield fish hatcheries, respectively. For the smolt release evaluation, we marked 4,587 and 4,179 fish at Sawtooth and Springfield hatcheries (Table 2), respectively. Smolt tagging was conducted by the Pacific States Marine Fisheries Commission in October and November 2018 according to their annual mass marking schedule. The tag number for smolts was informed by a power analysis (Supplement I available in the online version of this article) and the number of tags was higher for the Sawtooth group to account for a portion of the tags that are subject to a sort-by-code procedure at Lower Granite Dam as part of the Comparative Survival Study (see McCann et al. 2020).

Transport and Release

The Sawtooth and Springfield fish hatcheries are 36 and 303 km away by road from release sites on the Yankee Fork, respectively. For both the parr and smolt releases, we controlled for transport time and elevation

TABLE 1. Water chemistry profiles of sites involved in the proposed Crystal Springs Fish Hatchery for Chinook Salmon from water samples collected by the Shoshone-Bannock Tribes and analyzed by Intermountain Analytical Services, Pocatello, Idaho.

Site	Purpose	Collection date	Alkalinity, total as $CaCO_3$ (mg/L)	Hardness, $CaCO_3$ (mg/L)	pH
Crystal Springs well	Proposed hatchery water source	Jul 23, 2018	180	242	7.0
		Oct 2, 2018	190	264	7.1
		April 25, 2019	190	241	7.4
Springfield Fish Hatchery headbox	Out-of-basin group rearing water	Jul 23, 2018	200	251	6.9
		Oct 2, 2018	200	266	7.3
		Apr 25, 2019	200	258	7.4
Sawtooth Fish Hatchery	In-basin group rearing water	Jul 27, 2018	60	46	7.1
		Oct 05, 2018	80	80	6.8
		Apr 24, 2019	50	51	6.6
Yankee Fork Pond Series 1	Smolt release site	Jul 27, 2018	40	69	6.5
		Apr 24, 2019	40	54	6.7
Yankee Fork Pond Series 3	Parr release site	Oct 3, 2018	50	75	6.4

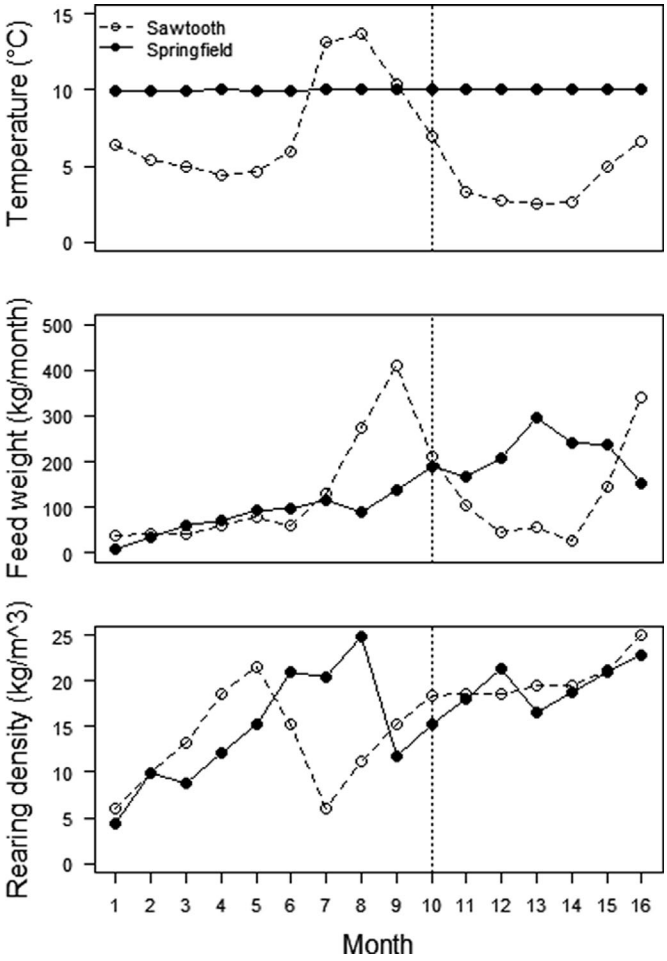

FIGURE 2. Rearing conditions for Chinook Salmon reared at Sawtooth Fish Hatchery (in basin) and Springfield Fish Hatchery (out of basin) from January 2018 (month 1) to April 2019 (month 16), including temperature (top panel), feed rate (middle panel), and rearing density (bottom panel). The horizontal dotted line indicates when a small portion of reared fish was released for parr evaluations.

changes experienced by the two groups by extending the route for the Sawtooth fish. The Springfield group was transported from Springfield Fish Hatchery over Willow Creek Summit (elevation 2,183 m) into the Salmon River basin at Challis, Idaho, then up the Salmon River to the Yankee Fork. The Sawtooth group was transported down the Salmon River and up to Willow Creek Summit before turning around and driving back up the Salmon River to the Yankee Fork (Figure 1). For both the parr and smolt releases, the transport trucks left their respective hatcheries between 0800 and 0830 hours and arrived at the Yankee Fork between 1200 and 1330 hours. Water temperatures did not vary substantially between the initiation and end of transportation because of cool ambient air temperatures during transport for both the parr and smolt releases, making tempering of transport water prior to release unnecessary.

We released the parr into Pond Series 3 of the Yankee Fork (Figure 1; hereafter, referred to as "parr release site") on October 3, 2019. Pond Series 3 is a restored, groundwater-dominated, low-velocity, beaver-influenced side channel, which facilitated the visual observation of the released fish. We released all of the smolts that were not used in physiological stress evaluations into the mainstem Yankee Fork from a tanker truck parked on a bridge spanning the river.

Biological Characteristics

To examine potential differences in fish size between the release groups at the parr stage, we measured the fork lengths of 1,166 and 4,987 individuals at the in-basin Sawtooth Hatchery and out-of-basin Springfield Hatchery, respectively. The large difference in sample size between groups was caused by technical difficulties with the digital measuring board during the marking session at Sawtooth Hatchery. In addition to measuring fork length, we also weighed 239 and 232 individuals at Sawtooth Hatchery and Springfield Hatchery, respectively. To test for differences between the biological characteristics, we compared the fork lengths and weights between the two groups. Because the variance differed between the two groups (variance ratio test: $F = 2.57$, $df_{numerator} = 4986$, $df_{denominator} = 1165$, $P < 0.001$), we compared fork lengths with a Behrens–Fisher t-test in the 'asht' package (Fay 2018) in R (R Core Team 2019), which is robust to differences in variance between the two groups under conditions when there is also a large difference in sample size (Zar 2010).

TABLE 2. Details of parr and smolts reared at two different hatcheries and released into the Yankee Fork Salmon River. The number of PIT tags included in the smolt release groups was determined by using a power analysis (see Supplement I).

Life stage	Hatchery	Group	N released	N tags	Release date
Parr	Springfield	Out of basin	4,998	4,998	Oct 3, 2018
	Sawtooth	In basin	5,000	5,000	Oct 3, 2018
Smolt	Springfield	Out of basin	101,577	4,179	Apr 25–26, 2019
	Sawtooth	In basin	94,462	4,587	Apr 25–26, 2019

The biological characteristics of the smolts were measured on the same fish that were sampled for stress physiology (see next subsection). After euthanizing the fish for blood sampling, each fish was individually blotted dry with a paper towel and measured for fork length (mm) and body weight (g). We used these measurements to calculate Fulton's condition factor (K) as $K = \frac{W}{L^3}$ where W = body weight in g and L is fork length in cm (Ricker 1975). It is typical during most hatchery smolt releases that a population of fish will display varying degrees of smolt development ranging from parr to fully developed smolts, especially in programs that release fish hundreds of kilometers from the ocean in early spring like those of the upper Salmon River basin. Thus, each fish was visually assessed for smolt index (1 = parr, 2 = transitional, 3 = smolt; modified from Gorbman et al. 1982). Following this index, "parr" have dark parr marks; transitional fish have fading parr marks and some silvering appearance; while smolts have few or no parr marks, silver coloration, some loss of scales, and clear fins. Following blood collection for the physiological evaluations (see next subsection), sex was determined by visual identification. Males were further visually identified as either immature or likely to mature the following autumn as an age-2 minijack based on testes morphology (Larsen et al. 2004). The biological characteristics of the fish that were sampled from the live wells during smolt physiology monitoring were compared across release groups using an unpaired t-test with significance set at $\alpha = 0.05$, and sex ratios were compared using chi-square test.

Stress Physiology

We evaluated the stress physiology of only the smolt groups in this study by measuring plasma cortisol, plasma glucose, blood lactate, hematocrit, blood Na^+, and blood Cl^- concentrations. Briefly, plasma cortisol has an important role (among others) in the "fight or flight" endocrine response to acute stress in most vertebrate taxa (Barton 2002) and smoltification (Barton et al. 1985). Plasma glucose and blood lactate, part of the secondary stress response, provide short- and long-term indicators, respectively, of energy mobilization. Hematocrit is a measure of red blood cell volume relative to total blood volume and may reflect swelling change in blood cell size, the number of blood cells, and/or hemodilution. Blood Na^+ and Cl^- concentrations measured over time reflect plasma ion homeostasis.

One thousand individuals from each hatchery were transported to the Yankee Fork on April 23, 2019, along the same route described for the main group releases. We distributed 800 of the total transported fish from each group among eight partitioned live wells (Figure 3). Excess fish (approximately 200 per group) were released at the sampling site. Four live wells were designated for physiological evaluations and four live wells were used to monitor acute mortality among undisturbed fish (see next subsection). All of the live wells were constructed from 100-gal Rubbermaid polyethylene stock tanks that were perforated on the sides and bottom with 9.5-mm holes. Each tank was partitioned into two compartments with perforated 6.3-mm UHMW sheeting and had a plywood lid hinged at the center to allow each compartment to be opened separately. We placed the live wells into the pond and leveled them with rocks such that the top 2 in (~51 mm) of the wells were above water to prevent escape during sampling (Figure 3). We placed 100 individuals from each hatchery group in each live well (200 total,

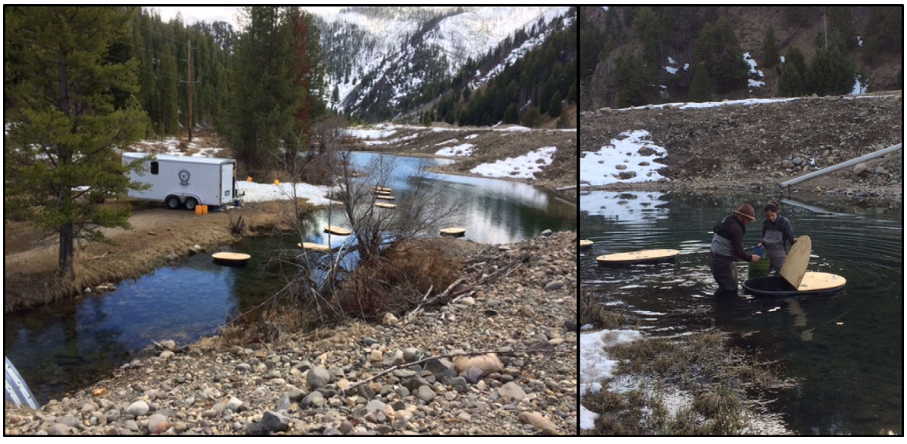

FIGURE 3. Setup of live wells and mobile fish sampling station in Pond Series 1 of the Yankee Fork Salmon River at the onset of smolt physiology sampling and acute mortality monitoring. Left panel: The four live wells lined up above the center line of the photograph held individuals used for blood sampling, whereas the four live wells distributed below the center line were designated for monitoring acute mortality. The placement of the live wells was constrained by depth and bottom substrate type. Right panel: Each live well was separated into two partitions, each with a separate opening to access, sample, and enumerate fish.

segregated by the partition) at a density of 13 kg/m^3—well within the range of acceptable rearing densities for Chinook Salmon (Ewing and Ewing 1995).

We collected blood samples from lethally sampled smolts to measure indices of stress according to a blocked design (Zar 2010). Twenty fish from each group were haphazardly collected from the hatchery raceway before transport and the fish transport truck after transport using a dip net, and then five fish were haphazardly collected from each live-well partition using a large aquarium net (five from each hatchery group) at the three postrelease times (4, 24, and 48 h) for a total of 20 fish from each group at each point. After netting, the fish were immediately sedated by immersion in a bath of metomidate hydrochloride (~5 mg/L; Aquacalm, Western Chemical, Ferndale, Washington, USA) and sampled within a minimum of ~1 min (first fish) and a maximum of ~20 min (last fish). Metomidate hydrochloride blocks corticosteroid synthesis in some fish species, including salmonids (Olsen et al. 1995; Davis and Griffin 2004), and is useful for minimizing the confounding effects of sampling on stress-related blood chemistry parameters. Depending on the postrelease time, sampling was conducted either at the respective hatchery facility or in a cargo trailer that was converted into a mobile wet lab at the release site on the Yankee Fork (Figure 3). We recognize that the ~20-min gap between sampling the first and last fish of each group may have had an effect on our metrics. However, because the groups were treated in an identical manner any effects of time delay would be similar between groups and would not reduce our ability to compare between them.

After severing the caudal vasculature with a single-edge razor blade, blood samples were collected in heparinized Natelson tubes (Fisher Scientific, Hampton, New Hampshire, USA), transferred to 0.5-mL microcentrifuge tubes, and kept on wet ice for no more than 4 h until further processing. It is important to note that during the first (4 h) sampling time, it was increasingly challenging to collect sufficient blood from some of the smaller and/or moribund fish in the Springfield group; thus the decision was made to target sampling of relatively larger, less moribund fish for subsequent sampling times (24 and 48 h) in this group to ensure the collection of sufficient blood for laboratory analyses. Whole blood was used for the determination of lactate (Lactate Plus lactate meter; Nova Biomedical, Waltham, Massachusetts, USA). A subsample of whole blood was transferred immediately after collection to microcapillary tubes, centrifuged (3,000 × *g*; 6 min) and used to measure hematocrit (HCT). Relative measures of blood Na^+ and Cl^- were determined using an i-STAT handheld analyzer and Chem8+ cartridges (Abbott Laboratories, Lake Bluff, Illinois, USA) according to the manufacturer's instructions. The iSTAT handheld analyzer was originally designed for use with mammalian rather than fish blood so it does have recognized limitations for providing accurate absolute measures in fish (Harter et al. 2014). However, as discussed in Trushenski et al. (2019), it does provide rapid ion values that are useful for relative comparisons of blood chemistry between groups. The remaining whole blood samples were then centrifuged (3,000 × *g*; 6 min), and the plasma was removed and frozen at −20°C before transfer to an ultra-low temperature freezer (−80°C) at the Northwest Fisheries Science Center, Seattle, Washington, for storage. The plasma samples were then analyzed for glucose and cortisol via Liquid Glucose [Hexokinase] Reagent (Sigma-Aldrich, St. Louis, Missouri, USA; method adapted to 96-well plates) and ELISA (Cayman Chemical, Ann Arbor, Michigan, USA), respectively, as described in Trushenski et al. (2019).

We statistically compared postrelease blood chemistry measurements across release groups using a type III ANOVA using the 'lmerTest' package in R (Kuznetsova et al. 2017), with hatchery group and sampling period as fixed categorical factors and live well as a random categorical factor (i.e., block). We qualitatively evaluated differences in blood chemistry pre- and posttransport because these two times were not blocked into four partitions per sample period.

Acute Mortality

Parr.— We evaluated acute mortality for parr in both closed and open environments. For the closed environment, we transferred 500 individuals from each hatchery group into two 0.5-m^3 perforated steel live wells at a stocking density of 15kg/m^3—within the range of accepted rearing densities for Chinook Salmon (Ewing and Ewing 1995). The live wells were placed side by side at the upper end of the side channel and leveled with rocks. The upper 2 in of the live wells were above water to prevent escape during mortality and behavior monitoring. A total of 250 fish from each truck were hand-counted into buckets and transferred to each live well. Therefore, each live box held 500 individuals that were mixed equally between the two hatchery groups and the groups were distinguishable by PIT tag codes. We monitored the behavior and acute mortality of fish in the live wells at 45 min and 3, 6, 21, 24, 28, 42, and 47 h postrelease. All live individuals were released from the live wells after 48 h.

The remaining 4,500 individuals from each group were released into the side channel through an 8-in (~203 mm) pipe immediately following the transfer of fish to live boxes. After release, we assessed relative acute mortality of the full parr release for 48 h by visually surveying the channel for carcasses. We walked from the channel's downstream confluence with the main-stem Yankee Fork upstream to the mouth of Cearly Creek (Figure 1), where surface flow into the side channel ended. Using polarized

sunglasses, one person walked on each bank and one person walked in the channel while searching for moribund or dead fish. We recorded fish behavior and retrieved mortalities, which we identified to either respective hatchery group by using a Biomark HPR plus handheld PIT tag reader. At least two channel surveys were conducted daily from October 3, 2018, to October 5, 2018.

Smolt.— We monitored acute mortality of smolts in the eight live wells described in the "stress physiology" subsection (Figure 3), and mortalities were enumerated at the same points as physiological sampling. We originally planned to enumerate mortalities without removing dead fish; however, after 24 h we found it necessary to remove dead individuals from some partitions to obtain accurate counts. At this point, and during subsequent sampling events, mortalities were removed from live wells with small aquarium nets.

Survival during Downstream Migration

We monitored parr and smolt survival from release to emigration from the Yankee Fork and Lower Granite Dam (Figure 1) using stationary PIT tag antennae in the lower Yankee Fork (site code YFK) and at main-stem Snake and Columbia River interrogation sites. Survival to Lower Granite Dam is a common performance metric for Snake River basin hatchery programs because it represents survival through the unimpounded portion of the migratory corridor and thus is unaffected by hydrosystem operational decisions. The Yankee Fork PIT array is a dual flat-panel, pass-over array that is located 3.1 rkm upstream from the confluence with the Salmon River and 5.9 rkm downstream from the parr release site. The main-stem interrogation sites include a large network of antennae on various dam structures and spillways throughout the Snake and Columbia rivers. We extracted tag detection files and PIT antennae interrogations from the Columbia Basin PIT Tag Information System (PTAGIS; www.ptagis.org) and analyzed the outputs in PitPro 4: PIT Tag Processor (Westhagen and Skalski 2009) using the Yankee Fork PIT array, Lower Granite Dam, Little Goose Dam, Lower Monumental Dam, Ice Harbor Dam, McNary Dam, John Day Dam, the Bonneville Dam Complex, and the Estuary Towed Array as detection sites. We planned to use the capture history output from PitPro 4: PIT Tag Processor (Westhagen and Skalski 2009) as the input to the Survival Under Proportional Hazards software program (SURPH 3.5.2; Lady et al. 2013) and use the ANODEV test for significance between groups with hatchery as the covariate. Yet, differences in survival between groups were so great that comparisons could be confidently made visually (see results) using the survival rate output from PitPro 4.

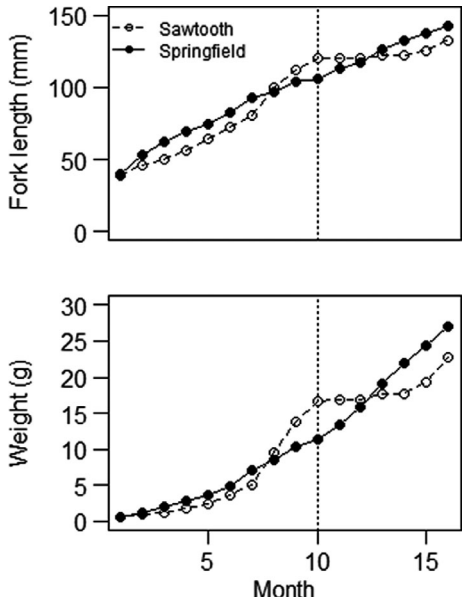

FIGURE 4. Monthly fork length (top panel) and weight (bottom panel) of Chinook Salmon reared at the Sawtooth Hatchery (in basin) and out-of-basin Springfield Hatchery from January 2018 (month 1) to April 2019 (month 16). The horizontal dotted line denotes the parr release.

RESULTS

Biological Characteristics

We observed seasonal variation in mean fork length (mm) and weight (g) between the two hatchery groups throughout rearing (Figure 4) and found a significant difference in fork length between the two parr groups during tagging in early September 2018 (Behrens–Fisher $t = -35.83$; $P < 0.0001$). Fork length of the out-of-basin Springfield group (mean $\pm$ SD = 91.0 $\pm$ 7.9 mm) was 7% lower than that of the in-basin Sawtooth group (97.6 $\pm$ 4.9 mm). At the time of transfer to the Yankee Fork, fish were loaded on the tanker truck at 16.9 fish per pound (individual fish weight of approximately 28.7 g) and 20 fish per pound (22.7 g) from the out-of-basin and in-basin groups, respectively. Thus, the out-of-basin group was marginally larger and the in-basin group marginally smaller than the target size of 18 fish per pound (25 g) at the time of release.

Throughout the stress physiology monitoring of smolts in April 2019, we quantified the size, smolt index, sex, and precocious male maturation proportion of the 100 lethally sampled individuals from each of the hatchery groups. The out-of-basin smolts were 14% longer and 42% heavier than the in-basin smolts, but they had a 7% lower condition factor (Table 3). The male : female sex ratio of the out-of-basin group was skewed toward males ($\chi^2_{0.05,1} = 5.76$, $P = 0.016$; Table 3), whereas the in-basin group was marginally, but not significantly, skewed toward females

TABLE 3. Biological characteristics of fish sampled from live wells during smolt physiology monitoring conducted April 23–25, 2019. The asterisks indicate significant differences between rearing groups for specific characteristics ($P < 0.0001$); SEM = standard error of the mean.

Metric	Out of basin	In basin
Total samples	100	100
Number of males	62	45
Number of females	38	55
% of sampled males maturing	30.6	40.0
Length (mm) ± SEM	145.6 ± 1.36	127.54 ± 0.72*
Weight (g) ± SEM	34.41 ± 1.18	24.05 ± 0.47*
Condition factor ± SEM	1.074 ± 0.011	1.146 ± 0.006*
% transitional (smolt index = 2)	58	92
% smolt (smolt index = 3)	42	8

($\chi^2_{0.05,1} = 1.00$, $P = 0.317$; Table 3). Visual assessments of testes morphology indicated that 30.6% and 40% of the out-of-basin and in-basin males had initiated maturation as an age-2 minijack, respectively. While 42% of the out-of-basin group were visually classified as smolts (smolt index = 3), only 8% of in-basin group were. The remaining individuals were classified as transitional (smolt index = 2), and no fish in either group were classified as parr (smolt index = 1). This relatively limited subsample indicated that the out-of-basin group had more advanced smolt development and less evidence of precocious male maturation than the in-basin group.

We observed a difference in the fin quality of fish from the two hatchery groups. Starting as early as mid-March 2019 (during precocious male maturation monitoring for a separate study; Larsen et al. 2020) until the end of the smolt release evaluations, some out-of-basin fish displayed poor fin quality. Most of the out-of-basin fish that were sampled for blood chemistry and those removed from live wells during acute mortality enumeration had eroded pectoral fins and frayed caudal fins (Figure 5). These observations suggest that the fish that were reared at the out-of-basin Springfield Hatchery were nipping and biting each other during some portion of hatchery rearing.

Stress Physiology

The blood chemistry measurements showed clear differences between the out-of-basin and in-basin groups after release into Yankee Fork water (Table 4; Figure 6). Hatchery group was a significant determinant of blood chemistry responses in all cases (Table 4). For some blood chemistry measures, the pattern of change across time also differed between groups, as indicated by a significant interaction term (Table 4).

The plasma cortisol concentrations of the two groups were similar prior to transport, but cortisol in the out-

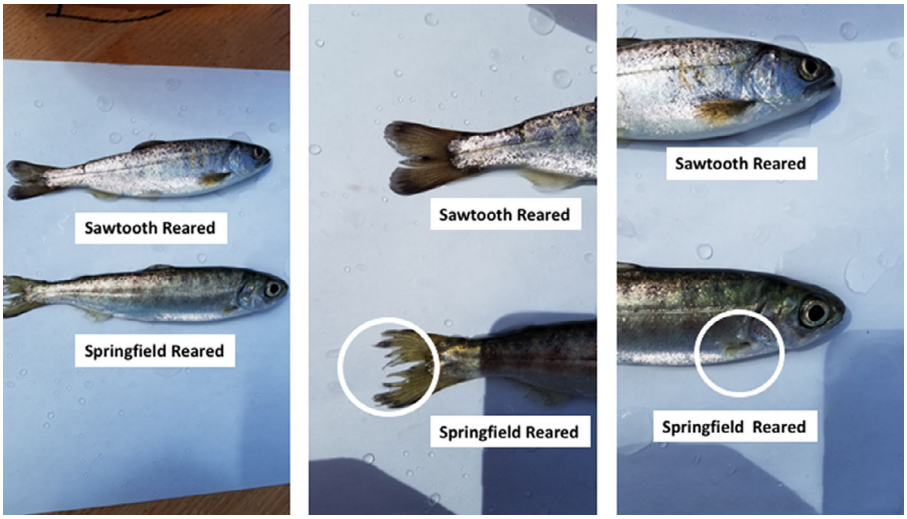

FIGURE 5. Visual comparison of haphazardly selected smolts from the in-basin group (top fish; Sawtooth Reared) and the out-of-basin group (bottom fish; Springfield Reared). The white circles show the existing fin damage in the Springfield group.

TABLE 4. Results of mixed effects ANOVA (type III) evaluating the response of juvenile Chinook Salmon blood chemistry metrics to differences in hatchery rearing group (out of basin, in basin) and time after release into live wells in the Yankee Fork Salmon River (sample period). The live wells were considered blocks and used as a random factor, and the model estimates from each ANOVA are shown in Figure 5.

Response	Source of variation	Sum of squares	Numerator df	Denominator df	*F*	*P*
Sodium (Na^+)	Group	19,395.4	1	108	266.8	<0.001
	Sample period	349.2	2	108	2.4	0.01
	Group × sample period	262.9	2	108	1.8	0.1689
Chloride (Cl^-)	Group	28,488	1	100	396.1	<0.001
	Sample period	241.4	2	100	1.7	0.19
	Group × sample period	1,232.2	2	100	8.6	<0.001
Lactate	Group	261.7	1	111	298.7	<0.001
	Sample period	1.61	2	111	0.9	0.40
	Group × sample period	0.11	2	111	0.1	0.94
Hematocrit	Group	2,047.0	1	107	128.9	<0.001
	Sample period	1,436.9	2	107	45.2	<0.001
	Group × sample period	1,53.4	2	107	4.8	0.01
Glucose	Group	739,257	1	113	259.2	<0.001
	Sample period	13,172	2	113	2.3	0.10
	Group × sample period	71,540	2	113	12.5	<0.001
Cortisol	Group	1,058,073	1	114	166.43	<0.001
	Sample period	59,295	2	114	4.66	0.01
	Group × sample period	184,931	2	114	14.54	<0.001

of-basin group was slightly more elevated than that in the in-basin group after transport (Figure 6A). The highest plasma cortisol concentration measured for the in-basin group occurred at 4 h after release at over fourfold higher than pretransport levels and then declined to pretransport levels at 24 h. In contrast, plasma cortisol concentrations of the out-of-basin group increased significantly after release and were 35-fold higher at 24 h postrelease than at pretransport levels and 14.9-fold higher than that of the in-basin group. At 48 h, the out-of-basin group's plasma cortisol level decreased from its highest measurement but remained 22-fold higher than pretransport levels. Thus, out-of-basin smolts displayed an extreme and sustained stress response.

Plasma glucose levels were higher in the out-of-basin group than the in-basin group during all of the sampling points (Figure 6B). Plasma glucose levels in the out-of-basin group increased dramatically after release, with the highest measurement occurring at 24 h at 141% higher than pretransport and 165% higher than the respective in-basin group's sample. Meanwhile, plasma glucose levels of the in-basin group increased after transport and release and were 63% higher than at pretransport levels at 4 h postrelease—its highest measured point. Out-of-basin group plasma glucose levels remained elevated between the 4- and 48-h sample period, whereas in-basin group plasma glucose declined, resulting in the significant interaction term in the type III ANOVA (Table 4).

Plasma lactate levels prior to transport were 127% higher in the out-of-basin group than the in-basin group (Figure 6C). In both groups, plasma lactate decreased after release, then stabilized; however, lactate remained significantly higher in the out-of-basin group (Table 4; Figure 5C). Hematocrit levels were also higher in the out-of-basin group than the in-basin group throughout the stress evaluation, but the difference between groups was more pronounced postrelease (Figure 6D; significant interaction term in Table 4). Hematocrit in both groups reached its highest measured concentration at 24 h, with the out-of-basin group hematocrit 18% higher than the in-basin group. The in-basin group hematocrit level was 18% higher at 24 h than the pretransport period.

Blood Na^+ concentrations in the in-basin group ranged 140–155 mmol/L across the entire study period (Figure 6E). Blood Na^+ concentration in the out-of-basin group was lower than that in the in-basin group prior to transport, then declined by 21% at 24 h and remained low for the remainder of sampling. Blood Cl^- concentrations followed a similar pattern for both groups—in-basin group blood Cl^- ranged 125–140 mmol/L over the study period, whereas blood Cl^- concentration of the out-of-basin group decreased by 29% at 24 h and remained depressed at 48 h (Figure 6F).

Acute Mortality

After 48 h, we found no mortalities in either group of the 1,000 parr that were placed into live wells. Similarly, we recovered only one mortality from the channel surveys. The one mortality was an out-of-basin fish that

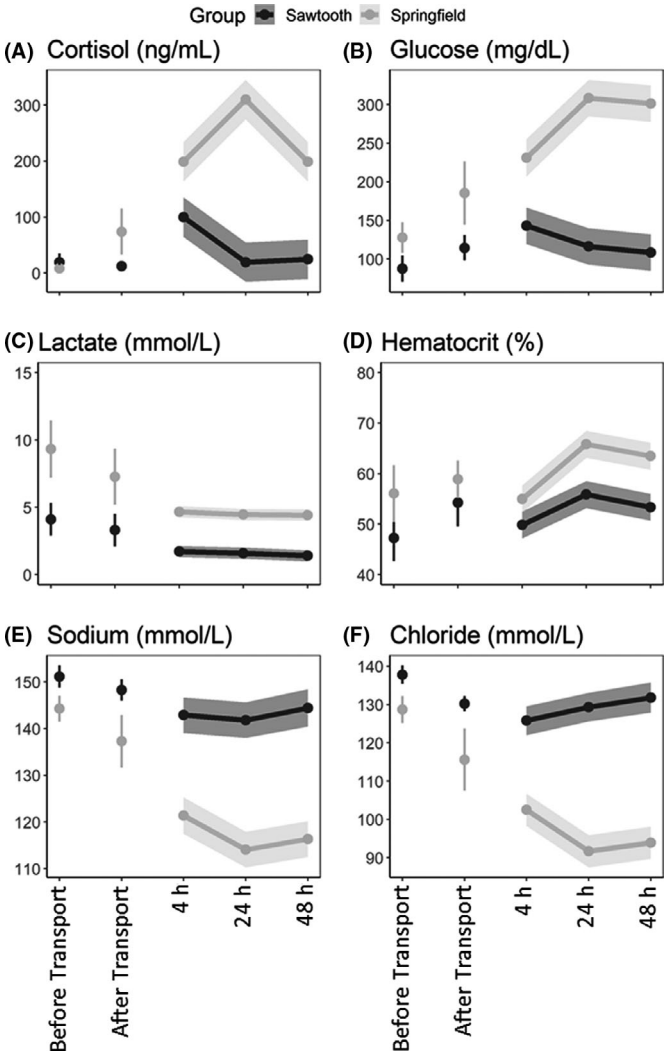

FIGURE 6. Interaction plots of Chinook Salmon smolt **(A)** plasma cortisol, **(B)** plasma glucose, **(C)** blood lactate, **(D)** hematocrit, **(E)** blood sodium, and **(F)** blood chloride. The preloading and posttransport values are the mean ± 1 SD of all fish sampled within a group at that period ($n = 20$) for the in-basin group (Sawtooth; dark gray) and out-of-basin group (Springfield; light gray). The values that are presented for 4-, 24-, and 48-h sample periods are estimates ± 95% CI (shaded regions) from the fitted statistical models and, thus, account for blocking by live well in the analysis.

was first observed behaving irregularly 3 h after release and was recovered 21 h after release near the release site. Otherwise, both groups of fish appeared healthy throughout the 48-h observation period. By the end of the 48-h period, parr were using habitat structure, including large woody debris, undercut banks, and aquatic macrophytes.

We observed high acute mortality of smolts from the out-of-basin group between 24 and 48 h, whereas we observed no mortality in the in-basin group. By 24 h after

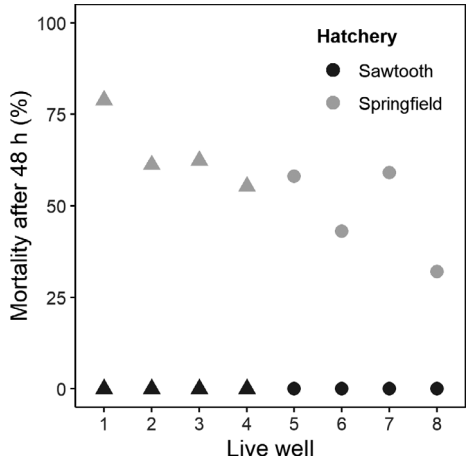

FIGURE 7. Acute mortality percentages of Chinook Salmon smolts reared at the out-of-basin Springfield Fish Hatchery (gray symbols) and in-basin Sawtooth Fish Hatchery (black symbols) groups. Live wells 1–4 had fish sampled for blood chemistry (triangles), whereas individuals were not removed for blood sampling from live wells 5–8 (circles).

release, the mortality percentage in the four acute mortality-designated live wells ranged from 15% to 30% (mean ± SD = 25 ± 7%). Mortality percentages over the whole 48 h evaluation ranged from 32% to 59% (48 ± 13%) in the designated live wells (Figure 7). The mortality percentages in the live wells where the fish were sampled for blood chemistry were higher than those in the unsampled live wells after accounting for individuals that were removed for blood sampling. Acute mortality in the sampled live wells ranged from 22% to 43% (33 ± 8%) after 24 h and 55–79% (64 ± 10%) over the course of the 48-h evaluation (Figure 7).

Survival during Downstream Migration

We detected 323 parr at the YFK array during the autumn migration period and a total of 382 across the fall, winter, and spring periods out of 9,998 tags across the two parr groups (Tables 2 and 5). The out-of-basin group comprised only 12% of total detections despite near equal numbers of tagged fish released. Survival of out-of-basin group to the Yankee Fork array was substantially lower than that of the in-basin group (Table 5). Upon examining the survival estimates produced by the Cormack–Jolly–Seber model in PitPro 4, it became apparent that further comparisons were unnecessary and unlikely to yield good model fits due to low detections of the out-of-basin group. Survival (±SE) to the Yankee Fork array of the out-of-basin and in-basin groups of parr was 3 ± 1% and 12 ± 1%, respectively. The differences in survival to Lower Granite Dam were even more pronounced, with only 0.1 ± 0.07% in the out-of-basin

TABLE 5. Number of tagged individuals detected and survival rate (%) of parr and smolts reared under different water hardness and temperature conditions at two different hatcheries and released into the Yankee Fork Salmon River. The detection points included PIT antenna arrays at main-stem Yankee Fork (YFK) and at dams along the Snake and Columbia rivers (FCRPS). The parr were released on October 3, 2018, and the smolts were released April 25–26, 2019 (See Table 2 for tagging details).

		YFK		FCRPS	
Life Stage	Hatchery	*N*	Survival (SE)	*N*	Survival (SE)
Parr	Out of basin	57	3 (1)	8	0.1 (0.1)
	In basin	325	12 (1)	94	3 (0.5)
Smolt	Out of basin	41	No estimate[a]	25	No estimate[b]
	In basin	159	No estimate[a]	1,267	44 (2)

[a]No usable estimate to the Yankee Fork array because of the high rates of tag collisions that occurred during smolt releases.
[b]The low number of tag detections at main-stem Snake and Columbia River interrogation sites precluded our ability to produce an estimate of survival.

group compared with $3 \pm 0.5\%$ in the in-basin group (Table 5).

Smolt survival for the out-of-basin group to Lower Granite Dam was so low that we were unable to obtain an estimate using a Cormack–Jolly–Seber model. Estimated survival (±SE) of the in-basin group to Lower Granite Dam was $44 \pm 2\%$ (Table 5). Overall, we detected only 25 individuals from the Springfield group at any main-stem Snake and Columbia River interrogation sites, in contrast to the 1,267 individuals from the Sawtooth group that were detected at these same sites.

DISCUSSION

The Crystal Springs Hatchery, designed for out-of-basin rearing of threatened Chinook Salmon for release in the upper Salmon River basin, was proposed to be built adjacent to the Springfield Hatchery that currently rears endangered Redfish Lake Sockeye Salmon. A major question raised by Trushenski et al. (2019) was whether high Sockeye Salmon stress and mortality were reflective of species-specific sensitivity to rapid changes in water chemistry or whether it might be a more general phenomenon among salmonid species. Our results suggest that sensitivity to water chemistry changes may be a more general phenomenon in salmonids, yet recognize that this case study involved variation in multiple factors between the hatchery groups, including differences in water chemistry as well as water temperature and associated rearing conditions. However, these differences could approximate the rearing conditions that Chinook Salmon are likely to experience during out-of-basin rearing prior to release. Thus, the goal of this case study was to expand on the results of Trushenski et al. (2019) to determine the viability of an out-of-basin rearing site for Chinook Salmon under a similar release scenario, and the results of this study provided useful insight toward achieving that goal.

Out-of-basin-reared Chinook Salmon from Springfield Hatchery expressed different biological characteristics, higher stress responses, higher acute mortality, and lower downstream survival compared with in-basin reared Chinook Salmon from Sawtooth Hatchery. Tests were conducted at both the parr and smolt stages of development to control for potential life stage-specific differences in sensitivity (Trushenski et al. 2019). At the parr stage of development, both in- and out-of-basin groups initially experienced negligible acute mortality upon release, but by the time these groups migrated to Lower Granite Dam the in-basin Sawtooth group had fourfold higher estimated survival than the out-of-basin Springfield group (i.e., delayed mortality). During the smolt release, the response was much more rapid and dramatic. The out-of-basin group expressed physiological evidence of extreme stress, had high acute mortality, and had an estimated survival probability to Lower Granite Dam below measurement limits, despite a PIT tagging rate with 90% statistical power to detect survival differences of 6% between the groups (Supplement I). In contrast, the in-basin group displayed a limited stress response following release, zero acute mortality, and an estimated survival probability to Lower Granite Dam similar to that of previous years' releases (0.30–0.64 survival from release from the Yankee Fork to Lower Granite Dam from 2010 to 2018; Shoshone-Bannock Tribes unpublished data) and lower than on-site releases from Sawtooth Fish Hatchery (Widener et al. 2020).

It is well established that rapid changes in water hardness can cause physiological stress to fish (reviewed in McDonald and Milligan 1997). In three genetic strains of Lake Trout *Salvelinus namaycush*, cortisol levels increased fourfold after release from hard water (100 mg $CaCO_3$/L) into soft water (5 mg $CaCO_3$/L), and the elevated plasma cortisol lasted for over 25 d (McDonald and Robinson 1993). The plasma cortisol levels of Redfish Lake Sockeye

Salmon (brood year 2016) that were raised at Springfield Fish Hatchery (water hardness ca. 240 mg $CaCO_3$/L) and released into Redfish Lake Creek, Salmon River basin, Idaho (water hardness 11–12 mg $CaCO_3$/L), changed from negligible concentrations prior to transport to between 600 and 800 ng/mL by 24 h after release into the soft water of the upper Salmon River basin (Trushenski et al. 2019). In the current investigation, brood year 2017 Yankee Fork Chinook Salmon were reared at the out-of-basin Springfield Fish Hatchery and released into the Yankee Fork Salmon River, Idaho (water hardness 56 mg $CaCO_3$/L). The Chinook Salmon experienced a similar temporal pattern of peak measured cortisol at 24 h after direct, unacclimated release into the Yankee Fork. However, plasma cortisol in Chinook Salmon reached its highest measured value at approximately 300 ng/mL, roughly 50% of peak plasma cortisol concentration experienced by Redfish Lake Sockeye Salmon from brood year 2016 (Trushenski et al. 2019). Previous studies have found that the magnitude of the stress response may vary with respect to diet, life stage, and rearing history (Martinez-Porchas et al. 2009). In addition to cortisol's recognized role in the acute stress response, it also has an important regulatory role in the smoltification process, becoming elevated during this period (Barton et al. 1985). The marginally higher cortisol levels observed in the out-of-basin compared with the in-basin fish at the hatchery prior to loading may have been reflective of the greater proportion of visually assessed smolts in that rearing group. Taken together, these differences in cortisol responses to marginally less extreme shifts in water chemistry compared with Redfish Lake Sockeye Salmon (Trushenski et al. 2019) underscore potential interspecific differences in response to stressors (McDonald et al. 1993) and the inherent challenges with comparing plasma cortisol levels across species and studies.

Other indicators of basal stress level were relatively similar prior to transport, but they increased dramatically and significantly in the out-of-basin group only. Plasma glucose levels were significantly higher in the out-of-basin fish but comparatively low in both rearing groups at the hatcheries prior to loading. However, in the out-of-basin fish, plasma glucose levels increased dramatically and significantly after transport and at all points after transfer to the Yankee Fork. In contrast, plasma glucose levels in the in-basin group remained low and unchanged throughout the investigation. These results further corroborate our conclusion that the out-of-basin fish were under significant stress following transfer to the Yankee Fork and were continuing to mobilize metabolic stores to cope with the environmental conditions that were beyond a normal stress response to transport alone.

Contrary to plasma glucose, plasma lactate levels were dramatically higher in the out-of-basin fish compared with the in-basin fish at the hatcheries prior to loading. The mechanisms are unclear but may reflect differences in nutritional status (Vijayan and Moon 1992), fish size, or water temperature at the time of sampling (Meka and McCormick 2005) or be an indicator of impaired health or evidence of anaerobic metabolism in response to some other factor(s) (Olsen et al. 1992). However, as discussed below, we found little pathological evidence to support the supposition of impaired health beyond differences in fin quality. Following transport and placement in the Yankee Fork, the plasma lactate levels of both the in-basin and out-of-basin reared fish decreased and then stabilized at levels lower than that of preloading, but more so in the in-basin-reared fish. This general decline may be reflective of mobilization and exhaustion of lactate levels following transport and transfer in both groups. Hematocrit levels were variable in both groups throughout the investigation but were consistently higher in the out-of-basin-reared fish compared with the in-basin-reared fish (Figure 6). Hematocrit levels of approximately 40–50% are relatively typical for healthy salmonids (Sandnes et al. 1988), but very high hematocrit levels, such as the 60–70% observed in out-of-basin fish, may be indicative of cell hypertrophy, which can lead to hemolysis and eventual loss of O_2 carrying capacity and asphyxiation (Soivio et al. 1974).

Hydromineral homeostasis is essential to the physiological health of fish (reviewed in Trushenski et al. 2019). In the present case study, measures of blood Na^+ and Cl^- levels were significantly different between the groups at the hatchery prior to loading. However, after trucking and throughout exposure to water in the Yankee Fork, the blood ion levels of the out-of-basin fish declined significantly and remained significantly depressed relative to those of the in-basin fish throughout the study. We recognize that the iSTAT blood analyzer does not provide accurate quantitative measures of blood ion concentrations in fish as it was designed specifically for use in mammalian systems (Harter et al. 2014). However, the utility of the iSTAT for making rapid, relative comparisons between groups has been demonstrated in many field studies with fish when conventional equipment may not be available (Cooke et al. 2008; Meland et al. 2010; Regan et al. 2016; Forrestal et al. 2017; McIntyre et al. 2018; Chow et al. 2019). Taken together, these results suggest that, similar to the findings of Trushenski et al. (2019) in Sockeye Salmon, the significant stress response experienced by the out-of-basin fish resulted in a dramatic loss of hydromineral homeostasis and is a likely source of mortality.

It is important to note that we selectively sampled larger fish from the out-of-basin group during the physiological stress sampling to obtain sufficient blood for analysis. We selectively sampled under the logic that obtaining some physiological information from these fish was superior to obtaining no information from dead fish, yet this

likely biased the size measurements of the out-of-basin group toward larger fish, with potential effects on the observed skewness in the sex ratio of smolts (out-of-basin fish were male-skewed vs. in-basin fish, which were slightly female-skewed). Additionally, the higher acute mortality rate that was observed in the sampled live wells (55–79% in out-of-basin fish) versus unsampled live wells (32–59% in out-of-basin fish) may have been an artifact of fish selection for blood sampling. Selectively sampling larger, healthier-looking out-of-basin fish from live wells for blood sampling may have biased mortality rates higher in these live wells because smaller, unhealthy, or moribund individuals remained in the live well. Still, the out-of-basin fish that were sampled for blood at 48 h were the healthiest fish available and therefore should be the fish most likely to recover from stress. Nevertheless, our results demonstrate that sampled out-of-basin fish were unable to return to preloading levels of the physiological factors we measured within 48 h. So, while the physiological measures may have been biased toward larger healthier out-of-basin fish, the fact that we still observed such profound differences suggests our results may likely underestimate the true effect for average-size fish within this group.

Out-of-basin-reared Chinook Salmon expressed different responses to rearing and release into the Yankee Fork depending on the life stage, which agrees with experiments conducted on Sockeye Salmon (Trushenski et al. 2019) and with other studies investigating differences in physiological responses among life stages of salmonid fishes (e.g., Barton et al. 1985; Carey and McCormick 1998). Unlike these previous studies, we did not directly measure physiological stress of parr in this study. Rather, we indirectly evaluated their response by quantifying acute mortality and survival to Lower Granite Dam. Neither the out-of-basin nor in-basin parr experienced acute mortality in the live wells, and only one mortality from the out-of-basin group was observed during the channel surveys (this individual appeared to have been concussed during release). Differences between the parr groups only became apparent during fall emigration from the release site and the Yankee Fork, as well as subsequent survival to Lower Granite Dam. The difference in number of tag detections at the two detection areas (i.e., Yankee Fork and dams throughout the Federal Columbia River Power System Hydrosystem) suggests that while the acute response to rearing differences was less extreme than for smolts, the long-term effect was still present. We do not know whether the lower survival of the out-of-basin parr was a direct result of the change in rearing conditions or whether the sublethal effects of these differences made individuals less competent during overwintering and downstream migration (i.e., tertiary response; Barton 2002).

We remain surprised that Chinook Salmon smolts in our out-of-basin group performed so poorly. The high rates for acute mortality and very low rates for survival during downstream migration that were experienced by Chinook Salmon in this study is unique among prior published investigations on the response of other species (e.g., Sockeye Salmon in Trushenski et al. [2019] and Lake Trout in McDonald and Robinson [1993]). Substantial evidence suggests that there are inter- and intraspecific differences in the ability of fish to tolerate changes in water chemistry, even among closely phylogenetically related species (Barton 2002) and genetic strains within species (McDonald and Robinson 1993). In our study, Chinook Salmon experienced 32–80% mortality after 48 h, which is much higher than that observed in Lake Trout (zero mortality; McDonald and Robinson 1993) or even Redfish Lake Sockeye Salmon reared at the same hatchery (Trushenski et al. 2019), where a single mortality was recorded in live wells. Moreover, the IDFG operates three anadromous steelhead *Oncorhynchus mykiss* hatcheries (Niagara Springs Fish Hatchery, Magic Valley Fish Hatchery, and Hagerman National Fish Hatchery) with water hardness that is similar (230–270 mg $CaCO_3$/L) to that at Springfield Fish Hatchery (Table 1). These hatcheries have reared and released steelhead smolts from multiple genetic stocks at sites in the upper Salmon River for almost 40 years, with survival to Lower Granite Dam sometimes exceeding 60% (Ainsworth et al. 1992; Dorman and Chapman 2002; Warren et al. 2017). In contrast, Sockeye Salmon that were exposed to approximately the same water hardness difference between rearing and release achieved only 16% survival to Lower Granite Dam (Trushenski et al. 2019), and in our study, downstream survival of Chinook Salmon experiencing the same conditions was so low that we were unable to estimate it due to lack of detections. While it is difficult to compare results across studies, these findings suggest that *Oncorhynchus* spp. may exhibit some variability in terms of their tolerance to abrupt changes in water hardness. However, further investigation using a "common garden" experimental construct with proper control for variation in rearing regime and water temperature is required for strong support of this hypothesis.

A challenge that we encountered in this study concerned differences in fin quality as a result of agonistic behavior and questions regarding the general health of fish from the two hatchery groups that may have confounded our results. While not ideal, agonistic behavior is not uncommon in hatchery salmonids, including Chinook Salmon (Wessel et al. 2006). Furthermore, beyond fin erosion, we found no visual evidence of pathology in the fish that we sampled for physiological stress. Preliberation health screens detected only one incidence of *Aeromonas hydrophila* in 12 pooled samples of five fish each for the out-of-basin parr group and no pathogens in 60 fish that were sampled from the out-of-basin smolt group (D. Munson, IDFG Eagle Fish Health Laboratory, personal

communication). Additionally, we provided all carcasses from the acute mortality evaluation to the IDFG for necropsy, which yielded no obvious signs of pathogen-associated impairments (Munson, personal communication). While fin erosion of out-of-basin reared smolts may have impaired swimming ability and could have acted as a compound stressor, we do not believe it was a significant contributor to the acute mortality that we observed.

In conclusion, out-of-basin hatcheries are relatively common throughout the Pacific Northwest but there is limited published information on how the combined effects of differences in environmental conditions between rearing and release influence fish condition, stress, survival and, in turn, the effectiveness of a hatchery program. Ultimately, the challenges that we encountered in our study may be rooted in our comparison of a single release group to another single release group without substantial replication. Therefore, our results should be viewed as a case study that raises important broadly applicable management questions and suggests patterns of which mechanisms should be explored further. To date, few studies describe thresholds where homeostatic mechanisms are challenged by a stressor or multiple stressors beyond a fish's ability to continue regulating internal processes. Where does a complete failure of ion-regulatory processes occur? If a complete failure does not occur, does the stress caused by multiple stressors have long-term effects on fish performance that may extend into and beyond their downstream migration period and ultimately affect recruitment of adults? Overall, the results of this study, when viewed in the context of other recent evaluations (Trushenski et al. 2019), bring to light the importance of understanding and accounting for intra-and interspecific differences in response to water chemistry changes and other rearing conditions (i.e., temperature) during hatchery program planning and implementation. In the end, an in-basin hatchery that mimics the water chemistry and temperature regimes in which these animals evolved or an out-of-basin hatchery that can match in-basin conditions may be better options for attaining optimal production and harvest goals. Finally, this investigation highlights the value of conducting preliminary "proof-of-concept" or "look-before-you-leap" studies, employing both physiological and behavioral/survival evaluations, in advance of large hatchery supplementation efforts. Based on the results of this study, comanagers from the Shoshone-Bannock Tribes, IDFG, and the Bonneville Power Administration have discontinued efforts to build the Crystal Springs Hatchery adjacent to the Springfield Hatchery as a smolt production facility. They are currently evaluating alternative sites for rearing and acclimation in the upper Salmon River basin for the recovery of threatened Chinook Salmon populations (NWPCC 2022). While this is a disappointing short-term setback to the Yankee Fork Supplementation Program, the alternative of building an out-of-basin hatchery facility that is not well suited to the future success of the restoration effort would likely have been a more daunting outcome.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.