EFFECTS OF FLOW, WATER QUALITY, AND HYPOXIA ON THREATENED SALISH SUCKER (*Catostomus* sp. cf. *catostomus*) AND JUVENILE COHO SALMON (*Oncorhynchus kisutch*)

by

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EFFECTS OF FLOW, WATER QUALITY, AND HYPOXIA ON THREATENED SALISH SUCKER (*Catostomus* sp. cf. *catostomus*) AND JUVENILE COHO SALMON (*Oncorhynchus kisutch*)

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the degree of   Master of Science

in   Zoology

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Abstract

The Salish sucker (*Catostomus* sp. cf. *catostomus*) is a federally Threatened species under Canada's *Species at Risk Act* and is restricted to 11 watersheds in British Columbia (lower Fraser River Valley) and six in Washington State. Agricultural development has been historically prominent in these areas, and hypoxia and the physical destruction of habitat have been identified as the most important threats to this species. Synergistic effects of reduced streamflow, nutrient inputs, and high temperatures are likely large determinants of seasonal hypoxia. The effects of stream flow on temperature and dissolved oxygen in Salish sucker critical habitat, and corresponding changes in habitat use, distribution, and growth of Salish sucker were studied. As salmonids are even more sensitive to impaired water quality than suckers, and maintaining salmonid populations is an additional major conservation concern in the lower Fraser Valley, the biological response of juvenile coho salmon was also studied as they co-occur in Salish sucker habitats. Through various flow manipulation and enclosure experiments I found that severely reducing flow in off-channel ponds resulted in low levels of dissolved oxygen (< 3 mg·L^{-1}). Salish sucker showed variable growth rate differences in flow and no-flow treatments, while juvenile coho salmon showed significantly decreased growth under the no flow treatment. A subset of fish were tagged with a passive integrated transponder (PIT), and their movement into an enclosed oxygenated refuge was studied. Salish sucker and coho salmon both made greater use of the oxygenated refuge under the no flow treatment. Salish sucker use of the oxygenated refuge was highest at night. It is likely that the availability of an

oxygenated refuge in all treatments mitigated negative impacts of reduced water quality on Salish sucker. The results of my thesis will help inform the potential costs and benefits of flow reduction or flow enhancement, and the potential impacts of altered flows in a changing climate.

Lay Summary

The lower Fraser River Valley of British Columbia is home to many native species of freshwater fishes. As this area coincides with high urban and agricultural pressure, negative impacts on fish communities have occurred. The Salish sucker (*Catostomus* sp. cf. *catostomus*) is listed as Threatened under the federal *Species at Risk Act* (SARA). Habitat loss and low dissolved oxygen have been identified as the main threats to the Salish sucker. I looked at how reduced streamflow affects water quality, and how this change in water quality affects Salish sucker growth and habitat use. I found that a reduction in streamflow resulted in impaired water quality, including very low dissolved oxygen levels that may be lethal to fish. Additionally, this reduction in water quality resulted in reduced habitat use and variable growth for Salish sucker. The results of my thesis will inform both current and future habitat and land use management.

Preface

This research was conducted in collaboration with the Canadian Freshwater Species at Risk Research Network (Fisheries and Oceans Canada), the Fisheries and Oceans Canada Pacific Region Species at Risk section, the B.C. Ministry of Environment, and Dr. Mike Pearson. I held primary responsibility for the study design, analysis of the data, and for the preparation of the manuscript for submission. Field work was led by myself with the assistance of Dr. Jordan S. Rosenfeld and Alyssa Nonis. Throughout the process I received valuable supervision and guidance from my supervisors, Dr. Jordan S. Rosenfeld and Dr. Eric B. Taylor, and from my supervisory committee member, Dr. Scott G. Hinch. A version of this thesis will be submitted for publication in April 2020 as "Effects of flow, water quality, and hypoxia on Threatened Salish sucker (*Catostomus* sp. cf. *catostomus*) and juvenile coho salmon (*Oncorhynchus kisutch*)." (Authors: Kaitlyn R. Zinn, Jordan S. Rosenfeld, and Eric B. Taylor). This study was conducted and written by KRZ under the supervision and guidance of JSR and EBT who helped with the conceptualization of the study and the preparation of the manuscript. All capture, tagging and handling procedures were approved by the University of British Columbia Animal Ethics Committee (animal care permit: A18-0093) in accordance with the Canadian Council of Animal Care. Other permits obtained for this research include Provincial fish collection permits (SU18-320475), Federal Species at Risk Act permits (18-PPAC-00011, 18-PPAC-00021), and a Special Use Permit from Metro Vancouver Regional Parks.

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Introduction

Freshwater ecosystems are home to over 10% of known species, while occupying less than 1% of the Earth's surface (Balian et al. 2008; WWF 2018). Freshwater species also represent the fastest declining fauna on the planet (Strayer and Dudgeon 2010; Darwall et al. 2018; Reid et al. 2019), with an estimated 83% decline in number of species since 1970 (WWF 2018). This disproportionate decline likely reflects the fact that human settlement is focused around lakes and streams, many of which are isolated and small in area, which concentrate the diffuse land use impacts that occur throughout their watersheds. Consequently, the key drivers of decreased freshwater biodiversity are intertwined with human development impacts, including overexploitation, urbanization, agriculture, and flow regulation (Arthington et al. 2016; WWF 2018). The dominant threats associated with human development include habitat modification, habitat fragmentation, pollution, disease, and climate change (WWF 2018).

Climate warming in particular is an overarching stressor that may act synergistically with other human impacts (Schindler 2001). Increased air and water temperatures adversely impact the physiology of coldwater taxa by directly elevating metabolic rates, and by lowering oxygen saturation concentrations available for aquatic life (Mimikou et al. 2000; Kalff 2002). Of particular concern is the interaction between climate warming and eutrophication. Anthropogenic nutrient enrichment in combination with increasing temperatures may result in severe eutrophication (Rogers et al. 2016), where increased respiration associated with algal and macrophyte blooms triggers

decreased dissolved oxygen (Smith 2003; Ficke et al. 2007; Whitehead et al. 2009). Hypoxia, or suppressed dissolved oxygen, is a widespread problem in aquatic systems subject to urban and agricultural impacts (Rabalais et al. 2010; Díaz and Rosenberg 2011; Jenny et al. 2016). These impacts will likely be exacerbated under climate warming, where warmer temperatures decrease saturation levels of dissolved oxygen while driving up fish respiration rates and overall biological oxygen demand. Depressed dissolved oxygen can lead to anoxia-related fish kills (Klapper 1991; Kalff 2002) and sublethal effects on growth, even for tolerant species (Whitehead et al. 2009).

Reduced streamflow is an associated driver of hypoxia, and seasonal hypoxia is commonly associated with declining summer flows. Reduced flow may result in elevated stream temperature because of a reduction in thermal inertia (Poole and Berman 2001; Kalff 2002), while diminished surface turbulence in riffles further reduces the diffusion rate of oxygen into the water column, making rivers especially susceptible to hypoxia during periods of warm dry weather associated with low flows. Consequently, declining streamflow may decrease further when accompanied by elevated temperatures and nutrient enrichment to cause severe hypoxia, that will likely get worse under climate change (Poff and Zimmerman 2010; Acreman et al. 2014). These effects are of particular concern for endemic taxa whose distribution strongly overlaps with human development, e.g. valley bottom lowland species.

The lower Fraser River Valley of British Columbia is an area of intense human settlement typical of highly developed landscapes where urban and agricultural development expands adjacent to rivers as water supply features and transportation corridors (Tockner and Stanford 2002; Hauer et al. 2016). This development is

coincident with abundant small streams that support native salmonids and several

Threatened fish species including the Salish sucker (*Catostomus* sp. cf. *catostomus*),

which is endemic to low gradient headwater streams in the region and adjacent portions

of Washington State. Many of these streams have been channelized, associated

wetlands have been drained, riparian vegetation removed, and many receive nutrient

and sediment runoff from urban and agricultural catchments. Consequently, hypoxia

and the physical destruction of habitat have been identified as the most important

threats to Salish sucker (Fisheries and Oceans Canada 2019).

Salish sucker ecology and conservation status

The ecological consequences of hypoxia to individual fish can be classified into

three broad categories: direct lethal effects under severe hypoxia; sublethal effects that

allow habitat use but result in reduce energy intake and growth (i.e. reduction in habitat

quality); and sublethal effects associated with effective loss of habitat to hypoxia,

resulting in density-dependent effects in the remaining usable habitat (reduction in

habitat availability). Although Salish sucker are likely better adapted to hypoxic

environments than the salmonids with which they often co-occur (Fisheries and Oceans

Canada 2019), sucker caught in traps are known to suffer mortality if they experience

severe hypoxia (Zinn, pers. obs). The scope and occurrence of sublethal effects (e.g.

reduced growth) is poorly understood, but of particular concern as sublethal effects may

be widespread even in the absence of direct mortality. To better understand the effects

of low water quality on sublethal effects and habitat use by Salish sucker, I performed

experimental field manipulations of flow and water quality. The goals of my thesis were to better understand 1) how stream flow affects water quality (dissolved oxygen and temperature), and 2) how a decline in water quality affects Salish sucker growth and habitat use. In addition to assessing the response of Salish sucker to flow and water quality, I included juvenile coho salmon (*Oncorhynchus kisutch*) in experimental treatments as a potentially more sensitive control taxa, and to better assess the effects of hypoxia on the broader fish community. My thesis work will help inform the potential costs and benefits of flow reduction or enhancement, and the potential impacts of altered flows in a changing climate.

Methods

Focal species

The Salish sucker is listed as a federally Threatened fish under Canada's *Species at Risk Act* (SARA; status updated from Endangered to Threatened August 2019). Salish sucker are restricted to 11 small watersheds in the lower Fraser Valley of British Columbia and six adjacent watersheds in Washington State, USA. The Salish sucker is a member of the Chehalis fauna, and its closest relative is the longnose sucker (*C. catostomus*), which has a widespread distribution across the northern half of North America (McPhail and Taylor 1999). The Salish sucker and the longnose sucker likely diverged from one another during the Pleistocene glaciation (McPhail and Taylor 1999). The Salish sucker is a short-lived species (up to five years) with a small home range and young reproductive age (McPhail 1987). The strongest determinant of adult Salish sucker presence is stream depth and the greatest fish activity is associated with dusk and dawn as demonstrated with tagging data (Pearson and Healey 2003). The two largest threats facing Salish sucker include hypoxia and the physical destruction of habitat (Fisheries and Oceans Canada 2019).

Study location

Field work took place between May 1st and September 23rd, 2018 in Pepin Creek, a second order tributary of the Nooksack River (Pearson and Healey 2003). The Nooksack River has its northern headwaters in southwestern British Columbia, near

Abbostford, and drains into the sea at Bellingham Bay, Salish Sea, in northern Washington State. Pepin Creek is one of several small streams occupied by Salish sucker in the lower Fraser Valley of British Columbia, which is a low elevation area of mixed land use including gravel extraction, farming, urban areas, parkland, and forest. It is largely groundwater fed in the summer. The study site was located in Aldergrove Regional Park (10 U 538669.25 m E 5428282.57 m N), where habitat restoration projects have created several ponds on the stream floodplain that were suitable for experimental manipulations (Pearson and Healey 2003). These ponds were intended to replicate the historical off-channel wetland habitat commonly inhabited by Salish sucker throughout their historical distribution, that have been lost as a consequence of agricultural drainage and floodplain channelization (Fisheries and Oceans Canada 2019).

The study site supports additional fish species including coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), rainbow trout (*Oncorhynchus mykiss*), coho salmon (*Oncorhynchus kisutch*), threespine stickleback (*Gasterosteus aculeatus*), Western brook lamprey (*Lampetra richardsoni*) invasive pumpkinseed (*Lepomis gibbosus*), and Endangered Nooksack dace (*Rhinichthys cataractae*).

Fish collection

Salish suckers for use in experiments were collected using "Fedes traps", large (60 X 100 cm) double-ended funnel traps made from 12 mm galvanized steel mesh (hardware cloth). Most suckers were collected directly from the main experimental pond

or nearby sites in Aldergrove Regional Park, and juvenile coho salmon were collected from the ponds and adjacent streams using standard minnow (Gee) traps. All traps were baited with dog food, cat food, or salmon roe. Traps were set for 24 hours overnight unless asphyxiation due to nighttime hypoxia was a concern in which case shorter daytime sets were used. Because I was interested in documenting potential sublethal effects of hypoxia on fish growth, I excluded any fish that were evidently in active reproductive condition, as spawning would result in a weight loss that would bias observed growth measurements. Consequently, any fish that were gravid were released outside of the experimental ponds.

Fish were anesthetized in a 100 mg·L^{-1} solution of MS22 (tricaine methanesulfonate) before marking or measurement of length (nearest mm) and weight (0.01g). Visible implant elastomer (Northwest Marine Technology Inc., Shaw Island, Washington, USA) was used to give each fish a unique mark so that individual growth could be determined over the duration of the study. A subset of the Salish suckers had passive integrated transponder (PIT) tags (Biomark® APT 12 tags) surgically implanted for recording movement, and a subset of the coho salmon had PIT tags injected with a syringe. Standard surgery protocols were followed for implanting PIT tags into the Salish suckers (Collins et al. 2013). Fish were held overnight for at least 24 hours after surgery to ensure no adverse health effects before being weighed and released into their respective experimental treatments.

Experimental design

To understand the effects of flow on water quality, fish growth, and habitat use, I performed two manipulative experiments in separate ponds. The first experiment involved capturing and marking suckers in a single backwater pond to track their growth over a four-week period where water flow was diverted into the pond. This was followed by another four-week treatment where flow into the pond was shut off. The second experiment applied contrasting flow and no flow treatments to either side of a second pond that was longitudinally split using an impermeable plastic barrier. One side of the pond was maintained as a control treatment with flow from the pond inlet, while the other side received no flow by means of the central dividing barrier (Figure 1). After an initial four-week period, the flow treatment applied to each side was reversed in a cross-over design, and the treatments were applied over a second four-week period. These two experiments will be referred to as the single-pond and split-pond experiments, respectively.

Single-pond experiment: sequential flow reduction

The intent of this experiment was to determine the effects of flow on water quality and growth of Salish suckers using a sequential flow reduction design. The experiment was performed in a single off-channel backwater pond (2,500 m^2 area, 110 m long) that had limited flow exchange with Pepin Creek through its single connection (i.e. it did not have a separate inlet and outlet; Appendix, Figure A1). The single combined inflow and

outlet connection with Pepin Creek made it ideal for a study of the effects of flow augmentation. Two paired six-inch and eight-inch diameter pipes were installed to divert flow from the mainstem creek into the end of the pond opposite from the single outlet to convert it into a flow-through system (Appendix, Figure A1). Flows were measured weekly and averaged 15.3 L·s^{-1}, as measured at the augmentation pipe outlets. Because Salish suckers are a Threatened species (Fisheries and Oceans Canada 2019), I ensured that fish would not be harmed in the event that the pond went hypoxic during the no flow treatment, while also ensuring that fish could be recovered so that their growth could be assessed. Consequently, a fence was installed at the outlet where the pond connected with Pepin Creek to retain fish in the pond, as well as to provide an oxygenated refuge (Appendix, Figure A1). Flow was diverted into the pond for four weeks (the "flow" treatment from July 15th to August 21st), followed by a four-week "no flow" treatment from August 21 to September 16th.

The fence at the pond outlet was made of six mm galvanized steel mesh (hardware cloth) attached to a frame of wooden posts driven into the stream bed. The fence and associated "box" were designed to prevent fish from escaping while providing a well oxygenated refuge so that fish wouldn't asphyxiate if the pond became hypoxic. The galvanized mesh was sealed at the bottom of the pond with sandbags and a 1.5 m by 2 m mesh box was attached to the fence and extended from the pond outlet into Pepin Creek, where constant stream flow ensured adequate water quality (dissolved oxygen in the refuge was recorded with a logger). A 45 cm by 45 cm square opening in the fence led directly into the mesh box, so that fish were free to swim between the pond and the oxygenated box (refuge; Appendix, Figure A1).

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Dissolved oxygen and temperature

To measure temporal variation in dissolved oxygen, MiniDOT® loggers were installed in the deepest part of the pond and in the oxygenated refuge protruding into the adjacent stream to track dissolved oxygen and temperature (Appendix, Figure A1). Loggers were mounted in the center of minnow (Gee) traps to elevate them 10-15 cm above the pond bottom to continuously recorded temperature and oxygen every 15 minutes. To characterize spatial variation in water quality, dissolved oxygen and temperature were measured along two systematic longitudinal transects at the pond surface and 10-15 cm above the bottom on August 19th-20th, during the third week of the flow treatment. Following the four-week flow treatment, the flow diversion was shut off to initiate the four-week no flow treatment. Loggers remained in place for the second treatment, and transect measurements were repeated on September 16th-17th, during the third week of the no flow treatment following the same protocol.

Fish growth

To track the growth of Salish suckers in the sequential flow and no flow treatments, a total of 116 fish were captured with Feddes traps over both treatments and individually marked with elastomer to monitor growth. Fish were recaptured at the end of the first (flow) treatment, measured for weight and length before fish were released for the second treatment, and any unmarked fish were elastomer tagged. At the end of the second (no flow) treatment, fish were recaptured and measured for length and weight to calculate growth.

Split-pond experiment: cross-over design

The split-pond experiment involved longitudinally dividing a smaller pond (~40 m^2 area, 8 m long) with an impermeable barrier that diverted flow to only one side of the pond (Figure 1). This allowed documentation of the direct effects of reduced flows on water quality (dissolved oxygen and temperature), and the response of Salish suckers and juvenile coho salmon in terms of habitat use, movement, and growth. Experimental flow treatments were again divided into two sequential four-week periods. The first treatment period ran from July 15th to August 17th, after which the barrier deflector at the upper end of the longitudinal impermeable divider was switched to divert flow to the opposite side of the pond (Figure 1). The experiment was then repeated for another four weeks from August 17th to September 16th in a cross-over design to obtain treatment replication and control for possible side effects (although potential side effects remain confounded with time effects; Figure 1). A diversion channel was used to control flow into the split pond from Pepin Creek. Flow into the pond averaged 6.5 L·s^{-1}, as measured weekly.

Dissolved oxygen and temperature

Dissolved oxygen and temperature loggers (MiniDOT®) were placed in minnow traps, as described earlier, and installed in the centre of either side of the split pond to track changes in water quality throughout the experiment (Appendix, Figure A1). Discharge entering the pond was measured weekly and adjusted as necessary to maintain consistency. As dissolved oxygen loggers only measured water quality at a

single location, point measurements of dissolved oxygen and temperature (at the water surface and bottom) were collected across the entire pond over a systematic grid at 1.5 m intervals along multiple transects to obtain a spatial snapshot of the distribution of water quality in each treatment. Transect measurements were collected once during the day and once at night in the third week of each four-week treatment period (August 13th-14th, and September 10th-11th, respectively).

Fish growth

To determine fish growth in different flow treatments, Salish suckers and juvenile coho salmon were stocked at equal densities (0.25 fish per m^2 for sucker, one fish per m^2 for juvenile coho salmon) on either side of the split pond (i.e. both flow and no flow sides). Fish were marked with visible implant elastomer so that individual growth could be measured at the end of each treatment period. All Salish suckers were surgically implanted with PIT tags following protocols from Collins et al. (2013). Juvenile coho salmon were PIT tagged with a syringe. Once marked, fish were held overnight to ensure no negative effects of handling occurred and were measured for length (mm) and weight (g) before being released into the pond. For the first treatment period five Salish suckers and 20 coho salmon were marked, measured, and released into each side of the pond. At the end of four weeks fish were recaptured using Feddes and minnow traps, and lengths and weights were recorded. Fish were held overnight while the longitudinal divider was moved to direct flow to the opposite side of the pond (Figure 1), and fish were re-stocked on their respective side of capture for the second treatment

period of the cross-over experiment. Five Salish suckers and 20 juvenile coho salmon were stocked on each side for the second four-week period where treatments were reversed by side (i.e. the flow side became the no flow side). Fish were re-stocked on their original sides to ensure a balanced design with all fish exposed to both water quality/flow treatments. A tear in the plastic divider was discovered at the end of the first time period that allowed some fish to cross-over between sides. Fish growth data was used in analysis only for fish that were recovered from the side they were stocked on, and all fish were returned to their original stocking side for the second time period.

Oxygenated refuge PIT antennae array

As with the single-pond experiment, a fence with an oxygenated refuge was constructed at the outflow of the pond where the outlet channel converged to approximately 2 m wide (Figure 1). The refuge box was divided longitudinally with a mesh screen that allowed oxygenated water from the flow side to cross over into the no flow side of the refuge. The fence and refuge were installed to retain fish within the experimental pond, while ensuring the presence of an oxygenated refuge in the event that the no flow treatment became hypoxic and (a design requirement for research on a listed species). To enter either side of the oxygenated refuge fish had to pass through two vertically oriented 45 X 45 cm square PIT tag antennae fitted to the entrance of each side of the refuge box (Figure 1), so that directional movement of fish could be monitored and compared between treatments. The PIT tag array was monitored using two Biomark® 24V antennae controllers powered by solar panels.

Because only two pit tag antennae were available, each side was fitted with both antennas for 24 hours over two successive days (had each side been fitted with only one antenna I would not have been able to determine the directionality of movement, so each side was monitored over sequential 24-hour periods). Four weekly sets of 24-hour PIT tag monitoring periods were collected during each four-week flow treatment period.

Data analysis

Where necessary, data were log-transformed to meet assumptions of normality and homogeneity of variance. When data could not be normalized, they were analyzed using a non-parametric Wilcoxon rank sum test. All statistical analysis for fish growth and water quality were conducted using R version 3.5.1 statistical software (R Core Team 2018). Fish refuge movement analyses were conducted using SAS (SAS Institute Inc. 2015).

Dissolved oxygen and temperature

Spatial transect measurements of dissolved oxygen and temperature were analyzed with a non-parametric Wilcoxon rank sum test to compare effects of flow and no flow treatments on water quality. To test for diurnal effects, flow effects on temperature and oxygen were analyzed separately for day and night time periods using a Wilcoxon rank sum test.

Daily mean, minimum, and maximum dissolved oxygen and temperature data were extracted from continuously recorded logger data using the average of each metric

across all days that logger sampling occurred. Flow treatment effects on temporally

averaged dissolved oxygen and temperature data were also analyzed separately by day

and night periods, using a non-parametric Wilcoxon rank sum test. To understand the

potential for seasonal changes in temperature to affect results, a one-way ANOVA was

used to assess differences in air temperature between sequential treatment periods

using average daily temperatures from the closest weather station (Abbotsford A;

Government of Canada 2019).

Instantaneous growth

Instantaneous growth rates of fish (% per day) in terms of both weight and length

were calculated as $\{(\log_e(\text{final measure}) - \log_e(\text{initial measure}))/\text{time (days)}\} \times 100$

(Ricker 1975) for Salish suckers and coho salmon. When data could not be normalized

by log transformation, the effects of flow on instantaneous growth in length and weight

were assessed using a non-parametric Wilcoxon rank sum test.

Oxygenated refuge use

Movement data were divided into daylight and nighttime intervals based on

sunset and sunrise times at the study site latitude. Three movement response metrics

were derived from PIT tag data: entries into the refuge, duration of time in the refuge,

and raw number of detections picked up by the PIT tag antennae. Entries into the

refuge were identified only when a fish stayed in the refuge for more than five seconds,

to exclude detections where fish were resting within the detection field of the PIT tag

antenna (e.g. sometimes coho salmon would hold position in front of the array). The three PIT tag response variables were analyzed as Generalized Linear Mixed Models (GLMM) in SAS (SAS Institute Inc. 2015), treating fish identity (PIT tag code) as a random effect to account for correlation in covariance structure (i.e. pseudoreplication) due to repeated measures on the same fish, with a Kenward-Roger degrees of freedom approximation. Entries and number of detections were integer count data with a moderately high proportion of zeros and were therefore modelled using a Poisson distribution and a log link. Duration also had a high proportion of zeros but had non-integer values so was modelled as a Tweedie distribution (Poisson distribution for zeros, Gamma distribution for non-zero values). Entries, duration and detections were first modelled as a global function of all fixed effects including species (two levels, Salish sucker and coho salmon), flow treatment (flow vs. no flow), period (first and second), and time of day (day vs. night), with fish identity as a random effect (repeated measure). Interactions (species by flow treatment and species by time of day) were included but removed from models when not significant. Separate GLMMs were also fit to Salish suckers and coho salmon data to better understand individual responses to the experimental treatments.

Results

Single-pond experiment

Flow effects on dissolved oxygen and temperature

Average dissolved oxygen measured along transects in the fourth week of each treatment was significantly higher in the flow treatment (9.11 mg·L^{-1}) than the no flow treatment (mean = 3.1 mg·L^{-1}; Wilcoxon rank sum test, W = 15124, p < 0.001). There was a strong diurnal signal, with flow reduction resulting in lower dissolved oxygen during both day (10.8 mg·L^{-1} flow vs. 3.5 mg·L^{-1} no flow; Wilcoxon rank sum; W = 4393.5, p < 0.001) and night (6.7 mg·L^{-1} vs. 2.7 mg·L^{-1}; Wilcoxon rank sum; W = 3210, p < 0.001; Figure 2).

Average dissolved oxygen measured by the logger placed at the deepest location in the pond (flow 30 days vs. no flow 30 days) was also significantly higher in the flow treatment (6.4 mg·L^{-1} flow vs. 1.5 mg·L^{-1} no flow; Wilcoxon rank sum, W = 5836900, p < 0.001). Daily minimum dissolved oxygen occurred at night and was also significantly higher during the flow treatment (4.0 mg·L^{-1} flow vs. 0.2 mg·L^{-1} no flow; Wilcoxon rank sum, W = 475, p < 0.001; Figure 2). In the refuge box, logger results indicated high and consistent average dissolved oxygen levels throughout the experiment (flow = 7.5 mg·L^{-1}, no flow = 7.9 mg·L^{-1}).

Average daily air temperature over the course of the single-pond experiment was significantly higher during the earlier flow treatment due to seasonal effects (20.7 vs. 16.5 °C; ANOVA, $F_{1,60}$ = 47.55, p < 0.001). Spatially extensive transect sampling also showed significantly higher average water temperature during transect measurements

in the flow treatment (15.9 °C) relative to the no flow treatment (14.3 °C; W = 15360, p < 0.001). Water temperatures also declined in the outlet refuge box during the no flow treatment period (logger data, 15.8 °C vs. 12.9 °C), indicating an effect of seasonal cooling on water temperature over the sequential flow reduction periods.

Daily average temperatures recorded by the dissolved oxygen logger (at 15 minute intervals) in the deepest part of the pond followed the same pattern as the transect data, with higher average temperature in the flow treatment (16.6 °C) relative to the no flow treatment (13.7 °C; Wilcoxon rank sum, W = 6234200, p < 0.001). Similarly, daily maximum temperatures were also higher during the flow treatment period (mean = 18.2 vs. 14.2 °C; Wilcoxon rank sum, W = 524, p < 0.001).

Fish growth

Salish sucker daily instantaneous growth in weight was significantly higher in the flow treatment (0.92 g·day^{-1} flow vs. 0.71 g·day^{-1} no flow; Wilcoxon rank sum, W = 296, p = 0.032; Figure 3). Instantaneous growth in length of Salish suckers was also higher in the flow treatment (0.28 vs. 0.23 mm·day^{-1} in no flow), although the difference was not statistically significant (Wilcoxon rank sum = 256, p = 0.27).

Split-pond experiment

Flow effects on dissolved oxygen and temperature

The split-pond treatments resulted in clear differences in water quality between sides. Transects capturing the spatial extent of water quality impacts revealed significantly higher dissolved oxygen in the flow treatment (6.9 flow vs. 2.9 mg·L^{-1} no flow; Wilcoxon rank sum, W = 4602.5, p < 0.001; Figure 4). As with the single-pond experiment, strong diurnal effects were present, with flow reduction resulting in lower average transect dissolved oxygen during both daylight hours (7.9 mg·L^{-1} flow vs. 4.4 mg·L^{-1} no flow; Wilcoxon rank sum, W = 1118, p < 0.001) and night (5.7 vs. 1.3 mg·L^{-1}; Wilcoxon rank sum, W = 1242, p < 0.001; Figure 2). There was no significant effect of sequential time period on dissolved oxygen measured at transects (5.4 mg·L^{-1} in period one vs. 4.7 mg·L^{-1} in period two; Wilcoxon rank sum, W = 3120.5, p = 0.14).

Average dissolved oxygen measured at loggers placed in the middle of each side of the pond (30 days per period) was significantly higher in the flow treatment (5.8 vs 3.3 mg·L^{-1}; Wilcoxon rank sum, W = 19839000, p < 0.001; Figure 2), as was daily minimum dissolved oxygen (3.8 vs. 1.3 mg·L^{-1}; Wilcoxon rank sum, W = 2498, p < 0.001). Loggers placed on both sides of the refuge box recorded consistently high dissolved oxygen throughout the experiment for both flow (period one = 7.5 mg·L^{-1}, period 2 = 7.5 mg·L^{-1}) and no flow treatments (period one = 7.4 mg·L^{-1}, period two = 7.0 mg·L^{-1}).

Average daily water temperatures measured in the center of each side of the pond were slightly elevated in the no flow treatment (14.7 °C flow vs. 15.3 °C no flow,

Wilcoxon rank sum, W = 11349000, p < 0.001), as were daily maximums (16.3 °C flow vs. 18.4 °C no flow; Wilcoxon rank sum, W = 1284, p = 0.25).

Fish growth

Instantaneous growth in weight of Salish suckers was not significantly different between the flow (mean = 0.24 g·day^{-1}) and no flow treatments (mean = 0.26 g·day^{-1}; ANOVA, $F_{1,8}$ = 0.25, p = 0.63; Figure 3). Growth in length of Salish suckers (0.10 mm·day^{-1} flow vs. 0.07 mm·day^{-1} no flow) also did not differ between flow treatments (ANOVA, $F_{1,8}$ = 2.79, p = 0.14). Sequential treatment period had no significant effect on growth in weight (ANOVA, $F_{1,8}$ = 0.57, p = 0.47) or length (ANOVA, $F_{1,8}$ = 2.79, p = 0.13).

The instantaneous growth in weight of coho salmon in the flow treatment (mean = 0.63 g·day^{-1}) was double that of growth in the no flow treatment (mean = 0.31 g·day^{-1}; Wilcoxon rank sum, W = 192, p = 0.045; Figure 3). The same pattern was found for instantaneous growth in length of coho salmon (0.25 mm·day^{-1} flow vs. 0.07 mm·day^{-1} no flow; Wilcoxon rank sum, W = 208, p < 0.001).

Oxygenated refuge use

The total number of daily detections in the oxygenated refuge were significantly higher in the no flow treatment than the flow treatment (GLMM for both species combined, $F_{1,285}$ = 11.0, p = 0.001; average 41,300 no flow detections vs. 9,800 flow detections), indicating elevated activity of PIT-tagged fish near or through the antenna

array under flow reduction. There was also a significant species effect, with fewer average daily Salish sucker detections per day (9,500) relative to coho salmon (42,300). When analyzed separately by species there was also a significant diurnal effect on Salish sucker activity, with significantly more detections at night ($F_{1,76}$ = 5.1, p = 0.026; average 15,900 night detections·day^{-1} vs. 5,800 day detections·day^{-1}; Figure 5). However, there was no significant effect of time of day on coho salmon detections for the coho salmon-only GLMM ($F_{1,206}$ = 0.9, p = 0.33; Figure 5), although the flow treatment effects on coho salmon remained significant ($F_{1,206}$ = 8.6, p = 0.04).

Daily entries into the oxygenated refuge were significantly higher in the no flow treatment for both species combined ($F_{1,300}$ = 9.1, p = 0.003; average no flow entries = 16.1 entries·day^{-1} vs. flow entries = 5.4 entries·day^{-1}; Figure 5), with no significant species effect ($F_{1,300}$ = 1.2 p = 0.27); however, there was a significant species by time of day interaction ($F_{1,300}$ = 8.1, p = 0.005), indicating different diurnal activity (entry) patterns for Salish suckers and coho salmon (Figure 5). Salish sucker entries into the refuge were significantly elevated at night (Salish sucker-only GLMM, $F_{1,75.3}$ = 6.2, p = 0.015; average night entries = 10.8 entries·day^{-1} vs. day entries = 3.4 entries·day^{-1}; Figure 5), while coho salmon entries were higher during the day (coho salmon-only GLMM, $F_{1,222}$ = 8.7, p = 0.004; average day entries = 22.5 entries·day^{-1} vs. night entries = 5.3 entries·day^{-1}; Figure 5). When modelled by species, Salish sucker entries were non-significantly higher in the no flow treatment ($F_{1,60.1}$ = 3.0, p = 0.09; average no flow entries = 10 entries·day^{-1} vs. flow entries = 3.4 entries·day^{-1}), while flow effects on coho salmon entries remained significant ($F_{1,133.8}$ = 6.9, p = 0.01; average no flow entries = 6.3 entries·day^{-1} vs. flow entries = 18.9 entries·day^{-1}).

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The combined GLMM analysis of duration of time spent within the oxygenated refuge showed that fish spent significantly more time in the refuge in the no flow treatment ($F_{1,298} = 12.2$, $p < 0.001$; average no flow duration = 1.9 hr·day^{-1} vs. flow duration = 0.6 hr·day^{-1}). There was also a significant species effect on duration (GLMM, $F_{1,298} = 5.4$, $p = 0.021$), with coho salmon (mean duration = 1.7 hr·day^{-1}) spending more time on average in the refuge than Salish suckers (mean duration = 0.7 hr·day^{-1}; Figure 5). There was no significant interaction between species and flow treatment ($F_{1,297} = 1.0$, $p = 0.32$), indicating that both species increased duration in the refuge under flow reduction. However, flow treatment effects were not significant for the sucker-only GLMM analysis ($F_{1,75} = 0.2$, $p = 0.67$), while flow effects remained significant for the coho salmon-only analysis ($F_{1,220} = 8.9$, $p = 0.003$).

Discussion

This study was motivated by a desire to understand the causation and impact of hypoxia commonly observed in Salish sucker critical habitat (Pearson 2015). My flow manipulation experiments unambiguously demonstrate the induction of severe hypoxia through water flow reduction. While this finding is not unexpected, it does highlight the context and drivers of hypoxia in Salish sucker streams, and the negative synergies between flow, eutrophication, and temperature. Flow reduction does not typically induce hypoxia in oligotrophic steams, unless temperatures become extremely high. Hypoxia under flow reduction is a classic signature of eutrophication, where elevated nutrients trigger algal blooms that result in high biological oxygen demand, leading to oxygen depletion (Paerl and Huisman 2009; Poff and Zimmerman 2010; Pardo and García 2016). The fact that severe hypoxia was observed at relatively low temperatures (e.g. 15-20 °C in the split-pond experiment) further highlights the likely role of nutrient inputs in stimulating elevated biological oxygen demand, independent of the reduction in dissolved oxygen saturation at higher temperatures. Eutrophication as a key driver of hypoxia is consistent with the relatively high nitrogen values in Pepin Creek (Fisheries and Oceans Canada 2019; Johnson et al. 2019), which is typical for salmonid and sucker rearing streams in the agricultural landscape of the lower Fraser Valley. Excessive primary production as the ultimate driver of hypoxia was also supported by the observation of high algal biomass and daytime supersaturation of dissolved oxygen in my flow reduction treatments.

Impacts of flow reduction on fish growth differed considerably between juvenile coho salmon and Salish suckers; this is not entirely surprising, given their known differences in ecology (e.g. a cold-water salmonid vs. a benthic cool-water species). The 50% reduction in juvenile coho salmon growth in the split-pond experiment is consistent with known effects of hypoxia on juvenile salmonid growth (Hermann et al. 1962; Brett and Blackburn 1981). The effects of flow and hypoxia on Salish sucker growth were more ambiguous, with significantly lower growth in the flow reduction treatment of the single-pond experiment, but not in the split-pond experiment. Interpretation of these contrasting outcomes requires careful consideration of the mechanisms whereby flow reduction and hypoxia can affect fish growth.

The first mechanism is an indirect trophic pathway if invertebrate abundance is reduced by elevated hypoxia, resulting in reduced prey consumption and growth (Connolly et al. 2004; Pardo and García 2016). Hypoxia often results in a shift to a more tolerant benthic invertebrate community, which may or may not entail a reduction in total prey abundance; reduction of invertebrate biomass is most likely when hypoxia is severe and prolonged (Kolar and Rahel 1993), and is more often associated with bathyal hypoxia in the hypolimnion of lakes (Arend et al. 2011; Weinke and Biddanda 2018). Although I did not systematically measure invertebrate abundance in my experiments, I qualitatively observed abundant macroinvertebrates in both flow and no flow treatments. The threespine stickleback, *Gasterosteus aculeatus* (a generalized insectivore that is hypoxia tolerant), was also abundant in the ponds irrespective of flow treatment, suggesting a robust prey base; consequently, there is no strong inference that hypoxia effects on prey were causative in reducing juvenile coho salmon growth.

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On the other hand, juvenile coho salmon feed on drifting aquatic and terrestrial invertebrates, and it is plausible that the flow reduction treatment diminished the total flux of available drifting prey (Connolly et al. 2004; Rosenfeld and Raeburn 2009), thereby contributing to reduced coho salmon growth.

Prolonged hypoxia may also create generalized stress in fish and induce a trade-off between investing in somatic maintenance vs. somatic growth. When ambient oxygen availability limits respiration, organisms may seek to maximize their limited growth capacity by reducing maintenance metabolism associated with tissue repair, protein turnover, or investment in immune response. An additional direct and well-documented effect of hypoxia is to reduce maximum ration; because the process of food digestion entails high aerobic demand (up to 70% of maximum metabolism; Alsop and Wood 1997), fish consumption and therefore growth decline steeply under hypoxia (Hermann et al. 1962). Reduced growth of coho salmon in the flow reduction treatment is consistent with the known effects of hypoxia on maximum ration and generalized stress; however, reduced prey flux is a confounding factor whose relative contribution to diminished growth is unclear.

The lack of a significant effect of flow and hypoxia on Salish sucker growth in the split-pond experiment indicates lower sensitivity to hypoxia; however, this inference must be tempered by the relatively low power of the Salish sucker treatment. To maintain Salish sucker densities within a realistic range, only five fish were stocked on either side of the split-pond (compared to ~20 coho salmon); this necessarily increased variance so that the minimum significant difference in Salish sucker growth between flow treatments (0.52 %) was greater than the treatment means. In contrast, decreased

Salish sucker growth in the single-pond experiment was consistent with the expected effects of hypoxia. Lower dissolved oxygen in the single-pond flow-reduction treatment was, however, confounded with lower temperature because the flow reduction treatment occurred at the end of the summer after the flow treatment. I originally intended the single-pond experiment to be a before-after controlled impact (BACI) experiment, using a nearby well-oxygenated reach as the temporal control, but were unable to capture a sufficient number of Salish suckers in the intended control habitat. Consequently, the relative impact of lower dissolved oxygen vs. lower temperature in driving decreased Salish sucker growth in the single-pond experiment remains ambiguous.

While there is some uncertainty in the significance of the growth response of Salish sucker to flow reduction, results indicate that they have a greater tolerance to hypoxia than juvenile salmonids. This is expected, because salmonids are generally coldwater-adapted with limited tolerance of poor water quality (Elliott 1976; Forseth et al. 2001). In contrast, the Salish sucker is a benthic fish that preferentially occupy deep slow water habitat, and occur disproportionately in headwater marshes and low gradient streams (Pearson and Healey 2003; Pearson 2004). These adaptations likely relate to lower basal metabolic rates associated with a more sedentary lifestyle (Stoffels 2015), and a higher haemoglobin affinity that can bind oxygen more effectively at low concentrations (Walker et al. 1989). Consequently, the meso-habitats that Salish suckers occupy in streams (i.e. pools, ponds, and deep marshes) are the most likely to experience hypoxia as stream flow declines and temperatures warm during the extended summer low flow season in western British Columbia and the Pacific Northwest. An extended summer low flow period has likely been a defining

characteristic of the Georgia Depression Ecoprovince for millennia (Demarchi 1996), likely resulting in selective pressure for some degree of hypoxia tolerance in Salish suckers. Tolerance of hypoxia may even allow some degree of refuge from predation by more hypoxia-sensitive predators like coastal cutthroat trout (Anjos et al. 2008; Hedges and Abrahams 2015). A stipulation remains that limits to hypoxia tolerance in most vertebrates, as evidenced by the periodic collection of dead Salish suckers when they are attracted to baited traps and subsequently asphyxiated in hypoxic habitats (Rosenfeld, Pearson, and Miners pers. obs.), exists. There is also strong circumstantial evidence for collapse of local Salish sucker populations that are associated with larger-scale hypoxia (Pearson 2004), but this is difficult to detect in natural settings.

While catostomids are relatively tolerant to hypoxia, they remain vulnerable to both lethal and sublethal effects (Cech et al. 1990). Sublethal effects of hypoxia include altered behaviour, presumably to mitigate the negative impacts of changes in water quality (Pihl et al. 1991; Matthews and Berg 1997; Pollock et al. 2007). Movement data of PIT-tagged fish in the split-pond experiment indicated an overall increase in activity and duration in the oxygenated refuge for both coho salmon and Salish suckers under flow reduction. Although juvenile coho salmon increased their refuge use during both day and night, their greatest refuge use occurred during daytime (when oxygen levels were highest), which was not expected. This behaviour was most likely related to flow effects on invertebrate drift flux, because I observed coho salmon drift-feeding in the cross-flow of oxygenated water entering the refuge from the flow treatment side, a behaviour that was not possible in the still water of the main flow reduction treatment. Greater Salish sucker use of the oxygenated refuge under hypoxia may have been

motivated by a need for greater oxygen to support digestive metabolism and growth, or it could have simply been associated with increased overall activity. Regardless, changes in movement patterns strongly suggest behavioural adaptation to hypoxia. Altered behaviour by coho salmon may have partially mitigated negative effects of reduced flows (Kramer 1987; Matthews and Berg 1997), but was not sufficient to prevent sublethal effects on growth (Brett and Blackburn 1981). On the other hand, increased activity and use of the oxygenated refuge by Salish sucker may have helped mitigate negative impacts on their growth. This was consistent with my finding that Salish suckers exited the refuge more at nighttime. Given their nocturnal habits, this is when I would expect them to feed.

Both flow manipulation experiments included the presence of a well-oxygenated refuge, to prevent the possibility of mortality of a legally-designated endangered species. While this design feature was essential, it also diminished power to detect adverse impacts of reduced flow, assuming that the presence of a well-oxygenated refuge could reduce the negative effects of hypoxia. There are several mechanisms whereby spatial heterogeneity in low dissolved oxygen could mitigate the negative impacts of overall hypoxia. Depending on species, fish can forage in hypoxic water for short bouts, analogous to mammals holding their breath; this may allow fish, for example, to penetrate into the poorly oxygenated hypolimnion of a shallow lake to forage on benthos. Even though fish may have tolerance to hypoxia, the distance that fish can penetrate into a hypoxic zone will have limits. If the size of a hypoxic patch is small then a fish may be able to forage throughout it, resulting in no effective loss of foraging habitat. However, once the spatial scale of a hypoxic patch exceeds the

effective foraging radius of a fish under hypoxia, habitat and prey resources in the patch

will become unavailable, leading to effective habitat loss. It is possible that the patch

size of my experiments (~20 m^2 for the split-pond and ~2,500 m^2 for the single-pond)

was below the threshold required to induce larger-scale effects on Salish sucker growth

when an oxygenated refuge is present. This highlights the importance of oxygenated

refuges in allowing persistence of fish under hypoxia (Woelfle-Erskine et al. 2017), and

the potential risks associated with large homogenous hypoxic zones.

Conservation and management implications

This study demonstrates the pre-eminence of flows in either preventing or

inducing hypoxia, particularly under eutrophic conditions. A key management

implication is that instream flow needs will be sensitive to water chemistry (nutrients) as

well as temperature. Flow requirements to maintain water quality and avoid hypoxia will

be higher in eutrophic waters than would be the case in the absence of nutrient inputs

(Nilsson and Renöfält 2008; Graeber et al. 2013; Pardo and García 2016). Similarly,

temperature-sensitive streams (i.e. those near an upper threshold of transition to a

warmwater community) may require higher flows to maintain temperatures below a

given upper limiting threshold (Sinokrot and Gulliver 2000; Null et al. 2017). Nutrients

that stimulate primary production or other organic matter inputs that increase biological

oxygen demand likely originate from a diversity of agricultural and urban sources, and

effectively managing these diffuse inputs will require an integrated approach at a

watershed scale.

My experiments also highlight the importance of well-oxygenated refuges (either at microhabitat or reach scales) as important drivers of individual survival and population persistence. Oxygenated refuges (or "sanctuary" reaches; Woelfle-Erskine et al. 2017) may be associated with springs at the bottom of mountain slopes where streams meet the valley bottom, oligotrophic or heavily shaded reaches, or groundwater discharge areas where streams cross alluvial or glacial deposits. Long-term conservation strategies will require the identification and protection of these areas, as well as wetland or other groundwater recharge areas that contribute disproportionately to maintaining summer baseflows. This likely includes headwater beaver pond complexes that can play an important role in storing and releasing flows, which were historically abundant.

Salish sucker are likely somewhat physiologically and behaviourally adapted to hypoxia, which could partially mitigate the negative effects of hypoxia if well-oxygenated refuges are present, i.e. fish can likely exploit small scale heterogeneity in dissolved oxygen to achieve homeostasis with minimal loss in growth or other sublethal effects (Sedell et al. 1990). However, this effect relies on heterogeneity in dissolved oxygen (i.e. discontinuous patches of hypoxia), and behavioural mitigation will be limited when hypoxic patch size is sufficiently large and hypoxia homogenous. Ultimately, a preference for slow, deep pool habitat and other adaptations to exploiting low gradient streams, while adaptive in an undisturbed environment, may be an ecological trap in a human-modified landscape if selected habitat is differentially vulnerable to hypoxia.

This is one of few experimental studies to consider two species with contrasting life histories and ecology. While reduced flows and associated hypoxia are a concern

for the Salish sucker, my experiments highlight that they are an even greater threat to juvenile salmonid production. Given climate change projections for increased temperatures and a longer summer low flow period in western North America (Arismendi et al. 2013), functional persistence of salmonids and Salish sucker will be increasingly dependent on management and restoration targeted at maintaining flows, and reducing stream temperatures and nutrient inputs that contribute to hypoxia.
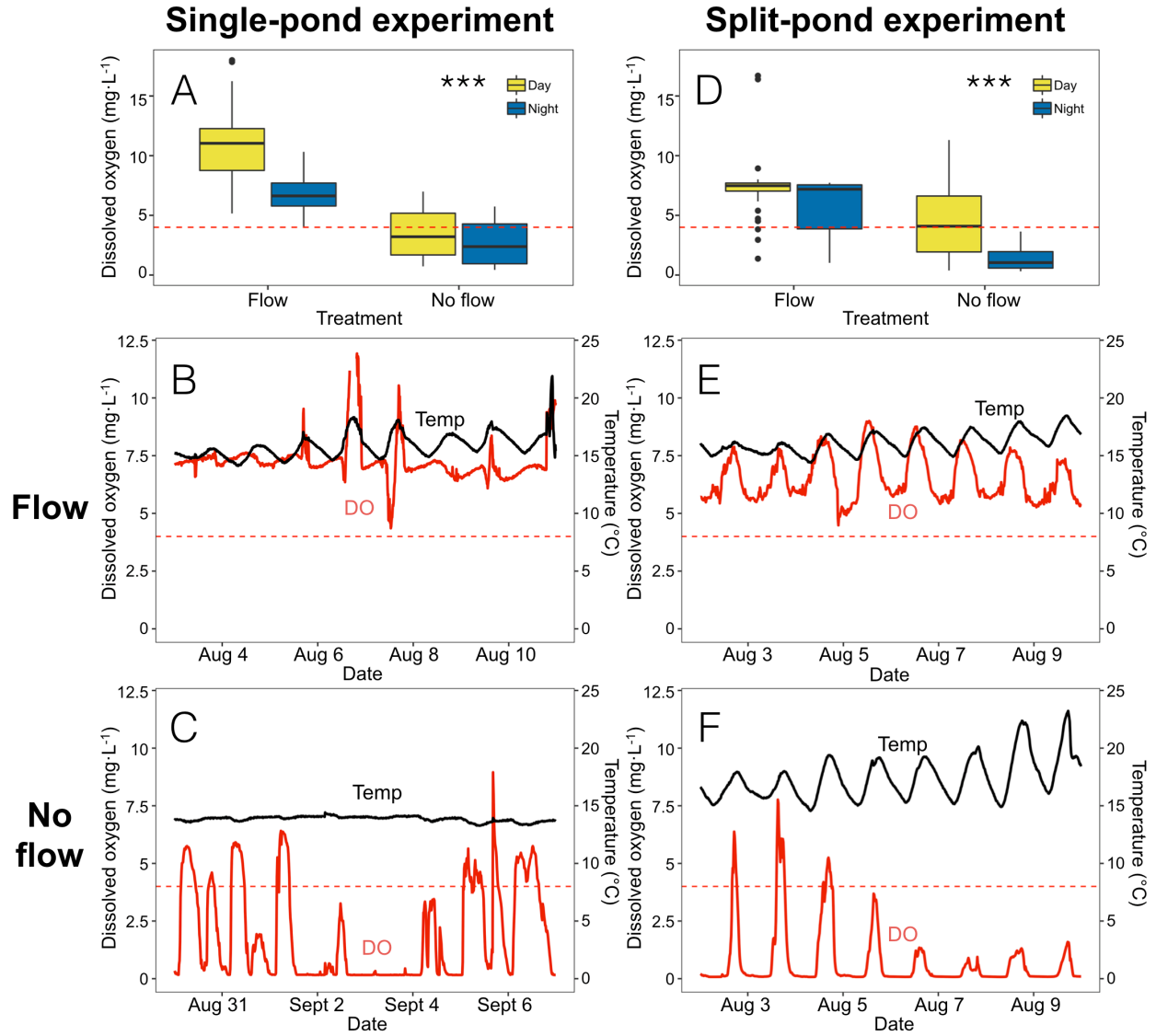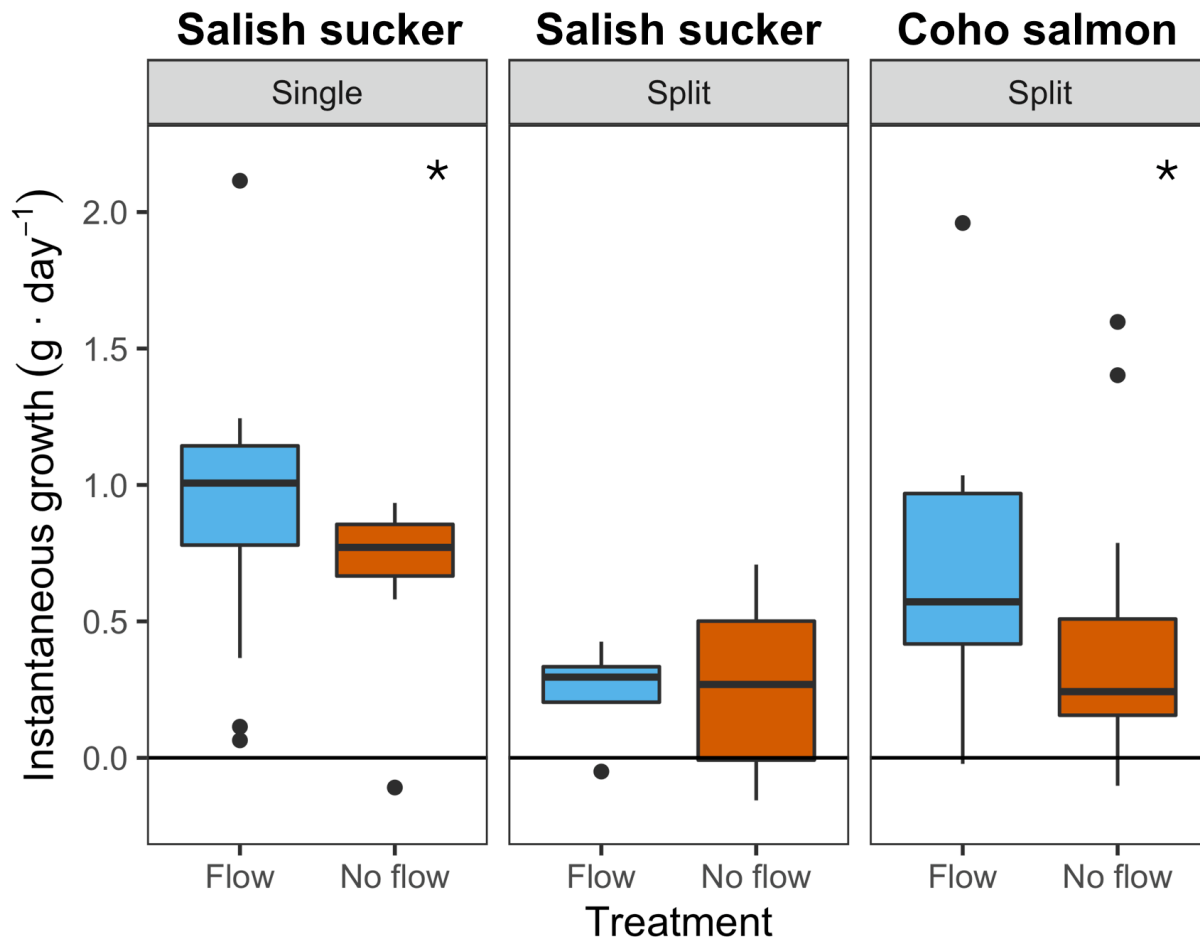
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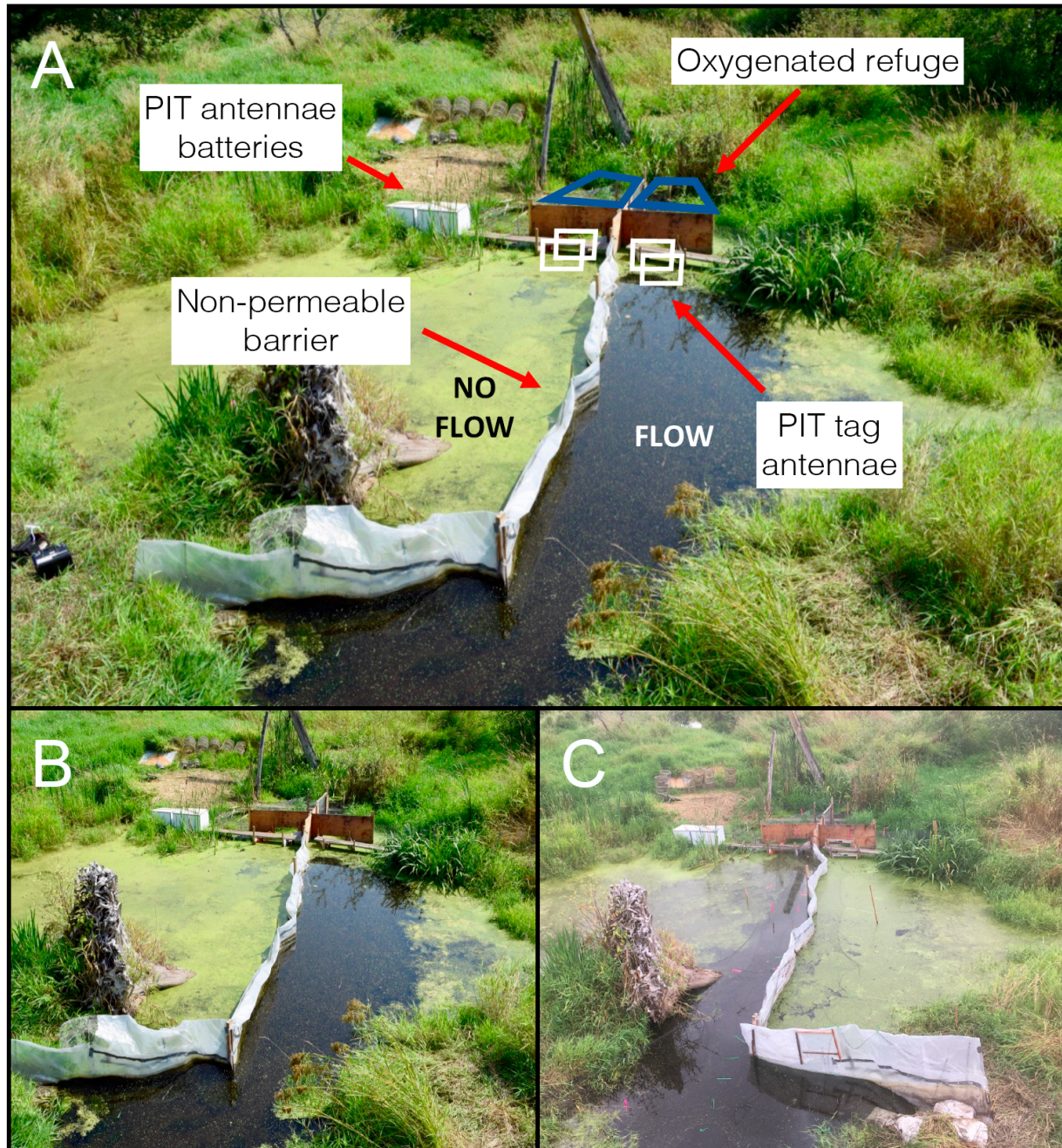
Figure 1. View of the split-pond experimental setup during the first study period (A; July 15th to August 17th, 2018). White arrow indicates water flow direction. Bottom panels are views of the split-pond experiment during the first study period (B; August 14th, 2018) and second period (C; September 5th, 2018), when the sides were switched and the experiment was repeated.

Figure 2. Summary of water quality for single (left) and split (right) pond experiments. The top pane is a summary of dissolved oxygen (mg·L^{-1}) transect point measurements for the relationship between dissolved oxygen (mg·L^{-1}) and water flow treatment by time of day (A: single-pond experiment, D: split-pond experiment). The bottom four panes are subsets of daily traces from MiniDOT loggers. The red lines represent dissolved oxygen (mg·L^{-1}; left axis) and the black lines represent temperature (°C; right axis). The single-pond flow subset (B) is from August 3rd to 10th, 2018. The single-pond no flow (C) subset is from August 29th to September 10th, 2018. The split-pond logger subsets are from the first period, both from August 2nd to 9th, 2018 (E: Flow, F: No flow). The dashed red line represents the 4 mg·L^{-1} threshold for Salish suckers as suggested by Fisheries and Oceans Canada (2016). ***Wilcoxon rank sum test, significant when *P* < 0.001.

Figure 3. The relationship between instantaneous growth (weight; g·day^{-1}) and flow (blue) and no flow (red) treatments for Salish suckers and juvenile coho salmon by experimental pond. Pane on the far left represents the single-pond experiment and the other two represent the split-pond experiment. *Wilcoxon rank sum test, significant when $P < 0.05$.
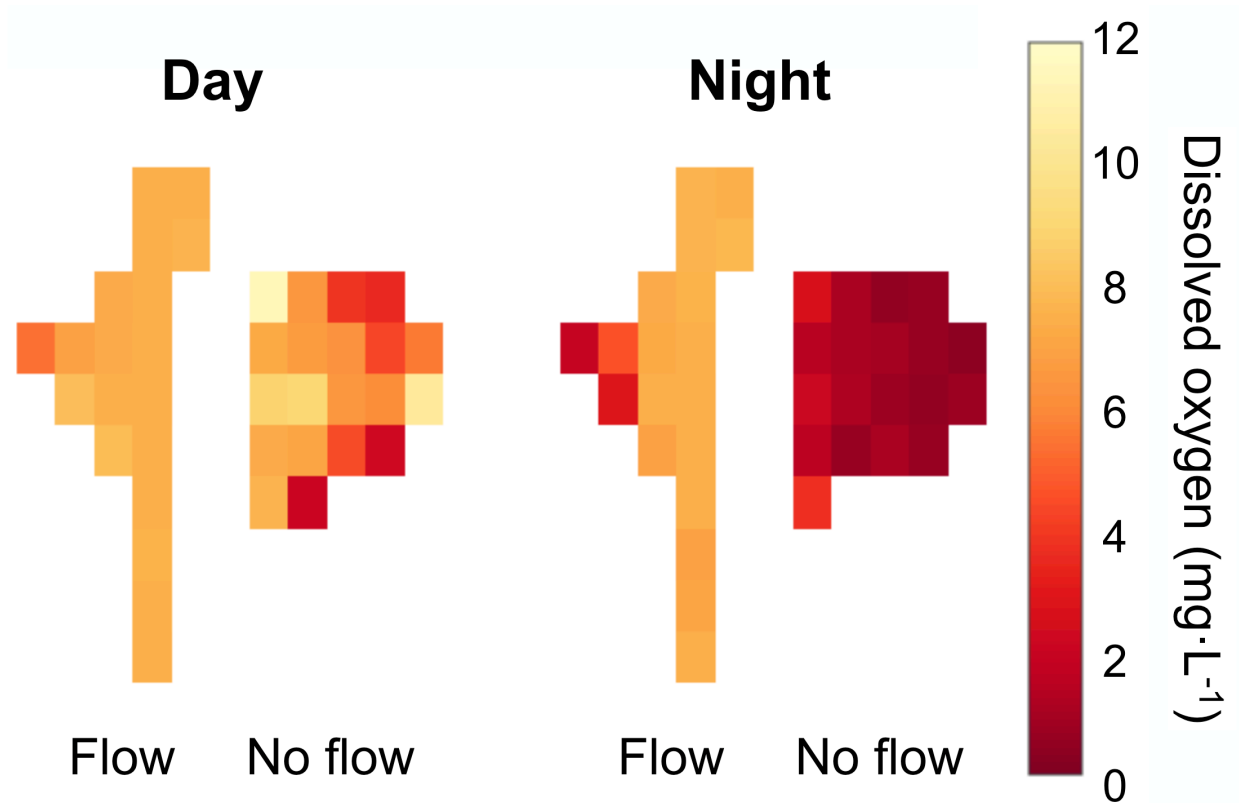
Figure 4. Heat map of split-pond transect dissolved oxygen (mg·L^{-1}) measurements for water flow and no flow treatments, split up by day (left) and night (right). This figure is from the first treatment period. Each cluster represents transects taken for each side of the pond from a birds-eye view.
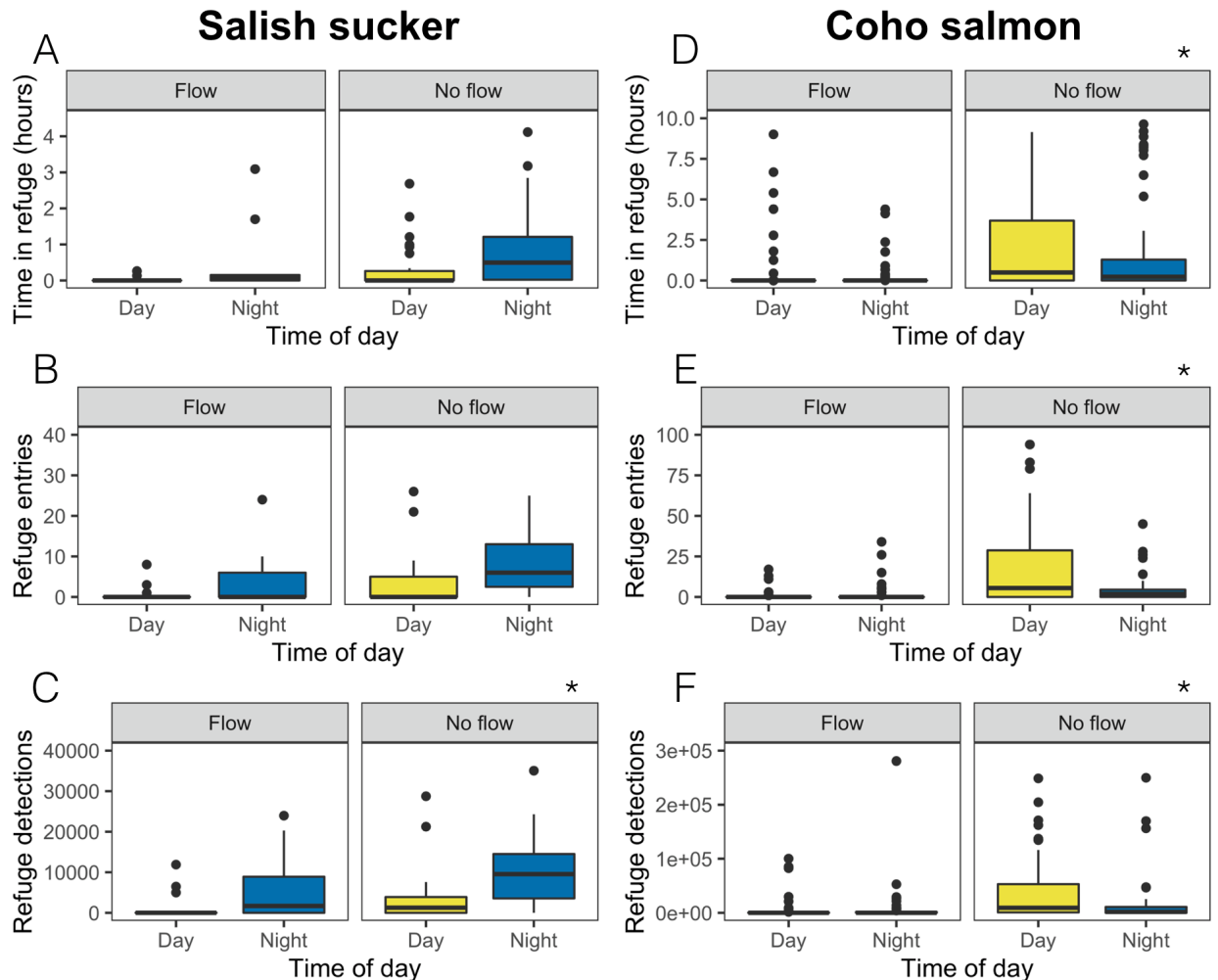
Figure 5. The relationship between refuge use and water flow treatment for Salish suckers (left) and coho salmon (right) by time of day (day = yellow, night = blue; A: Salish sucker time spent in oxygenated refuge (hours), B: Salish sucker refuge entries, C: Salish sucker raw PIT refuge detections, D: coho salmon time spent in oxygenated refuge (hours), E: coho salmon refuge entries, F: coho salmon raw PIT refuge detections). Grey bars represent flow treatment for each panel. *GLMM, significant (flow effect) when $P < 0.05$.

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Appendix Figures

Figure A1. View of single-pond (top – A) and split-pond experiments (bottom – B). The single-pond experiment "water flow" treatment ran from July 15th to August 21st, 2018. The "no water flow" treatment ran from August 21st to September 16th, 2018. The split-pond experiment's first period from July 15th to August 17th, 2018. The sides were then switched, and the second treatment period ran from August 17th to September 16th, 2018. Black arrows indicate flow and grey lines indicate fences put in to prevent fish escape. Scale is approximate.