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Article

Changes in infectious agent profiles and host gene expression during spawning migrations of adult sockeye salmon (*Oncorhynchus nerka*)

Laura K. Elmer[a,b], **Arthur L. Bass**[b], **Stephen D. Johnston**[b], **Karia H. Kaukinen**[c], **Lisa A. Kelly**[a], **Shaorong Li**[c], **Amy K. Teffer**[b], **Kristi M. Miller**[c], **Steven J. Cooke**[a], **and Scott G. Hinch**[b]

[a]Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, ON, Canada; [b]Pacific Salmon Ecology Laboratory, Department of Forestry, University of British Columbia, Vancouver, BC, Canada; [c]Molecular Genetics Laboratory, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada

Corresponding author: **Laura K. Elmer** (email: lauraelmer193@gmail.com)

Abstract

During spawning migrations, adult Pacific salmon (*Oncorhynchus* spp.) must undergo a suite of morphological and physiological changes to prepare for spawning. Modern genomics approaches can be used to conduct nonlethal assessments of physiology and infectious agent presence in migrating salmon. We investigated gene expression changes and shifts in the prevalence and load of 18 infectious agents in the gill tissue of adult sockeye salmon (*Oncorhynchus nerka*) during the final 50 km of their freshwater migration. We found 77% of fish successfully migrated to spawning grounds. Fish exhibited a decrease in thermal stress gene expression following lake migration, consistent with the hypothesized benefits of thermal refugia during spawning migrations. We also found evidence of cooler water selection (~1.5 °C) during lake migration for fish with higher infection burden, suggesting behavioural changes for fish suffering higher infection burdens and potential benefits of thermal refuges for reducing the negative impacts of infection for migrating salmon. Collectively, our findings provide further evidence that fish behaviourally regulate exposure to stressful conditions such as high temperature that may undermine survival.

Key words: salmon, freshwater, infectious agents, thermoregulation, gene expression

Introduction

During spawning migrations, adult Pacific salmon (*Oncorhynchus* spp.) return to natal spawning grounds with incredible fidelity and precise timing (Keefer et al. 2004; Hinch et al. 2006). Hormonal shifts in the ocean initiate this migration (Crossin et al. 2009), prompting adult Pacific salmon to undergo a suite of morphological and physiological changes that allow them to move from ocean feeding grounds to freshwater spawning habitats (Hinch et al. 2006; Hruska et al. 2010; Flores et al. 2012). As these salmon approach the riverine environment, they begin to cease feeding and shift to catabolism of endogenous energy reserves (Rand and Hinch 1998; Kiessling et al. 2004; Miller et al. 2009). Physiological and functional shifts in osmoregulatory tissue, including gills and kidneys, facilitate movement from saltwater into freshwater (Shrimpton et al. 2005; Flores et al. 2012). Finally, upon arriving at spawning grounds, fish undergo further physiological changes in association with final maturation and senescence (Miller et al. 2009; Hruska et al. 2010).

Riverine environments often present Pacific salmon with higher, more variable water temperatures, which can negatively impact salmon migration success (e.g., Hinch and Martins 2011; Atlas et al. 2021; Teffer et al. 2021). Water temperature is often described as the master factor influencing fish survival (Brett 1971; Fry 1971) and high-water temperatures can be devastating for adult Pacific salmon during spawning migrations (Cooke et al. 2004; Hinch et al. 2012). For some salmon populations, thermal refuges are present along their migration route. Thermal refuges can include lakes, reservoirs, and cool water tributaries, which allow fish to evade warm river water and mitigate the negative effects of thermal stress (Goniea et al. 2006). Such behavioural thermoregulation is thought to have numerous benefits for Pacific salmon and has been shown to increase survival to spawning grounds (Newell and Quinn 2005; Mathes et al. 2010; Keefer and Caudill 2015). In an era of persistent climate change and warming water temperatures, thermal refugia will likely become increasingly important for the ultimate productivity of salmon populations (McDaniels et al. 2010).

Infectious agents are a natural component of ecosystems (Windsor 1998) and are intrinsically linked to the survival and fitness of host salmon populations (Miller et al. 2014). At least 23 infectious agents are now known to infect sockeye salmon (*Oncorhynchus nerka*) across various life stages in

British Columbia (Miller et al. 2014; Bass 2018; Thakur et al. 2019), and likely many more remain undiscovered (Mordecai et al. 2019, 2021). Many infectious agents are opportunistic and do not invade or cause harm or disease unless their host is in a compromised immune state (Pickering and Pottinger 1989; Tort 2011), infectious agent presence is therefore not necessarily indicative of fish in a disease state. High water temperatures can affect both host immunity and the productivity of certain infectious agents (Jeffries et al. 2012; Chiaramonte 2013; Dittmar et al. 2014; Paull and Johnson 2014; Teffer et al. 2019, 2021). In general, the host responds to an infection when the agent is causing damage to the cells (Matzinger 2002). Because the earliest responses occur at the molecular level as shifts in gene expression, shifts in gene activity relative to the state of an infection can be observed often before clinical signs or tissue damage are evident (Andres-Terre et al. 2015). This is especially useful when nonlethal methods are necessary (such as for threatened species). Miller et al. (2017) conducted meta-analyses on transcriptome data from infection challenges with several species of RNA viruses and developed a gene biomarker panel predictive of disease development across RNA-viral species (viral disease development (VDD) panel). Activation of the VDD panel in salmon not only differentiates viral carrier states from disease states but has also been successfully applied to discover previously uncharacterized viruses (Mordecai et al. 2019, 2021) and to study early disease processes (Di Cicco et al. 2018).

Modern molecular approaches allow the assessment of hundreds, or even thousands, of genes with known functions that can reflect physiological processes (Connon et al. 2018; Houde et al. 2019*b*) or indicate specific stress (e.g., Zhang et al. 2009; Tomalty et al. 2015; Houde et al. 2019*a*), injury, or infection (e.g., Miller et al. 2017). The number of studies investigating genomic responses to various stressors is increasing and, like with the development of the VDD panel, curated gene biomarker panels have been developed that are predictive of temperature stress (Akbarzadeh et al. 2018), osmotic stress (Houde et al. 2019*a*), and hypoxia stress (Akbarzadeh et al. 2020) in Pacific salmon species. High-throughput quantitative polymerase chain reaction (qPCR) techniques make it easier to study gene expression changes and infectious agent presence and abundance in salmonids (Miller et al. 2016). There have been several recent studies that have investigated how gene expression patterns can reveal potential causes of mortality or likelihood of survival in migrating adult salmon (Evans et al. 2011; Miller et al. 2011; Drenner et al. 2018; Bass et al. 2019), and how stressors such as high temperature can affect gene expression (Jeffries et al. 2012; Teffer et al. 2017).

Studies investigating genomic and physiological changes during migration generally involve sampling a group of salmon at one location, and another group of different individuals at a second location and time point (Kiessling et al. 2004; Miller et al. 2009; Evans et al. 2011; Flores et al. 2012). Few studies have taken an individual-based approach to investigating physiological changes throughout the migration of Pacific salmon. However, Hruska et al. (2010) provides an example of such an individual-based, repeated-measures study, investigating limited blood plasma physiological indicators (lactate, cortisol and major plasma ions) associated with rapid senescence of sockeye salmon on spawning grounds. To our knowledge, there have been no studies that have examined how tissue gene expression patterns change in free-ranging individual fish during the last portion of their spawning migrations, as Hruska et al. (2010) examined using basic blood parameters. Repeat sampling of individual fish can allow us to relate physiological changes to behaviour and survival, and thus we can draw more specific conclusions about the interplay between physiological shifts and migratory patterns of Pacific salmon.

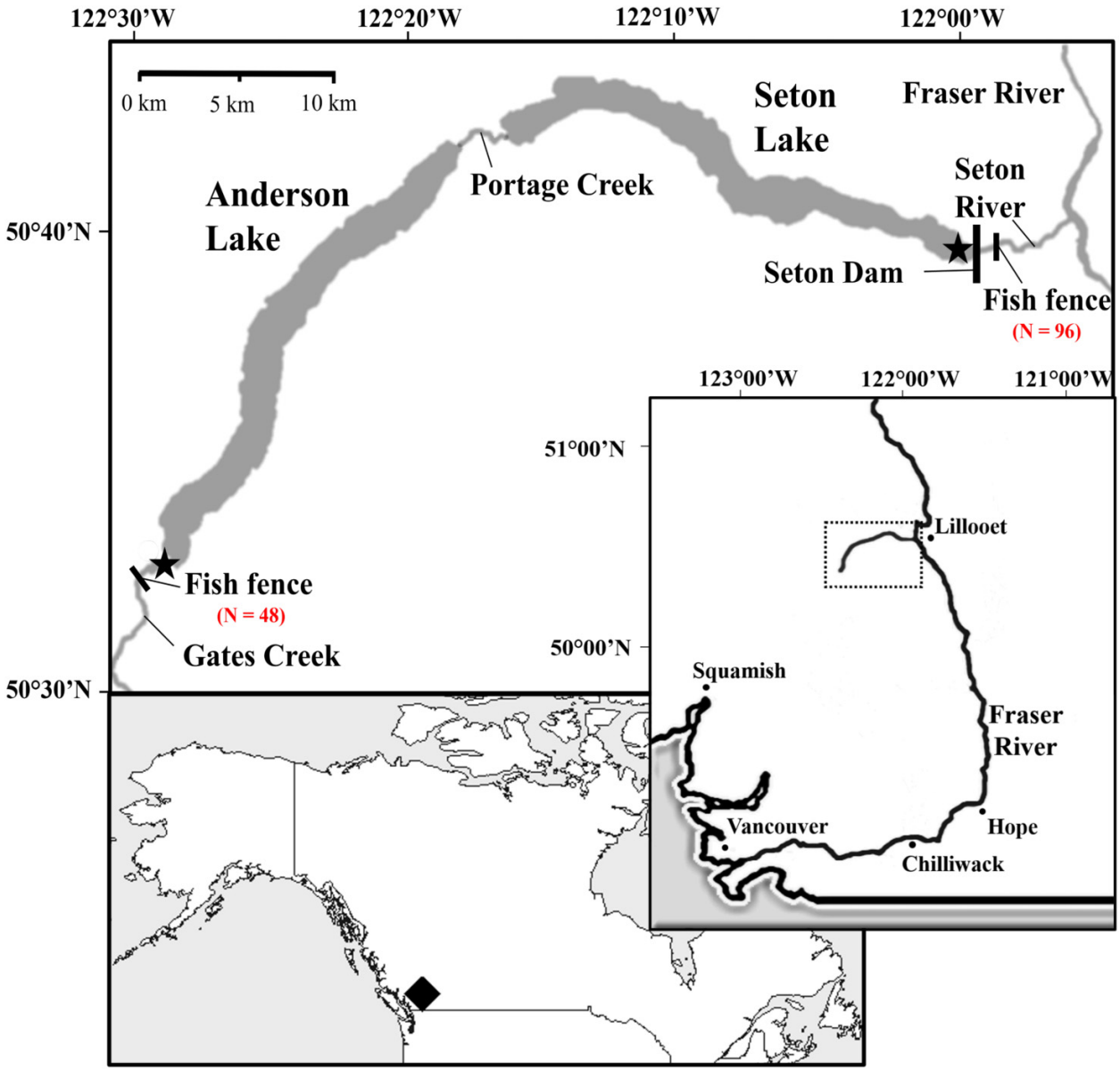
We investigated shifts in host gene expression and infectious agents of adult Gates Creek sockeye salmon in the Seton River, British Columbia (BC). We used an individual-based, repeated-measures approach. Sockeye salmon were captured, tagged, and gill-biopsied upon entering the Seton River, BC, with a subset of these fish then rebiopsied upon arrival at Gates Creek spawning grounds, 50 km from the initial capture location after an average 13-day migration through a stratified lake system. Nonlethal gill biopsies were analyzed using high-throughput quantitative polymerase chain reaction (HT-qPCR) techniques to measure the presence and loads of 18 infectious agents and the relative expression of 25 host gene biomarkers associated with immune function, viral disease response, and thermal stress. We conducted a two-phase study: first, to identify gene expression signatures and infectious agents associated with the migration success and behaviour (i.e., thermal selection) of sockeye salmon. Second, for those fish that successfully migrated to spawning grounds and were able to be resampled, we aimed to characterize how gene expression and infectious agent profiles change as fish migrate. We present novel data showing both gene expression and infectious agent community changes of repeat-sampled sockeye salmon in the wild.

Methods

Study site

This study examined Gates Creek sockeye salmon, a summer-run Fraser River sockeye salmon population. All fish collection and tagging took place on the Seton River, a tributary of the Fraser River (British Columbia, Canada). Gates Creek sockeye salmon migrate approximately 364 km upstream from the Pacific Ocean through the Fraser River, and then travel an additional 50 km through the "Seton system" (Seton River, Seton Lake, and Anderson Lake) to ultimately reach spawning habitat in Gates Creek (Fig. 1). A dam exists approximately 4.5 km upstream from the Seton–Fraser confluence, so migrating fish must navigate the Seton Dam fish ladder before passing through both Seton and Anderson lakes to reach Gates Creek. Seton Lake and Anderson Lake are connected by Portage Creek, which is approximately 3 km in length. Water temperatures in Seton and Anderson lakes range from approximately 6 °C at their deepest to 15 °C at the surface in August (Roscoe et al. 2010). Previous tracking studies have estimated that Gates Creek sockeye salmon migrate from the mouth of the Fraser River to the mouth of the Seton River in about 10 days (Crossin et al. 2009), and then migrate for an additional 50 km over 10 days in the Se-

Fig. 1. Map of the study area in British Columbia, Canada. The Seton system is shown in the upper map. The location of the Seton Dam and the fish fences are shown, and locations of PIT receivers on the Seton Dam fishway and at Gates Creek mouth are indicated by stars. The number of fish captured, sampled, and tagged at Seton fish fence is shown in red at the fish fence site. Similarly, the number of these fish recaptured and resampled at Gates Creek fish fence is also shown in red at the fence site. A map of the lower Fraser River is shown in the lower right-hand corner; the location of the Seton system is outlined by a dashed box on this map. Map of Canada is shown in the lower left-hand corner, and the location of the study area is shown by a black diamond.



ton system to reach spawning grounds (Minke-Martin et al. 2018). A fish collecting fence has been seasonally used to capture and tag fish as part of fishway and dam effectiveness monitoring studies (Bett et al. 2022) in the Seton River, and is constructed immediately below Seton Dam (Fig. 1). An artificially enhanced spawning channel is operated 800 m upstream from the mouth of Gates Creek, and a fish weir (fence) is constructed across the creek to divert fish towards the enhanced spawning channel entrance (Fig. 1). This entrance is blocked by a gate that is manually operated to allow fish to enter either the enhanced spawning channel or to pass further upstream into Gates Creek.

Fish collection, biopsy, and tagging at Seton River fish fence

Gates Creek sockeye salmon were captured on the Seton River using a fish collection fence (Fig. 1). This fence consisted of removable pickets, which, when fully operational, will block any upstream movement of fish in the Seton River.

A small gap in the fence encouraged migrants to enter an enclosed holding area. The fence was operational throughout the night for 10 h to intercept and capture sockeye salmon during their migration but allowed salmon unimpeded passage during daytime hours.

Fish were removed from the holding area via dipnet and transferred to a tagging trough with a continuous supply of river water. Gross somatic energy (GSE) was estimated using a fish FatMeter (Model FM 692, Distell, West Lothian, Scotland, UK), which measures lipid concentration at two locations on the body of the fish (Crossin and Hinch 2005). Pacific salmon rely on endogenous energy reserves to fuel their upstream spawning migrations and thus energy use and allocations are an important consideration for these fish, and GSE measurements allow us to estimate their energy reserves. Previous studies on the Seton River have found GSE to be negatively associated with survival to Gates Creek (Bass et al. 2018). GSE measurements obtained at this site have previously been used to discriminate sockeye salmon populations (Casselman et al. 2016); thus, we were able to identify Gates Creek sockeye salmon from other straying sockeye salmon entering the Seton River system (fish with an average GSE measurement $\leq$2.7 MJ/kg). Only Gates Creek sockeye salmon, as identified through GSE measurements, were tagged as part of this study.

Fish were assigned a "wound score" based on any visible injuries and scale loss (scored from 0 to 3 based on the presence and severity of any injuries; 0 = no injuries present, 1 = <5% total body scale loss and/or presence of small surficial wounds; 2 = 5%–20% scale loss and/or shallow wounds, 3 = >20% scale loss and deep wounds exposing flesh). Sea lice, lamprey, and other predator wounds were commonly found on these fish, as well as other wounds of unknown origin. Bass et al. (2018) previously found sea lice wounds and wounds of unknown origin to be negatively associated with survival for Gates Creek sockeye salmon in the Seton River. Fish with gillnet scars were not tagged as part of our study. Previous studies have found that 19%–27% of sockeye salmon intercepted in the Seton River had markings consistent with previous gillnet entanglement in the Fraser River (Bass et al. 2018; Kanigan et al. 2019).

All fish in this study were gill-biopsied, gastrically tagged for tracking, and externally tagged for visual identification. Gill biopsies were performed by taking a small tissue sample (approximately 3 mm from the tips of 2–3 gill filaments), which was then transferred into 1.5 mL RNAlater solution (Qiagen, MD, USA) and stored at −80 °C. Fish were then tagged with 32 mm half duplex (HDX) passive integrated transponder (PIT) tags (Oregon RFID, Portland, OR, USA). To allow these PIT tags to be inserted gastrically, they had been inserted into smoothed acetal Delrin tube sections 1.59 cm in diameter, 3.81 cm in length, and were then inserted into the abdominal cavity of the fish using smoothed plungers. These gastric tagging and biopsy procedures have previously been validated for use with adult sockeye salmon, and no impact on survival was found (Cooke et al. 2004). A subset of fish also had temperature loggers (iButton Thermochron model DS1921Z or DS1922L; Maxim Integrated, San Jose, CA, USA) installed on their internal tag that were programmed to record the temperature of the fish every 30 min. Approximately 10 fish were chosen for iButtons every day throughout the study period, distributed evenly across each tagging day. All internal tags with iButtons were waterproofed using Plasti Dip (International, St. Louis Park, MN, USA). Finally, fish were also externally tagged using a fluorescent yellow 7.62 cm T-bar anchor tag (Floy Tag & Mfg. Inc., Seattle, TA, USA) to allow visual identification of tagged fish. Each of these external tags had a unique identification number specific to each individual fish.

After tagging and sampling was complete, all fish were released on the upstream side of the fish fence to allow them to complete their migration. A total of 103 fish were tagged and sampled at Seton River fish fence for this study from August 8th to August 20th, 2017.

PIT telemetry

Three PIT antennas were located along the Seton system migration route to track the migration of sockeye salmon from the Seton River fish fence to Gates Creek spawning grounds (Fig. 1). Two antennas were installed at Seton Dam, one at the fish ladder entrance, and the second at the fish ladder exit (see Casselman et al. 2016). These two antennas allowed assessment of dam passage success and passage efficiency. The final antenna was installed approximately 100 m upstream from the mouth of Gates Creek. This was a pass-through antenna constructed from 1.5-inch PVC piping that measured 16 m in length and 1 m in diameter. This antenna spanned the creek and thus any fish that entered Gates Creek would swim over this receiver. Fish detected at this antenna were considered to have successfully migrated to spawning grounds.

Detection efficiency of PIT receivers was estimated by the number of fish detected at each receiver, divided by the total number of fish known to have that passed receiver. Fish were known to pass each receiver independent of detection at that receiver by either capture in upstream fisheries, recapture at Gates Creek spawning grounds, collection of carcasses at spawning grounds, or detection at another upstream receiver. Detection efficiencies of the Seton Dam fishway entrance, Seton Dam fishway exit, and Gates Creek PIT receivers were determined to be 98%, 98.8%, and 99.5%, respectively.

Recapture of tagged fish at spawning grounds

Similar to the Seton River fish fence, the Gates Creek fish fence is constructed of removable pickets that block all upstream migration for sockeye salmon. Two "gates" were installed into the fence, one opened allowing fish into the artificially enhanced spawning channel, and the other opened to allow fish further upstream into Gates Creek. Sockeye salmon migrating into Gates Creek hold behind this fence when both gates are closed. Gates were closed at night for approximately 10 h.

Tagged fish were recaptured and biopsied for a second time at spawning grounds. Because fish naturally hold in a pool at the Gates Creek fish fence, tagged fish were recognizable by their external anchor tag and were recaptured from this pool using dipnets. Fish were immediately transferred to a tagging trough and reassessed for wounds, scale loss, and fin

damage, and a gill sample was taken and preserved in 1.5 mL RNAlater solution (Qiagen, MD, USA). These recaptured fish were released upstream of the fence into the spawning channel. A total of 48 tagged fish were recaptured and resampled in this way at spawning grounds.

In addition to recapturing live fish as they entered Gates Creek, surveys were also conducted to look for carcasses of tagged fish. Weekly creek walks and visual inspection of all sockeye salmon carcasses in both Gates Creek and the artificially enhanced spawning ground allowed us to recover tagged fish and any temperature loggers from these fish. A total of 42 temperature loggers were recovered in this way. Spawning success of female carcasses could also be determined. A failed female spawner was identified by egg retention, whereas female carcasses with approximately 50%–100% of their eggs missing were considered successful spawners for this study.

Thermal experience through Seton and Anderson lakes was evaluated using temperature logger data from recovered carcasses at Gates Creek ($N = 42$). We determined entrance into Seton Lake as the first temperature recording following detection of the fish at the PIT receiver located at the fishway exit of Seton Dam. We determined exit from Anderson Lake by the first detection of the fish at the PIT receiver at the mouth of Gates Creek. We removed data as fish migrated through Portage Creek as fish are not able to thermally regulate in this section of creek like they can in lakes. Temperatures as fish migrated through Portage Creek were determined by looking at the thermograph for each individual fish. The time span of fish as they migrated through the creek was estimated as it is much higher in temperature (typically 19–20 °C) compared with the temperatures at which fish choose to migrate through in the lake (typically below 16 °C). The average water temperature experienced by each fish during migration was then calculated for both Seton and Anderson lakes separately, as well as both lakes combined ("average lake migration temperature"). See Casselman et al. (2016) and Minke-Martin et al. (2018) for more details on how we are able to determine location through iButton thermographs in the Seton system.

Laboratory analyses

All laboratory analyses were performed at Pacific Biological Station (Nanaimo, BC, Canada). Gill samples were analyzed for RNA expression of select host gene biomarkers and infectious agent RNA copy numbers using high-throughput nanofluidic qPCR (Fluidigm® BioMark™ Dynamic Array, CA, USA). For a comprehensive overview of this technology see Miller et al. (2014, 2016). In total, 18 infectious agents previously detected in sockeye salmon tissues and 25 host gene biomarkers were assayed in the gill tissue of tagged fish; see Tables 1 and 2 for an overview of gene biomarker assays and infectious agent assays run, respectively. All infectious agent and gene biomarker assays were run in duplicate. Host biomarkers included 7 genes associated with salmonid thermal stress (Houde et al. 2019*a*), 5 genes coactivated in a mortality related signature (MRS; Miller et al. 2011), 10 genes upregulated with VDD (Miller et al. 2017), and 3 genes associated with immune function and general stress. Assays to two recently discovered viruses, one with close similarity to the Qin virus family and the other a putative Picovirus (G. Mordecai, unpublished data), that had been detected in juvenile sockeye salmon in other drainages were additionally run on a QuantStudio platform.

Gill tissue was thawed and removed from RNAlater solution and transferred to microtubes containing stainless-steel beads for homogenization. This tissue was then homogenized using TRI reagent (Thermo Fisher Scientific Inc.) and vigorous shaking in a MM301 mixer mill (Retsch Inc., Newtown, PA, USA), then separated using 1-bromo-3-chloropropane and centrifugation (1500*g*, 6.5 min). The aqueous phase was aliquoted into 96-well plates and RNA extraction was performed using MagMAX-96 for Microarrays Total RNA Isolation Kits (Ambion Inc.) with a Biomek NXP automated liquid-handling machine using the "spin method" as per the manufacturer's instructions. DNase treatment was incorporated at this step to reduce any DNA contamination. RNA quantity (A260; ng mL^{-1}) and quality (A260/A280) was measured using a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA). All samples were then normalized to 62.5 ng μL^{-1} using the Biomek NXP automated liquid-handling machine. RNA quantity was checked again following normalization. Reverse transcription of 1 μg normalized RNA was performed using a SuperScript VILO MasterMix Kit (Invitrogen, Carlsbad, CA) to create cDNA using PCR cycling conditions of 25 °C for 10 min, 42 °C for 60 min, and 85 °C for 5 min. Specific target amplification (STA) of select gene and infectious agent biomarkers was performed using 1.25 μL cDNA, assay 200 nmol L^{-1} primer pairs (see Tables 1 and 2), TaqMan Preamp Master Mix (Applied Biosystems, Foster City, California) and cycling at 95 °C for 10 min followed by 15 cycles of 95 °C for 10 s and 60 °C for 4 min, following Fluidigm guidelines. This allowed preamplification of all assays, a necessary step before qPCR due to the small assay volume (7 nL wells) required. Following STA, any unincorporated primers were removed using ExoSAP-IT (Affymetrix, Santa Clara, California) and cycling at 37°C for 15 min, 80°C for 15 min. Finally, samples were diluted 5-fold using DNA Suspension Buffer (Teknova, Hollister, California).

For qPCR, both samples (preamplified cDNA product from every gill biopsy) and assays (qPCR assays for all host and infectious agent biomarkers) were prepared and loaded onto the Fluidigm chip. A sample mix was prepared using preamplified cDNA, 1× TaqMan Universal Master Mix (Life Technologies), and 1× GE Sample Loading Reagent (Fluidigm PN 85000746). An assay mix was then prepared with 9 μm primer pairs, 2 μm probes, and assay loading reagent (Fluidigm PN 85000736). All assays were run in duplicate. Sample and assay mixes were then pipetted into the chip and were loaded using an IFC controller HX (Fluidigm). Assay mixes, both infectious agents and gene biomarkers, were pipetted onto the chip in duplicate. Finally, qPCR was conducted (50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min). Probes (with FAM dye) quantified target amplicons during the qPCR cycling.

Two serial dilutions were also added to the dynamic array to allow for calculation of assay efficiency. The first was a serial dilution of pooled cDNA. This pooled cDNA contained 1

Table 1. Sockeye salmon host biomarkers evaluated using qPCR.

Assay name	Gene category	Gene name	Accession #	Primer and probe sequences
JUN	General stress	Transcription factor AP-1	CA056351	F: TTGTTGCTGGTGAGAAAACTCAGT
				R: CCTGTTGCCCTATGAATTGTCTAGT
				P: AGACTTGGGCTATTTAC
78d16.1	HKG	S100 calcium-binding protein	CA056739	F: GTCAAGACTGGAGGCTCAGAG
				R: GATCAAGCCCCAGAAGTGTTTG
				P: AAGGTGATTCCCTCGCCGTCCGA
COIL-P84-2	HKG	Coiled-coil domain-containing protein 84	CA053789	F: GCTCATTTGAGGAGAAGGAGGATG
				R: CTGGCGATGCTGTTCCTGAG
				P: TTATCAAGCAGCAAGCC
MRPL40	HKG	39S ribosomal protein L40, mitochondrial precursor	CK991258	F: CCCAGTATGAGGCACCTGAAGG
				R: GTTAATGCTGCCACCCTCTCAC
				P: ACAACAACATCACCA
RIG1	Immune	Retinoic acid inducible gene I	NM_001163699	F: ACAGCTGTTACACAGACGACATCA
				R: TTTAGGGTGAGGTTCTGTCCGA
				P: TCGTGTTGGACCCCACTCTGTTCTCTC
C7	Immune/MRS	Complement factor	CA052045	F: ACCTCTGTCCAGCTCTGTGTC
				R: GATGCTGACCACATCAAACTGC
				P: AACTACCAGACAGTGCTG
ATP5G3	MRS	ATP synthase	CB493164	F: GGAACGCCACCATGAGACA
				R: CGCCATCCTGGGCTTTG
				P: AGCCCCATTGCCTC
HTA	MRS	HIV-1 Tat interactive protein	–	F: CTTGTAACAGTTCGACATGGCTTATT
				R: TGGTGAAGCATTTCTGTATGTCAA
				P: TCTGTACTGAGCATCCCCGCACATTACA
MMP25	MRS	Matrix metalloproteinase-25 precursor		F: TGCAGTCTTTTCCCCTTGGAT
				R: TCCACATGTACCCACACCTACAC
				P: AGGATTGGCTGGAAGGT
PRAS	MRS	*Oncorhynchus mykiss* G-protein (P-ras) mRNA, complete cds	CA059617	F: GCAGGATGAGCAGAGGAAGAA
				R: GGCCTGGGCAATGTAACACT
				P: CCCCCTAAAGATGCAG
SCG	MRS	Secretogranin-2	CA053613	F: GGATGTGAAGAATCCAACACTGAT
				R: ACACCACTTCAAACTAGCCATACATT
				P: CGGCTGTATGTGCACTG
hsp70	Thermal	Heat shock 70 kDa protein	C249R043	F: TCAACGATCAGGTCGTGCAA
				R: CGTCGCTGACCACCTTGAA
				P: CCGACATGAAGCACTGG
hsp90a-15	Thermal	Heat shock protein 90 alpha	CA062155	F: ATGACCCTCAGACACACTCCAA
				R: CCTCATCAATACCCAGTCCTAGCT
				P: CGCATCTACAGAATGA
HSP90alike_6	Thermal	Heat shock protein 90 alpha like	C020R155	F: TTGGATGACCCTCAGACACACT
				R: CGTCAATACCCAGGCCTAGCT
				P: CCGAATCTACCGGATGAT
SERPIN9	Thermal	Serpin H1 precursor (HSP47)	C236R132	F: GAGGTCAGCGACCCAAAGAC
				R: GCCGTAGAGGCGGTTACTGAT
				P: CGGAACGTCACATGGA
SERPIN20	Thermal	Serpin H1 precursor (HSP47)	CA063723	F: ACTATGACCACTCGAAGATCAACCT
				R: CCCATTCGTTGATGGAGTTCA
				P: AGGGACAAGAGGAGC
SFRS2	Thermal	Splicing factor, arginine/serine-rich 2	CB493433	F: TCCAGATGGCCCGTTACG
				R: CACCACCGCCTCCATGAT
				P: TCCCCCAGATTCT

Table 1. (*concluded*).

Assay name	Gene category	Gene name	Accession #	Primer and probe sequences
ef2_14	Thermal (−)	Elongation factor 2	CB498321	F: AGGTCACAGCCGCCCTTAG
				R: ACACAGTCTCTGTCTGCACACACA
				P: CGACTGCGTCTCAGGT
52RO	VDD	52 kDa Ro protein-2 - 52Ro	CX141267	F: TGCACTATTGCCCAGTAACCAT
				R: TGCAAGAGGAGATGCCAACA
				P: AGTAGGATTCACAGAGAGTT
CA054694	VDD	Mitochondrial ribosomal protein (VAR1)	CA054694	F: CACCTGAGGTACTGAAGATAAGACA
				R: TTAAGTCCTCCTTCCTCATCTGGTA
				P: TCTACCAGGCCTTAAAG
DEXH	VDD	ATP-dependent RNA helicase	FN396359	F: CCATAAGGAGGGTGTCTACAATAAGAT
				R: CTCTCCCCCTTCAGCTTCTGT
				P: TGGCGCGCTACGTG
GAL3	VDD	Galectin-3-binding protein precursor	CB515011	F: TTGTAGCGCCTGTTGTAATCATATC
				R: TACACTGCTGAGGCCATGGA
				P: CTTGGCGTGGTGGC
HERC6	VDD	Probable E3 ubiquitin-protein ligase HERC6	CA060884	F: AGGGACAACTTGGTAGACAGAAGAA
				R: TGACGCACACACAGCTACAGAGT
				P: CAGTGGTCTCTGTGGCT
IFI44A	VDD	IFN-induced protein 44-1	GS365948	F: CGGAGTCCAGAGCAGCCTACT
				R: TCCAGTGGTCTCCCCATCTC
				P: CGCTGGTCCTGTGTGA
IFIT5	VDD	Interferon-induced protein with tetratricopeptide repeats 5	CA051350	F: CCGTCAATGAGTCCCTACACATT
				R: CACAGGCCAATTTGGTGATG
				P: CTGTCTCCAAACTCCCA
MX_ONTS	VDD	Interferon-induced GTP-binding protein Mx	CB516446	F: AGATGATGCTGCACCTCAAGTC
				R: CTGCAGCTGGGAAGCAAAC
				P: ATTCCCATGGTGATCCGCTACCTGG
RSAD	VDD	Radical S-adenosyl methionine domain-containing protein 2	CA038316	F: GGGAAATTAGTCCAATACTGCAAAC
				R: GCCATTGCTGACAATACTGACACT
				P: CGACCTCCAGCTCC
STAT1	VDD	Signal transducer and activator of transcription 1-alpha/beta	CA050950	F: TGTCACCGTCTCAGACAGATCTG
				R: TGTTGGTCTCTGTAAGGCAACGT
				P: AGTTGCTGAAAACCGG

Note: A total of 28 biomarkers were assayed, including 3 house-keeping genes (HKG). Biomarkers are categorized as genes involved with viral disease development (VDD), morality-related signatures (MRS), thermal stress, immune response, or general stress. Forward (F) and reverse (R) primer sequences and probe (P) sequences are given.

μL of every sample from this study. A serial 5-fold dilution of the pooled cDNA was then prepared and was included on the final dynamic array to determine host gene biomarker assay efficiency. A second serial dilution of artificial positive constructs (APC clones) of infective agents was also included on the dynamic array that can be used to determine infectious agent RNA copy numbers and assay efficiency (Miller et al. 2016). A probe (with NED™ dye, Applied Biosystems, Foster City, CA, USA) was also included to detect APC contamination in samples. Positive and negative controls were included on each dynamic array for RNA extraction, cDNA synthesis, STA, and qPCR stages.

Output was scored using the BioMark Real-Time PCR analysis software. For an in-depth overview of scoring procedures, see Miller et al. (2016). For all host gene biomarkers and infectious agent biomarkers, the BioMark Real-Time PCR analysis software generated a quantification cycle threshold (C_t) value. All assays were performed in duplicate, and thus C_t values for host gene or infectious agent biomarkers in every fish were averaged. For infectious agents, assays not detected in duplicate were failed. Relative expression of host genes was derived according to Pfaffl (2001) using two reference genes and efficiency correction. For infectious agents, the number of RNA copies per sample was calculated using the standard curves of APC clone dilutions that were run on each dynamic array. Any APC contaminated samples were detected by fluorescence of the NED probe, and these were removed. Infectious agent RNA copy number was log transformed for all analyses.

Statistical analyses

All statistical analyses were performed using R statistical software (R Core Team 2015). First, analyses investigating variables associated with migration success, spawning success, and thermal selection are described, followed by a description of analyses investigating changes in gene expres-

Table 2. Infectious agents assayed in sockeye salmon during this study.

Assay name	Type	Full name	Accession #	Primer and probe sequences	Assay reference
c_b_cys	Bacterium	*Candidatus* Branchiomonas cysticola	JQ723599	F: AATACATCGGAACGTGTCTAGTG R: GCCATCAGCCGCTCATGTG P: CTCGGTCCCAGGCTTTCCTCTCCCA	Mitchell et al. (2013)
fl_psy	Bacterium	*Flavobacterium psychrophilum*	–	F: GATCCTTATTCTCACAGTACCGTCAA R: TGTAAACTGCTTTTGCACAGGAA P: AAACACTCGGTCGTGACC	Duesund et al. (2010)
rlo	Bacterium	*Rickettsia*-like organism	EU555284	F: GGCTCAACCCAAGAACTGCTT R: GTGCAACAGCGTCAGTGACT P: CCCAGATAACCGCCTTCGCCTCCG	Lloyd et al. (2011)
ic_mul	Ciliate	*Ichthyophthirius multifiliis*	IMU17354	F: AAATGGGCATACGTTTGCAAA R: AACCTGCCTGAAACACTCTAATTTTT P: ACTCGGCCTTCACTGGTTCGACTTGG	Miller et al. (2016)
sp_des	Mesomycetozoea	*Sphaerothecum destructuens*	AY267346	F: GGGTATCCTTCCTCTCGAAATTG R: CCCAAACTCGACGCACACT P: CGTGTGCGCTTAAT	Miller et al. (2016)
lo_sal	Microsporidian	*Loma salmonae*	HM626243	F: GGAGTCGCAGCGAAGATAGC R: CTTTTCCTCCCTTTACTCATATGCTT P: TGCCTGAAATCACGAGAGTGAGACTACCC	Miller et al. (2016)
pa_ther	Microsporidian	*Paranucleospora theridion*	FJ59481	F: CGGACAGGGAGCATGGTATAG R: GGTCCAGGTTGGGTCTTGAG P: TTGGCGAAGAATGAAA	Nylund et al. (2010)
ce_sha	Myxozoan	*Ceratomyxa shasta*	AF001579	F: CCAGCTTGAGATTAGCTCGGTAA R: CCCCGGAACCCGAAAG P: CGAGCCAAGTTGGTCTCTCCGTGAAAAC	Hallett and Bartholomew (2006)
pa_kab	Myxozoan	*Parvicapsula kabatai*	DQ515821	F: CGACCATCTGCACGGTACTG R: ACACCACAACTCTGCCTTCCA P: CTTCGGGTAGGTCCGG	Miller et al. (2016)
pa_min	Myxozoan	*Parvicapsula minibicornis*	AF201375	F: AATAGTTGTTTGTCGTGCACTCTGT R: CCGATAGGCTATCCAGTACCTAGTAAG P: TGTCCACCTAGTAAGGC	Miller et al. (2016)
de_sal	Protozoan	*Dermocystidium salmonis*	U21337	F: CAGCCAATCCTTTCGCTTCT R: GACGGACGCACACCACAGT P: AAGCGGCGTGTGCC	Miller et al. (2016)
ic_hof	Protozoan	*Ichthyophonus hoferi*	AF467793	F: GTCTGTACTGGTACGGCAGTTTC R: TCCCGAACTCAGTAGACACTCAA P: TAAGAGCACCCACTGCCTTCGAGAAGA	White et al. (2013)
psav-2	Virus	Pacific salmon arenavirus 2	–	F: AACATGAAGGGCGATTCGTT R: CAGCCCGCGGACTGAGT P: CAAGTGATGTAAGCTTG	Mordecai et al. (2020)
ascv	Virus	Atlantic salmon calicivirus	–	F: ACCGACTGCCCGGTTGT R: CTCCGATTGCCTGTGATAATACC P: CTTAGGGTTAAAGCAGTCG	Mordecai et al. (2021)
psnv	Virus	Pacific salmon nidovirus	–	F: GGATAATCCCAACCGAAAAGTTT R: GCATGAAATGTTGTCTCGGTTTAA P: CGATCCCGATTATC	Mordecai et al. (2020)
ctv-2	Virus	Cutthroat trout virus 2	–	F: CCACTTGTCGCTACGATGAAAC R: CGCCTCCTTTGCCTTTCTC P: ATGCCGGGCCATC	Mordecai et al. (2021)
ortho	Virus	OrthoMyxoV	–	F: GGAAGCAGTGGACGCTAACC R: TCGCGAAGGTCTCTCAATGTC P: ATTCTTCTCATCAAAGGCA	G. Mordecai (unpublished)
cav	Virus	Chinook aquareovirus	–	F: AACTTTCGGCTTTCTGCTATGC R: GAGGACAAGGGTCTCCATCTGA P: TTAATTGCGGTACTGCTC	Mordecai et al. (2020)

Note: A total of 18 infectious agents were evaluated through qPCR. Forward primer (F), reverse prime (R), and probe (P) sequences are given.

sion and infectious agents for repeat sampled fish during their migration through the Seton system. We calculated relative infection burden (RIB) for each individual as per Bass et al. (2019). RIB allows us to collapse all infectious agent load data for an individual into a single metric that represents the overall burden (loads) of infectious agents on an individual. For each fish, the RNA copy number of the each positively detected infectious agent is divided by the maximum RNA copy number within the population of fish sampled for that infectious agent. This is repeated for every infectious agent detected within the population of fish sampled, and all calculated values are then summed. RIB can range from 0 to N, where N is the total number of infectious agents detected in a study.

Migration success, spawning success, and thermal selection

The first aim of our study was to investigate gene expression signatures and infectious agents associated with migration success and behaviour. A total of 103 Gates Creek sockeye salmon were sampled and tagged at Seton fish fence and migration was tracked to the Seton Dam fishway PIT receivers and the Gates Creek PIT receiver. Of these, seven tagged fish were captured by fisheries between Seton fish fence and Gates Creek and so were removed from further analyses. Migration success to pass Seton Dam was determined by detection at the Seton Dam fishway exit receiver. Migration success to Gates Creek spawning grounds was determined by detection at the PIT receiver near the mouth of Gates creek. The data were formatted as binary data representing either presence or absence of each fish at the receivers. Because success to pass Seton Dam was so high (only four fish did not successfully pass the Seton Dam exit receiver) we were not able to adequately test which factors were associated with migration success to pass the dam. To test which factors were associated with migration success to Gates Creek, we created binomial logistic regression models. To determine which infectious agent variables, if any, should be included in this final model for migration success to Gates Creek, we first performed χ^2 tests or Welch's t tests to determine if the presence or load, respectively, of each individual infectious agent alone had a significant impact on migration success. No infectious agents were found to be significant in these individual tests (at $P < 0.05$), so they were not included in the final model. RIB was included in the final model to represent overall infection burden for each individual fish. Our final model also included the explanatory covariates GSE, fork length, tagging date, and wound score as previous work has shown that these factors have impacted Gates Creek sockeye salmon survival (Bass et al. 2018). Sex was also included in these models due to its importance in many studies on sockeye salmon survival (Hinch et al. 2021).

To identify gene expression signatures associated with migration success we performed random forest classification (RFC) using R package "*randomForest*" (Liaw and Wiener 2002). RFC is a supervised machine learning technique that combines the predictions of multiple independent decision tree models to build classification rules, and ultimately forms a composite model with high predictive accuracy (Breiman 2001; Cutler et al. 2007). Our initial RFC model used 500 classification trees to quantify relationships between our categorical dependent variable (migration success to Gates Creek) and 25 predictor variables (expression of the 25 host genes assayed in this study). Predictor variable importance was evaluated using two measures. The first, "mean decrease in accuracy", was measured by the decrease in classification accuracy resulting from removal of that single predictor from the model (Breiman 2001). The second measure, the "Gini index", is the total decrease in node impurities from splitting on the predictor variable averaged over all trees (Breiman et al. 1984). We then developed a reduced RFC model that removed genes with no importance to survival. Our reduced model was developed by removing genes based on their mean decrease in accuracy values. Starting with the gene with the lowest "mean decrease in accuracy" value, we removed genes one at a time until the final model contained no genes with a mean decrease in accuracy lower than 2.

We investigated the impact of RIB on migration temperature through Seton Lake, Anderson Lake, and both lakes combined using linear regression models. Sex, GSE, wound score, tagging date, and fork length were also included as covariates for consistency with the other models. We investigated relationships between individual infectious agent load at Seton fence and average migration temperature through Seton and Anderson lakes through linear regression models. No infectious agents were found to be significantly associated with subsequent migration temperature through Seton or Anderson lakes (at $P < 0.05$) so were not included in the final linear regression model. We also investigated the length of time fish spent in lake thermal refuge habitat using linear regression models. This model included length of time spent in lake habitat (Seton and Anderson lakes combined) as the response variable, and RIB, sex, GSE, wound score, tagging date and fork length as explanatory covariables.

Spawning success was determined through recovery of tagged female carcasses at Gates Creek; these data were formatted as binary data representing either successful or failed spawning. To investigate variables associated with spawning success, we constructed logistic regression models that included wound score, tagging date, and RIB of fish captured and resampled at the Gates Creek fish fence. Again, we tested for any effects individual infectious agent presence or load might have on spawning success using χ^2 tests and Welch's t tests, respectively. However, no infectious agents were found to be significant predictors of spawning success in these individual tests and so were not included in the final logistic regression model.

Changes in gene expression and infectious agents over migration

As the second aim of this study was to look at differences in gene expression from time point 1 at the Seton fish fence (T1) compared with time point 2 at Gates Creek spawning channel (T2), any individuals that did not have two successful sam-

ples at T1 and T2 were removed from these analyses. The final dataset therefore consisted of fish ($N = 48$) with two gill samples (thus $N = 96$ gill samples) taken at different time points in their freshwater migration.

To test for differences in host gene expression between the two sampling locations (Seton fence and Gates Creek) we performed PERMANOVA analysis using R package "vegan" (Oksanen et al. 2019). Here, the normalized expression values for all 25 gene biomarkers represented the multivariate response dataset and sampling location (Seton fence or Gates Creek) represented the explanatory variable. PERMANOVA was used to test if the mean centroid of gene expression differed at Seton fence compared with Gates Creek. PERMANOVA was performed using the *adonis*() function in "vegan" R package (Oksanen et al. 2019). We accounted for repeated measures by restricting permutations within individual subjects. We also tested for differences in the variance, or dispersion, of gene expression between Seton fence and Gates Creek using the *betadisper*() function in "vegan".

We tested for differences in prevalence of infectious agents at Seton fish fence compared with Gates Creek fish fence by first formatting infectious agent as dichotomous variables to represent either presence or absence of each agent. We first investigated the change in presence of each infectious agent between Seton fence and Gates Creek using McNemar's χ^2 test for paired data. For any infectious agent that exhibited a significant shift in presence between these two locations, we next investigated which explanatory variables, if any, were associated with this change. Logistic regression models were constructed and used to look for an effect of sex, wound score, tagging date and average lake migration temperature on presence of each infectious agent. Fish identification number was included as random effect variables (intercepts) to account for repeated measures.

To investigate changes in infectious agent load (RNA copies) and RIB over migration through the Seton system, we first used Wilcoxon's signed-ranks test for paired data to compare the loads of each infectious agent at Seton fence with Gates Creek. RNA copy number and RIB was log transformed for all analyses. For any infectious agent that exhibited a significant change in load between these two locations, we next investigated if any explanatory variables were associated with this change. To achieve this, we first calculated the difference in infectious agent load between Seton fence and Gates Creek. We then constructed linear regression models for each infectious agent that included difference in load as the response variable, and sex, wound score, average lake migration temperature, tagging date and migration time to Gates Creek as explanatory covariables.

Results

Migration survival to Gates Creek spawning grounds

A total of 103 sockeye salmon were sampled and tagged at the Seton River fish fence (48 females and 55 males). Of these, 7 fish were captured by fisheries between the Seton River fish fence and Gates Creek and so were removed from the dataset. A total of 96 fish (45 females and 51 males) were therefore included in further analyses. Mean GSE across all fish was 1.19 ± 0.47 and mean fork length was 58.9 ± 2.87 cm. A total of 48 tagged fish were recaptured and sampled a second time at the Gates Creek fish fence (50.0% of the total number of fish tagged at Seton fish fence and 64.9% of the estimated number of tagged fish to successfully migrate to Gates Creek). We recovered 26 tagged female carcasses at Gates Creek and found that 13 of these females (50%; 95% confidence interval = 32%–68%) successfully spawned.

Success in ascending the Seton Dam fishway (determined by detection at the fishway exit PIT receiver) was 95.8% (95% confidence interval = 89%–99%), and fish took an average of 13.1 ± 13.3 h to reach the fishway exit receiver. Overall migration success to Gates Creek PIT receiver was 77.1% (95% confidence interval = 67%–85%), and average migration time to Gates Creek was 12.6 ± 3.6 days. Our logistic regression model testing variables associated with migration success to Gates Creek had a relatively weak fit ($r^2 = 0.07$), but indicated that severe wound score was negatively associated with survival (Table 3). While 87.5% of fish with no visible wounds ($n = 32$) survived to Gates Creek, only 55.6% of fish with a wound score of 3 ($n = 9$) survived to spawning grounds. Fish with wound scores of 1 ($n = 35$) and 2 ($n = 20$) also had lower survival to Gates Creek (75.5% and 76.5%, respectively); however, these fish were not significantly less likely to survive to Gates Creek (Table 3).

Our initial Random Forest model classified gene expression to migration success with an overall accuracy of 75.7% and an out-of-bag (OOB) error rate of 24.3%. The primary predictors of migration success identified by this initial Random Forest model (in decreasing order of importance) were HSP70, GAL3, C7, and RIG1. Six genes were found to have negative mean decrease in accuracy values in this model: SFR2, STAT1, HSP90a15, HERC6, SERPIN9, and IFIT5 (in increasing order). Our final, reduced Random Forest model eliminated all genes with a mean decrease in accuracy lower than 2 from the model. This reduced model classified gene expression to migration success with an overall accuracy of 75% and an OOB error rate of 19.6%. The primary predictors of migration success identified by our Random Forest model (in decreasing order of importance) were HSP70, 52RO, IFI44A, and RIG1 (Fig. 2).

We found no variables in this study to be significant predictors of spawning success at Gates Creek (Table 3); however, this logistic regression model was found to have a weak fit ($r^2 = 0.01$).

Thermal selection during lake migration

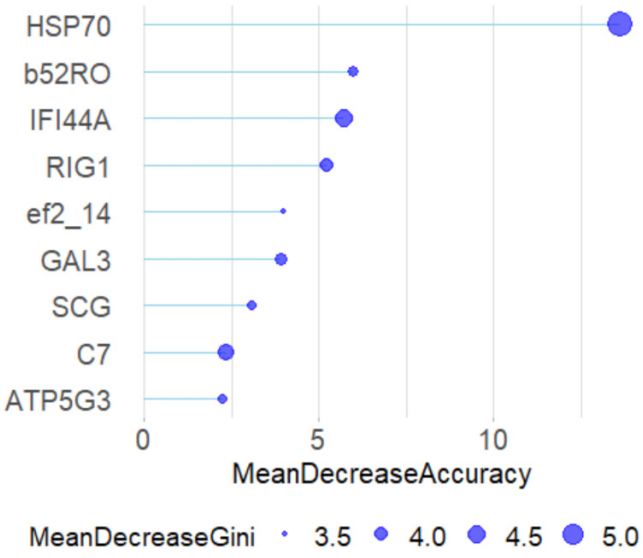
A total of 42 temperature loggers were recovered from carcasses at Gates Creek in 2017. The average temperature of fish in the Seton River (from tagging release to first detection at the Seton Dam fishway exit) was 18.4 ± 1.13 °C. The average temperature at which fish migrated through Seton Lake was estimated to be 15.1 ± 1.9 °C, and average migration temperature through Anderson Lake was estimated to be 12.6 ± 1.0 °C. Through Portage Creek, which separates Seton and Anderson lakes, fish migrated at an average tem-

Table 3. Model results for logistic and linear regression models testing the effects of wound, sex, GSE, tagging date, RIB, and fork length on migration success and migration temperature for Gates Creek sockeye salmon.

				Wound score								
Response variable	Model type	*N*	Model parameter	1	2	3	Sex	GSE	RIB	Fork length	Tag date	R^2
Success to Gates Creek	Logistic regression	96	β	−0.98	−0.81	−1.92	0.25	−0.43	−0.22	0.03	−0.02	0.07
			SE	0.73	0.93	0.92	0.61	0.50	0.76	0.11	0.08	
			z	−1.35	−0.87	−2.08	0.40	−0.86	−0.28	0.24	−0.26	
			P	0.18	0.38	**0.038**	0.69	0.39	0.78	0.81	0.79	
Spawning success	Logistic regression	26	β	0.39	0.21	0.20	–	–	0.60	–	−0.03	0.01
			SE	0.58	0.75	0.94	–	–	0.71	–	0.07	
			z	0.66	0.28	0.21	–	–	0.85	–	−0.44	
			P	0.51	0.78	0.83	–	–	0.40	–	0.66	
Average migration temp. through lakes	Linear regression	42	β	−0.21	−0.76	0.43	0.17	−0.31	−0.79	0.02	0.10	0.23
			SE	0.31	0.48	0.50	0.26	0.36	0.41	0.04	0.04	
			t	−0.68	−1.56	0.86	0.64	0.88	−1.91	0.41	2.21	
			P	0.50	0.12	0.40	0.53	0.39	**0.02**	0.69	**0.03**	
Time spent in lake thermal refuge	Linear regression	42	β	0.22	0.14	−0.34	−0.97	0.58	2.26	−0.22	−0.43	0.19
			SE	1.73	2.70	2.81	1.48	2.00	2.33	0.24	0.25	
			t	0.13	0.05	−0.12	−0.66	0.29	0.97	−0.93	−1.76	
			P	0.90	0.96	0.90	0.52	0.77	0.34	0.36	0.09	

Note: Significant findings ($P < 0.05$) are boldened. *R*-squared (R^2) values for logistic regression models are McFadden's *r*-squared, and R^2 values for linear regression models are adjusted *r*-squared.

Fig. 2. Results from reduced random forest classification model of genes used to predict migration success of sockeye salmon. Plot shows both the mean decrease in accuracy and mini decrease in Gini index for each gene. This reduced model was created by eliminating any genes from the initial model with a mean decrease in accuracy value less than 2.



perature of 20.6 ± 1.4 °C. Migration time through Portage Creek ranged from 2.5 to 108 h (average migration time was 14.4 ± 6.7 h).

We investigated factors associated with migration temperature though Seton and Anderson lakes using a linear regression model ($r^2 = 0.23$). We found RIB was negatively associated with overall lake migration temperature though Seton and Anderson lakes ($P = 0.02$; Table 3). Figure 3 shows a visual plot of the relationship between overall lake migration temperature and RIB, but this plot is not part of the model results shown in Table 3. Tagging date was also found to be significantly associated with overall lake migration temperature ($P = 0.03$; Table 3). We did not find any significant correlations between individual infectious agent loads and migration temperature through Seton or Anderson lakes. We also looked for any relationship in the length of time spent in lake (thermal refuge) habitat and RIB, but found no significant effect (Table 3).

Changes in gene expression over migration

We found that the mean centroid of host gene expression differed significantly between Seton fence and Gates Creek (PERMANOVA; $P = 0.001$), and additionally found that variance in gene expression increased for fish at Gates Creek (betadisper; $P \leq 0.0001$). Samples taken at thermal stress biomarker HSP90alike_6, SERPIN9, and SERPIN20 decreased in relative expression for fish at Gates Creek compared with Seton fence (Fig. 4), whereas VDD biomarkers HERC6, RSAD, MX, IFIT5, 52RO, STAT1, DEXH, CA054694, and GAL3 increased in relative expression as fish approach spawning grounds (Fig. 4).

Changes in infectious agent load and prevalence from Seton fence to Gates Creek

A total of 7 infectious agents were detected in sockeye salmon sampled at either Seton fence or Gates Creek (Fig. 5). Two infectious agents significantly increased in presence from Seton fence to Gates Creek: *Flavobacterium psychrophilum* ($P \leq 0.0001$) and *Ichthyophthirius multifiliis* ($P = 0.016$; Fig. 5). At Seton fence, *F. psychrophilum* was detected in 28.6% of fish, which increased to 83.9% at Gates Creek. Similarly, *Ichthyophthirius multifiliis was detected in* 28.6% at Seton fence and 53.6% at Gates Creek. No explanatory variables were found to be associated with infectious agent presence in this study.

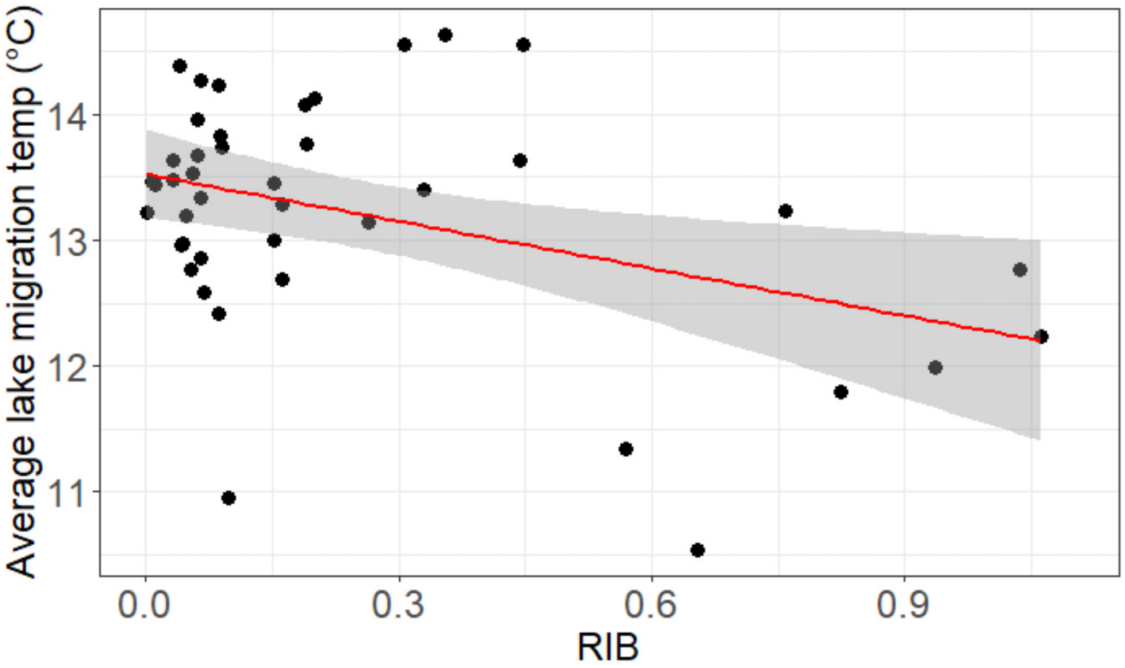
Wilcoxon's signed-ranks test for paired data was used to compare infectious agent loads and RIB at Seton fence compared with Gates Creek. Three infectious agents showed significant elevation in loads from the Seton fence to Gates Creek: *Candidatus* Branchiomonas cysticola ($P = 0.0003$), *F. psychrophilum* ($P < 0.0001$), and *Parvicapsula minibicornis* ($P < 0.0001$; Fig. 5). We did not find any variables to be associated with the changes in load of *Ca*. B. cysticola, *F. psychrophilum* or *Parvicapsula minibicornis* (Table 4).

Overall RIB of sockeye salmon was significantly higher in fish sampled at Gates Creek (0.54 ± 0.41) compared with the Seton fence (0.44 ± 0.57; Wilcoxon's signed-ranks test, $P = 0.045$). Linear mixed effects models were constructed to investigate if wound severity, sex, lake migration temperature, or migration time were associated with the change in RIB exhibited between the Seton fence and Gates Creek; no variables were found to be significantly associated with the change in RIB (Table 4).

Discussion

In this study, we were successful in repeat-sampling 48 Gates Creek sockeye salmon at two locations along their freshwater migrations to spawning grounds. We found that both gene expression and infectious agent profiles changed significantly over this final 50 km section of migration. Specifically, we found a decrease in expression of genes associated with thermal stress, and an increase in expression of genes associated with VDD. Migration success between sampling locations was comparable to that of previous years (Bass et al. 2018) with 77% of fish successfully reaching Gates Creek. Many variables were assessed for potential impacts on migration success and although we found some association with severe wounds and certain gene signatures with survival, the relationships were weak, thus highlighting the complexities underlying en route mortality during Pacific salmon spawning migrations. We also investigated thermal selection as fish migrated through Seton and Anderson lakes. Fish migrated at much cooler temperatures through the lakes (averaging 15.1 and 12.6 °C through Seton and Anderson lakes, respectively) compared with temperatures they experienced in Seton River (18.4 °C) and Portage Creek (20.6 °C), thus supporting previous studies showing that sockeye salmon selectively utilize the lake strata, choosing to migrate at optimal water temperatures through the lakes (Mathes et al. 2010; Armstrong

Fig. 3. Relationship between relative infection burden (RIB) and the average migration temperature (as determined from temperature logging iButtons) through both Seton and Anderson lakes. The red line represents the mean response, and the shaded area represents the 95% confidence level interval for predictions from the linear model. RIB was found to be a significant predictor of average lake migration temperature for Gates Creek sockeye salmon in our mixed effects linear regression model. The relationship shown here was found to be significant (linear regression; slope $= -1.25$, $r^2 = 0.16$, $P = 0.0091$). This figure shows a simple linear regression isolating RIB against average lake migration temperature for each fish. We note that this regression is not part of the model results reported in Table 3.



et al. 2016; Minke-Martin et al. 2018). Additionally, our data suggest that fish with greater overall RIB occupied slightly cooler temperatures in these lakes compared with fish with lower RIB, possibly suggesting a beneficial effect of cooler water temperatures for fish with higher infection levels.

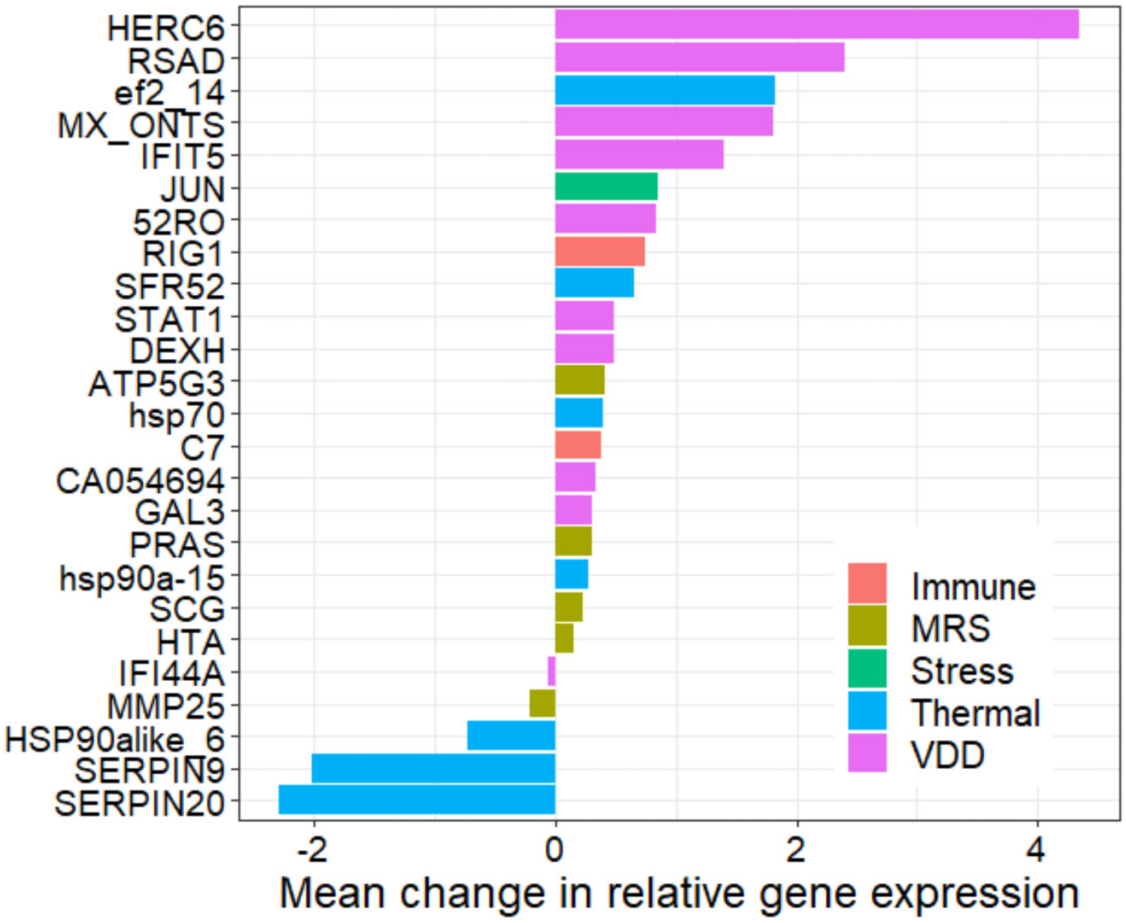
Migration success

We found passage of Seton Dam to be very high in this study (96% success rate to reach Seton Dam fishway exit), which is comparable to previous findings for Gates Creek sockeye PIT-tagged in the Seton River between 2014 and 2016 (where dam passage success ranged from 96% to 98%; Harrower et al. 2019). Previous studies conducted on the Seton River assessing dam passage efficiency of Gates Creek sockeye salmon have found dam operation to be the most important factor associated with fishway passage success. The location of siphon releases (Burnett et al. 2014*b*, 2017; Casselman et al. 2016), burst swimming activity (Burnett et al. 2014*a*), and discharge conditions (Roscoe and Hinch 2008) have all been previously shown to influence sockeye salmon passage. Since 2014, Seton Dam operations during peak migration for Gates Creek sockeye salmon have been optimized to allow very high passage for these fish (Harrower et al. 2019).

We found 77% of fish successfully migrated to Gates Creek in this study, which is consistent with previous tracking studies on the Seton. Previous studies reported migration success from the Seton fish fence to Gates Creek to range from 77% to 78% between 2014 and 2016 (Bass et al. 2018; Elmer et al. 2022). The only factor found to be associated with survival in this study was severe wound score. Fish with deep, severe wounds exposing flesh were less likely to survive to Gates Creek compared with fish with no visible wounds. This finding is comparable to Bass et al. (2018), who found survival of Gates Creek sockeye salmon tagged in the Seton River was negatively impacted by severe gillnet scars and the presence of sea lice scars visible on the fish. Wounds leave fish susceptible to infection and can therefore be associated with mortality (Svendsen and Bøgwald 1997), or may be indicative of stress-induced immune reduction preventing wound healing (MacKinnon 1998).

Our reduced RFC model identified important genes associated with migration success in this study. Of the 25 genes assayed in this study, HSP70 was found to be the most important to predicting migration success. HSP70 protein is a heat stress chaperone important for molecular tolerance to thermal stress (Iwama et al. 1999; Tsan and Gao 2004) and for which elevated gene expression has been shown in response to increased water temperatures for Pacific salmon (von Biela et al. 2020). Temperature is a critically important environmental factor for fish (Brett 1971; Fry 1971) and extreme high water temperatures can have devastating consequences on survival during Pacific salmon spawning migrations (Cooke et al. 2004; Hinch et al. 2012). We do not know the thermal experience of these fish prior to capture at the Seton River fish fence; however, exposure of some fish to supraoptimal

Fig. 4. The mean change in relative expression of 25 genes of individual fish as they migrated from Seton fish fence to Gates Creek. A total of 48 Gates Creek sockeye salmon were captured and sampled at Seton River fish fence and then recaptured and resampled at Gates Creek spawning grounds. Change in gene expression was calculated by subtracting the expression of each gene at Seton fence from the expression at Gates Creek for each fish. Biomarkers are categorized based on their functions: thermal stress, viral disease development (VDD), mortality-related signature (MRS), immune response, and general stress.



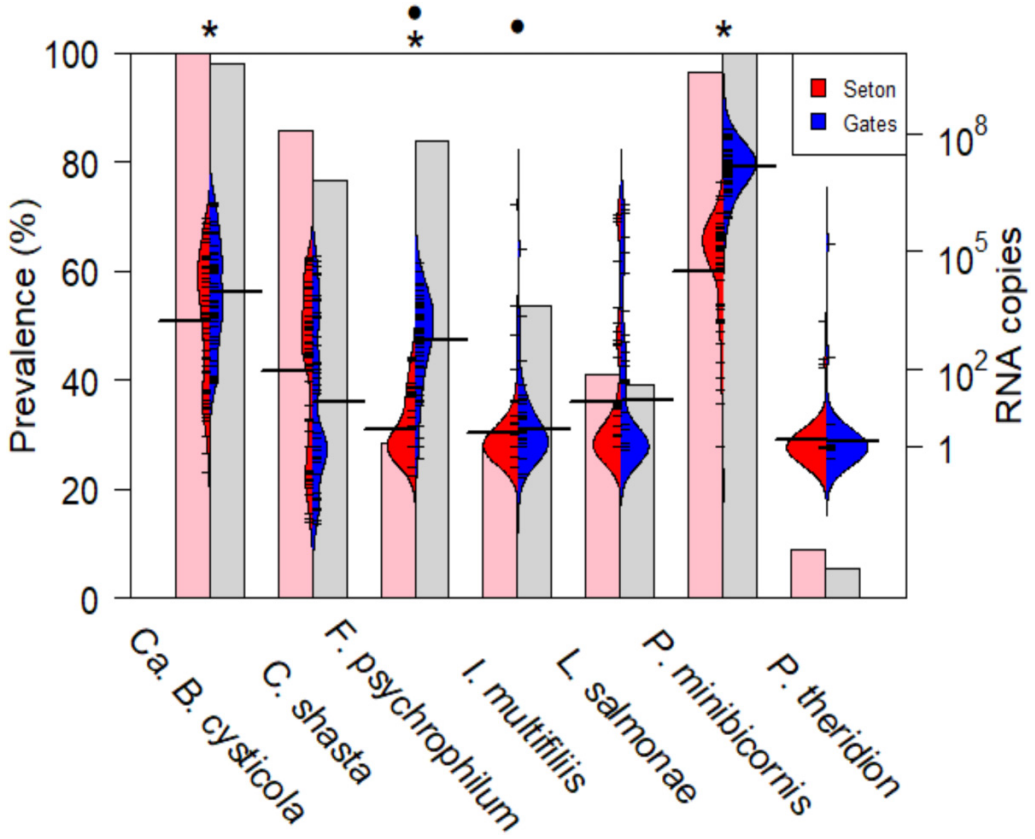
river temperatures may activate HSP70 expression as well as causing physiological damage that reduces probability of survival to spawning grounds. The next three most important genes for survival prediction were the VDD response genes 52RO and IFI44A, and immune stimulation gene RIG1. Association of these genes with survival may be a reflection of infection or immune stress that compromises physiology and behaviour of the fish. A major limitation to our study exists as our gene panel was restricted to only 25 genes, which greatly limits the extent to which we can investigate gene expression changes associated with migration survival. Gene expression signatures predictive of migration success for sockeye salmon have previously been recognized by Miller et al. (2011); however, this study involved analysis of thousands of genes on a microarray panel and gene expression was analyzed at an early period in their migration.

Despite assessing many variables, gene signatures, and infectious agents for associations with migration success, only weak associations were found with severe wounds and gene expression. Processes underlying en route mortality are highly complex and we often need to move away from assessing the consequences of single stressors in isolation, and instead investigate the impacts of multiple stressors and their cumulative impacts on survival (Cohen 2012; Miller et al. 2014; Deinet et al. 2020). In this study, no single variable could be associated with the 23% en route mortality we reported. It may be that explanatory variables are having interactive or cumulative effects on survival, other variables are impacting survival that were not measured, or perhaps experiences occurring earlier in migration are having delayed impacts on survival in the final portion of their spawning migration. This highlights the difficulties faced by fisheries managers tasked with predicting migration survival and escapement estimates for Pacific salmon species.

Evidence of behavioural thermoregulation in response to infectious agent burden

Seton and Anderson lakes offered cooler migration temperatures for Gates Creek sockeye salmon compared with the surrounding rivers and creeks. We found that Gates Creek sockeye salmon migrated at cooler temperatures through Seton and Anderson lakes (averaging 15.1 and 12.6 °C, respectively) compared with temperatures they experienced in Seton River (18.4 °C) and Portage Creek (20.6 °C). Such thermal

Fig. 5. Barplots and Beanplots showing the prevalence and load, respectively, of infectious agents detected at Seton fence and Gates Creek sockeye in 2017. Small hash marks represent individual fish and the larger horizontal line represents the group mean at either Seton fence or Gates Creek locations. Data from Seton fence are shown on the left-hand side of the plots (red). Data from Gates Creek are shown on the right-hand side (blue). Significant differences in infectious agent RNA copy number (load) between Seton fence and Gates Creek are indicated with an asterisk (*) and significant differences in prevalence are indicated by a dot (•).



refuge has previously been reported for the Seton lakes system (Roscoe et al. 2010; Bass 2018; Minke-Martin et al. 2018; Elmer et al. 2022) as well as other systems across the Pacific coast (Mathes et al. 2010; Armstrong et al. 2016). We found that fish migrated through Anderson Lake at cooler average temperatures compared with Seton Lake, which is comparable to previous findings for Gates Creek sockeye salmon in the Seton system (Bass 2018). We suspect that this finding is a result of differences in thermocline depth between Seton and Anderson lakes: in Anderson Lake, the thermocline was situated between 10 and 30 m in depth, whereas in Seton Lake, the thermocline was found to be situated deeper between 25 and 45 m in depth (Roscoe et al. 2010).

Previous work has demonstrated behavioural thermoregulation following warm water temperatures (Goniea et al. 2006; Armstrong et al. 2016), lower levels of somatic energy (Roscoe et al. 2010), gillnet injuries (Bass 2018), and fisheries interactions (Elmer et al. 2022). Here, higher RIB of fish in the Seton River (before they entered lake environments) was correlated with behavioural thermoregulation, where highly infected fish sought cooler migration temperatures in both Seton and Anderson lakes. There was no relationship between RIB and the length of time fish spent in lake thermal refuge habitat, indicating that fish did not slow or delay migration in cooler water in response to high infection burden, but instead just migrated through the lakes at slightly cooler water temperatures (~3 °C). Given that rates of change in infectious loads have been found to be a function of both temperature and time (Teffer et al. 2018, 2019), delaying migration to occupy cooler environments would be less efficient than maintaining ground speeds at greater (cooler) depths. This behaviour serves to reduce both time- and temperature-mediated increases in infection burden. Mathes et al. (2010) demonstrated that early-arriving Weaver Creek sockeye salmon only survived to spawning grounds if they utilized the cooler temperatures of Harrison Lake, and the authors predicted that this behaviour slowed development of infectious agents, in particular *Parvicapsula minibicornis*; however, this hypothesis was not conclusively proven.

Here, we found a negative correlation between RIB and subsequent selection of migration temperature. We suspect that this finding might be a behavioural response evolved to hinder temperature-mediated development of certain infectious agents. The relationship between RIB and lake migration temperature, although significant, was not particularly strong nor dramatic (average temperature during lake migration only ranged from 10.5 to 14.5 °C). We suspect that this weak

Table 4. Results of linear regression models investigating variables associated with change in infectious agent load and RIB as fish migrated from the Seton fish fence to Gates Creek spawning grounds.

			Wound score							
Response variable	*N*	Model parameter	1	2	3	Sex	Temp.	Migration time	Tagging date	R^2
Change in *Ca.* B. cysticola load	48	β	−0.32	−1.01	−0.44	−0.59	−0.73	−0.12	−0.16	0.07
		SE	0.33	0.54	0.40	0.23	0.37	0.06	0.08	
		t	−0.94	−1.86	−1.10	−2.54	−1.98	−1.95	−2.07	
		P	0.37	0.10	0.30	0.31	0.08	0.08	0.07	
Change in *F. psychrophilum* load	48	β	−0.79	1.36	0.42	1.32	0.74	0.08	0.70	0.23
		SE	2.36	3.83	2.82	1.63	2.61	0.44	0.56	
		t	−0.34	0.36	0.15	0.81	0.28	0.17	1.25	
		P	0.75	0.73	0.89	0.44	0.78	0.87	0.2423	
Change in *Parvicapsula minibicornis* load	48	β	0.58	3.33	3.64	1.38	2.12	0.95	−0.05	0.06
		SE	3.84	6.24	4.61	2.66	4.26	0.71	0.92	
		t	0.15	0.53	0.79	0.52	0.50	1.34	−0.05	
		P	0.88	0.61	0.45	0.62	0.63	0.22	0.96	
Change in RIB	42	β	−0.23	−0.14	−0.28	−0.23	0.31	−0.01	−0.17	0.08
		SE	0.56	0.91	0.67	0.39	0.62	0.10	0.13	
		t	−0.41	−0.15	−0.41	−0.59	0.51	−0.10	−1.26	
		P	0.69	0.88	0.69	0.57	0.63	0.91	0.245	

Note: Adjusted *R*-squared (R^2) values are also shown for all models.

relationship is due to extremely complex host-infectious agent dynamics and species-specific responses of infectious agents to temperature. For example, *F. psychrophilum* shows greater virulence below 16 °C (Starliper 2011), whereas other infectious agents show increased virulence with higher temperatures, such as *Ichthyophthirius multifiliis* (Noe and Dickerson 1995), *Parvicapsula minibicornis* (Crossin et al. 2008), *Tetracapsuloides bryosalmonae* (Bettge et al. 2009), and *Ceratomyxa shasta* (Chiaramonte 2013). Depending on the infectious agents present in an adult salmon, migrating at cooler temperatures in thermal refuge habitat may therefore not always be beneficial. Small temperature variations may also have little impact on virulence or disease outcome (Gregg et al. 2011). Added complexity is provided by effects of water temperature on fish behaviour and physiology; high water temperatures can compromise host immune function (Magnadottir et al. 1999; Dominguez et al. 2004).

For fish that were able to be recaptured and resampled at Gates Creek spawning grounds, we could investigate changes in RIB as fish migrated between Seton fish fence and Gates Creek. Average RIB at Gates Creek was higher compared with Seton fence, which further supports previous studies showing an increase in infection burden throughout freshwater migration (Bass et al. 2017; Bass 2018; Teffer et al. 2021). However, we did not find a large increase in RIB between locations, likely reflecting the already close proximity of fish to spawning grounds when initially sampled at Seton fence (~310 km already completed of an approximately 364 km total freshwater migration). In our repeated-measures study, a major limitation exists in that our dataset only includes individuals that survived to spawning grounds. Those individuals that died en route are not accounted for and thus we do not know the infectious agent profiles of these unsuccessful migrants at the time of death. It might be possible that these unsuccessful migrants experienced greater increases in RIB, which may have contributed, or been a function of, their early death.

Changes in gene expression during Seton system migration

We found a large difference in gene expression of sockeye salmon in the Seton River compared with Gates Creek spawning grounds. Specifically, three thermal biomarkers—HSP90alike_6, SERPIN9, and SERPIN20—decreased in expression between sampling locations. These genes are a known cluster of thermal-responsive biomarkers previously demonstrated to be strong indicators of thermal stress (Akbarzadeh et al. 2018; Houde et al. 2019*a*). Indeed, in this study we found that fish migrated at cooler temperatures in the lake environments compared with in Seton River and Portage Creek, and the reduced expression of thermal gene biomarkers likely reflects this behaviour. We suspect that use of thermal refuge in Seton Lake resulted in reduced expression of thermal gene biomarkers, which is indicative of thermal stress recovery of sockeye salmon in the wild. Indeed, thermal holding studies have demonstrated a similar recovery, both in expression in thermal gene biomarkers and in reduction in mortality, when temperatures are artificially lowered (Teffer et al. 2021).

As sockeye salmon migrated from Seton fence to Gates Creek, we also observed increases in expression of genes previously shown to be predictive of VDD (Miller et al. 2017). In fact, all 10 VDD biomarkers assayed for in this study were upregulated as sockeye salmon migrated through the Seton system, with biomarkers IFIT5, MX_ONTS, RSAD, HERC6, and STAT1 showing the greatest differential. While none of the viruses on our panel were detected, over a dozen previously uncharacterized viruses have been discovered by sequencing fish carrying the VDD signature (Mordecai et al. 2019, 2020; G. Mordecai, unpublished data). Two previously uncharacterized viruses were recently discovered in sockeye salmon with the VDD signature, a Qin virus and a Picornavirus (G. Mordecai, unpublished data); however, these viruses were not detected in any fish in this study.

While it is plausible that there is an uncharacterized virus responsible for the activation of the VDD signature at Gates Creek, we cannot preclude the possibility that thermal stress experienced by fish sampled at Seton resulted in a general downregulation of intracellular immunity. A holding study by Teffer et al. (2021) demonstrated a weakening in expression of immune-related genes, including those involved in viral responses, in fish experiencing thermal stress, and an elevation of expression for genes associated with thermal stress and wounding. These data imply that thermal stress can weaken an animal's ability to fight infection, a finding that complements that of previous studies (Dittmar et al. 2014; Bailey et al. 2017). An alternate hypothesis is that intracellular immune response may be enhanced as a function of maturation, senescence, or preparation for spawning grounds as these fish transition through the final stretch of their spawning migration. We know that Pacific salmon undergo a suite of physiological (Hinch et al. 2006; Hruska et al. 2010; Flores et al. 2012), morphological (Burgner 1991), and genomic (Miller et al. 2009; Evans et al. 2011) changes during their spawning migrations. There is also evidence of inherent immunological changes during spawning migrations for Pacific salmon (Schouten et al. 2013; Dolan et al. 2016). However, Dolan et al. (2016) found that both innate and adaptive immune responses diminish as fish approach spawning grounds, while Schouten et al. (2013) found certain aspects of adaptive immune responses to be retained, or even increase, as fish approach spawning. Immune responses during spawning migrations are highly complex, and we see a great variety in immune gene expression changes over migration. The VDD response we found in this study is likely complicated and could be a combined function of innate and adaptive immune responses over migration, viral activity, an increase in nonviral infectious agents, or other unknown functions.

Changes in infectious agent profiles during Seton system migration

We determined the presence and load of 18 infectious agents known or suspected to infect Fraser River sockeye salmon. Seven infectious agents were detected in Gates Creek sockeye salmon sampled at Seton River and again at Gates Creek spawning grounds. Of these, two bacterial species, two myxozoan, two microsporidian, and one protozoan species

were detected; no surveyed viruses were detected. The prevalence and load distributions of these infectious agents is comparable to previous findings for Gates Creek sockeye salmon sampled in the Seton River (Bass 2018). Three infectious agents increased in load as sockeye salmon travelled the 50 km from the Seton River fish fence to Gates Creek spawning grounds: *Candidatus* Branchiomonas cysticola, *F. psychrophilum*, and *Parvicapsula minibicornis*. Previous studies have also reported the load of *Parvicapsula minibicornis* to increase following initial exposure in the lower Fraser River (Jones et al. 2003; Wagner et al. 2005). Similarly, *Ca*. B. cysticola has been found to increase in load over days surviving in sockeye salmon (Teffer et al. 2017) and *F. psychrophilum* has been shown to increase in prevalence towards spawning grounds (Miller et al. 2014; Bass 2018). Certain infectious agents may affect survival and migration rate for Pacific salmon, and previous research for sockeye salmon has suggested associations between *F. psychrophilum* and reduced survival (Teffer et al. 2017), and *Parvicapsula minibicornis* and reduced swimming performance (Wagner et al. 2005; Bradford et al. 2010).

Again, we emphasize that this study only resampled those fish that successfully migrated to Gates Creek spawning grounds, and the gene expression and infectious agent profiles of fish that suffered en route mortality are not known. We might therefore expect that these unsuccessful migrants experienced even greater increases in loads of specific infectious agents during the study period. In a comparison between sockeye salmon that died in the Seton River fence and live-sampled sockeye from the same location, Elmer (2020) found that fish that perished had significantly higher loads (RNA copy numbers) of *F. psychrophilum* and *Parvicapsula minibicornis* than live-sampled fish. This correlative finding suggests that high loads of these infectious agents are associated with en route mortality but cannot ascribe causality or the mechanisms of mortality. In the present study, we found both *F. psychrophilum* and *Parvicapsula minibicornis* increased in load for fish arriving at spawning grounds, thus confirming temporal increases in the loads of these agents within migrating individuals in the wild. We acknowledge that only gill tissue was analyzed for infectious agents in this study. Gill tissue is not always the primary infective tissue for certain infectious agents, and other organs might show different loads or infectious agent composition altogether. However, Teffer et al. (2017) demonstrated high overall correlation of infectious agent load between gill and pooled organ tissues from sockeye salmon and Chinook salmon (*Oncorhynchus tshawytscha*), including the infectious agents identified in our study. *Ceratomyxa shasta* was our only infectious agent that Teffer et al. (2017) found not to be well correlated between gill and pooled organ tissue, so it is possible that *C. shasta* burden is actually higher than it appears from just analyzing gill tissues alone.

Conclusions

Here, we present the first repeated-measures study to investigate changes in gene and infectious agent profiles of wild sockeye salmon as they complete their freshwater migration. We applied a functional genomics approach to address physiological correlates with survival and to characterize physiological changes associated with successful spawning migrations. Most notably, we found that fish showed diminished expression of thermal stress genes and increased expression of genes associated with intracellular immune response as they migrated across two lakes. Broad-scale assessments of infective agents revealed an increase in overall infection burden as sockeye salmon approach spawning grounds. While none of these infectious agents detected at the initial time of capture were associated with survival to spawning grounds for Gates Creek sockeye salmon, these fish would have already completed approximately 300 km of their freshwater migration, and it is possible that infectious impacts on survival may occur earlier in their migration journey. Importantly, our study suggests additional support for the benefits of thermal refuges along Pacific salmon migration routes for those experiencing high burdens of infection.

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Author information

Author ORCIDs
Laura K. Elmer https://orcid.org/0000-0003-3182-8786

Author contributions
Conceptualization: LKE, ALB, SJC, SGH
Data curation: LKE
Formal analysis: LKE, ALB, AKT
Funding acquisition: SJC, SGH
Investigation: LKE, SDJ, KHK, LAK, SL, KMM
Methodology: LKE, ALB, SJC, SGH
Resources: KMM, SJC, SGH
Software: KMM
Supervision: ALB, KHK, SL, KMM, SJC, SGH
Visualization: LKE, KMM, SGH
Writing – original draft: LKE, ALB, SJC, SGH
Writing – review & editing: LKE, ALB, SDJ, KHK, LAK, SL, AKT, KMM, SJC, SGH

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References
Akbarzadeh, A., Gunther, O.P., Houde, A.L., Li, S.R., Ming, T.J., Jeffries, K.M., et al. 2018. Developing specific molecular biomarkers for thermal stress in salmonids. BMC Genomics, **19**. doi:10.1186/s12864-018-5108-9.

Akbarzadeh, A., Houde, A.L.S., Sutherland, B.J.G., Günther, O.P., and Miller, K.M. 2020. Identification of hypoxia-specific biomarkers in salmonids using RNA-sequencing and validation using high-throughput qPCR. G3 (Bethesda), **10**: 3321–3336. doi:10.1534/g3.120.401487.

Andres-Terre, M., McGuire, H.M., Pouliot, Y., Bongen, E., Sweeney, T.E., Tato, C.M., and Khatri, P. 2015. Integrated, multi-cohort analysis identifies conserved transcriptional signatures across multiple respiratory viruses. Immunity, **43**: 1199–1211. doi:10.1016/j.immuni.2015.11.003.

Armstrong, J.B., Ward, E.J., Schindler, D.E., and Lisi, P.J. 2016. Adaptive capacity at the northern front: sockeye salmon behaviourally thermoregulate during novel exposure to warm temperatures. Conserv. Physiol. **4**: cow039. doi:10.1093/conphys/cow039.

Atlas, W.I., Seitz, K.M., Jorgenson, J.W.N., Millard-Martin, B., Housty, W.G., Ramos-Espinoza, D., et al. 2021. Thermal sensitivity and flow-mediated migratory delays drive climate risk for coastal sockeye salmon. Facets, **6**: 71–89. doi:10.1139/facets-2020-0027.

Bailey, C., Segner, H., Casanova-Nakayama, A., and Wahli, T. 2017. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. Fish Shellfish Immunol. **63**: 424–437. doi:10.1016/j.fsi.2017.02.039.

Bass, A.L., Hinch, S.G., Teffer, A.K., Patterson, D.A. and Miller, K.M. 2017. A survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in south-western British Columbia determined by high-throughput quantitative polymerase chain reaction. J. Fish Dis., **40**: 453–477.

Bass, A.L. 2018. Fisheries gear and biological context drive fishing-related incidental mortality in Pacific salmon spawning migrations. Ph.D. thesis, University of British Columbia, Vancouver, BC, Canada.

Bass, A.L., Hinch, S.G., Casselman, M.T., Bett, N.N., Burnett, N.J., Middleton, C.T., and Patterson, D.A. 2018. Visible gill-net injuries predict migration and spawning failure in adult sockeye salmon. Trans. Am. Fish. Soc. **147**: 1085–1099. doi:10.1002/tafs.10103.

Bass, A.L., Hinch, S.G., Teffer, A.K., Patterson, D.A., and Miller, K.M. 2019. Fisheries capture and infectious agents are associated with travel rate and survival of Chinook salmon during spawning migration. Fish. Res. **209**: 156–166. doi:10.1016/j.fishres.2018.09.009.

Bett, N.B., Hinch, S.G., Bass, A.L., Braun, D.C., Burnett, N.J., Casselman, M.T., et al. 2022. Using an integrative research approach to improve fish migrations in regulated rivers: a case study on Pacific salmon in the Fraser River, Canada. Hydrobiologia, **849**: 385–405. doi:10.1007/s10750-020-04371-2.

Bettge, K., Wahli, T., Segner, H., and Schmidt-Posthaus, H. 2009. Proliferative kidney disease in rainbow trout: time- and temperature-related renal pathology and parasite distribution. Dis. Aquat. Organ. **83**: 67–76. doi:10.3354/dao01989.

Bradford, M.J., Lovy, J., Patterson, D.A., Speare, D.J., Bennett, W.R., Stobbart, A.R., and Tovey, C.P. 2010. *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. Can. J. Fish. Aquat. Sci. **67**: 673–683. doi:10.1139/F10-017.

Breiman, L. 2001. Random forests. Mach. Learn. **45**: 5–32. doi:10.1023/A:1010933404324.

Breiman, L., Friedman, J., Stone, C., and Olshen, R. 1984. Classification and regression trees. CRC Press LLC, Boca Raton, Florida.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. **11**: 99–113. doi:10.1093/icb/11.1.99.

Burgner, R.L. 1991. Life history of sockeye salmon *Oncorhynchus nerka*. *In* Pacific salmon life histories. *Edited by* C. Groot and C. Margolis. UBC Press, Vancouver, Canada. pp. 3–117.

Burnett, N.J., Hinch, S.G., Bett, N.N., Braun, D.C., Casselman, M.T., Cooke, S.J., et al. 2017. Reducing carryover effects on the migration and spawning success of sockeye salmon through a management experiment of dam flows. River Res. Appl. **33**: 3–15. doi:10.1002/rra.3051.

Burnett, N.J., Hinch, S.G., Braun, D.C., Casselman, M.T., Middleton, C.T., Wilson, S.M., and Cooke, S.J. 2014a. Burst swimming in areas of high flow: delayed consequences of anaerobiosis in wild adult sockeye salmon. Physiol. Biochem. Zool. **87**: 587–598. doi:10.1086/677219.

Burnett, N.J., Hinch, S.G., Donaldson, M.R., Furey, N.B., Patterson, D.A., Roscoe, D.W., and Cooke, S.J. 2014b. Alterations to dam-spill discharge influence sex-specific activity, behaviour and passage success of migrating adult sockeye salmon. Ecohydrology, **7**: 1094–1104.

Casselman, M.T., Middleton, C.T., Minke-Martin, V., Drenner, S.M., Bett, N.N., Burnett, N.J., et al. 2016. BRGMON-14 Effectiveness of Cayoosh flow dilution, dam operation, and fishway passage on delay and survival of upstream migration of salmon in the Seton–Anderson watershed. Annual Report - 2015. Report prepared for St'át'imc Eco-Resources Ltd. and BC Hydro., The University of British Columbia, Vancouver, BC.

Chiaramonte, L.V. 2013. Climate warming effects on the life cycle of the parasite Ceratomyxa shasta in salmon in the Pacific Northwest. M.Sc. thesis, Oregon State University, Corvallis, OR, USA.

Cohen, B. 2012. 'The uncertain future of Fraser River sockeye' in final report, Cohen Commission of Inquiry into the decline of sockeye salmon in the Fraser River (Canada). DFO Overview: Organization, Science, Policies.

Connon, R.E., Jeffries, K.M., Komoroske, L.M., Todgham, A.E., and Fangue, N.A. 2018. The utility of transcriptomics in fish conservation. J. Exp. Biol. **221**: jeb148833. doi:10.1242/jeb.148833.

Cooke, S.J., Hinch, S.G., Farrell, A.P., Lapointe, M.F., Jones, S.R.M., Macdonald, J.S., et al. 2004. Abnormal migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. Fisheries, **29**: 22–33. doi:10.1577/1548-8446(2004)29%5b22:AMTAHE%5d2.0.CO;2.

Crossin, G.T., and Hinch, S.G. (2005) A non-lethal method for assessing the somaticenergy content of freely migrating adult Pacific salmon. Trans. Am. Fish. **134**: 184–191.

Crossin, G.T., Hinch, S.G., Cooke, S.J., Cooperman, M.S., Patterson, D.A., Welch, D.W., et al. 2009. Mechanisms influencing the timing and success of reproductive migration in a capital breeding semelparous fish species, the sockeye salmon. Physiol. Biochem. Zool. **82**: 635–652. doi:10.1086/605878.

Crossin, G.T., Hinch, S.G., Cooke, S.J., Welch, D.W., Patterson, D.A., Jones, S.R.M., et al. 2008. Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration. Can. J. Zool. **86**: 127–140. doi:10.1139/Z07-122.

Cutler, D.R., ,Edwards, T.C., Jr., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J., and Lawler, J.J. 2007. Random Forests for classification in ecology. Ecology, **88**: 2783–2792. doi:10.1890/07-0539.1.

Deinet, S., Scott-Gatty, K., Rotton, H., Twardek, W.M., Marconi, V., McRae, L., et al. 2020. The Living Planet Index (LPI) for freshwater migratory fish – Technical Report. World Fish Migration Foundation, The Netherlands.

Di Cicco, E., Ferguson, H.W., Kaukinen, K.H., Schulze, A.D., Li, S.R., Tabata, A., et al. 2018. The same strain of Piscine orthoreovirus (PRV-1) is involved in the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. Facets, **3**: 599–641. doi:10.1139/facets-2018-0008.

Dittmar, J., Janssen, H., Kuske, A., Kurtz, J., and Scharsack, J.P. 2014. Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). J. Anim. Ecol. **83**: 744–757. doi:10.1111/1365-2656.12175.

Dolan, B.P., Fisher, K.M., Colvin, M.E., Benda, S.E., Peterson, J.T., Kent, M.L., and Schreck, C.B. 2016. Innate and adaptive immune responses in migrating spring-run adult Chinook salmon, *Oncorhynchus tshawytscha*. Fish Shellfish Immunol. **48**: 136–144. doi:10.1016/j.fsi.2015.11.015.

Dominguez, M., Takemura, A., Tsuchiya, M., and Nakamura, S. 2004. Impact of different environmental factors on the circulating immunoglobulin levels in the Nile tilapia, *Oreochromis niloticus*. Aquaculture, **241**: 491–500. doi:10.1016/j.aquaculture.2004.06.027.

Drenner, S.M., Hinch, S.G., Furey, N.B., Clark, T.D., Li, S.R., Ming, T.B., et al. 2018. Transcriptome patterns and blood physiology associated with homing success of sockeye salmon during their final stage of marine migration. Can. J. Fish. Aquat. Sci. **75**: 1511–1524. doi:10.1139/cjfas-2017-0391.

Duesund, H., Nylund, S., Watanabe, K., Ottem, K.F., and Nylund, A. 2010. Characterization of a VHS virus genotype III isolated from rainbow trout (*Oncorhychus mykiss*) at a marine site on the west coast of Norway. Virol. J. **7**: 19. doi:10.1186/1743-422X-7-19.

Elmer, L.K. 2020. Fisheries escape, temperature, and infectious agents: multiple stressors impacting wild sockeye salmon spawning migrations. Ph.D. thesis, Carleton University, Ottawa, ON, Canada.

Elmer, L.K., Moulton, D.L., Reid, A.J., Farrell, A.P., Patterson, D.A., Hendriks, B., et al. 2022. Thermal selection and delayed migration by adult sockeye salmon (*Oncorhynchus nerka*) following escape from simulated in-river fisheries capture. Fish. Res. **251**: 106321. doi:10.1016/j.fishres.2022.106321.

Evans, T.G., Hammill, E., Kaukinen, K., Schulze, A.D., Patterson, D.A., English, K.K., et al. 2011. Transcriptomics of environmental acclimatization and survival in wild adult Pacific sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Mol. Ecol. **20**: 4472–4489. doi:10.1111/j.1365-294X.2011.05276.x.

Flores, A.M., Shrimpton, J.M., Patterson, D.A., Hills, J.A., Cooke, S.J., Yada, T., et al. 2012. Physiological and molecular endocrine changes in maturing wild sockeye salmon, *Oncorhynchus nerka*, during ocean and river migration. J. Comp. Physiol. B, **182**: 77–90. doi:10.1007/s00360-011-0600-4.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. *In* Fish physiology: environmental relations and behaviour. *Edited by* W. Hoar and D. Randall. Academic Press, New York, USA. pp. 1–98.

Goniea, T.M., Keefer, M.L., Bjornn, T.C., Peery, C.A., Bennett, D.H., and Stuehrenberg, L.C. 2006. Behavioral thermoregulation and slowed migration by adult fall Chinook salmon in response to high Columbia River water temperatures. Trans. Am. Fish. Soc. **135**: 408–419. doi:10.1577/T04-113.1.

Gregg, J.L., Vollenweider, J.J., Grady, C.A., Heintz, R.A., and Hershberger, P.K. 2011. Effects of environmental temperature on the dynamics of Ichthyophoniasis in juvenile Pacific herring (*Clupea pallasii*). J. Parasitol. Res. 563412.

Hallett, S.L., and Bartholomew, J.L. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. Dis. Aquat. Organ. **71**: 109–118. doi:10.3354/dao071109.

Harrower, W.L., Bett, N.N., and Hinch, S.G. 2019. BRGMON-14: Effectiveness of Cayoosh flow dilution, dam operation, and fishway passage on delay and survival of upstream migration of salmon in the Seton–Anderson watershed. Prepared for St'a´t'imc Eco- Resources Ltd. and BC Hydro. The University of British Columbia, Vancouver, BC.

Hinch, S.G., and Martins, E.G. 2011. A review of potential climate change effects on survival of Fraser River sockeye salmon and an analysis of interannual trends in en route loss and pre-spawn mortality. Cohen Commission Technical Report 9, Vancouver, B.C.

Hinch, S.G., Bett, N.N., Eliason, E.J., Farrell, A.P., Cooke, S.J., and Patterson, D.A. 2021. Exceptionally high mortality of adult female salmon: a large-scale pattern and a conservation concern. Can. J. Fish. Aquat. Sci. **78**: 639–654. doi:10.1139/cjfas-2020-0385.

Hinch, S.G., Cooke, S.J., Farrell, A.P., Miller, K.M., Lapointe, M.F., and Patterson, D.A. 2012. Dead fish swimming: a review of research on the early migration and high premature mortality in adult Fraser River sockeye salmon *Oncorhynchus nerka*. J. Fish Biol. **81**: 576–599. doi:10.1111/j.1095-8649.2012.03360.x.

Hinch, S.G., Cooke, S.J., Healey, M.C., and Farrell, A.P. 2006. Behavioural physiology of fish migrations: salmon as a model approach. *In* Behaviour and physiology of fish. *Edited by* K. Sloman, S. Balshine and R Wilson. Academic Press, New York, USA. pp. 239–295.

Houde, A.L.S., Akbarzadeh, A., Günther, O.P., Li, S., Patterson, D.A., Farrell, A.P., et al. 2019a. Salmonid gene expression biomarkers indicative of physiological responses to changes in salinity and temperature, but not dissolved oxygen. J. Exp. Biol. **222**: jeb198036. doi:10.1242/jeb.198036.

Houde, A.L.S., Schulze, A.D., Kaukinen, K.H., Strohm, J., Patterson, D.A., Beacham, T.D., et al. 2019b. Transcriptional shifts during juvenile Coho salmon (*Oncorhynchus kisutch*) life stage changes in freshwater and early marine environments. Comp. Biochem. Physiol. Part D Genomics Proteomics, **29**: 32–42.

Hruska, K.A., Hinch, S.G., Healey, M.C., Patterson, D.A., Larsson, S., and Farrell, A.P. 2010. Influences of sex and activity level on physiological changes in individual adult sockeye salmon during rapid senescence. Physiol. Biochem. Zool. **83**: 663–676. doi:10.1086/652411.

Iwama, G.K., Vijayan, M.M., Forsyth, R.B., and Ackerman, P.A. 1999. Heat shock proteins and physiological stress in fish. Am. Zool. **39**: 901–909. doi:10.1093/icb/39.6.901.

Jeffries, K.M., Hinch, S.G., Sierocinski, T., Clark, T.D., Eliason, E.J., Donaldson, M.R., et al. 2012. Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). Ecol. Evol. **2**: 1747–1764. doi:10.1002/ece3.274.

Jones, S.R.M., Prosperi-Porta, G., Dawe, S.C., and Barnes, D.P. 2003. Distribution, prevalence and severity of *Parvicapsula minibicornis* infections among anadromous salmonids in the Fraser River, British Columbia, Canada. Dis. Aquat. Organ. **54**: 49–54. doi:10.3354/dao054049.

Kanigan, A.M., Hinch, S.G., Bass, A.L., and Harrower, W.L. 2019. Gillnet fishing effort predicts physical injuries on sockeye salmon captured near spawning grounds. N. Am. J. Fish. Manage. **39**: 441–451. doi:10.1002/nafm.10282.

Keefer, M.L., and Caudill, C.C. 2015. Estimating thermal exposure of adult summer steelhead and fall Chinook salmon migrating in a warm impounded river. Ecol. Freshw. Fish, **25**: 599–611. doi:10.1111/eff.12238.

Keefer, M.L., Peery, C.A., Jepson, M.A., Tolotti, K.R., Bjornn, T.C., and Stuehrenberg, L.C. 2004. Stock-specific migration timing of adult spring-summer Chinook salmon in the Columbia River basin. N. Am. J. Fish. Manage. **24**: 1145–1162. doi:10.1577/M03-170.1.

Kiessling, A., Lindahl-kiessling, K., and Kiessling, K. 2004. Energy utilization and metabolism in spawning migrating Early Stuart sockeye salmon (*Oncorhynchus nerka*): the migratory paradox. Can. J. Fish. Aquat. Sci. **61**: 452–465. doi:10.1139/f04-006.

Liaw, A., and Wiener, M. 2002. Classification and regression by random forest. R News, **2**: 18–22.

Lloyd, S.J., LaPatra, S.E., Snekvik, K.R., Cain, K.D., and Call, D.R. 2011. Quantitative PCR demonstrates a positive correlation between a *Rickettsia*-like organism and severity of strawberry disease lesions in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis. **34**: 701–709. doi:10.1111/j.1365-2761.2011.01285.x.

MacKinnon, B. 1998. Host factors important in sea lice infections. ICES J. Mar. Sci. **55**: 188–192. doi:10.1006/jmsc.1997.0361.

Magnadottir, B., Jonsdottir, H., Helgason, S., Bjornsson, B., Jorgensen, T.O., and Pilstrom, L. 1999. Humoral immune parameters in Atlantic cod (*Gadus morhua* L.) I. The effects of environmental temperature. Comp. Bioch. Phys. B Biochem. Mol. Biol. **122**: 173–180. doi:10.1016/S0305-0491(98)10156-6.

Mathes, M.T., Hinch, S.G., Cooke, S.J., Crossin, G.T., Patterson, D.A., Lotto, A.G., and Farrell, A.P. 2010. Effect of water temperature, timing, physiological condition, and lake thermal refugia on migrating adult Weaver Creek sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **67**: 70–84. doi:10.1139/F09-158.

Matzinger, P. 2002. The Danger model: a renewed sense of self. Science, **296**: 301–305. doi:10.1126/science.1071059.

McDaniels, T., Wilmot, S., Healey, M., and Hinch, S.G. 2010. Vulnerability of Fraser River sockeye salmon to climate change: a life cycle perspective using expert judgments. J. Environ. Manage. **91**: 2771–2780. doi:10.1016/j.jenvman.2010.08.004.

Miller, K.M., Gardner, I.A., Vanderstichel, R., Burnley, T., Angela, D., Li, S., et al. 2016. Report on the performance evaluation of the fluidigm biomark platform for high-throughput microbe monitoring in salmon. DFO Canadian Science Advisory Secretariat Research Document 2016/038. Ottawa, ON, Canada.

Miller, K.M., Gunther, O.P., Li, S.R., Kaukinen, K.H., and Ming, T.J. 2017. Molecular indices of viral disease development in wild migrating salmon. Conserv. Physiol. **5**: cox036. doi:10.1093/conphys/cox036.

Miller, K.M., Li, S.R., Kaukinen, K.H., Ginther, N., Hammill, E., Curtis, J.M.R., et al. 2011. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science, **331**: 214–217. doi:10.1126/science.1196901.

Miller, K.M., Schulze, A.D., Ginther, N., Li, S.R., Patterson, D.A., Farrell, A.P., and Hinch, S.G. 2009. Salmon spawning migration: metabolic shifts and environmental triggers. Comp. Biochem. Physiol. D Genomics Proteomics, **4**: 75–89.

Miller, K.M., Teffer, A.K., Tucker, S., Li, S., Schulze, A.D., Trudel, M., et al. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evol. Appl. **7**: 812–855. doi:10.1111/eva.12164.

Minke-Martin, V., Hinch, S.G., Braun, D.C., Burnett, N.J., Casselman, M.T., Eliason, E.J., and Middleton, C.T. 2018. Physiological condition and migratory experience affect fitness-related outcomes in adult female sockeye salmon. Ecol. Fresh. Fish, **27**: 296–309. doi:10.1111/eff.12347.

Mitchell, S.O., Steinum, T.M., Toenshoff, E.R., Kvellestad, A., Falk, K., Horn, M., and Colquhoun, D.J. 2013. *Candidatus* Branchiomonas cysticola is a common agent of epitheliocysts in seawater-farmed Atlantic salmon *Salmo salar* in Norway and Ireland. Dis. Aquat. Org. **103**: 35–43. doi:10.3354/dao02563.

Mordecai, G.J., Di Cicco, E., Günther, O.P., Schulze, A.D., Kaukinen, K.H., and Li, S., 2020. Emerging viruses in British Columbia salmon discovered via a viral immune response biomarker panel and metatranscriptomic sequencing. BioRxiv, 948026. doi:10.1101/2020.02.13.948026.

Mordecai, G.J., Di Cicco, E., Günther, O.P., Schulze, A.D., Kaukinen, K.H., Li, S., et al. 2021. Discovery and surveillance of viruses from salmon in British Columbia using viral immune-response biomarkers, metatranscriptomics, and high-throughput RT-PCR. Virus Evol. **7**: veaa069. doi:10.1093/ve/veaa069.

Mordecai, G.J., Miller, K.M., Di Cicco, E., Schulze, A.D., Kaukinen, K.H., Ming, T.J., et al. 2019. Endangered wild salmon infected by newly discovered viruses. eLife, **8**: e47615. doi:10.7554/eLife.47615.

Newell, J.C., and Quinn, T.P. 2005. Behavioral thermoregulation by maturing adult sockeye salmon (*Oncorhynchus nerka*) in a stratified lake prior to spawning. Can. J. Zool. **83**: 1232–1239. doi:10.1139/z05-113.

Noe, J.G., and Dickerson, H.W. 1995. Sustained growth of *Ichthyophthirius multifiliis* at low temperature in the laboratory. J. Parasitol. **81**: 1022–1024. doi:10.2307/3284065.

Nylund, S., Nylund, A., Watanabe, K., Arnesen, C.E., and Karlsbakk, E. 2010. *Paranucleospora theridion* (Microsporidia, Enterocytozoonidae) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*). J. Eukary. Microbiol. **57**: 95–114. doi:10.1111/j.1550-7408.2009.00451.x.

Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. 2019. vegan: community ecology package, R package version 2.4-0.

Paull, S.H., and Johnson, P.T.J. 2014. Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. Ecol. Lett. **17**: 445–453. doi:10.1111/ele.12244.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29**: e45. doi:10.1093/nar/29.9.e45.

Pickering, A., and Pottinger, T. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. **7**: 253–258. doi:10.1007/BF00004714.

R Core Team 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rand, P.S., and Hinch, S.G. 1998. Swim speeds and energy use of upriver-migrating sockeye salmon (*Oncorhynchus nerka*): simulating metabolic power and assessing risk of energy depletion. Can. J. Fish. Aquat. Sci. **55**: 1832–1841. doi:10.1139/f98-068.

Roscoe, D.W., and Hinch, S.G. 2008. Fishway passage, water diversion and warming temperatures: factors limiting successful spawning migration of Seton–Anderson watershed sockeye salmon. Final Report for the Bridge Coastal Restoration Program, Project 07. **BRG01**. 101 pp.

Roscoe, D.W., Hinch, S.G., Cooke, S.J., and Patterson, D.A. 2010. Behaviour and thermal experience of adult sockeye salmon migrating through stratified lakes near spawning grounds: the roles of reproductive and energetic states. Ecol. Fresh. Fish, **19**: 51–62. doi:10.1111/j.1600-0633.2009.00388.x.

Schouten, J., Clister, T., Bruce, A., Epp, L., and Zwollo, R. 2013. Sockeye salmon retain immunoglobulin-secreting plasma cells throughout their spawning journey and post-spawning. Immunology, **40**: 202–209.

Shrimpton, J.M., Patterson, D.A., Richards, J.G., Cooke, S.J., Schulte, P.M., Hinch, S.G., and Farrell, A.P. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. J. Exp. Biol. **208**: 4069–4078. doi:10.1242/jeb.01871.

Starliper, C.E. 2011. Bacterial coldwater disease of fishes caused by *Flavobacterium psychrophilum*. J. Advanced Res. **2**: 97–108. doi:10.1016/j.jare.2010.04.001.

Svendsen, Y.S., and Bøgwald, J. 1997. Influence of artificial wound and non-intact mucus layer on mortality of Atlantic salmon (*Salmo salar* L) following a bath challenge with *Vibrio anguillarum* and *Aeromonas salmonicida*. Fish Shell. Imm. **7**: 317–325. doi:10.1006/fsim.1997.0087.

Teffer, A.K., Hinch, S.G., Miller, K.M., Jeffries, K.M., Patterson, D.A., Cooke, S.J., et al. 2018. Infections, fisheries capture, temperature and host responses: multi-stressor influences on survival and behaviour of adult Chinook salmon. Can. J. Fish. Aquat. Sci. **75**: 2069–2083. doi:10.1139/cjfas-2017-0491.

Teffer, A.K., Hinch, S.G., Miller, K.M., Jeffries, K.M., Patterson, D.A., Cooke, S.J., et al. 2019. Cumulative effects of thermal and fisheries stressors reveal sex-specific effects on infection development and early mortality of adult coho salmon (*Oncorhynchus kisutch*). Physiol. Biochem. Zool. **92**: 505–529. doi:10.1086/705125.

Teffer, A.K., Hinch, S.G., Miller, K.M., Patterson, D.A., Bass, A., Cooke, S.J., et al. 2021. Host–pathogen–environment interactions determine survival outcomes of adult sockeye salmon (*Oncorhynchus nerka*) released from fisheries. Mol. Ecol. **31**: 134–160. doi:10.1111/mec.16214.

Teffer, A.K., Hinch, S.G., Miller, K.M., Patterson, D.A., Farrell, A.P., Cooke, S.J., et al. 2017. Capture severity, infectious disease processes and

sex influence post-release mortality of sockeye salmon bycatch. Cons. Physiol. **5**: cox017.

Thakur, K.K., Vanderstichel, R., Kaukinen, K., Nekouei, O., Laurin, E., and Miller, K.M. 2019. Infectious agent detections in archived Sockeye salmon (*Oncorhynchus nerka*) samples from British Columbia, Canada (1985–94). J. Fish Dis. **42**: 533–547. doi:10.1111/jfd.12951.

Tomalty, K.M.H., Meek, M.H., Stephens, M.R., Rincón, G., Fangue, N.A., May, B.P., and Baerwald, M.R. 2015. Transcriptional response to acute thermal exposure in juvenile Chinook salmon determined by RNAseq. G3 (Bethesda), **7**: 1335–1349. doi:10.1534/g3.115.017699.

Tort, L. 2011. Stress and immune modulation in fish. Dev. Comp Immunol. **35**: 1366–1375. doi:10.1016/j.dci.2011.07.002.

Tsan, M.F., and Gao, B. 2004. Heat shock protein and innate immunity. Cell. Mol. Immunol. **1**: 274–279.

von Biela, V.R., Bowen, L., McCormick, S.D., Carey, M.P., Donnelly, D.S., Waters, S., et al. 2020. Evidence of prevalent heat stress in Yukon River Chinook salmon. Can. J. Fish. Aquat. Sci. **77**: 1878–1892. doi:10.1139/cjfas-2020-0209.

Wagner, G.N., Hinch, S.G., Kuchel, L.J., Lotto, A.G., Jones, S.R.M., Patterson, D.A., et al. 2005. Metabolic rates and swimming performance of adult Fraser River sockeye salmon (*Oncorhynchus nerka*) after a controlled infection with *Parvicapsula minibicornis*. Can. J. Fish. Aquat. Sci. **62**: 2124–2133. doi:10.1139/f05-126.

White, V.C., Morado, J.F., Crosson, L.M., Vadopalas, B., and Friedman, C.S. 2013. Development and validation of a quantitative PCR assay for *Ichthyophonus* spp. Dis. Aquat. Organ. **104**: 69–81. doi:10.3354/dao02579.

Windsor, D.A. 1998. Most of the species on Earth are parasites. Int. J. Parasitol. **28**: 1939–1941. doi:10.1016/S0020-7519(98)00153-2.

Zhang, Z., Ju, Z., Wells, M.C., and Walter, R.B. 2009. Genomic approaches in the identification of hypoxia biomarkers in model fish species. J. Exp. Mar. Biol. Ecol. **381**: 180–187. doi:10.1016/j.jembe.2009.07.021.