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Host-pathogen-environment interactions predict survival outcomes of adult sockeye salmon (*Oncorhynchus nerka*) released from fisheries

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Abstract

Incorporating host-pathogen(s)-environment axes into management and conservation planning is critical to preserving species in a warming climate. However, the role pathogens play in host stress resilience remains largely unexplored in wild animal populations. We experimentally characterized how independent and cumulative stressors (fisheries handling, high water temperature) and natural infections affected the health and longevity of released wild adult sockeye salmon (*Oncorhynchus nerka*) in British Columbia, Canada. Returning adults were collected before and after entering the Fraser River, yielding marine- and river-collected groups, respectively ($N = 185$). Fish were exposed to a mild (seine) or severe (gill net) fishery treatment at collection, and then held in flow-through freshwater tanks for up to four weeks at historical (14°C) or projected migration temperatures (18°C). Using weekly nonlethal gill biopsies and high-throughput qPCR, we quantified loads of up to 46 pathogens with host stress and immune gene expression. Marine-collected fish had less severe infections than river-collected fish, a short migration distance (100 km, 5–7 days) that produced profound infection differences. At 14°C, river-collected fish survived 1–2 weeks less than marine-collected fish. All fish held at 18°C died within 4 weeks unless they experienced minimal handling. Gene expression correlated with infections in river-collected fish, while marine-collected fish were more stressor-responsive. Cumulative stressors were detrimental regardless of infections or collection location, probably due to extreme physiological disturbance. Because river-derived infections correlated with

single stressor responses, river entry probably decreases stressor resilience of adult salmon by altering both physiology and pathogen burdens, which redirect host responses toward disease resistance.

KEYWORDS
fisheries, gene expression, infectious agents, *Oncorhynchus nerka*, stress, temperature

1 | INTRODUCTION

Infectious agents are integral components of wild animal ecology that remain understudied in the context of wildlife conservation, especially considering their ubiquity and potential importance (Chapman et al., 2021; Johnson et al., 2015; Sofonea et al., 2017). Wild animal disease dynamics include an array of host-pathogen, pathogen-environment, host-environment, and pathogen-pathogen relationships (Alizon et al., 2013; Engering et al., 2013; Mitchell et al., 2005). For example, migration is a common life history trait of wild animals that can influence disease development at individual and population scales (Altizer et al., 2011). As the environment around the host changes, so does the nature of host-pathogen relationships, thereby affecting pathogen species composition, host immune responses, and whole animal performance (Altizer et al., 2011; Evans et al., 2011). Migration can also amplify the effects of stressors experienced by wild animals (Lennox et al., 2016), with disease outcomes that presumably depend on host responses and recovery as well as within-host pathogen community composition (Altizer et al., 2013; Mitchell et al., 2005). Our knowledge of how multiple infections and cumulative stressors affect wild animal population dynamics is limited, especially within the context of migratory species.

Environmental and anthropogenic stressors, such as increasing temperatures and human-animal interactions, are increasingly experienced by wild animals as our climate changes, with potentially cumulative effects (Crain et al., 2008; Isaak et al., 2012; Poloczanska et al., 2013). Multiple stressors are therefore important to study in the context of wild animal conservation. Stress-associated impacts on physiology vary according to the characteristics of the animal (species, life history stage, etc.), the type of stressor and the level of biological organization; hence, cumulative stressors can have additive, synergistic, or antagonistic effects on wild animals, which can reduce their survival, fitness, and adaptive capacity and impede management efforts focused on single stressors (Baker et al., 2013; Petitjean et al., 2019). Stressors can influence infection development through a variety of mechanisms including immunological suppression (Tort, 2011), but whether the presence of infectious agents influences resiliency to individual and cumulative stressors in the wild has proved logistically difficult to test. Effectively characterizing pathogen influences on stressor resilience of wild animals requires the measurement of stress responses under "natural" coinfection conditions (i.e., coinfections of endemic agents; Paterson, 2013). Most data describing the pathogenicity of infectious agents are from studies that isolate a single pathogen. The virulence of a single infectious agent, however, may be diminished or enhanced by the presence of another, so collective pathogen community composition is more relevant to proximal virulence (e.g., current host survival and reproductive success) as well as the evolution of virulence factors (Sofonea et al., 2015, 2017). Predictions of population resilience to individual and cumulative stressors can be improved by identifying how pathogen diversity and cumulative infection intensity are associated with host survival outcomes.

Pacific salmon (*Oncorhynchus* spp.) are ideal model species for such an investigation given their migratory life histories, multiple infections, and cumulative stressors affecting their survival (Groot & Margolis, 1991; Miller et al., 2014; Teffer et al., 2017). Pacific salmon begin their lives in fresh water as eggs, then migrate as juveniles to the marine environment to feed and grow, and finally return to natal freshwater spawning grounds to spawn and then die (Groot & Margolis, 1991). Prespawning adult mortality can have population-level impacts with economic, ecological, and cultural repercussions, in addition to lost individual fitness and reduced spawning biomass (Carey et al., 2021; Hinch et al., 2012; Jacob et al., 2010; Spromberg & Scholz, 2011; Willson & Halupka, 1995). Adult Pacific salmon cease feeding prior to river entry, using endogenous energy reserves to fuel migration, maturation, and other biological processes like immunity (Miller et al., 2009; Rand et al., 2006). Recent work has demonstrated correlations between infection development, reduced immune defences, and early mortality of adult Pacific salmon in fresh water (e.gDolan et al., 2016; Miller et al., 2014; Teffer et al., 2017, 2018), but causal linkages have yet to be established, especially considering multiple stressors and spatially variant coinfection dynamics (marine vs. freshwater).

Pacific salmon physiology and disease ecology have been well studied in the Fraser River watershed, British Columbia (BC), Canada. Surveys have captured snapshots of infectious agent communities in adult salmon that shift throughout the spawning migration (Bass, 2018; Bass et al., 2017). Pathogen richness and loads generally increase after river entry where adult salmon are exposed to an array of freshwater pathogens (e.g., myxozoan parasites; Atkinson et al., 2011; Bartholomew et al., 1997; Bass, 2018; Bass et al., 2017). Infection intensities of bacterial and parasitic agents generally continue to increase with time spent and distance traveled in rivers (Bass et al., 2017; Miller et al., 2014; Teffer et al., 2017, 2018). The array of infectious agents carried by migrating salmon can have detrimental impacts on hosts if the environment becomes more stressful. High temperatures, for example, alter infection development and host physiology (Bettge et al., 2009; Bruneaux et al., 2016; Farrell et al.,

2008) and can compound the effects of additional stressors like fishery capture and release (Gale et al., 2013). The Fraser River has experienced climate-driven warming in recent decades (Patterson et al., 2007) and is Canada's largest salmon producer. Several Pacific salmon species comigrate during fishery openings, so nontarget species are frequently caught and released, which can have harmful delayed impacts on health, maturity, and survival, especially if rivers are warm (Baker et al., 2013; Patterson et al., 2017; Raby et al., 2015; Teffer et al., 2017).

To identify the role of pathogens in host resilience to multiple stressors during spawning migration, adult sockeye salmon (*Oncorhynchus nerka*) were collected prior to or after river entry and experimentally treated with thermal and (or) fishery treatments. To account for temporal shifts in hormonal states (Jeffries et al., 2011), marine- and river-captured fish were matched in maturation trajectories by timing collections with projected migration rates for the dominant stock (Adams-Shuswap) and genetically confirming stock identity. We then compared host responses to mild (seine) or severe (gillnet) fishery bycatch simulation and then held fish until the onset of spawning at optimal (historic; 14°C) or projected (18°C) water temperatures (Ferrari et al., 2007; Patterson et al., 2007).

Our objectives were twofold. First, we identified differences in survival rates of adult sockeye salmon based on internal (infection profiles, genomic responses) and external factors (thermal and capture stress, collection environment). Second, we characterized the interplay between infection profiles and host genomic responses over time relative to collection context, thermal experience, and longevity (e.gBass et al., 2019; Teffer et al., 2018, 2019). Our methodological approach paired experimental fisheries treatments and thermal modulation with repeated tissue biopsies to assess infectious agent presence and load, genetic biomarkers of stress and immune function, and physiological markers of stress. We hypothesized that: (i) infection burdens would be lower and survival rates higher in marine-captured fish relative to river-captured fish (Bass, 2018; Bass et al., 2017), (ii) survival would be reduced under individual and cumulative stressor treatments relative to treatment controls and independent of collection location (Donaldson et al., 2011; Gale et al., 2013; Raby et al., 2015), and (iii) host genomic responses would reflect environmental conditions (acute fisheries and/or chronic thermal stress) as well as infection burdens.

2 | MATERIALS AND METHODS

2.1 | Fish collection and treatment

We focused fishing effort during the "late run" sockeye salmon migration in the Fraser River, which was dominated by the Adams-Shuswap stock complex during our collection period, confirmed by DNA analysis (Beacham et al., 2004). On 11–12 September 2014, 153 sockeye salmon were collected by a commercial purse seiner in the Strait of Georgia (15°C; 49.232 N, 123.271 W; Figure 1). It is unlikely that marine-captured fish entered and exited the river prior to collection given previously documented high mortality of adult Pacific salmon exposed to seawater following freshwater exposure (Cooperman et al., 2010; Hinch et al., 2008). Fish were transported in live-wells filled with seawater to a dock at the Fisheries and Oceans Canada (DFO) West Vancouver Laboratory, West Vancouver, BC (40 min transport), where they were transferred using dipnets to truck-mounted tanks filled with cold (~10°C), filtered, UV-treated water for transport to the DFO Cultus Lake Salmon Research Laboratory, Cultus Lake, BC (1.25 h transport). Transport tanks were fitted with air stones and continually monitored to ensure temperature was maintained and dissolved oxygen levels did not decrease over the duration of transport.

At the Cultus Lake Laboratory, fish were sequentially distributed among 12 holding tanks filled with sand-filtered, UV-treated water from the neighbouring Cultus Lake at equal temperature to the lower Fraser River during collection (14°C). Densities within holding tanks depended on tank size, which included large (8000–10,000 L), medium (4000 L) and small (1400 L) tanks; large tanks held ≤22 fish, while medium tanks held ≤13 fish, and small tanks ≤5 fish. All tanks were covered and fitted with air stones and a submersible pump (large tanks only) that produced a slow current around the tank periphery, encouraging fish to swim in place during holding (approximately 1 body length/s). The velocity of water entering small tanks produced a similar current with no pump needed. Tank replicates included one large and one small or medium tank per temperature-treatment group. Fish were left undisturbed for one week to allow recovery from transport and to simulate the approximate migration time from the collection location to the lower Fraser River. Beginning on 17 September, the temperature was incrementally increased over 48 h from 14°C to 18°C in half (six) of the tanks, producing two temperature groups with either a cool (14°C) or warm (18°C) thermal experience; both temperatures are ecologically relevant: 14°C is the historical average temperature that Late run Fraser River sockeye experienced during up-river migrations and 18°C is considered a physiological thermal extreme (Farrell et al., 2008; Jeffries et al., 2014). Adult salmon are encountering critical temperatures with increasing frequency, with fish now experiencing almost double the number of critical temperature exposure days (>18°C) due to climate change and recent changes in river migration entry timing (Islam et al., 2019; Morrison et al., 2002; Patterson et al., 2007).

One week after collection (19 September), one third of the marine-captured fish from each temperature group was exposed to a fishery treatment that simulated capture and release from a gillnet. Following methodologies applied successfully in previous studies (Teffer et al., 2017, 2018, 2019), the treatment proceeded as follows: a fish was removed from its holding tank using a dipnet and immediately submerged in a small (1,400 L) treatment tank within the dipnet. The opening of the dipnet faced a taut monofilament gillnet (mesh size: 5.25-inch, 13.3 cm) mounted in a wide frame. Upon exiting the dipnet, the fish was "caught" in the gillnet and entanglement was maintained for 20 s. If the fish escaped, the timer was stopped until entanglement had been achieved. After 20 s of sustained entanglement, the fish and gillnet were pulled from the

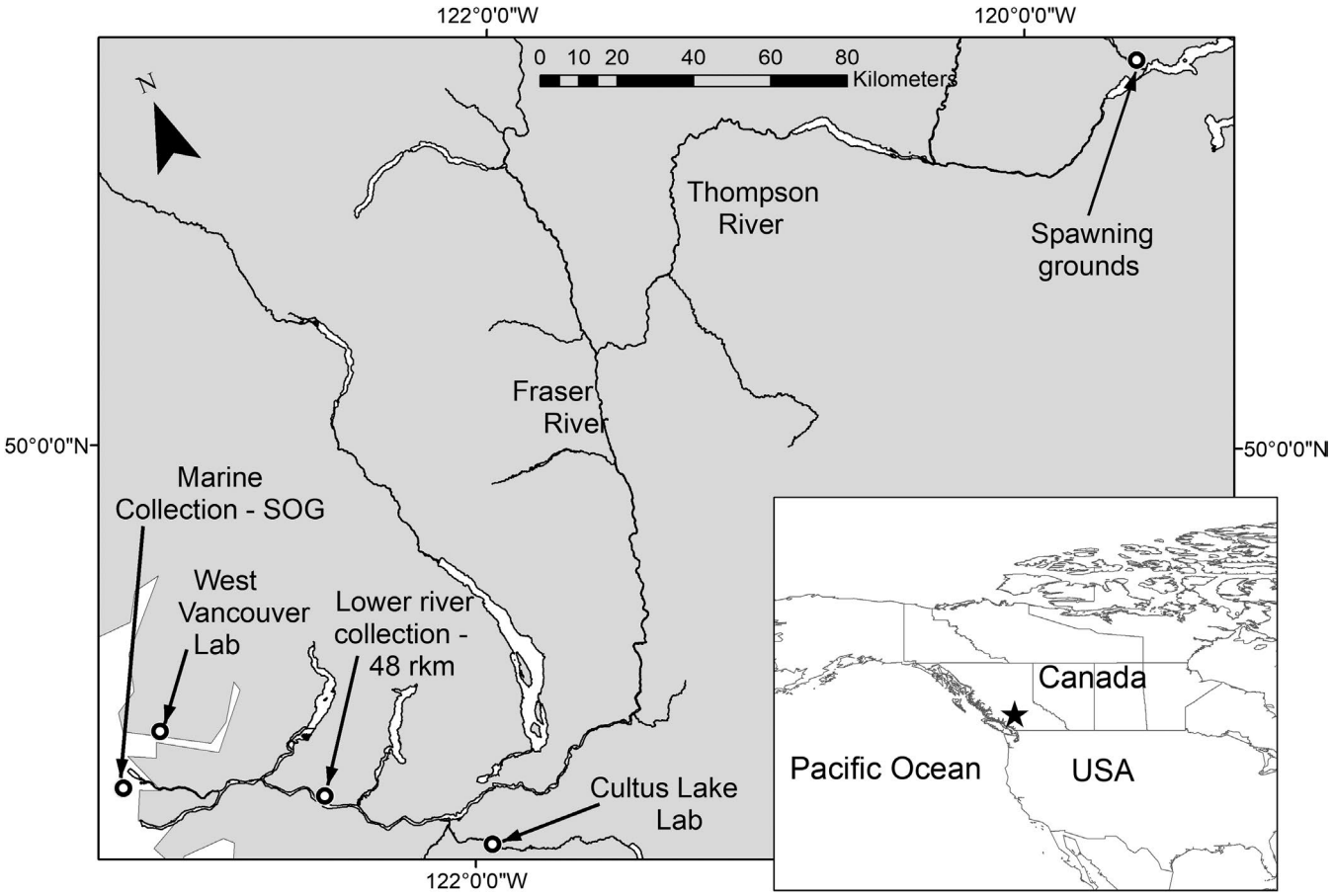

FIGURE 1 The southern portion of the Fraser River watershed, BC, Canada, showing collection locations in the Pacific Ocean (Strait of Georgia – SOG), and lower Fraser River (48 river km), transfer location for marine-captured fish from boat to truck tanks (West Vancouver Laboratory), Cultus Lake Laboratory holding facility and spawning grounds for the Adams-Shuswap sockeye salmon population under study

water and placed into a dipnet for 1 min of air exposure while the fish was detangled from the gillnet (simulating bycatch release by fishers). The fish was then submerged in a foam-lined, flow-through sampling trough (water flowing over gills and body) where a small amount of gill tissue (2–3 filament tips, ~0.5 mg) was taken using sterile end clippers (sample preservation details below), 2 ml of blood was extracted from the caudal vasculature (21-gauge needle with lithium heparinized vacutainer, Becton-Dickson; data not shown), a Floy "spaghetti" style tag (Seattle, WA) was secured in the dorsal musculature. The fish was then placed into a recovery tank (3,000 L) for up to 30 min before being returned to its holding tank. Water temperature throughout the treatment, biopsy, and recovery were consistent with that of the fish's holding tank. The remaining marine-captured fish were divided into two control groups: one biopsied and one left undisturbed until the termination of the study. Biopsied controls followed the same tissue and blood sampling protocol described for gillnet-treated fish but proceeded directly from holding tanks to the sampling trough (no gillnet or air treatment). The biopsy procedure took <2 min overall and included <10 s of total air exposure.

During 24–26 September, 183 sockeye salmon were collected from the lower Fraser River near Fort Langley (15–17°C), approximately 50 river km (distance measurement following the centre of the river, hereafter abbreviated rkm) from the Fraser River estuary. River-captured fish were not gillnet-treated in the laboratory but instead collected with either a gillnet (treatment; $N = 125$) or a beach seine (control; $N = 58$) to reduce experimental handling. Beach seines have been previously demonstrated as a minimally invasive fishing gear, associated with high survival of released catch relative to other gear types (Bass et al., 2018; Donaldson et al., 2012; Raby et al., 2015). Disparity in sample sizes between gear types was unavoidable due to river conditions at the time of collection that were more favourable to gillnet capture. Beach seines were deployed from shore, encircling and corralling fish into shallow (0.5–1 m depth) water without beaching them. Gillnets were deployed in deeper water near the middle of the river for <20 min sets. Gillnet- and seine-collected fish were removed from nets following best fishery practices (e.g., quick removal of fish from gillnets by fishers "picking" fish from the gill net by boat, dip-net removal of fish from the seine) and placed into net pens anchored in the river until biopsy and (or) transfer to truck-mounted tanks.

Subsets of gillnet-collected ($N = 70$) and seine-collected ($N = 25$) fish were biopsied riverside for gill tissue and blood and tagged following the same protocols described for marine-captured fish

(sampling trough supplied with fresh river water) prior to transport to the Cultus Lake Laboratory. The remaining fish were not biopsied, serving as nonhandled controls; however, to identify "treatment" (gear type), the adipose fin was clipped from gillnet-collected controls using scissors within a cylindrical recovery bag submerged in water (duration ≤10 s, no air exposure). Truck-transport conditions were identical to those described for marine-collected fish, but transit time was approximately 40 min. Upon arrival at the laboratory, fish were sequentially distributed among 12 holding tanks of equal temperature to the river during collection (14°C), separate from marine-captured fish. Transport mortalities ($N = 16$) were immediately biopsied for gill and blood, examined for gross pathology (lesions, organ discoloration, macroparasites), and morphometrics recorded including length (post-orbital hypural, ±1.0 cm), total weight (±1.0 g) and organ weights as well as tag ID if applicable and gear type. An operculum biopsy punch was preserved in 90% EtOH for stock identification using microsatellite analysis (Beacham et al., 2004).

Tank temperatures were held relatively constant, allowing for some diurnal variation (±1.5°C). However, we did incorporate behavioural thermoregulation of adult Pacific salmon during freshwater migration into the thermal experience of held fish. In the wild, individuals temporarily reside near the thermocline of corridor lakes (Newell & Quinn, 2005). Therefore, beginning approximately 10 days after treatment (30 September–1 October for marine-sourced, 5–6 October for river-sourced), all tank temperatures were decreased to 10°C for 48 h and then increased back to experimental temperatures (14°C or 18°C) and maintained for the remainder of the holding period.

Biopsy of all fish was repeated weekly until study termination on 16–18 October, resulting in four weeks of gill biopsies for marine-captured fish and three weeks for river-captured fish, plus a terminal gill biopsy at death for all fish. Sampling troughs and recovery tanks were sanitized after all fish in each tank had been processed to prevent transmission of infectious agents among tanks. Throughout the experiment, tanks were monitored for water quality, temperature, and fish morbidity at ≤4 h intervals from 0800–2400 h. Fish that became moribund (gulping, loss of equilibrium) during the study, and all surviving fish at the termination of the study (16–18 October, marking beginning of the spawning period for the Adams-Shuswap stock complex), were sacrificed using cerebral concussion and cervical dislocation and gill biopsied. Gill samples were immediately stored in 1.5 ml RNA*later* solution (Ambion, Inc) and stored at 4°C for 24 h, then −20°C for up to two months, and then −80°C for three months until analysis.

2.2 | Laboratory analyses

Gill samples were processed at the DFO Pacific Biological Station in Nanaimo, BC using high-throughput quantitative polymerase chain reaction (HT-qPCR) on the Fluidigm BioMark Dynamic Array microfluidics platform (Fluidigm). This technology allows for the simultaneous quantification of 96 molecular assays (i.e., targeting either host or infectious agent genes) on 96 tissue samples; the platform has been analytically validated against traditional qPCR for its use in infectious agent screening (Miller et al., 2016), applied in multiple field surveys of wild salmon populations (Bass et al., 2017; Nekouei et al., 2018; Thakur et al., 2018; Tucker et al., 2018), and paired with evaluations of host gene expression (Jeffries et al., 2014; Miller et al., 2014, 2017; Teffer et al., 2017, 2019). Gill has been identified as a representative tissue for nonlethal evaluation of transcriptomics including host infection burdens, which comprise most microorganisms detected in multitissue qPCR surveys of Pacific salmon (Jeffries et al., 2021; Teffer et al., 2017; Teffer & Miller, 2019). Here, we used this tool to characterize the development of multiple infections in gill during a ≥5-week period simultaneously with the expression of a suite of host stress and immune genes to describe how differences in initial infection burdens, infection development, and host responses in gill contribute to the early mortality of Pacific salmon. A suite of 17 infectious agents were evaluated in gills based on a survey of Late run Adams sockeye salmon conducted in the same year as the present study (Bass, 2018). The survey screened for 45 infectious agents, including viruses, bacteria and various parasites, in multitissue pools of wild sockeye salmon throughout their migration to spawning grounds. Agents detected by the survey, including high and low prevalence and potentially pathogenic organisms, were included in our analysis (Table 1). We evaluated biomarkers of host stress and immunity ($N = 27$ genes) that comprised aspects of osmotic stress, heat shock, innate and adaptive immunity, tissue repair and others, evaluated simultaneously with two host reference genes and 17 infectious agents (Table 1). Our infectious agent screening approach is used to quantify RNA rather than DNA of infectious agents to measure variation in "productivity" (e.g., RNA transcription and maintenance) of active infections based on the expression of each target gene. As target gene types differed among assays depending on the infectious agent (i.e., surface protein, ribosomal, etc. see Miller et al., 2016), relative loads can only be compared within agent species, not across.

Tissue samples were trimmed in the laboratory for size uniformity and then homogenized in sterile microtubes with stainless steel beads using 600 µl TRI-reagent 148 (Ambion Inc.), 75 µl 1-bromo-3-chloropropane and a MM301 mixer mill (Restch Inc., Newtown, PA, USA). Centrifugation (6.5 min) separated the aqueous phase, which was aliquoted into 96-well plates for RNA purification. The "spin method" for Magmax™-96 for Microarrays Kits (Ambion Inc.) was used to purify RNA following manufacturer's instructions, using a Biomek FXP liquid handler (Beckman-Coulter) and including a DNase treatment after the first wash. RNA quality and quantity were assessed using spectrophotometry (A_{260}, $A_{260/280}$) and samples were normalized to 1 µg RNA prior to cDNA synthesis. Samples with low RNA yield (<62.5 ng/µl) were removed from analyses. Invitroge SuperScript VILO cDNA Synthesis Kit synthesized cDNA under cycling conditions 25°C for 10 min, 42°C for 60 min and 85°C for 5 min. As per manufacturer's recommendations (BioMar), preamplification of cDNA was completed in a multiplex PCR including

TABLE 1 Assay information for host biomarkers of stress and immunity, reference genes and infectious agents evaluated using qPCR, including gene functions, EST/Accession numbers, primer and probe sequences, and sources

Name	Abbreviation	Assay type	EST/Accession#	Forward primer
Cell receptor	b2m	Acquired immunity	AF180490	F - TTTACAGCGCGGTGGAGTC
Cell receptor	CD4	Acquired immunity	AY973028	F - CATTAGCCTGGGTGGTCAAT
Cell receptor	CD83	Acquired immunity	AY263794	F - GATGCACCCCTTGAGAAGAA
Major histocompatibility complex IIβ	MHCIIb	Acquired immunity	AF115533	F - TGCCATGCTGATGTGCAG
Antiviral protein	Mx	Antiviral		F - AGATGATGCTGCACCTCAAGTC
Retinoic acid inducible gene I	RIGI	Antiviral	NM_001163699	F - ACAGCTGTTACACAGACGACATCA
ATP synthase lipid-binding protein	ATP5G3C	Cellular energy	CB493164	F - GGAACGCCACCATGAGACA
chemokine receptor	CXCR4	Immune regulation	CA054133	F - GGAGATCACATTGAGCAACATCA
Cytokine	IL8	Immune regulation	AJ279069	F - AGAATGTCAGCCAGCCTTGT
Cytokine	IL11	Immune regulation	AJ535687	F - GCAATCTCTTGCCTCCACTC
Cytokine	IL15	Immune regulation	AJ555868.1	F - TTGGATTTTGCCCTAACTGC
Cytokine	IL1R	Immune regulation	AJ295296	F - ATCATCCTGTCAGCCCAGAG
Interferon-α	IFNa	Immune regulation	AY216595	F - CGTCATCTGCAAAGATTGGA
Complement factor	C7	Innate immunity	CA052045	F - ACCTCTGTCCAGCTCTGTGTC
Immunoglobulin	IgMs	Innate immunity	S63348, AB044939	F - CTTGGCTTGTTGACGATGAG
Sodium potassium ATPase subunit	NKA_a1b	Ion regulation	CK879688	F - GCTACATCTCAACCAACAACATTACAC
Transferrin	TF	Iron regulation	D89083	F - TTCACTGCTGGAAAATGTGG
78d16.1	78d16.1	Reference gene	CA056739	F - GTCAAGACTGGAGGCTCAGAG
COIL-P84-2	COIL-P84-2	Reference gene	CA053789	F - GCTCATTTGAGGAGAAGGAGGATG
Glucocorticoid receptor	GR–2	Stress		F - TCCAGCAGCTATGCCAGTTCT
Heat shock cognate 70	HSC70	Stress	CA052185	F - GGGTCACACAGAAGCCAAAAG
Heat shock protein 90	HSP90	Stress	CB493960, CB503707	F - TGGGCTACATGGCTGCCAAG
Transcription factor	JUN	Stress	CA056351	F - TTGTTGCTGGTGAGAAAACTCAGT
Matrix metalloproteinase	MMP13	Wound healing	213514499	F - GCCAGCGGAGCAGGAA
Aeromonas hydrophila	ae_hyd	Bacterium		F - ACCGCTGCTCATTACTCTGATG
Aeromonas salmonicida	ae_sal	Bacterium		F - TAAAGCACTGTCTGTTACC
Candidatus Branchiomonas cysticola	c_b_cys	Bacterium		F - AATACATCGGAACGTGTCTAGTG
Flavobacterium psychrophilum	fl_psy	Bacterium		F - GATCCTTATTCTCACAGTACCGTCAA
Rickettsia-like organism	rlo	Bacterium		F - GGCTCAACCCAAGAACTGCTT
Ceratonova shasta	ce_sha	Parasite		F - CCAGCTTGAGATTAGCTCGGTAA
Cryptobia salmositica	cr_sal	Parasite		F - TCAGTGCCTTTCAGGACATC
Dermocystidium salmonis	de_sal	Parasite		F - CAGCCAATCCTTTCGCTTCT
Ichthyophthirius multifiliis	ic_mul	Parasite		F - AAATGGGCATACGTTTGCAAA
Loma salmonae	lo_sal	Parasite		F - GGAGTCGCAGCGAAGATAGC
Myxobolus arcticus	my_arc	Parasite		F - TGGTAGATACTGAATATCCGGGTTT
Paranucleospora theridion	pa_ther	Parasite		F - CGGACAGGGAGCATGGTATAG
Parvicapsula minibicornis	pa_min	Parasite		F - AATAGTTGTTTGTCGTGCACTCTGT
Parvicapsula pseudobranchicola	pa_pse	Parasite		F - CAGCTCCAGTAGTGTATTTCA
Sphaerothecum destruens	sp_des	Parasite		F - GCCGCGAGGTGTTTGC
Pacific salmon parvovirus	pspv	Virus		F - CCCTCAGGCTCCGATTTTTAT
Erythrocytic necrosis virus	ven	Virus		F - CGTAGGGCCCCAATAGTTTCT

Note: Assays referenced as "In house" refer to assays developed at the Molecular Genetics Laboratory, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC.

Reverse primer	Probe	Source
R - TGCCAGGGTTACGGCTGTAC	P - AAAGAATCTCCCCCCAAGGTGCAGG	Haugland et al. (2005)
R - CCCTTTCTTTGACAGGGAGA	P - CAGAAGAGAGAGCTGGATGTCTCCG	Raida and Buchmann (2008)
R - GAACCCTGTCTCGACCAGTT	P - AATGTTGATTTACACTCTGGGGCCA	Raida et al. (2011)
R - GTCCCTCAGCCAGGTCACT	P - CGCCTATGACTTCTACCCCAAACAAAT	Raida and Buchmann (2008)
R - CTGCAGCTGGGAAGCAAAC	P - ATTCCCATGGTGATCCGCTACCTGG	Eder et al. (2009)
R - TTTAGGGTGAGGTTCTGTCCGA	P - TCGTGTTGGACCCCACTCTGTTCTCTC	Larsen et al. (2012)
R - CGCCATCCTGGGCTTTG	P - AGCCCCATTGCCTC	Miller et al. (2016)
R - GCTGCTGGCTGCCATACTG	P - TCCACGAAGATCCCCA	In house
R - TCTCAGACTCATCCCCTCAGT	P - TTGTGCTCCTGGCCCTCCTGA	Raida and Buchmann (2008)
R - TTGTCACGTGCTCCAGTTTC	P - TCGCGGAGTGTGAAAGGCAGA	Raida and Buchmann (2008)
R - CTGCGCTCCAATAAACGAAT	P - CGAACAACGCTGATGACAGGTTTTT	Raida et al. (2011)
R - TCTGGTGCAGTGGTAACTGG	P - TGCATCCCCTCTACACCCCAAA	Raida et al. (2011)
R - GGGCGTAGCTTCTGAAATGA	P – TGCAGCACAGATGTACTGATCATCCA	Ingerslev et al. (2009)
R - GATGCTGACCACATCAAACTGC	P - AACTACCAGACAGTGCTG	In house
R - GGCTAGTGGTGTTGAATTGG	P - TGGAGAGAACGAGCAGTTCAGCA	Raida et al. (2011)
R - TGCAGCTGAGTGCACCAT	P - ACCATTACATCCAATGAACACT	Nilson et al. (2007)
R - GCTGCACTGAACTGCATCAT	P - TGGTCCCTGTCATGGTGGAGCA	Raida and Buchmann (2009)
R - GATCAAGCCCCAGAAGTGTTTG	P - AAGGTGATTCCCTCGCCGTCCGA	In house
R - CTGGCGATGCTGTTCCTGAG	P - TTATCAAGCAGCAAGCC	In house
R - TTGCCCTGGGTTGTACATGA	P - AAGCTTGGTGGTGGCGCTG	Yada et al. (2007)
R - GCGCTCTATAGCGTTGATTGGT	P - AGACCAAGCCTAAACTA	In house
R - TCCAAGGTGAACCCAGAGGAC	P - AGCACCTGGAGATCAA	In house
R - CCTGTTGCCCTATGAATTGTCTAGT	P - AGACTTGGGCTATTTAC	In house
R - AGTCACCTGGAGGCCAAAGA	P - TCAGCGAGATGCAAAG	Tadiso et al. (2011)
R - CCAACCCAGACGGGAAGAA	P - TGATGGTGAGCTGGTTG	Lee et al. (2006)
R - GCTACTTCACCCTGATTGG	P - ACATCAGCAGGCTTCAGAGTCACTG	Keeling et al. (2013)
R - GCCATCAGCCGCTCATGTG	P - CTCGGTCCCAGGCTTTCCTCTCCCA	Mitchell et al. (2013)
R - TGTAAACTGCTTTTGCACAGGAA	P - AAACACTCGGTCGTGACC	Duesund et al. (2010)
R - GTGCAACAGCGTCAGTGACT	P - CCCAGATAACCGCCTTCGCCTCCG	Lloyd et al. (2011)
R - CCCCGGAACCCGAAAG	P - CGAGCCAAGTTGGTCTCTCCGTGAAAAC	Hallett and Bartholomew (2006)
R - GAGGCATCCACTCCAATAGAC	P - AGGAGGACATGGCAGCCTTTGTAT	Miller et al. (2016)
R - GACGGACGCACACCACAGT	P - AAGCGGCGTGTGCC	Miller et al. (2016)
R - AACCTGCCTGAAACACTCTAATTTTT	P - ACTCGGCCTTCACTGGTTCGACTTGG	Miller et al. (2016)
R - CTTTTCCTCCCTTTACTCATATGCTT	P - TGCCTGAAATCACGAGAGTGAGACTACCC	Miller et al. (2016)
R - AACTGCGCGGTCAAAGTTG	P - CGTTGATTGTGAGGTTGG	Miller et al. (2016)
R - GGTCCAGGTTGGGTCTTGAG	P - TTGGCGAAGAATGAAA	Nylund et al. (2010)
R - CCGATAGGCTATCCAGTACCTAGTAAG	P - TGTCCACCTAGTAAGGC	Hallett and Bartholomew (2009)
R - TTGAGCACTCTGCTTTATTCAA	P - CGTATTGCTGTCTTTGACATGCAGT	Jørgensen et al. (2011)
R - CTCGACGCACACTCAATTAAGC	P - CGAGGGTATCCTTCCTCTCGAAATTGGC	Miller et al. (2016)
R - CGAAGACAACATGGAGGTGACA	P - CAATTGGAGGCAACTGTA	Miller et al. (2016)
R - GGAGGAAATGCAGACAAGATTTG	P - TCTTGCCGTTATTTCCAGCACCCG	Purcell et al. (2016)

all primers to be evaluated by qPCR (200 nM primer mix, TaqMan Preamp Master Mix; Applied Biosystems). Due to the nanofluidic properties of the Fluidigm BioMar, preamplification is necessary to achieve adequate sensitivity. Cycling conditions for the preamplification were 95°C for 10 min then 15 cycles of 95°C for 10 s and 60°C for 4 min, which was followed by ExoSap-it Product Clean-up (Affymetrix Inc.) cycled at 37°C for 15 min then 80°C for 15 min, and then a 5-fold dilution (TEKnova suspension buffer, Hollister). A pool of gill samples from $N = 20$ fish sacrificed riverside during the collection of river-sourced fish was included on all chips as a positive control prior to and following preamplification (e.g., cDNA positive control, preamplification control); negative controls were also included at each step in the protocol. A serial dilution of artificial positive constructs (APC clones) matching the primer-probe sequence for each infectious agent under evaluation was added to the dynamic array just prior to qPCR and tagged with a secondary probe (NE reporter dye) to identify potential contamination of samples. Samples (TaqMan Universal Master-Mix, Life Technologies; GE Sample Loading Reagent, Fluidigm, preamplified cDNA) and assays (in duplicate; 10 μM primers, 3 μM probes for Taqman assays) were loaded onto dynamic arrays using the integrated fluidics controller HX (Fluidigm) and qPCR was completed following 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. All assay and sample combinations were analysed in independent 7 nl wells on the dynamic arrays.

The BioMark Real-Time PCR analysis software was used to manually score output, following protocols described in Miller et al. (2016). Infectious agents that were not positive in duplicate were failed and quantification cycles (Cq) for duplicates were averaged for host genes and infectious agents. Host biomarkers were normalized to the average of the two reference genes and are reported as relative expression following the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Infectious agent Cq was subtracted from 40 (maximum Cq), producing what is referred to herein as "relative load," and is therefore a representation of RNA expression. Note that relative loads are platform-specific, as the range of Cq values differ between the BioMark and traditional qPCR platforms. Further information regarding the protocols described above can be found in Teffer et al. (2017) and details regarding the BioMark platform's applications in infectious agent screening and validation are described in Miller et al. (2016).

2.3 | Statistical analyses

Longevity was calculated as the total days surviving after treatment (marine-captured: laboratory treatment; river-captured: collection); note that the holding period was shorter for river-captured fish due to the lag between collection dates. Sex-specific differences in infection development (Bass et al., 2017; Teffer et al., 2017) and migration success (Martins et al., 2012) under suboptimal conditions prompted the investigation of sex as a cofactor. Differences in survival between capture locations (source), sexes, treatments (gillnet-treated/captured, biopsied controls, nonbiopsied controls), and temperatures were characterized using survival analysis (Cox proportional hazards and Kaplan-Meier curves, survival package) and linear models (LM) in the R statistical software (R Core Team, 2015; Therneau, 2014). For Cox regression analyses that included both sources, days surviving was censored at 21 days post-treatment to avoid the bias of extended holding time for low temperature marine-sourced fish (i.e., earlier capture and longer survival), while models including only one source group and Kaplan-Meier curves used all days surviving. For marine-sourced fish, survival analyses were performed relative to nonhandled and handled (biopsied) controls to identify potential impacts of experimental handling and biopsy on survival. For river-sourced fish, nonhandled controls and biopsied fish were included among both gillnet- and seine-captured groups, which allowed "experimental handling and biopsy" to be included as a cofactor in the survival analyses of river-sourced fish.

Overall effects of sex were tested in a model that stratified source, treatment, and temperature to determine if sex should be included in subsequent models. In cases where sex was not identified as significant, sexes were pooled for subsequent analyses. Source effects were then evaluated, stratifying all data by treatment and temperature. Treatment, temperature, and biopsy (experimental handling) effects were then evaluated within each source group. For Cox proportional hazards analyses, hazard ratios (exponents of coefficients; e^{β}) for significant effects are presented, which correspond to the daily hazard of mortality, as well as model r^2 and likelihood ratio tests for model significance. Where assumptions of the Cox regressions could not be met, coefficients ($\beta \pm$ standard error) for significant parameters and interactions are presented from linear models.

To assess the relative influences of source, temperature, gillnetting, sex, and time on infection metrics, we used linear mixed effects (LME) models with a random intercept that accounted for resampling of individuals over time (i.e., fish ID as a random effect for repeated measures). Interactions of time (weeks post-treatment) with source, temperature, and gillnetting tested for differences in infection development depending on each factor. A top-down approach for model selection was used to identify significant interactions and factors associated with relative infections burden (RIB, a composite metric of infectious loads and richness, see Bass et al., 2019), richness, and infectious loads of highly prevalent agents (Zuur et al., 2009). A $p < .05$ a priori cutoff was applied for likelihood ratio tests comparing models including and excluding each variable and interaction term, starting with a full model that included all possible factors and interactions and then removing those with low *t*-values and high *p*-values in a stepwise fashion. Significance and coefficients therefore pertain to the role of each variable or interaction in describing the infection metric data within the final model. The final model included only significant interaction terms and factors, as well as main effects that served as components of significant interactions.

Survival analysis with time-dependent covariates tested whether enhanced RIB or individual infections increased the estimated daily hazard of mortality. Monitored temporal changes in RIB

and individual infectious agent presence and loads in gill were incorporated into a survival analysis with Cox proportional hazards. All models included treatment and sex as constant cofactors to account for their associated variance. Model assumptions were evaluated: influential observations were removed and models with proportional hazard violations were stratified by treatment or sex or complemented by an interaction of the offending factor with time. Models were constructed separately for marine- and river-collected fish and within temperature groups due to the strong influence of these factors on survival. Separate models for each infection metric were performed because each is measured on a unique scale (i.e., qPCR assays designed to different genes with varying functions). Infection data varied over time at the resolution of experimental day: nonlethal gill samples were generally taken at ~7-day increments, but gill data from dying fish in the interim between sampling events were also incorporated, with the lowest temporal increment set at one day. Experimental start was the same as that described above for the general survival analysis. Time-dependent covariates included RIB (log-transformed), presence/absence (1 = positive detection, 0 = no detection), and high loads (1 = greater than the mean load of positive detections, 0 = less than the mean load or negative detection). Previous studies have identified adult salmon as generally showing physiological and immunological responses when infectious loads increase beyond the mean load of the host population (e.g., Teffer et al., 2019). Sample sizes of positive detections across all individuals and repeated samples (N) and exponents of coefficients (e^{β}, daily hazard of mortality) for significant ($p < .05$) infection metrics are reported for significant models (likelihood ratio test, $p < .05$). Infectious agent community composition among biopsied fish was qualitatively evaluated by normalizing the loads of each positive detection to the maximum for each species (i.e., the first step in the RIB calculation). Normalized loads were then summed for each agent in each source-temperature-treatment group and totals plotted as a function of time (i.e., relative contributions to total normalized loads of all agents in each temperature-treatment group at each weekly interval).

Permutational multivariate analysis of variance (PERMANOVA) was used to assess contributions of source, sex, RIB, temperature, and gillnetting (including a temperature-gillnet interaction term) to overall variation in gene expression data (i.e., multivariate response). Twenty-two biomarkers of stress and immunity comprised aspects of heat shock responses, osmotic imbalance, innate and adaptive immunity and tissue repair (Table 1). Relative expression of all biomarkers was used as the response matrix for the PERMANOVA during each week of the study. Data from samples taken at morbidity were included in the analysis within the week that the animal died. Nonbiopsied marine controls and seine-collected river fish were excluded from the PERMANOVA as the longevity of these groups was biased toward study termination, producing unbalanced sample sizes with disparate response profiles. Samples taken at death from nonbiopsied gillnet-collected fish were included in the analysis because they showed similar survival patterns to biopsied fish, which improved our power to detect thermal and sex-specific differences. Unsupervised principal component analysis (PCA) was used to relate individual biomarker expression to each factor using linear models with component axes (PC) as response variables and factors used in PERMANOVAs as predictors, including a temperature-treatment interaction where sample sizes permitted. PCs were included that explained >10% of the variance (i.e., eigenvalues) and contributed to significant ($p < .05$) linear models. Significant factors in linear models are discussed with respect to biomarkers most positively and negatively loaded (i.e., eigenvectors) on the corresponding PC axis. Only fish identified as part of the Adams-Shuswap stock complex were included in analyses of survival, infection metrics, and gene expression to avoid potential stock biases.

3 | RESULTS

3.1 | Survival

Percentages of fish surviving to the spawning period of this population are presented first, followed by the results of the survival analyses. Survival was highest at 14°C for both marine and freshwater-collected groups. Marine-captured controls (with and without biopsy) and gillnet-treated males survived 100% at 14°C, while gillnet-treated females survived 92% (Figure 2, Table 2). Low sample sizes for several groups warrant caution in sex-specific comparisons of survival for river-captured fish (see Table 2). Among river-captured seine-collected fish held at 14°C, males and females survived 100% if not biopsied, while biopsy reduced survival of females (F; $N = 1$) to 0% and males (M; $N = 4$) to 75%. Among river-captured gillnet-collected fish held at 14°C, both sexes survived better if not biopsied ($F = 55\%$, $M = 82\%$) than if biopsied ($F = 44\%$, $M = 50\%$). At 18°C, gillnetting and biopsy reduced the survival of marine-captured males and females to 0%, while nonbiopsied controls survived relatively well ($F = 73\%$, $M = 100\%$). Similarly, 0% of river-captured fish held at 18°C survived if biopsied (regardless of gear type) and few nonbiopsied gillnetted ($F = 8\%$, $M = 0\%$) or seine-collected fish ($F = 8\%$, $M = 17\%$) survived to study termination.

Survival analysis identified a significant effect of collection location, where river-captured fish were 3.3 times ($p < .001$) more likely to die (each day) than marine-captured fish (model $r^2 = 0.14$, $p < .001$). No significant effect of sex on survival was identified overall ($p = .255$) or within source groups ($p > .400$), so sexes were pooled in subsequent survival models. Among river-captured fish, high temperature ($e^{\beta} = 47.98$, $p < .001$) and gillnetting ($e^{\beta} = 5.30$, $p = .04$) significantly decreased the likelihood of survival, with no significant interaction ($p = .098$). However, the effect of biopsy ($e^{\beta} = 5.16$, $p = .012$) was similar to that of gillnetting, which we interpret as no effect beyond experimental handling and biopsy. Furthermore, a significant interaction between temperature and biopsy ($e^{\beta} = 0.26$, $p = .015$) suggested that the negative biopsy effect on survival was reduced (or masked) at high temperature (model $r^2 = 0.48$, $p < .001$).

Among marine-captured fish, excellent survival at 14°C and extremely poor survival at 18°C violated Cox model assumptions

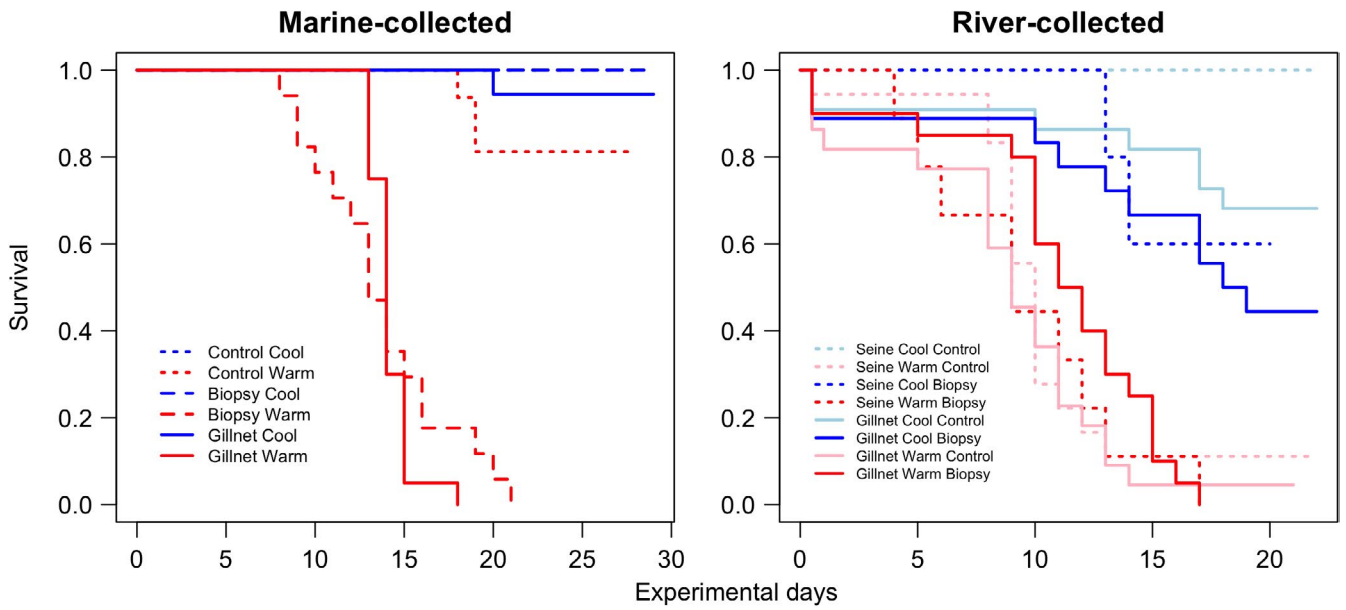

FIGURE 2 Kaplan-Meier curves describing the survival of adult sockeye salmon held in fresh water at 14°C (blue) or 18°C (red) for up to four weeks. The left panel shows survival of fish captured in the Strait of Georgia. The right panel shows survival of fish collected from the lower Fraser River. Line type denotes treatment (left plot: solid, gillnetted and air exposed; dashed, biopsied control; dotted, nonbiopsied control; right plot: solid, gillnet-collected; dashed, seine-collected; lighter colours, nonbiopsied; darker colours, biopsied). To simulate behavioural thermoregulation, tank temperatures were decreased to 10°C for 48 h on 30 September–1 October for marine-captured and 5–6 October for river-captured fish, and then increased back to experimental temperatures

and prompted the use of simple linear models for survival analysis. Qualitatively, survival among marine-captured treatments was similar at 14°C (only one female held at 14°C died prior to study termination). A linear model including both temperature groups of marine-captured fish identified significant interactions of temperature with gillnetting ($\beta = -11.99 \pm 1.15$, $p < .001$) and biopsy ($\beta = -13.39 \pm 1.23$, $p < .001$), supporting decreased resilience of marine-captured fish to handling stress at high temperature only. Longevity of marine-captured fish was also independently reduced by high temperature ($\beta = -1.75 \pm 0.84$, $p = .041$). Among fish held at 14°C, no impact of either biopsy or gillnetting was apparent ($p > .05$), while at 18°C, biopsy ($\beta = -12.54 \pm 1.07$, $p < .001$) and gillnetting ($\beta = -12.05 \pm 1.03$, $p < .001$) similarly reduced survival relative to nonhandled controls. Gillnetting, however, had no effect beyond experimental handling (significance of gillnet effect relative to biopsy: $p = .587$).

3.2 | Infection metrics in gill

Prevalence was highest overall for "*Ca*. B. cysticola", *I. multifiliis*, *F. psychrophilum*, and RLO (Table S1). *C. shasta* and *P. minibicornis* were prevalent only in river-captured fish, especially in later weeks. *P. theridion*, *L. salmonae*, *P. pseudobranchicola* (primarily cool water), *A. hydrophila* and *A. salmonicida* (river) were moderately prevalent, whereas *D. salmonis* (primarily first biopsy, river), *M. arcticus* and *S. destruens* were minimally prevalent. Overall prevalence by treatment and source can be found in Table S2.

Richness, *F. psychrophilum*, *I. multifiliis*, and "*Ca*. B. cysticola" loads increased at a faster rate in river-captured fish (i.e., significant interaction of source with time; Figure 3, model coefficients in Table 3). RIB and "*Ca*. B. cysticola" increased at a faster rate in warm water, whereas *I. multifiliis* and RLO were consistently higher in warm water (no interaction). "*Ca*. B. cysticola" increased at a faster rate in gillnetted fish from both marine and river locales, but positive effects of gillnetting on RIB were only evident in river-captured fish. *F. psychrophilum* was higher in females than males. *C. shasta* and *P. minibicornis* loads were only increased by time (evaluated in river-sourced fish only). Low intraclass correlation coefficients were apparent for *I. multifiliis*, RLO, *C. shasta* and *P. minibicornis*, suggesting high temporal variability within individuals.

Survival analysis with time-dependent infection covariates demonstrated associations of various infectious agents and RIB with early mortality (Table 4). This analysis could not be completed for marine-captured cool water fish due to extremely low mortality (insufficient data). For marine-captured fish held in warm water, only *A. hydrophila* was associated with a higher risk of mortality (presence effect decreased with time), whereas the presence of *F. psychrophilum* was associated with slightly decreased mortality risk. For river-captured fish in cool water, the presence of *L. salmonae* (stratified by sex to satisfy proportional hazards), high loads of *F. psychrophilum*, and the presence and high loads of *P. minibicornis*, *I. multifiliis* and RLO, and high RIB all increased mortality risk. For river-captured fish in warm water, the presence of *A. hydrophila*, *L. salmonae*, and *P. minibicornis*, high loads of *I. multifiliis* and the presence and high loads of *A. salmonicida*, RLO and *C. shasta* and high RIB were associated with increased mortality risk.

Infectious agent community composition was dynamic over time, but with few drastic shifts in composition and a high degree of similarity between source-temperature-treatment groups

TABLE 2 Sample sizes, longevity (mean ± standard deviation) and length (post-orbital hypural, cm) by sex for adult Adams-Shuswap sockeye salmon captured from either marine or riverine waters, held at cool (14°C) or warm (18°C) temperatures for up to 4 weeks

Source	Temperature	Treatment	Sex	N	Longevity (d)	Length (cm)	Survival (%)
Marine	14°C	Gillnet	F	13	27.7 ± 2.5	49.2 ± 2.0	92
			M	5	28.6 ± 0.9	50.8 ± 0.7	100
		Control					
		Biopsy	F	7	29.0 ± 0.0	49.5 ± 2.2	100
			M	6	28.7 ± 0.5	50.5 ± 2.5	100
		No biopsy	F	15	28.0 ± 0.0	50.4 ± 3.3	100
			M	2	28.0 ± 0.0	51.3 ± 0.1	100
	18°C	Gillnet	F	14	14.3 ± 1.3	48.7 ± 1.5	0
			M	6	14.0 ± 0.9	49.1 ± 1.8	0
		Control					
		Biopsy	F	12	13.2 ± 3.4	49.4 ± 1.5	0
			M	5	15 ± 4.8	49.2 ± 2.6	0
		No biopsy	F	11	25.5 ± 4.4	49.1 ± 2.3	73
			M	5	28.0 ± 0.0	50.3 ± 1.6	100
River	14°C	Gillnet					
		Biopsy	F	21[a]	13.9 ± 7.0	49.8 ± 1.2	44
			M	2	20.5 ± 2.1	49.5 ± 1.0	50
		No biopsy	F	11	16.1 ± 8.1	50.2 ± 6.8	55
			M	11	20.3 ± 3.6	49.7 ± 1.9	82
		Seine					
		Biopsy	F	3[a]	9.3 ± 4.0	51.1 ± 5.7	0
			M	4	18.3 ± 3.5	51.1 ± 2.8	75
		No biopsy	F	8	21.5 ± 0.5	52.3 ± 7.5	100
			M	2	22.0 ± 0.0	51.3 ± 0.4	100
	18°C	Gillnet					
		Biopsy	F	18[a]	8.9 ± 4.1	49.3 ± 1.9	0
			M	9	13.0 ± 2.4	50.3 ± 2.1	0
		No biopsy	F	12[a]	8.8 ± 6.1	49.9 ± 6.3	8
			M	10	8.7 ± 3.6	48.8 ± 1.5	0
		Seine					
		Biopsy	F	6	10.3 ± 4.5	49.8 ± 1.1	0
			M	3	8.0 ± 3.6	50.6 ± 0.5	0
		No biopsy	F	12	10.0 ± 4.8	49.7 ± 2.5	8
			M	6	11.7 ± 4.8	52.2 ± 1.3	17

Note: Gillnet treatment included entanglement and air exposure in the laboratory (marine) or as the means of collection (river); biopsy refers to weekly gill biopsy from group subsets. Lethal sampling of a subset of fish at 7 days reduced sample sizes to 16, one, nine, and 10 for river-collected females from cool gillnet, cool seine, warm gillnet and warm seine groups.

[a]Lethal sampling of a subset of fish at 7d reduced sample sizes to 16, 1, 9, 10, respectively.

(Figure 4). Several compositional changes were temperature- and source-dependent, with less distinction between gear types. Greater species richness in river-captured fish allowed for more variation in composition with time, demonstrating losses and decreased loads of several agents within the first two weeks and subsequent increases in the representation of myxozoan and other parasites as well as some bacteria (*A. salmonicida*, RLO). In cool water, representation of *F. psychrophilum* was consistently reduced in later weeks among marine-sourced fish, due to both mortality of infected fish and apparent clearance of the bacteria in survivors (Figure S1), but was well-represented throughout time among river-captured fish. In warm water, however, losses of *F. psychrophilum* representation at later sampling dates were evident and concurrent with enhanced representation of *A. salmonicida* (river) and *A. hydrophila* (marine).

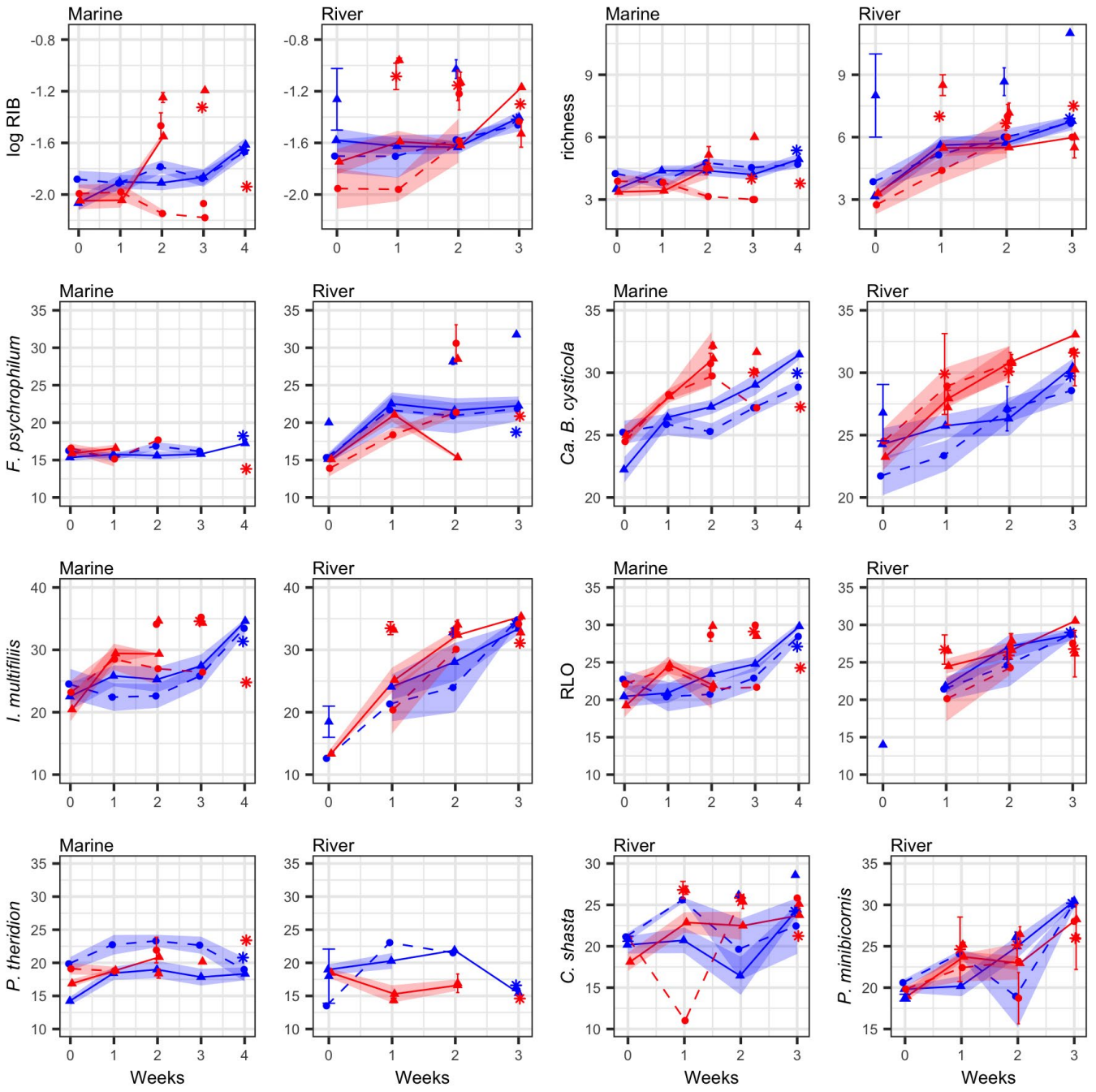

FIGURE 3 Relative infection burden (RIB) and loads (40 - Cq) of prevalent agents in adult sockeye salmon gill during a 4-week freshwater holding period following capture from marine or river environments. Colors indicate temperature (blue, 14°C; red, 18°C), lines and symbols indicate treatment (solid, triangle, gillnet; dashed, circle, seine). Ribbons describe loads from fish that were biopsied weekly while independent points represent controls that were not repeat biopsied. Mean ±s.e.m. including zeros. Abbreviations are listed in Table 1

3.3 | Host stress and immune responses

There was a consistent relationship between gene expression and temperature regardless of capture location, though among river fish, RIB was also a consistent driver of gene expression and some specific gene responses were shared between RIB and thermal stress. Intracellular immunity genes showed decreasing relevance to stressor responses in later weeks in both marine- and river-captured fish.

For marine-captured fish, high temperature was the primary factor contributing to variation in gene expression during weeks 0–3 (r^2 range: 0.24–0.42), increasing in importance over time (Table 5, Figure 5). The effect of gillnet treatment was weakly temperature-dependent during weeks 0–1 (interactions $p \leq .022$), but after only cool-temperature fish remained (weeks 3–4), its independent effect increased ($r^2 = 0.08–0.19$). RIB was only significantly associated with gene expression of marine-captured fish during week 2 ($r^2 = 0.17$, $p < .001$).

TABLE 3 Parameters (β ± s.e.m.) of significant ($p < .05$) factors associated with infection metrics measured in adult sockeye salmon during five weeks of freshwater residence

Metric	R	R:T	R:G	H	H:T	G	G:T	S	T	ΔAICc	ICC
RIB[a]	0.21 ± 0.07		0.18 ± 0.09, $p = 0.043$	−0.10 ± 0.05	0.08 ± 0.03, $p = 0.003$	−0.06 ± 0.06	0.06 ± 0.02, $p = 0.025$		0.04 ± 0.02	5.12	0.27
Richness	−0.02 ± 0.21	1.09 ± 0.12, $p < 0.001$							0.25 ± 0.06	1.39	0.22
I. multifiliis	−5.95 ± 1.79	3.70 ± 1.02, $p < 0.001$		3.35 ± 0.87, $p < 0.001$					2.68 ± 0.38	2.82	<0.001
RLO				2.29 ± 0.63, $p < 0.001$					2.17 ± 0.26, $p < 0.001$	3.80	<0.001
F. psychrophilum	1.05 ± 0.71	3.13 ± 0.43, $p < 0.001$						1.12 ± 0.56, $p = 0.043$	0.11 ± 0.23	8.44	0.33
Ca. B. cysticola	−1.14 ± 0.54	0.62 ± 0.26, $p = 0.019$		1.09 ± 0.53	1.36 ± 0.25, $p < 0.001$	−0.47 ± 0.54	0.84 ± 0.21, $p < 0.001$		1.07 ± 0.17	3.38	0.50
C. shasta[b]									1.43 ± 0.53, $p = 0.007$	3.57	<0.001
P. minibicornis[b]									2.70 ± 0.44, $p = 0.001$	3.65	<0.001

Note: Factors evaluated included river exposure (R), high temperature (H; 18°C vs. 14°C), gillnet entanglement (G; entanglement and air exposure), sex (S) and time (T; weeks), with significant interactions. ICC is the intraclass correlation coefficient of the model.

[a]log transformed.

[b]River fish only.

TABLE 4 Exponents of coefficients (e^{β}, daily hazard of mortality) and significance (P) from a time-dependent covariate analysis of infection metrics measured with qPCR in the gill of adult sockeye salmon

Infectious agent or metric	Marine: warm[a]					River: cool					River: warm				
		Presence		Load			Presence		Load			Presence		Load	
	N	e^{β}	p	e^{β}	p	N	e^{β}	p	e^{β}	p	N	e^{β}	p	e^{β}	p
A. hydrophila	12	7.0	<.001[b]	8.2	<.001	2					2	27.4	.009		
A. salmonicida	0					3					6	17.5	<.001	14.2	<.001
F. psychrophilum	45	0.2	.008			41			19.7	.022	31				
Rickettsia-like organism	68					24	7.6	.038	16.1	.002	26	6.2	.018	4.6	.004
C. shasta	4					42					35	3.8	.005	6.7	<.001
P. minibicornis	2					28	16.7	.010	34.4	.004	35	3.4	.034		
I. multifiliis	85					34	16.2	.040	28	.008	38			5.1	.008
L. salmonae	13					7	19.1[c]	.017			8	6.3	.001		
Relative Infection Burden	95	NA	NA			51	NA	NA	42434	.005	59	NA	NA	12.6	<.001

Note: Presence and loads of individual infectious agents were measured in weekly gill tissue from marine- and river-captured fish held in cool (14°C) or warm (18°C) water for up to four weeks. Relative infection burden is a composite metric of richness and load of all agents. Only significant ($p < .005$) parameters from significant models (log-likelihood $p < .05$) are shown. Sample sizes (*N*) describe the total positive detections in all fish within the group over time for each agent or metric.

[a]All marine warm models stratified by treatment.

[b]Effect decreased with time ($p = .038$).

[c]Sex stratified.

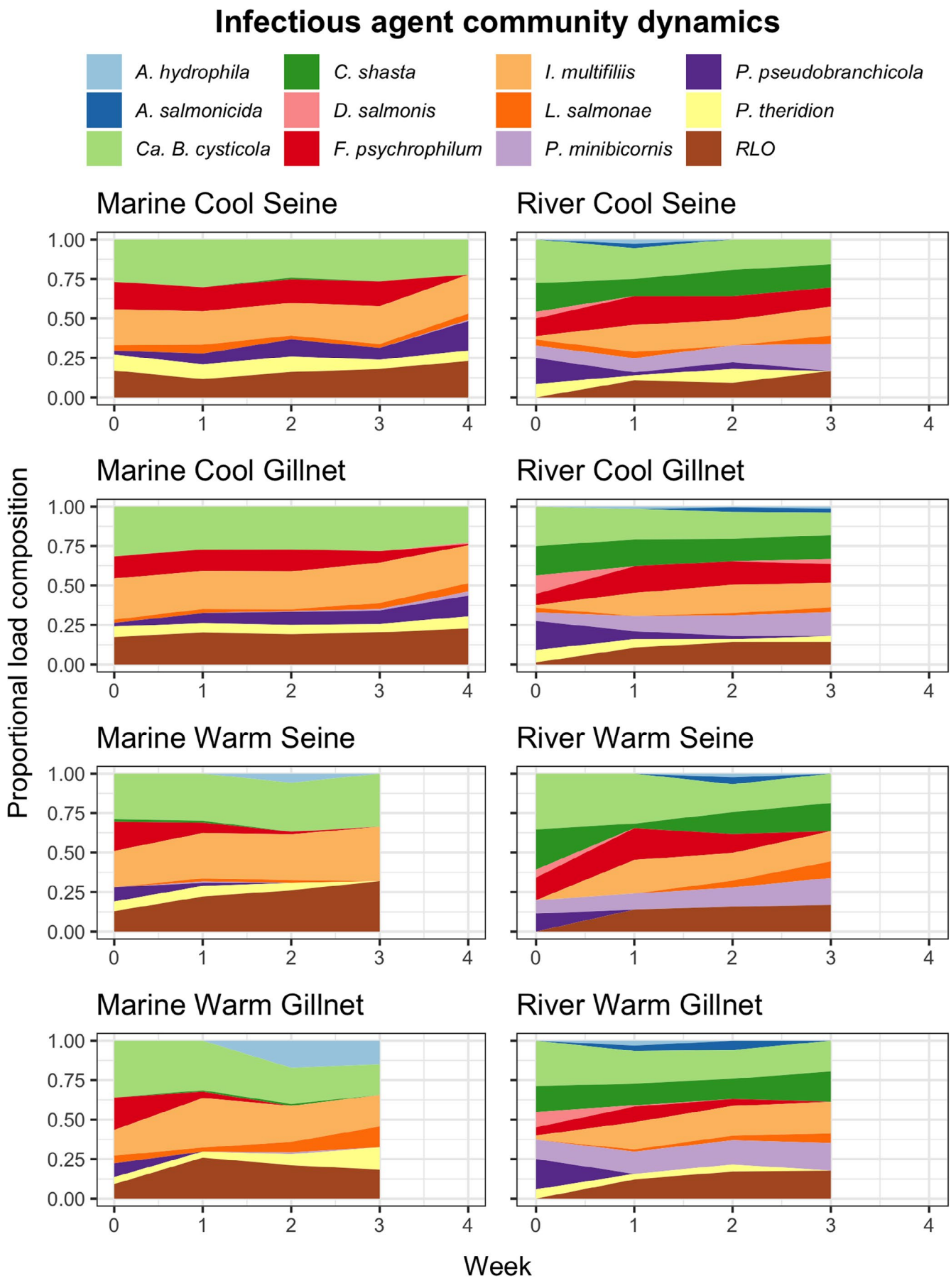
Infectious agent community dynamics
A. hydrophila
A. salmonicida
Ca. B. cysticola
C. shasta
D. salmonis
F. psychrophilum
I. multifiliis
L. salmonae
P. minibicornis
P. pseudobranchicola
P. theridion
RLO
Marine Cool Seine
River Cool Seine
Marine Cool Gillnet
River Cool Gillnet
Marine Warm Seine
River Warm Seine
Marine Warm Gillnet
River Warm Gillnet
Proportional load composition
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
Week

FIGURE 4 Temporal shifts in proportional load composition of infectious agents measured using HT-qPCR in repeated gill biopsies over 3–4 weeks. Colors indicate temperature (blue, 14°C; red, 18°C), lines and symbols indicate treatment (solid, triangle = gillnet; dashed, circle = seine). Proportional load composition was calculated by normalizing individual agent loads by the maximum load for each agent, summing normalized loads within agent species, and then plotting sums as a proportion of the total sum from all agents in each temperature-source-treatment group at each week

The temperature-gillnet interaction term at week 0 was negatively associated with PC1 for marine-captured fish, while high temperature negatively associated with PC2, demonstrating cellular and osmotic stress (HSP90, GR2, NKA_a1b), iron regulation (TF) and tissue repair (MMP13) in thermally stressed fish. Thermally stressed gillnetted fish also associated with the expression of JUN, HSC70, and all other biomarkers, which loaded negatively on PC1. Gillnetting was positively associated with PC3 along with stress indices, intracellular immunity, and antiviral activity, whereas females were negatively associated with PC3, correlating with the expression of cytokines, immune receptors (CD83), and antiviral genes (IFNa, Mx). By the end of week 1, thermally stressed fish were negatively associated with PC1 and positively associated with PC2, which corresponded to the expression of HSP90, MMP13 and C7, whereas cool-temperature fish were more strongly associated with antiviral indicators, cellular receptors and cellular energy generation. At week 2, thermally stressed fish were positively associated with PC1 and PC2, corresponding to gene loadings indicating tissue repair, cellular stress, cytokine and chemokine activity, iron regulation (TF) and complement (C7). RIB was also positively associated with PC1, but negatively with PC2, suggesting greater energy needs and osmotic stress (NKA_a1b) in addition to the associations described for thermally stressed fish. Weeks 3 and 4 included only cool-temperature fish due to mortality at high temperature (i.e., low sample sizes due to fewer surviving fish), and week 4 included survivors sacrificed at the start of the spawning period (study termination). Gillnetted fish were positively loaded on PC2 at week 3 indicating tissue repair and inflammation (IL8, MMP13), iron regulation, complement, and increased cellular stress and energy needs. At week 4, gillnetted fish loaded negatively on PC1 with indices of inflammation (IL8), cellular stress (JUN, HSC70) and some cellular receptors, which loaded opposite to the expression of Mx and other antiviral components.

For river-captured fish, temperature was not evaluated at week 0 (no thermal application at capture) but gained importance in weeks 1 and 2 (r^2 = 0.06 and 0.22, respectively). Gillnetting was marginally associated with gene expression ($r^2 \leq 0.06$) only at week 0 and 1 and with no interaction with temperature. RIB was the primary factor associated with gene expression of river-collected fish, increasing the amount of variation explained with time (r^2 range: 0.04–0.51).

For river-captured fish at collection (treatment), gillnetted fish were negatively associated with PC2 and PC3, indicating expression profiles consistent with cellular stress (GR2, JUN, HSC70), osmotic imbalance (NKA_a1b), iron regulation (TF), antiviral activity (RIG1) and extracellular receptor (CD4) genes. During week 1, RIB was positively associated with PC1, indicating inflammation (IL11, IL8, CXCR4), iron regulation, tissue repair (MMP13) and complement (C7) as characteristics of fish with high RIB, while adaptive immunity (b2m, MHCIIb), RIG1 and protein repair (HSC70) were associated with low RIB. At week 2, RIB was again strongly positively associated with PC1, demonstrating a similar profile to that described for week 1. Thermally stressed fish and females were both positively associated with PC2, suggesting that these fish were recruiting aspects of cellular stress response, inflammation, tissue repair, and iron regulation, while neglecting most cellular immune aspects. In the final week of holding, including only cool water fish and survivors sacrificed at study termination, RIB was strongly negatively associated with PC1, reflecting the same gene set correlations as in previous weeks.

4 | DISCUSSION

This study experimentally quantified how environmental conditions, pathogen burden, and host genomic responses collectively influence the longevity of wild adult Pacific salmon during their once-in-a-lifetime spawning migration. Pathogen community dynamics and host genomic responses to experimentally applied cumulative stressors differed between fish collected from marine and freshwater locations. Our results provide evidence to implicate river-derived infections as causal factors of stressor-mediated early mortality of sockeye salmon during spawning migration. As predicted and previously shown (Bass, 2018; Bass et al., 2017), fish collected from the lower Fraser River carried heavier gill infections than fish collected from the Strait of Georgia. In the context of a total river migration of 480 km in addition to thousands of km at sea, this result is striking in that a relatively short migration section (approximately 100 km, 5–7 days lag in sampling) can produce such profound differences in infection burden. Increased RIB was primarily due to the accumulation of river-derived infections (e.g., myxozoan parasites) upon freshwater entry. Also consistent with our initial hypothesis, marine-collected fish that bypassed the lower river had higher survival in cool water than river-exposed fish, which survived poorly (~50%) and died sooner unless they were collected under the most benign conditions (seine) with no additional handling. Handling effects are emphasized in our results because survival of fish exposed to gillnetting and air exposure showed no difference from biopsied controls, highlighting the detrimental effects of any level of handling.

Supporting our predictions about stressor tolerance, river-captured fish had lower survival rates than marine-captured fish when exposed to a single stressor (handling or high temperature). Thermal stress tolerance of adult sockeye salmon was drastically reduced in river-collected fish as only nonhandled marine-collected fish survived well at high temperature (~80%). Relative to seine-collected controls, gillnetting and repeated handling (biopsy) reduced the survival of river-captured fish, though sample sizes were

low. Furthermore, river-captured fish demonstrated stronger relationships between infection profiles, host gene expression, and early mortality than marine-captured fish. These results support our prediction that enhanced pathogen burdens due to river exposure would reduce host stressor resilience, but only in the context of single stressors. Conversely, we found no difference in survival rates between marine- and river-collected groups when exposed to cumulative thermal and fisheries stressors (gillnet or seine). Host physiological and immune data from this and previous studies (Teffer et al., 2017, 2018, 2019) suggest that the mechanisms of adult Pacific salmon mortality under cumulative stressors are probably associated with physiological impairment and a decreased threshold for multiple infections. Most notably, nearly all handled fish held in warm water died prior to the spawning period for this population, regardless of capture locale. These results are highly relevant as climate change continues to warm salmon bearing rivers across their range (Isaak et al., 2012; Reed et al., 2011).

4.1 | Fishery and thermal stressor effects on fish survival

Our survival results align with those described by Martins et al. (2012), in that model-averaged survival for Adams sockeye (the same stock complex that is studied in the present study) at 14°C was 90%–100% (standard error range) in seawater and 60%–100% in the river. At 18°C, however, Martins et al. (2012) estimate survival between 80%–90% in seawater and 15%–25% in the river, which more closely resembles survival rates of nonbiopsied controls in the present study. This discrepancy suggests that experimental holding may compound handling effects at high temperature. Regarding improved survival of fish released in the ocean versus the river, differences in salinity and temperature of the recovery environment, predation, repeat capture probability, and gear type (Raby et al., 2015), as well as infection burdens (present study), collectively influence survival likelihood and will vary by capture locations. Far more fish died in the first 24 h following collection from the river than from the marine environment in the present study. This finding points to proximal causes of mortality in a subset of river-exposed fish, such as cardiac collapse or anaerobiosis (Eliason et al., 2011; Fenkes et al., 2016; Raby et al., 2015). Linkages between infections, immune suppression (Tort, 2011), and host aerobic or osmotic capacity should be assessed in future studies as observed responses could be associated with compromised osmoregulatory or aerobic capacity from previous and current infections (i.e., disease effects on aerobic resiliency to fishery stress; Ewing et al., 1994; Nematollahi et al., 2003; Bradford et al., 2010a; Bradford et al., 2010b).

4.2 | Stressor effects on infection trajectories and host responses

Temperature has been coined the "master" factor (Fry, 1971) due to its strong influence on fish physiology and behaviour (Pacific salmon e.gJain & Farrell, 2003; Jeffries et al., 2012, 2014; Kocan et al., 2009; MacNutt et al., 2004), including the potential to exacerbate fishery impacts (Gale et al., 2013). Elevated river temperatures similar to those applied in this study are already impacting sockeye salmon populations during freshwater migrations (Patterson et al., 2007). Regardless of collection location (and hence initial infection status), handled fish held at an ecologically relevant 18°C did not survive to the spawning period of their population (≥4 weeks). This result has drastic implications for the fate of released sockeye bycatch in the lower Fraser River when temperatures are high (even with minimal handling) and supports the closure of fisheries when waters are warm to improve annual post-release survival (Martins et al., 2012). Additionally, given that river-captured fish survived poorly at high temperature even in the absence of handling, river-derived infections are probably causal factors of natural mortality at high temperature as well.

High temperatures have been shown here and previously to accelerate infection development (Bradford et al., 2010; Miller et al., 2014; Teffer et al., 2018; Wagner et al., 2005), modulate immune gene expression (Jeffries et al., 2012; Teffer et al., 2018), and occur concurrently with increased mortality of wild adult Pacific salmon during freshwater migration (Hinch et al., 2012; Keefer et al., 2008; Martins et al., 2012). Thermal stress enhanced bacterial and parasitic infections that either maintained high loads over time or showed accelerated infection development (i.e., higher loads at later time points). Enhanced infection development is a common response of infectious agents to increased temperature (e.gBettge et al., 2009; Kocan et al., 2009; Mitchell et al., 2005) in combination with host cellular stress responses (Jeffries et al., 2014; Kassahn et al., 2009). Thermally driven amplification of RIB did not differ between marine- and river-captured fish, suggesting that collective infection development is independent of initial infection status. RIB was initially low for fish from both capture locales at high temperature, followed by a sharp increase especially among gillnetted fish and early mortalities. Infectious agent community composition, richness, and structure did vary between marine- and river-captured fish, and thermal impacts on loads differed among agent species. The mechanisms of mortality therefore depend at least partially on capture locations, as well as immune impairment (Dittmar et al., 2014) and variable direct thermal impacts on pathogens (e.gAihua & Buchmann, 2001; Groberg et al., 1978; Udey et al., 1975).

Thermally stressed fish showed greater expression of stress and immune genes such as complement, iron metabolism, and inflammatory responses rather than adaptive immunity and antiviral responses. Our survival results suggest that these responses are inadequate to prevent mortality at high temperature, given that nearly all thermally stressed fish died early. Chronic stress is known to be immunosuppressive, including negative impacts of cortisol on antibody production and inflammatory responses through glucocorticoid receptor suppression (Tort, 2011; Zwollo, 2018). Indeed, thermally stressed marine-captured fish showed an initial positive association with GR2 expression in gill but then little correlation in

TABLE 5 Results from (a) permutational multivariate analysis of variance and (b) principal component analysis (PCA) of the expression of 22 stress and immune gene biomarkers (Table 1) in adult sockeye salmon from marine or river environments

	PerMANOVA			
A	**H**	**G**	**H:G**	**RIB**
Marine				
Wk 0	$r^2 = 0.24$, $p < .001$	$r^2 = 0.07$, $p < .001$	$r^2 = 0.06$, $p < .001$	
Wk 1	$r^2 = 0.29$, $p < .001$	$r^2 = 0.02$, $p = .040$	$r^2 = 0.03$, $p = .022$	
Wk 2	$r^2 = 0.42$, $p < .001$			$r^2 = 0.17$, $p < .001$
Wk 3[b]	NA	$r^2 = 0.19$, $p < .001$	NA	
Wk 4[a,b]	NA	$r^2 = 0.08$, $p = .028$	NA	
River				
Wk 0	NA	$r^2 = 0.06$, $p = .003$	NA	$r^2 = 0.04$, $p = .048$
Wk 1	$r^2 = 0.06$, $p = .006$	$r^2 = 0.05$, $p = .032$		$r^2 = 0.23$, $p < .001$
Wk 2	$r^2 = 0.22$, $p < .001$			$r^2 = 0.32$, $p < .001$
Wk 3[a,b]				$r^2 = 0.51$, $p = .001$

	PC1					
B	**V**	**LM**	**H**	**G**	**H:G**	**RIB**
Marine						
Wk 0	25	$r^2 = 0.14$, $p = .019$	1.23 ± 0.84, $p = .151$	0.83 ± 0.91, $p = .365$	-3.14 ± 1.15, $p = .009$	
Wk 1	35	$r^2 = 0.44$, $p < .001$	-3.88 ± 0.77, $p < .001$	-1.62 ± 0.76, $p = .037$		
Wk 2	41	$r^2 = 0.70$, $p < .001$	3.27 ± 0.56, $p < .001$			4.40 ± 0.89, $p < .001$
Wk 3[b]	28	$r^2 = 0.04$, $p = .287$				
Wk 4[a,b]	26	$r^2 = 0.34$, $p = .004$	NA	-2.47 ± 0.75, $p = .003$	NA	
River						
Wk 0	26	$r^2 = 0.09$, $p = .062$				
Wk 1	31	$r^2 = 0.59$, $p < .001$				5.14 ± 0.82, $p < .001$
Wk 2	36	$r^2 = 0.66$, $p < .001$				8.29 ± 1.27, $p < .001$
Wk 3[a,b]	41	$r^2 = 0.69$, $p < .001$				-10.46 ± 1.85, $p < .001$

Note: Linear models (LM) were used to identify factors contributing to the variation in each PC axis (V = % variance explained by PC). Models describe weekly variation in gene expression in association with stressors (high temperature 18°C [H], gillnet entanglement [G], and their interaction [H:G]), relative infection burden in gill (RIB), and sex (S). Nonsignificant ($p > .05$) models and factor parameters ($\beta \pm$ s.e.m.) are not shown, or in grey if components of significant interactions in LMs.

[a]Includes survivors.

[b]Cool water only.

following weeks when inflammatory biomarkers were more prominently featured. An acute stress response, as would be expressed follow handling, can divert immunity toward innate responses (Demers & Bayne, 1997; Zwollo, 2018). Regarding the mechanisms of cumulative stressor effects on the survival of marine-captured fish, acute stress responses may be maladaptive in thermally stressed salmon that are already immune-compromised (Jeffries et al., 2012) and heavily infected (present study).

River-derived infections may reduce the ability of hosts to maintain low infection burdens following acute fishery stress given that infection development following gillnetting (at optimal temperature) was accelerated among river-exposed fish only. Our results showed that river-captured fish demonstrated divergence from marine-captured fish in stress and immune gene expression, with profiles associating with infections more than stressors. Conversely, gene expression profiles of marine-captured fish were more strongly

PC2						PC3			
V	LM	H	G	RIB	S	V	LM	G	S
14	$r^2 = 0.70$, $p < .001$	−3.39 ± 0.38, $p < .001$				12	$r^2 = 0.39$, $p < .001$	1.21 ± 0.54, $p = .030$	−1.17 ± 0.36, $p = .002$
17	$r^2 = 0.11$, $p = .037$	1.73 ± 0.67, $p = .012$				10	$r^2 = 0.06$, $p = .132$		
20	$r^2 = 0.16$, $p = .035$	1.68 ± 0.66, $p = .015$		−2.21 ± 1.04, $p = .040$		10	$r^2 = 0.01$, $p = .362$		
17	$r^2 = 0.30$, $p = .017$	NA	2.49 ± 0.71, $p = .002$			16	$r^2 = 0.18$, $p = .073$		
15	$r^2 = 0.10$, $p = .131$					13	$r^2 = 0.00$, $p = .952$		
15	$r^2 = 0.18$, $p = .008$	NA	−1.82 ± 0.53, $p = .002$			10	$r^2 = 0.21$, $p = .003$	−1.44 ± 0.41, $p = .001$	
22	$r^2 = 0.07$, $p = .145$					11	$r^2 = 0.02$, $p = .328$		
27	$r^2 = .49$, $p < .001$	3.53 ± .69, $p < .001$			1.71 ± 0.71, $p = .025$	8	$r^2 = 0.01$, $p = .389$		
21	$r^2 = 0.09$, $p = .252$					15	$r^2 = 0.05$, $p = .322$		

influenced by temperature and showed divergent responses to fishery stress. The survival and host responses of marine-captured fish support additive effects of thermal and capture stress previously documented in sockeye and coho (*Oncorhynchus kisutch*) salmon (Gale et al., 2011; Teffer et al., 2019), but this effect may only occur in a low infection scenario. Overall, a divergence in expression profiles depending on river-exposure suggests that alternate host response tactics to capture and thermal stressors are contingent on capture location (marine or freshwater), with river entry associated with enhanced infection burdens and decreased longevity.

Heightened demands on the immune system following river entry are evidenced by the strong correlation between infection burden and immune gene expression in river-exposed fish. Up to eight different bacterial and parasitic agents were associated with early mortality of river-exposed fish, with a greater influence of parasitic agents (*P. minibicornis*, *I. multifiliis*, *L. salmonae*) in cool water

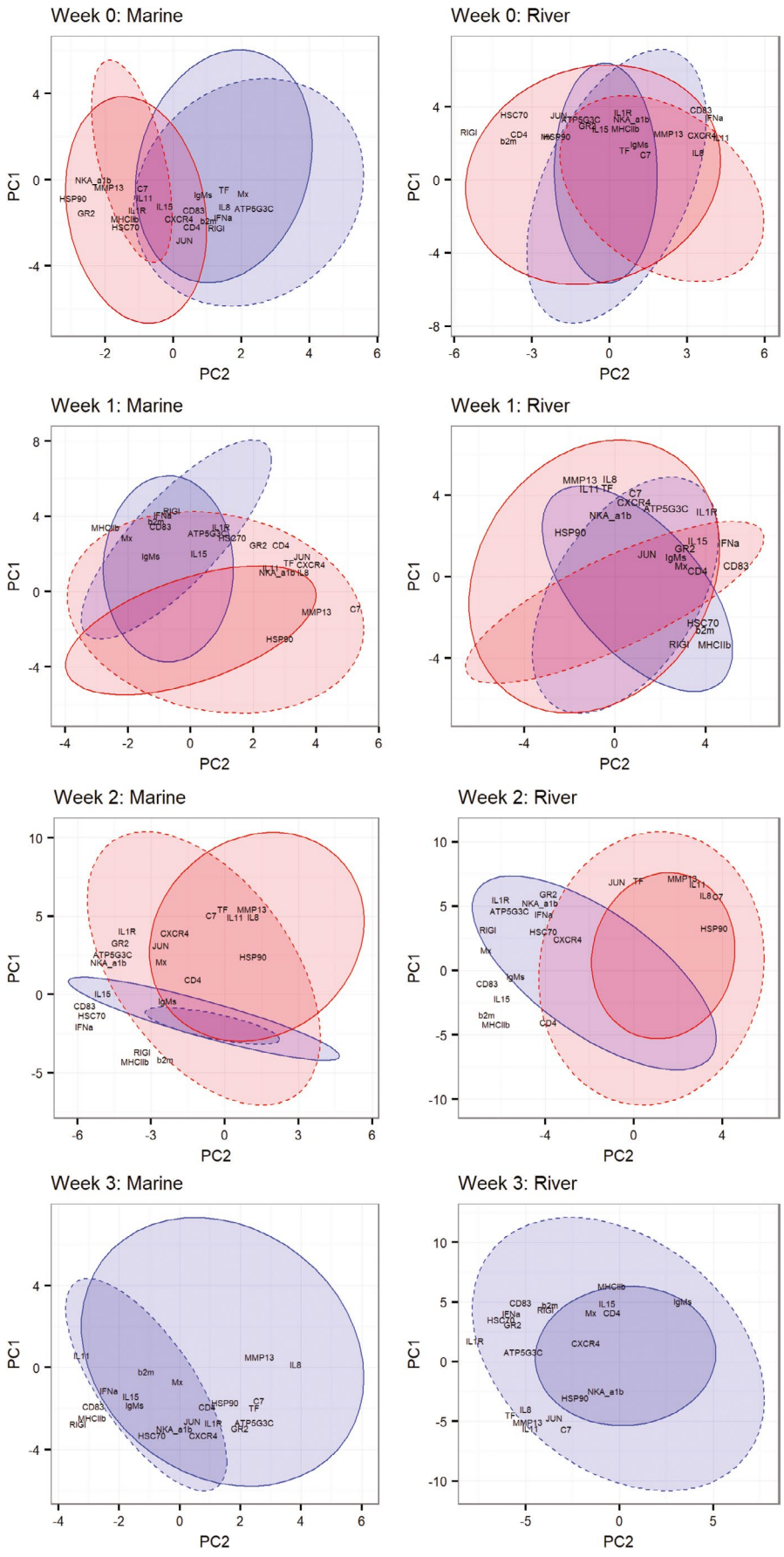

FIGURE 5 Principal component analysis of stress and immune gene expression in adult sockeye salmon gill at 14°C (blue) and 18°C (red) from seined (dashed) or gillnetted (solid) fish (95% confidence intervals). Abbreviations are listed in Table 1

and bacterial agents (*A. hydrophila*, *A. salmonicida*) in warm water. Each of these parasitic agents have been associated with migratory failure, premature mortality, and (or) thermal stress in Pacific salmon populations (Bradford et al., 2010; Crossin et al., 2008; Miller et al., 2014; Traxler et al., 1998). Mortality of other *Oncorhynchus* species experimentally challenged with *A. hydrophila* and *A. salmonicida* was previously demonstrated at high temperature (Groberg et al., 1978). Interestingly, relationships with mortality do not necessarily align with positive temperature-load correlations, as thermal stress also increased the loads of several agents that were not associated with host mortality. Therefore, although the loads of some agents, including bacteria, may be useful virulence indicators at high temperature, other agents may have pathogenic properties at low infection intensities. For example, the tolerance thresholds for infection development for some agents may be decreased in hosts at high temperature due to physiological impairment (Alcorn et al., 2002) or an inhospitable host environment (Thomas & Blanford, 2003). Interactions between pathogens may also be altered by thermal stress through changes in the production of public goods or cross-reactive immune responses to agents with differing thermal tolerances (Alizon et al., 2013). Our data are limited to changes in qPCR load levels and not metabolites, so the nature of potential interpathogen relationships is beyond the scope of this study. We can only comment on changes in community composition that preceded mortality, such as the influx of *Aeromonas* species among thermally stressed fish concurrently with elimination of *F. psychrophilum* and high levels of host mortality.

Early mortality of marine-sourced fish was associated with *A. hydrophila* but only in warm water. This Gram-negative bacteria causes haemorrhagic septicaemia in fish and gastroenteritis in humans (Harikrishnan & Balasundaram, 2005; Janda & Abbott, 2010). Among marine-captured fish, *A. hydrophila* only occurred at high temperature and only in fish that tested negative for *F. psychrophilum*, a negative correlation that partially explains the positive effect of *F. psychrophilum* on marine fish survival in warm water. Competitive exclusion (Sofonea et al., 2015), however, is unlikely since coinfections of these bacteria occurred in river-exposed fish at both temperatures, and frequently at high *F. psychrophilum* loads. *A. salmonicida* was the more prevalent *Aeromonas* species in the gills of river-exposed fish and was associated with early mortality at high temperature; *A. salmonicida* showed no clear load relationship with *F. psychrophilum*. Our results also demonstrate mortality of river-captured fish in association with RLO, the bacterial agent of Strawberry Disease and an endosymbiont of *I. multifillis* that is rarely directly linked to salmon mortality (Lloyd et al., 2008; Sun et al., 2009). Further research should be directed toward describing the relationship between bacterial and other agents in the context of temperature in freshwater, especially given warming trajectories in salmon-bearing rivers (Ferrari et al., 2007; Petersen & Kitchell, 2001) that may alter pathogen community structure in migrating adults.

The life history of an infectious agent plays a major role in disease dynamics. As has been observed previously (Bass, 2018; Bass et al., 2017), the freshwater myxozoans *C. shasta* and *P. minibicornis* were highly prevalent in fish collected in the river, but not entirely absent in marine-collected fish. Resilience of spores in the Fraser River plume in the Strait of Georgia or retention of myxozoan infections from juvenile life stages may explain marine detections (probably the former, see Mahony et al., 2017; Thakur et al., 2018; Tucker et al., 2018). These agents require an intermediate freshwater polychaete host, which releases infective myxozoan spores into the river during salmon migrations (Bartholomew et al., 1997, 2006). Greater prevalence of these myxozoans in river-exposed fish contributed to heavier overall infection burdens and richness; continued river exposure if fish were not transported to the laboratory would further increase infective dosage (Benda et al., 2015; Ray et al., 2012). New infectious agents accumulated during river entry may elicit host responses that were not activated for carrier-state infections, and this enhanced response may be deleterious due to cross-reactive immune responses (Alizon et al., 2013; Alvarez-Pellitero, 2008). The demands of osmoregulatory transition and new infections probably reduce the capacity of adult salmon to effectively respond to thermal, fishery, and other stressors, especially given limited endogenous resources (Kiessling et al., 2004).

4.3 | Broader implications and future directions

Our results characterize differences in post-release survival between marine or freshwater environments depending on gear type, but also demonstrate differences between naturally accumulated low and heavy infection groups. We chose to allow river-captured fish to complete early river migration and acquire a realistic "dose" of river-derived infections to improve the ecological context of our findings. The experience of early river migration requires simultaneous osmoregulatory transition and predator and fishery avoidance with associated energetic costs, which may have contributed to the physiological differences observed between collection groups. The experience of river-captured fish in this study accurately simulated capture and release in the lower river, which was crucial to ascertaining the role of natural infections in host responses to cumulative stressors. Seine nets are a preferred gear type for fishers in the marine environment; working with commercial fishers for this study meant that marine-collected fish needed to be experimentally gillnetted in the laboratory for our comparison. River-captured fish were also gillnetted in relatively cool river water and then held in warm or cool water, whereas treatment temperatures for marine-captured fish matched holding temperatures. Our results demonstrate substantially elevated infectious loads in the gills of an adult population of sockeye salmon following river entry despite these logistical constraints, which was associated with altered genomic responses to stressors and pathogen community trajectories. Future studies could attempt to sample and track migrating fish in the wild and recapture them at upstream points to identify how the context of river migration, such as energetic costs and additional pathogen exposures upstream, factor into host responses and survival outcomes.

Several key findings can be more broadly applied in the context of disease ecology. Thermal stress was again identified as a "master factor" influencing host resilience (Fry, 1971) but our data add multiple infection burdens and altered pathogen community composition to the array of known thermal impacts on wild exothermic host species. Projected temperature increases in freshwater, marine, and terrestrial habitats (Isaak et al., 2012; Poloczanska et al., 2013; Walther et al., 2002) can therefore be expected to alter host-pathogen relationships, not only within hosts as demonstrated by this study, but at regional spatial scales if host or geographic ranges are subsequently shifted (Altizer et al., 2013; Engering et al., 2013). The dependence of genomic stress responses on infection status is intriguing and alludes to the allocation of host resources to responses that maximize host survival odds in the absence of infections (e.g., protein stabilization and repair) versus under heavy infection burdens (e.g., immune modulation, inflammation). Hosts are therefore modulating genomic responses to external conditions based on internal factors – specifically immune activity and pathogen dynamics – thereby supporting a basic host-pathogen(s)-environment framework. However, the plurality of "pathogen" adds complexity to this triangle as host-pathogen relationships are the product of coevolution, and may be unbalanced by shifts in both pathogen diversity (species, genotypes) and temperature (Mitchell et al., 2005; Sofonea et al., 2017). Our results provide a foundation of data that can be used to test specific hypotheses regarding interpathogen dynamics and the mechanisms of host resource allocation to manage stress under different infection levels.

Infections are a natural component of ecosystems and can drive the evolutionary basis of wild animal migrations. However, anthropogenic changes to these conditions may alter the effectiveness of life history strategies (Altizer et al., 2011). As climate change continues to impact freshwater and marine thermal regimes, pathogen virulence will also change, challenging our ability to manage wild salmon productivity and maintain indigenous, commercial, and recreational fisheries (Altizer et al., 2013; Jacob et al., 2010; McDaniels et al., 2010; Reed et al., 2011). Our study offers insight into the mechanisms of premature mortality of adult sockeye salmon. We identified significant differences in the survival and infection burdens of sockeye salmon based on river exposure. Multiple infections responded to thermal stress with increases in the loads of most pathogens evaluated, whereas gillnetting only increased infections among river-exposed fish. Subtle differences in community composition over time and between capture locations, temperatures, and gear types suggested that the infectious agent communities in surviving fish are responsive to stressors, increase with time, but were stabilized at the population level by host mortality under heavy infections (Bass et al., 2017; Dolan et al., 2016; Teffer et al., 2017, 2018). Host resilience to single stressors was reduced after river entry and probably pathogen-mediated, whereas cumulative stressors are detrimental regardless of river exposure and initial pathogen loads. Importantly, nearly all handled fish held at an ecologically relevant elevated water temperature died prior to the spawning period, even with behavioural thermoregulation (a temporary decrease in temperature). Given these results, fisheries managers should continue to reduce or cease fishing pressure when rivers are warm and focus fishing effort in the marine environment using minimally invasive gear types. Subject to the socioeconomic constraints of fisheries, including cost, culture, and logistics, strategic fishing prior to river entry or only when rivers are cool will probably reduce en route losses of wild sockeye salmon and more effectively preserve populations in a warming future.

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DATA AVAILABILITY STATEMENT

All data used in the analyses presented in this manuscript can be publicly accessed through University of Victoria's Dataverse Research Data Collections at https://doi.org/10.5683/SP3/NFKUGG.

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