

Infections, fisheries capture, temperature, and host responses: multistressor influences on survival and behaviour of adult Chinook salmon¹

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Abstract: Infectious disease dynamics of wild Pacific salmon are poorly understood and may play a prominent role in recent declines of Chinook salmon (*Oncorhynchus tshawytscha*) populations. Multiple stressors influence migration success of adult salmon, such as rising river temperatures and capture and release by fisheries, and likely modulate infection development. To understand how these factors impact survival and migration behaviour of adult salmon in fresh water, we conducted simultaneous holding and telemetry studies with gillnet treatments and nonlethal biopsy. Laboratory fish were held and treated in either cool (9 °C) or warm (14 °C) water. High temperature reduced survival but did not amplify simulated gillnetting effects. Gillnetting reduced migration rate and distance traveled upriver and increased infection burdens, but had no effect on longevity. Heavy infections were associated with reduced longevity and faster migrations. Blood properties and immunity were associated with stressors, survival, and infections. These results improve our predictive capability regarding how stressors can reduce migration success and longevity of Pacific salmon, demonstrating multiple impacts of infections and adding to growing knowledge of disease dynamics in wild fish populations.

Résumé : La dynamique des maladies infectieuses chez les saumons du Pacifique sauvages n'est pas bien comprise et pourrait jouer un rôle prédominant dans les baisses récentes des populations de saumons quinnats (*Oncorhynchus tshawytscha*). Différents facteurs de stress influencent le succès de migration des saumons adultes, comme l'augmentation de la température des rivières et la capture suivie de la remise à l'eau dans les pêches, et ces facteurs modulent probablement le développement d'infections. Pour comprendre l'incidence de ces facteurs sur la survie et le comportement migratoire de saumons adultes en eau douce, nous avons mené des études de rétention et de télémétrie simultanées avec des traitements au filet maillant et des biopsies non létales. En laboratoire, des poissons ont été maintenus et traités en eau froide (9 °C) ou chaude (14 °C). La température élevée réduit la survie, sans toutefois amplifier les effets simulés du filet maillant. Le traitement au filet maillant réduit la vitesse et la distance de migration parcourue vers l'amont et accroît les charges infectieuses, mais n'a pas d'effet sur la longévité. Des infections à forte charge infectieuse sont associées à une longévité réduite et des migrations plus rapides. Les propriétés du sang et l'immunité sont associées à des facteurs de stress, à la survie et aux infections. Ces résultats améliorent la capacité de prédire comment des facteurs de stress peuvent réduire le succès de migration et la longévité des saumons du Pacifique, démontrant différents impacts des infections et rehaussant les connaissances sur la dynamique des maladies dans les populations de poissons sauvages. [Traduit par la Rédaction]

Introduction

Little is known about the infectious disease dynamics of wild Pacific salmon (*Oncorhynchus* spp.) and their associated impacts on population productivity (Riddell et al. 2013; Miller et al. 2014). The unique life history of Pacific salmon comprises multiple life stages and long-distance migrations spanning thousands of kilometres across marine and freshwater habitats (Groot and Margolis 1991). These characteristics add complexity to host–parasite relationships and make infectious disease development and associated mortality and migration failure difficult to observe in the wild

(Bakke and Harris 1998). Challenging environmental conditions and gradients likely influence infection development and host stress and immune responses to enhance the likelihood of mortality during migration (Snieszko 1974; Miller et al. 2014).

Chinook salmon (*Oncorhynchus tshawytscha*) is one of several species of Pacific salmon to have experienced population declines within Canada (Riddell et al. 2013) and the United States (Myers et al. 1998; Heard et al. 2007; Hoekstra et al. 2007). In British Columbia, Canada, habitat degradation and overfishing contributed to declines of Fraser River Chinook salmon starting in the 1950s, with some recovery following enhanced regulations put

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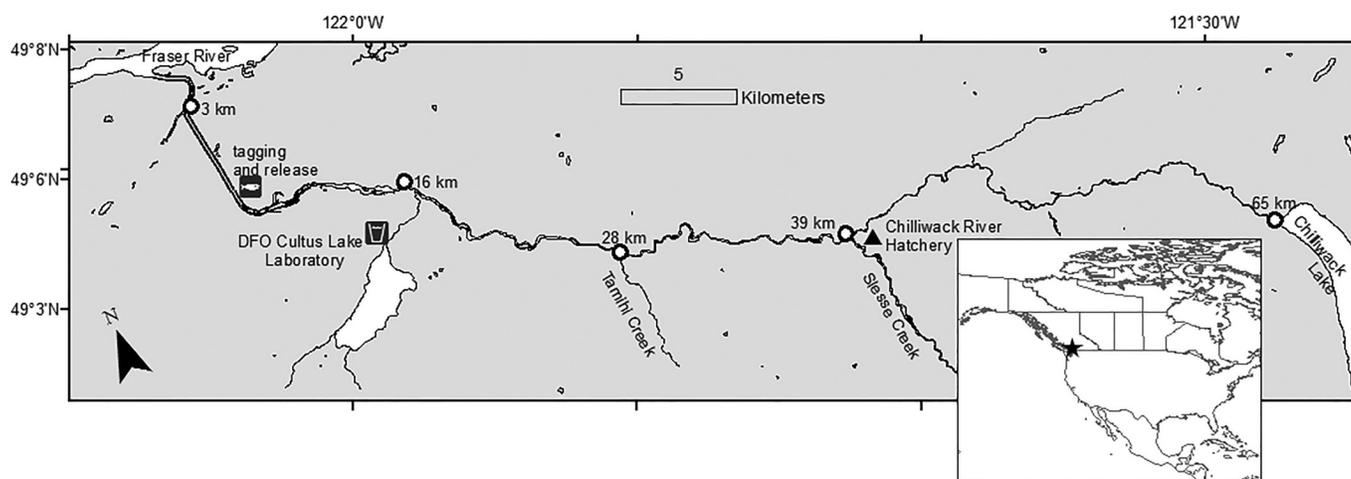
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Fig. 1. Map of Chilliwack River including Chilliwack River Hatchery (collection site), DFO Cultus Lake Laboratory (holding facility), tagging and release location for tagged fish, and telemetry receiver locations.



forth by the Pacific Salmon Treaty in the 1980s (Fraser et al. 1982; DFO 1999). Since then, however, declines have been observed over recent generations (Riddell et al. 2013). Continued losses despite management efforts suggest that some causes are beyond those alleviated by current regulatory influence. Infectious disease processes enhanced by mounting environmental and anthropogenic stressors may help to explain these continued declines through their impacts on survival and migration success (Miller et al. 2011; Teffer et al. 2017).

Although many factors can contribute to salmon population declines (Hoekstra et al. 2007) with influences affecting all life stages, en route and prespawn mortality of adult Pacific salmon during their once-in-a-lifetime spawning migration can have drastic population-level effects (Bowerman et al. 2016), including increased extinction risk (Reed et al. 2011; Spromberg and Scholz 2011). Adult salmon cease feeding prior to entering fresh water and rely on endogenous energy reserves to fuel migration and spawning, after which rapid senescence results in natural death (Groot and Margolis 1991; Kiessling et al. 2004; Hruska et al. 2010). This life history strategy places a great deal of weight on migration and spawning success, as failure to arrive at spawning grounds and spawn reduces an individual's fitness to zero. Efforts to improve our predictive capabilities with respect to early mortality of adult salmon can aid fishery managers in decision-making and must be informed by studies describing its associated mechanisms (Macdonald et al. 2010) and cumulative effects of multiple factors (Miller et al. 2014).

Stressors beyond those historically encountered during migration, such as high river temperatures (Morrison et al. 2002; Martins et al. 2011; Altizer et al. 2013) and fishery nonretention (i.e., capture and release; Baker and Schindler 2009; Raby et al. 2015; Teffer et al. 2017), can elicit a stress response in returning adults. High temperature, for example, can cause osmoregulatory impairment, increased metabolic stress, and altered immune gene expression in adult sockeye salmon (*Oncorhynchus nerka*) (Jeffries et al. 2012). A stress response can therefore alter host-pathogen relationships by reducing the resilience and resistance of hosts to infections, depending on the type and magnitude of the stressor (Barton 2002; Bowers et al. 2008; Mateus et al. 2017); additionally, chronic high temperature can directly increase pathogen replication (Ewing et al. 1986; Bettge et al. 2009) or infectious dose in the environment (Stocking et al. 2006). Fishery nonretention is an example of an acute stressor and occurs when captured fish are not the target species of a fishery and are released (i.e., bycatch, discards). Air exposure and handling during the release process contribute to oxygen deprivation, equilibrium

loss, physiological stress, and reduced antimicrobial defenses (Svendsen and Bøgvold 1997; Gale et al. 2011; Raby et al. 2015). Water temperature plays a large role in determining postrelease survival of Pacific salmon in addition to its strong independent effects on physiology and survival (Martins et al. 2012; Jeffries et al. 2012b; Gale et al. 2013). Co-occurrence of these stressors is expected to increase as water temperatures experienced by adult Pacific salmon continue to rise due to climate change (Ferrari et al. 2007; Isaak et al. 2012). The degree to which individual and combined stressors impact disease development in wild adult salmon will be a function of fish condition as well as the responses and interactions of an array of infectious agents.

Pacific salmon, like all organisms, accumulate infectious agents throughout their lives, with a spike in pathogen richness following freshwater re-entry (Benda et al. 2015; Bass et al. 2017). These infections then develop at different rates depending on resistance factors, such as pathogen recognition capability (Bayne and Gerwick 2001; Alvarez-Pellitero 2008; Dolan et al. 2016), physiological resilience (including injury, aerobic capacity, and energy density; Hinch et al. 2012; Gale et al. 2014; Mateus et al. 2017), as well as pathogen type and co-infection (Cox 2001; Hori et al. 2013). Our knowledge of stress-dependent disease dynamics of wild Pacific salmon is in its infancy, especially regarding multiple stressors experienced by migrants (but see Miller et al. 2014; Teffer et al. 2017), with far more disease information available from culture settings (Kurath and Winton 2011). However, recent technological developments have brought new tools, such as high-throughput quantitative polymerase chain reaction (HT-qPCR), to the forefront of disease ecology. This technology facilitates rapid quantification of dozens of gene transcripts at once, targeting both the host and infectious agents in very small amounts of tissue, for a comprehensive evaluation of multiple infections and pathways to disease development in wild animals.

A recent study used HT-qPCR to describe the pathogen community carried by adult Chinook salmon during their return migration in southwestern British Columbia, sampling multiple populations and sites in marine and freshwater habitats (Bass et al. 2017). This work, in addition to identifying 20 unique bacteria, viruses, protozoa, and other microparasites in the tissues of 82 adult Chinook salmon, demonstrated sex-specific differences in infectious loads and correlated infection intensities of several agents with indices of morbidity and advanced senescence. Furthermore, prevalence and loads of different agents were spatially variable across marine and freshwater collection sites and temporally variable within one site (Chilliwack River Hatchery, Chilliwack, British Columbia; Fig. 1). This variability could be due to pathogen-induced mortality

(loss of diseased hosts from the population due to pathologies from infectious agents as well as deleterious host immune responses), effective host immune responses (reduced loads and detection probabilities over distance or time), or life cycle characteristics of individual pathogens (freshwater or seawater tolerance, timing of shedding or spore release). Empirical study of how pathogen loads change over time within individuals is needed to clarify these trends.

The findings of Bass and colleagues (2017) provide baseline data for an assessment of infection responses of adult Chinook salmon to high river temperature and capture and release from fisheries. Adult Chinook salmon en route to spawning grounds are frequently captured as bycatch in sockeye and other salmon fisheries and must be released. The extent to which physiological stress and injuries associated with this interaction alter migration behaviour, infection development, and longevity of Chinook salmon is unknown. The Fraser River is the largest producer of Chinook salmon in Canada, which are distributed throughout most of the watershed (Fraser et al. 1982), returning in three run-timing groups (spring, summer, fall) that comprise four major stock complexes (Parken et al. 2008). The fall timed run is dominated by “ocean type” Harrison River fish that generally spawn from mid-October to mid-November. Harrison and Pitt river fish comprise the source population for the fall Chinook run returning to the Chilliwack River Hatchery, which was sampled on two occasions (10 and 21 October 2014) by Bass and colleagues (2017). Given that temperatures in the Fraser River are increasing as a result of climate change and will likely affect fall run Chinook salmon in coming decades (Patterson et al. 2007; Hague et al. 2011), cumulative impacts of fishery and thermal stressors are increasingly relevant.

We conducted a two-phase study to examine independent and cumulative effects of high water temperature and fisheries capture and release on infection development, physiology, migratory behaviour, and survival of adult Chinook salmon. The first phase of our study used a laboratory holding approach with repeated biopsy of individuals to track changes in infections and host responses over time. The second phase worked to compensate for potential impacts of holding. We tagged and released biopsied fish into the lower Fraser River to relate infectious loads with longevity and behaviour in the river and the likelihood of returning to hatchery “spawning grounds.” For both experiments, we exposed a subset of fish to a standardized gillnet entanglement treatment, a frequently used gear-type used in the Fraser River. Furthermore, laboratory fish were treated and held in either cool water typical of historical river temperatures during migration or warm water representing a climate change scenario, allowing for evaluation of interacting effects of multiple (fishery and thermal) stressors. Our objectives were to (i) evaluate the effects of gillnet entanglement and air exposure on survival, behaviour, and migration success, (ii) evaluate the impact of thermal stress on survival and potential interactions with a fishery stressor, (iii) determine how stressors modulate pathogen productivity over time, (iv) relate stressors, survival, infection intensities, and host immune and stress responses to characterize differences in response profiles of survivors relative to early mortalities, and (v) use pathogen loads to predict migration behaviour in the river, evaluating sex-specific effects and any influence of fishery stressors.

Methods

To better understand the stress-related disease dynamics of adult Chinook salmon, we conducted laboratory holding and telemetry studies of adult Chinook salmon from the Chilliwack River Hatchery in October and November of 2013. Returning adult salmon enter the hatchery from a neighbouring creek where they can hold or move freely between the river and raceway until

maturity, at which time they are collected from raceways, sacrificed, and spawned by hatchery personnel. Although differences in the pathogen dynamics and immune competency between wild and hatchery-produced salmon may exist and are currently under investigation (K. Miller, unpublished data), hatchery-produced fish are released as juveniles and experience similar environmental conditions in both marine and freshwater habitats as wild fish do (Naish et al. 2007). Therefore, pathogen burdens of hatchery-produced and wild adult salmon are likely similar early after freshwater re-entry, making hatchery-produced fish an effective model for understanding pathogen dynamics in salmon populations.

For both holding and telemetry studies, fish in good condition (vibrant, minimal scale loss) were dip-netted from raceways and immediately placed into truck-mounted tanks for transport to the Cultus Lake Salmon Research Laboratory, Cultus Lake, British Columbia, or to the telemetry release location (~40 min transport; Fig. 1). Collection for laboratory holding took place on 9 and 10 October 2013, while collection for tagging took place throughout the freshwater residence period of this population (3, 7, 17, and 24 October 2013). Transport tanks were filled with cold (8–9 °C), sand-filtered, and UV-treated water, equipped with air stones, and monitored continuously for dissolved oxygen and temperature.

Laboratory holding

Upon arrival at the Cultus Lake laboratory, fish were sequentially distributed among 12 holding tanks (8000–10 000 L) of equal temperature to the hatchery (~9 °C); tanks were equipped with air stones and a submerged pump, which provided a slow current around the tank periphery to encourage fish to swim during holding (approximately 1 body length·s⁻¹). After 48 h acclimation, the temperature of half of the tanks was increased incrementally over 48 h from 9 to 14 °C. Two temperature groups represented either a “cool” thermal experience, which reflected current Chilliwack River and hatchery temperatures during migration (~9 °C), or a “warm” thermal experience (~14 °C) that represented potential maximum temperatures encountered during migration and those expected to affect Chinook salmon populations under projected changes to the hydrology of the Fraser River watershed (Barnes and Magnusson 2000; Morrison et al. 2002).

Four days after collection (time point henceforth referred to as “T1”), three tanks from each temperature group were exposed to standardized gillnet entanglement and air exposure treatment; each fish was dip-netted from its holding tank and immediately submerged in a treatment tank, with the opening of the dip-net facing a taught 8-inch (1 inch = 2.5 cm) monofilament gill net mounted within a wide frame; upon exiting the bag of the dip-net, fish immediately swam into and were “caught” in the gill net. After 20 s of sustained entanglement under water, the fish and gillnet were removed from the water and held in air for 1 min while the fish was disentangled from the net. Following air exposure, the fish was placed into a foam-lined trough with water flowing continually over the body and gills. Blood was extracted from the caudal vasculature (21-gauge needle, lithium heparinized Vacutainer), a small gill biopsy (two to three gill filament tips) was taken using sterile end cutters and preserved in 1.5 mL RNAlater solution, a “spaghetti”-style tag (Northwest Marine Technology, Shaw Island, Washington) was secured in the dorsal musculature, and any external wounds were recorded. The fish was placed into a recovery tank for up to 30 min and then put back into its holding tank. Time in the trough was ~2 min, and water temperature throughout treatment, biopsy, and recovery was the same as the holding temperature. Control fish were similarly dip-netted from holding tanks but immediately submerged in the sampling trough for blood and gill sampling following the procedures described above, with no gillnet or air treatment.

Holding tanks were monitored at ≤4 h increments from 0800 to 2400 for fish condition and water quality. Any fish displaying

signs of morbidity (lethargy, gulping, loss of equilibrium) was removed and euthanized by cerebral concussion and cervical dislocation. Four days after nonlethal biopsy and treatment, all surviving fish were sacrificed (time point henceforth “T2”); this time frame was in alignment with the telemetry component of the study, given that most tagged fish completed their migration from the release location to the hatchery within 4 days. Blood was again extracted from the caudal vasculature, and tissue samples (~0.5 mg) were collected from various organs using sterile tools and included gill (two to five gill filament tips), muscle (at lateral line even with dorsal fin, including skin and red and white muscle), liver, spleen, heart (ventricle), head kidney, and brain (every other fish). Blood and tissue sampling was conducted as above for prematurely morbid fish (only moribund or freshly dead) and all sacrificed fish. Tissue samples were preserved in 1.5 mL RNAlater solution (whole brain in 3 mL), allowed to fix at 4 °C for 24 h, and then frozen at -80 °C until analysis. Fish length, mass, and individual organ masses were recorded as well as aspects of gross pathology and condition, such as the presence of macroparasites and lesions, scale loss and injuries, and organ discoloration.

Telemetry

For the telemetry component of our study, we aimed to test whether fisheries capture and release and infectious agents (measured in nonlethal gill samples taken at release) were associated with longevity, migratory success, and migration time. Longevity was defined as the number of days surviving in the Chilliwack River following release, migratory success was defined as detection at the Tamihi Rapids radio receiver (river kilometre (rkm) 28; Fig. 1), which was considered the downstream extent of suitable spawning habitat (snorkeling observations, Fisheries and Oceans Canada (DFO) Stock Assessment, personal communication, 2013), and migration time was calculated as the difference between the time of the first detection at rkm 28 and the time of release.

Fish were transported by truck from the hatchery to a release location 8 km upstream from the mouth of the Chilliwack River (Fig. 1). Temperature in the river during the study ranged from 8 to 12 °C. No more than 15 fish were held in a transport tank at one time, and dissolved oxygen was monitored to maintain proper concentration (8–11 mg·L⁻¹) during transport and as fish were removed from the tank for tagging. No fish were held in the transport tank for longer than 2.5 h. Tagged fish were released into the Chilliwack River with the expectation that they would be motivated to return to the hatchery from where they were captured. Treatments (gill net and control) were alternated between fish. Simulated gillnet capture and gill and blood biopsy were conducted as described for the holding study. Gillnet treatment took place in a 1000 L tank that was continuously fed with river water. Control fish (not gillnet treated) were taken directly to the tagging trough for biopsy. Following biopsy, a gastric radio tag (Pisces 5, Signa Eight Inc., Newmarket, Ontario; 43 mm length × 16 mm diameter, 15.2 g in air) was inserted into the stomach of all fish, just past the esophageal sphincter with the wire hanging out of the mouth. A visual identification “spaghetti” tag was looped through the musculature posterior to the dorsal fin for recovery of fish at the hatchery and by anglers.

Six fixed radio telemetry receivers (Orion, Sigma Eight Inc.), each equipped with a three-element Yagi antenna, were positioned along the Chilliwack River, similar to the layout used by Nelson et al. (2005; Fig. 1). Additionally, a receiver was placed immediately upstream of the hatchery to detect fish moving further upstream, and another was placed immediately downstream of the hatchery attraction channel to detect fish entering and leaving. Detection ranges were tested using a radio tag to ensure complete coverage of the river channel. Stationary receiver effi-

ciency was calculated for the four middle receivers based on detections of tagged fish (>95%) at adjacent upstream and downstream receivers — this could not be estimated for the furthest downstream or upstream receivers. Mobile tracking was performed on a weekly basis throughout October and the first week of November using a Lotek SRX 600 (Newmarket, Ontario, Canada) with a truck-mounted five-element Yagi antenna. At study completion, the entire river between the hatchery and confluence with the Fraser River was walked with a mobile receiver to determine final locations of radio tags. Mobile tracking data were used to determine maximum upstream detection location, to calculate antenna efficiency, and to visualize detection data to aid in interpretation of movement patterns.

Telemetry data were filtered to remove nontarget frequency-code combinations. False positives for a given frequency-code combination were filtered automatically in R statistical software (R Core Team 2015), removing detections that occurred less than 5 s apart (the tag burst rate) or more than 2 min apart. Next, plots of detections by time for each individual were observed to identify remaining false positives. Longevity in the river was determined by observing individual detection plots. We assumed that rapid downstream detections (equating to a rough approximation of river velocity) indicated moribund or deceased individuals. These rapid downstream movements were easily recognizable in plots and were not followed by subsequent upstream movements. Survival times were assigned to the first detection in the series of downstream detections. An intensive recreational fishery overlaps with the fall Chinook migration on the Chilliwack River. If reported tags could not be censored at any fixed station prior to capture (i.e., fish captured prior to any detections at stationary receivers), they were removed from further analyses.

Laboratory analysis

Haematocrit was measured in the field by centrifuging (2 min at 10 000g; LW Scientific ZIPocrit; Georgia, USA) blood in heparinized microcapillary tubes (Drummond Scientific, Pennsylvania, USA). Vacutainers of whole blood were centrifuged at 7000g for 7 min (Clay Adams Compact II centrifuge; New York, USA), and extracted plasma was flash-frozen in liquid nitrogen. All plasma analyses were conducted at the DFO West Vancouver Laboratory, West Vancouver, British Columbia. Plasma chloride, sodium, potassium, lactate, and glucose concentrations and osmolality were measured following protocols described in Farrell et al. (2001), and cortisol, estradiol, and testosterone were measured using enzyme-linked immunosorbent assay (ELISA) kits (Neogen Corporation, Kentucky, USA) according to manufacturer’s protocols.

Gene expression was measured using the Biomark platform for HT-qPCR (Miller et al. 2016) in the Molecular Genetics Laboratory at the DFO Pacific Biological Station, Nanaimo, British Columbia. Primer and probe sequences and assay efficiencies can be found in Table S1². We first screened for 45 infectious agents in a pool of terminally sampled organ tissues (holding study only); positively detected pathogens were then measured in nonlethally and lethally sampled gill tissue along with 17 host immune genes (*C3*, *C7*, *CD4*, *CD83*, *GR2*, *IFNa*, *IgMs*, *IL11*, *IL15*, *IL1R*, *MHCI*, *MHCIIb*, *MMP13*, *Mx*, *RIG.I*, *TF*) and two reference genes. Processing protocols for HT-qPCR followed those described in Bass et al. (2017). To summarize, each tissue sample was homogenized independently using a MM301 mixer mill (Restch Inc., Pennsylvania, USA); aliquots of aqueous phase from seven lethally sampled organs (holding study only) were pooled for each individual (one organ tissue pool per fish) prior to RNA purification, including lethally sampled gill. All gill samples (nonlethally and lethally sampled) were also homogenized and extracted independently to quantify host and pathogen gene expression at the start of holding and telemetry studies

²Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2017-0491>.

Table 1. (A) Sample size and percent mortality of held Chinook salmon by treatment and sex and (B) sample size and number arriving at spawning grounds by sex and treatment group for radio-tagged Chinook salmon released into the Chilliwack River, British Columbia.

(A) Sample size and percent mortality by treatment and sex.

Treatment	Female		Male	
	<i>n</i>	% mortality	<i>n</i>	% mortality
Cool*	14	36	75	37
Warm*	15	27	46	28
Cool, control	7	86	19	37
Cool, gill net	2	50	28	46
Warm, control	4	100	17	71
Warm, gill net	7	71	16	69

(B) Sample size and number arriving at spawning grounds by sex and treatment group.

Treatment	Female		Male	
	<i>n</i>	<i>n</i> arriving at spawning (%)	<i>n</i>	<i>n</i> arriving at spawning (%)
Gill net	14	1 (7)	44	17 (39)
Biopsy	20	0 (0)	34	26 (76)

*Mortality prior to T1 treatment.

and at the close of the holding study. RNA quantity and quality were assessed by spectrophotometry using A_{260} and $A_{260/280}$. Following RNA normalization across samples (0.5 μg for gill, 1 μg for pooled tissues), cDNA was synthesized (Invitrogen SuperScript VILO, California, USA), and all primers were incorporated into a multiplex PCR prior to qPCR as per manufacturer's protocols for the Biomark platform, followed by ExoSAP-IT PCR Product Cleanup (MJS BioLynx Inc., Ontario, Canada) and fivefold dilution (TEKnova suspension buffer, California, USA). Assay and samples mixes were loaded into qPCR chambers using the integrated fluidics circuit controller, and qPCR was completed using the "GE 96 \times 96 Standard v1.pcl." (TaqMan) cycling. Positive and negative controls were included at each step in the protocol, including serial dilutions of host template and artificial clone constructs containing the primer sequence of the 45 pathogen assays under examination (Miller et al. 2016). Two probes were quantified by the qPCR; the first detected contamination by clone constructs, which could then be removed from subsequent analysis; the second quantified target primers of host and pathogen genes. Infectious agent assays not detected in duplicate were failed. Quantification cycle (Ct) is reported as the average of assay duplicates, and relative expression of host biomarkers was derived according to Pfaffl (2001) using two reference genes. Infectious agent loads are presented as RNA copy number calculated using standard curves of clone dilutions.

Statistical analysis

High levels of mortality prior to initial nonlethal biopsy and treatment (see Results) raised questions about the role of pathogens in mortality following collection and transport; therefore, we compared infectious loads in the gill of early mortalities (prior to T1) with those of fish that survived to T1 (low temperature controls) using nonparametric Kruskal–Wallis rank sum tests to account for uneven sample sizes. This analysis and all of those described for held fish were restricted to males only due to the low number of females collected for the laboratory holding study (~20%; Table 1A), which likely reflected hatchery sex ratios during collection. Infectious agent metrics under evaluation included relative loads (RNA copy number) of pathogens, infectious agent richness (number of positively detected agents), and relative infection burden (RIB; Bass 2018) in pooled tissues. RIB is a composite metric of multiple infection burden using qPCR data and was derived by the following equation:

$$\text{RIB} = \sum_{i \in m} \frac{L_i}{L_{\text{max}_i}}$$

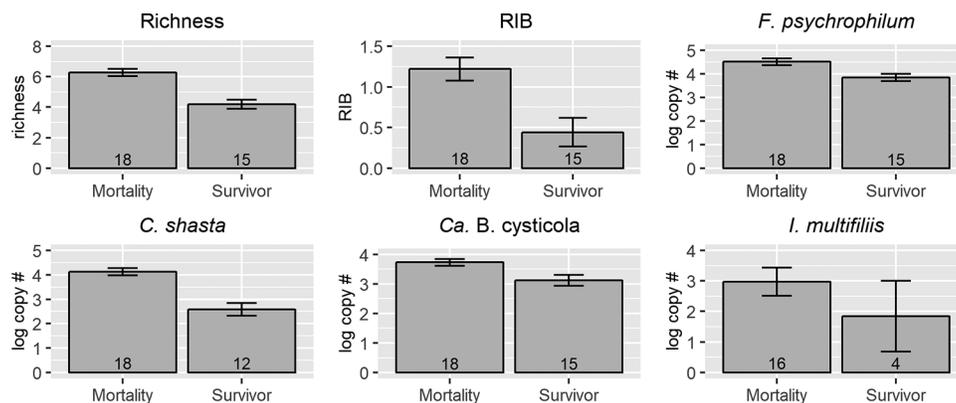
where for a given fish, the relative load of the *i*th infectious agent (L_i) is divided by the maximum load within the population for the *i*th infectious agent (L_{max_i}) and then summed across all agents (m) infecting the given fish. Relative loads (RNA copy number) and RIB were log-transformed prior to analysis, and all tests comparing relative loads omitted negative detections, using only positive detections from prevalent pathogens.

The impact of gillnet treatment and high temperature on the survival of held fish was quantified using survival analysis and Cox proportional hazards (survival, R statistical package; Therneau 2014). We used linear mixed effects models to quantify the effects of gillnet treatment and high temperature on changes in infectious agent loads, richness, and RIB measured in gill tissue at T1 and T2 (repeated measures). Random effects either allowed the response variable (infectious agent metrics) to vary across individuals but remain constant over time (random intercept) or allowed the response variable and its relationship with time to vary among individuals (random intercept and slope). Optimal random effects were chosen for each metric using the full set of fixed effects (temperature, gillnet treatment, time, and all two-way interactions) by comparing second-order Akaike's information criteria (AIC_c) and significance of model differences with analysis of variance (ANOVA; $P < 0.05$ corrected for "testing on the boundary"; Zuur et al. 2009). Random intercept variance or intercept and slope variance and their correlation coefficients are reported for each model. Top-down model selection was then applied to identify fixed factors contributing to variation in disease metrics, whereby beginning with the full model (all factors and interactions), low *t* value and high *P* value parameters were removed and the reduced model compared using ANOVA; factors that did not significantly increase model likelihood ($P < 0.05$) and reduce AIC_c when included were excluded from the subsequent model. The final model included only parameters that significantly contributed to variation in the data or were components of a significant interaction term (Zuur et al. 2009); reported ΔAIC_c values correspond to differences between the full model and the fully reduced model.

To characterize the relative contribution of high temperature, gillnet treatment, and fate (survival 4 days = travel time to spawning grounds) to the variation in blood properties measured in held males at T1 (immediately following gillnet treatment and after 48 h of increasing temperature), we used ANOVA with blood properties as response variables. Response variables were log-transformed if necessary to meet assumptions of normality, and all interaction terms were included. Unbalanced sample sizes were unavoidable due to mortality at 14 °C (9 °C: control survivor $n = 7$, gillnet survivor $n = 15$, control mortality $n = 5$, gillnet mortality $n = 10$; 14 °C: control survivor $n = 4$, gillnet survivor $n = 4$, control mortality $n = 9$, gillnet mortality $n = 10$). ANOVA was also used to characterize the explanatory power of thermal and fisheries stressors on blood properties measured in surviving males at T2 with an interaction term (9 °C: control $n = 12$, gill net $n = 15$; 14 °C: control $n = 5$, gill net $n = 5$).

A binomial generalized linear model (GLM) was used to identify contributing factors leading to early mortality of held fish based on information from plasma stress indices, gill pathogen burdens, and immune gene expression, as well as influences of high temperature and gillnet treatment. To condense information describing stress, maturation, and immunity at T1, we conducted principal components analysis (PCA) of parameters measured in blood and another of immune gene expression in gill and then used Monte Carlo randomization tests, which permuted raw data to assess the significance of eigenvalues at $P < 0.05$ (McCune

Fig. 2. Differences in average pathogen richness, relative infection burden (RIB), and relative loads (log RNA copy number) of prevalent pathogens of male Chinook salmon that died <4 days after collection (mortality; $n = 21$) or survived to T1 (survivor (biopsied controls); $n = 22$). Error bars represent standard errors; all differences were significant at $P < 0.05$, except for *I. multifiliis* ($P = 0.298$).



et al. 2002; McGarigal 2015) to identify components to include in the GLM. If necessary, parameters were log-transformed prior to PCA to meet assumptions of normality. Pathogen burdens in gill were condensed into RIB for inclusion in the GLM. Coefficient estimates (β) and standard errors of significant parameters ($P < 0.05$) identified by the GLM are reported. Linear regression was then used to describe relationships between RIB and condensed gene expression and blood property data (i.e., significant PCA axes identified by GLM). Blood and gene expression data from fish that survived to T2 were also incorporated into PCAs, and significant components were used as response variables in linear regressions with RIB (gill or pooled tissues), temperature, and gillnet treatment (with an interaction term) to assess the extent to which infection burden correlated with host condition and immune activity following exposure to stressors and their individual and combined influence on host physiology.

For the telemetry study, we aimed to test whether fisheries capture and (or) infectious agents (determined from nonlethal gill samples) were associated with longevity, migratory success, and migration time. Longevity was defined as the number of days surviving in the Chilliwack River following release, migratory success was defined as detection at the Tamihi Rapids radio receiver (rkm 28, downstream extent of suitable spawning habitat), and migration time was the difference between the time of the first detection at rkm 28 and the time of release. These response variables were tested by comparing models containing infectious agent indices that were identified in the holding study as being associated with mortality: richness, RIB, and loads of *Flavobacterium psychrophilum*, *Ceratonova shasta*, and "*Candidatus* Branchiomonas cysticola". Treatment (gill net versus control) was included in all models, and sex and body size (fork length) were included where applicable. Five candidate models, varied by the inclusion of an infectious agent variable, were compared using the information-theoretic approach (AIC_c ranking) to determine the best fit for each modeling objective (Burnham and Anderson 2002). For longevity, all accelerated failure time (AFT) models included treatment and sex. We fit the longevity data with the Weibull, Gaussian, exponential, log-logistic, lognormal, and logistic distributions and compared both AIC_c values and plots of the negative log of the Cox-Snell residuals over time. The Weibull distribution was selected as the best fit for the longevity data. Owing to its skewed distribution, RIB was log-transformed for this and following analyses. Analysis was performed with the survival package. For modeling migration time, AFT modeling was again employed, but in this case the log-logistic distribution was determined to fit the data best. Only males could be included in the migration time analysis, since only one female arrived at the spawning grounds. We included both treatment and body size in these models. Body

size was included to account for differences in energy use during swimming and ability to navigate hydraulic challenges (Hinch and Rand 1998; Crossin et al. 2004). Similar to the AFT approach, we created five GLMs to fit the migratory success (binomial) data and compared them by AIC_c . Again, we included both treatment and body size in these models.

Results

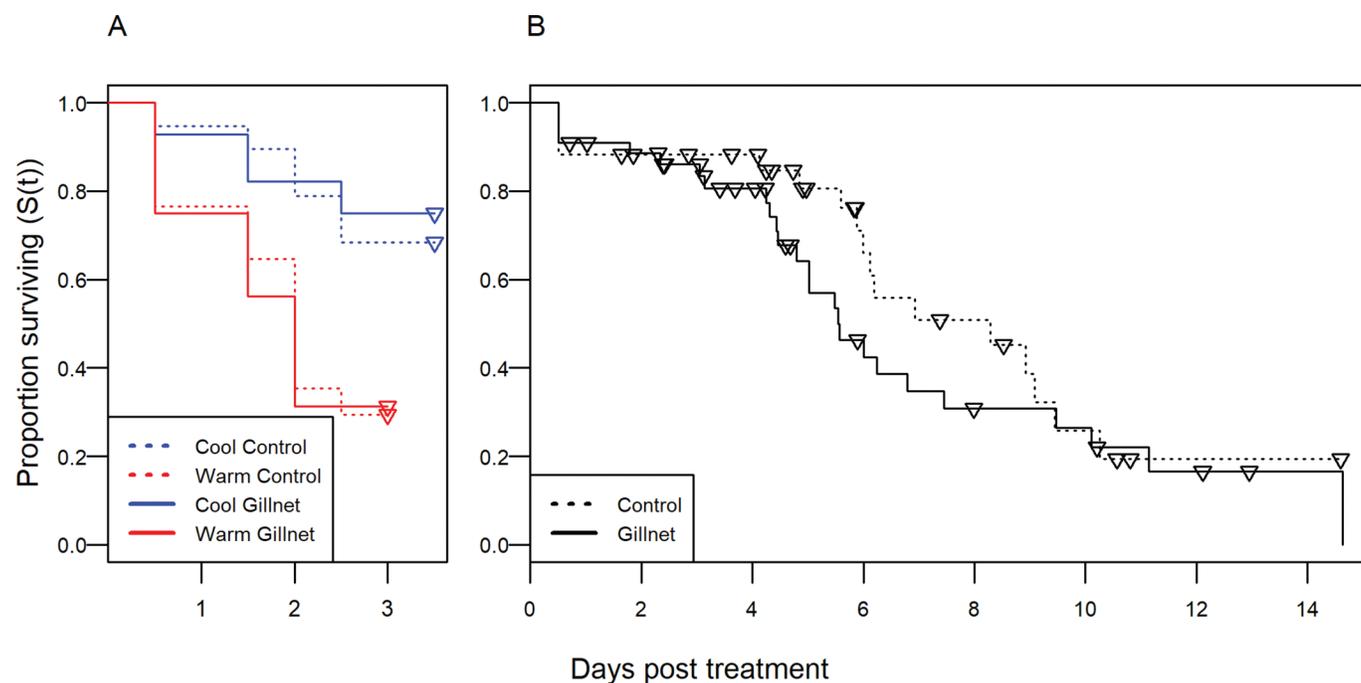
Holding study

In cool water (9 °C), males that died prior to T1 (<4 days after collection, $n = 18$) had higher RIB ($\chi^2 = 10.8$, $P = 0.001$) and infectious agent richness ($\chi^2 = 16.7$, $P < 0.001$) and higher loads of *C. shasta* ($\chi^2 = 14.8$, $P < 0.001$), *F. psychrophilum* ($\chi^2 = 7.6$, $P = 0.006$), and "*Ca. B. cysticola*" ($\chi^2 = 7.8$, $P = 0.005$) in gill relative to those that survived (biopsied controls, $n = 15$; Fig. 2). Loads of *Ichthyophthirius multifiliis* ($\chi^2 = 1.1$, $P = 0.298$) did not significantly differ between early mortalities and survivors.

Even though the duration of the holding study was brief, survival was not consistent across groups (Table 1A). Low sample sizes for females precluded any statistical analysis of sex-specific impacts on survival, and percent mortality reported here for held females should be interpreted with caution. Pretreatment mortality was ~30% for both sexes and temperatures. For fish that survived to T1, percent mortality was higher for females than males in cool (9 °C; 86%) and warm (14 °C; 100%) control groups but similar between sexes following gillnet treatment at both temperatures. Among males, percent mortality was higher in warm water than in cool, similar for gillnetted and control fish in warm water (~70%), and slightly higher for gillnetted fish in cool water (control = 37%, gillnetted = 46%). Survival analysis (males only) identified a significant effect of temperature on survival where fish held in warm water experienced 1.2 times the hazard of mortality (standard error = 3.3) as those held in cool water ($P = 0.019$; Fig. 3A). Neither gillnet treatment nor the interaction between high temperature and gillnetting significantly impacted survival during the holding period.

Infectious agent prevalence for held fish is shown in Table 2. For linear mixed effects models (Fig. 4), a random intercept was best applied for richness ($\Delta AIC_c = 0.22$, $P = 0.385$; $\sigma^2 = 0.60$) and "*Ca. B. cysticola*" loads ($\Delta AIC_c = 0.49$, $P = 0.075$; $\sigma^2 = 0.21$), while random slope and intercept models were better suited to *I. multifiliis* ($\Delta AIC_c = 2.97$, $P = 0.013$; intercept $\sigma^2 = 2.85$, slope $\sigma^2 = 3.86$, correlation coefficient = -0.88), *C. shasta* ($\Delta AIC_c = 1.49$, $P = 0.041$; intercept $\sigma^2 = 0.79$, slope $\sigma^2 = 0.85$, correlation coefficient = 0.78), *F. psychrophilum* ($\Delta AIC_c = 7.25$, $P = 0.003$; intercept $\sigma^2 = 0.46$, slope $\sigma^2 = 0.25$, correlation coefficient = -0.82), and RIB ($\Delta AIC_c = 10.19$, $P < 0.001$; intercept $\sigma^2 = 0.64$, slope $\sigma^2 = 0.51$, correlation coeffi-

Fig. 3. (A) Kaplan–Meier curve of survival of male Chilliwack River Chinook salmon following experimental temperature manipulation and gillnet entanglement treatment. Colour indicates holding temperature (9 °C = blue; 14 °C = red) and treatment is indicated by line type (20 s gillnet entanglement and 1 min air exposure = solid, control = dashed). Sample sizes can be found in Table 1. (B) Kaplan–Meier curve of the longevity of radio-tagged Chinook salmon receiving either a gillnet (solid) or control (dashed) treatment. Censored individuals are indicated by triangles. [Colour online.]



cient = -0.84). Fixed effects impacting richness included gillnet treatment ($\beta = 0.54 \pm 0.28$, $P = 0.050$), temperature ($\beta = -1.53 \pm 0.70$), time ($\beta = 1.60 \pm 0.28$), and an interaction between temperature and time ($\beta = 1.29 \pm 0.49$, $P = 0.009$, $\Delta AIC_c = 6.63$); no P values are presented for the independent effects of temperature and time due to the significance of their interaction, which prevented further reduction of the model. *Flavobacterium psychrophilum* was influenced by gillnet treatment ($\beta = 0.35 \pm 0.12$, $P = 0.004$) and time ($\beta = 1.01 \pm 0.08$, $P < 0.001$, $\Delta AIC_c = 7.25$). RIB was also significantly associated with gillnet treatment ($\beta = 0.27 \pm 0.11$, $P = 0.012$) and time ($\beta = 0.69 \pm 0.07$, $P < 0.001$, $\Delta AIC_c = 5.28$). Time was the only significant fixed effect describing *C. shasta* ($\beta = 0.66 \pm 0.15$, $P < 0.001$, $\Delta AIC_c = 6.58$), *I. multifiliis* ($\beta = 0.81 \pm 0.38$, $P = 0.036$, $\Delta AIC_c = 10.19$), and “*Ca. B. cysticola*” ($\beta = 0.45 \pm 0.11$, $P < 0.001$, $\Delta AIC_c = 3.18$).

A primary factor explaining the variation in blood properties of males at T1 was fate (i.e., survival to study termination). For fish that would die within 4 days, cortisol ($F = 27.5$, $P < 0.001$), glucose ($F = 4.8$, $P = 0.032$), and lactate ($F = 27.6$, $P < 0.001$) were elevated at T1, while testosterone ($F = 13.7$, $P = 0.001$), osmolality ($F = 14.8$, $P < 0.001$), sodium ($F = 11.5$, $P = 0.001$), and chloride ($F = 20.6$, $P < 0.001$) were depressed. For both survivors and mortalities at T1, lactate ($F = 17.8$, $P < 0.001$), potassium ($F = 17.8$, $P < 0.001$), and estradiol ($F = 9.5$, $P = 0.003$) were increased at 14 °C, while osmolality ($F = 7.2$, $P = 0.009$), sodium ($F = 6.5$, $P = 0.014$), and testosterone ($F = 8.2$, $P = 0.006$) were increased immediately following gillnet treatment. However, some aspects of the stress response differed depending on fate. At T1, only survivors showed an increase in cortisol (interaction: $F = 6.6$, $P = 0.013$) following gillnet treatment and a greater increase in estradiol at high temperature (interaction: $F = 5.7$, $P = 0.021$) relative to mortalities. Among survivors at T2, high temperature was the only factor significantly contributing to the variation in blood properties, with increased potassium ($F = 25.2$, $P < 0.001$), lactate ($F = 8.6$, $P = 0.006$), cortisol ($F = 5.2$,

$P = 0.030$), and estradiol ($F = 7.2$, $P = 0.011$), but no significant impact of gillnet stress ($P > 0.05$).

Patterns in immune gene expression, plasma stress indices, and temperature were associated with short-term survival of males (<4 days after T1; binomial GLM: null deviance = 88.5, $df = 63$; residual deviance = 40.0, $df = 55$; $AIC_c = 61.33$). Twelve infectious agents were positively detected in the gill of 64 adult male Chinook salmon sampled at T1; RIB values ranged between 0.001 and 2.643, with a mean of 0.289. Data from blood properties measured at T1 were incorporated into the GLM as the first two components of the PCA (variation explained: PC1: 35%, PC2: 20%; $P < 0.001$; Fig. 5); gene expression data at T1 were also represented in the GLM by the first two components of the PCA (PC1: 24%, PC2: 22%; $P < 0.001$). At T1, low plasma ions and testosterone as well as high lactate, cortisol, and glucose characterized fish that died within 4 days (plasma PC1: $\beta = -1.09 \pm 0.38$; $P = 0.004$). Higher relative expression of genes associated with wound healing (*MMP13*, *IL11*), as well as complement (*C7*) and iron metabolism (*TF*), but decreased relative expression of cell-mediated immunity (*MHCI*, *MHCII*, *CD4*) and *IL15* (gene expression PC2: $\beta = 0.79 \pm 0.33$; $P = 0.016$) were characteristic of fish that would die within 4 days. High temperature was associated with early mortality ($\beta = 4.05 \pm 2.00$; $P = 0.042$), but gillnet treatment and the interaction between stressors were not significant factors in the model. RIB in gill was marginally but nonsignificantly associated with mortality ($\beta = 1.81 \pm 1.03$; $P = 0.081$). Linear regression of gill RIB with PC1 of blood properties showed a negative relationship ($\beta = -1.55 \pm 0.33$; adjusted $r^2 = 0.25$; $P < 0.001$), where heavy infections corresponded to the characteristics identified by the GLM as associated with premature mortality. The relationship of gill RIB to immune gene expression data (PC2) was also highly significant and associated with characteristics of early mortality ($\beta = 1.54 \pm 0.33$; adjusted $r^2 = 0.25$; $P < 0.001$).

Table 2. Positive detections and prevalence of pathogens measured in the gill of adult fall run Chilliwack Chinook salmon that were tagged and released or held.

Infectious agent	Group	Male		Female	
		Positive detections	% prevalence	Positive detections	% prevalence
<i>Flavobacterium psychrophilum</i>	Tagged	70	100	32	100
	Held T1	73	100	20	100
	Held T2	40	100	8	100
"Candidatus Branchiomonas cysticola"	Tagged	70	100	32	100
	Held T1	73	100	20	100
	Held T2	40	100	8	100
<i>Ceratonova shasta</i>	Tagged	70	100	32	100
	Held T1	65	89	19	95
	Held T2	38	95	8	100
<i>Ichthyophthirius multifiliis</i>	Tagged	37	53	26	81
	Held T1	32	44	12	60
	Held T2	39	98	7	88
Rickettsia-like organism	Tagged	7	10	1	3
	Held T1	11	15	4	20
	Held T2	29	73	4	50
<i>Aeromonas salmonicida</i>	Tagged	6	9	1	3
	Held T1	12	16	7	35
	Held T2	19	48	4	50
<i>Parvicapsula minibicornis</i>	Tagged	19	27	5	16
	Held T1	16	22	5	25
	Held T2	12	30	2	25
<i>Cryptobia salmocitica</i>	Tagged	13	19	8	25
	Held T1	9	12	3	15
	Held T2	12	30	3	38
<i>Kudoa thyrsites</i>	Tagged	12	17	6	19
	Held T1	9	12	5	25
	Held T2	7	18	3	38
<i>Dermocystidium salmonis</i>	Tagged	9	13	3	9
	Held T1	3	4	2	10
	Held T2	8	20	1	13
Viral erythrocytic necrosis virus	Tagged	11	16	8	25
	Held T1	7	10	0	0
	Held T2	2	5	0	0
<i>Loma salmonae</i>	Tagged	2	3	7	22
	Held T1	2	3	0	0
	Held T2	1	3	0	0
<i>Tetracapsuloides bryosalmonae</i>	Tagged	4	6	3	9
	Held T1	1	1	0	0
	Held T2	0	0	0	0
Piscine reovirus	Tagged	1	1	0	0
	Held T1	0	0	1	5
	Held T2	0	0	1	13
<i>Sphaerothecum destruens</i>	Tagged	2	3	1	3
	Held T1	0	0	2	10
	Held T2	0	0	0	0

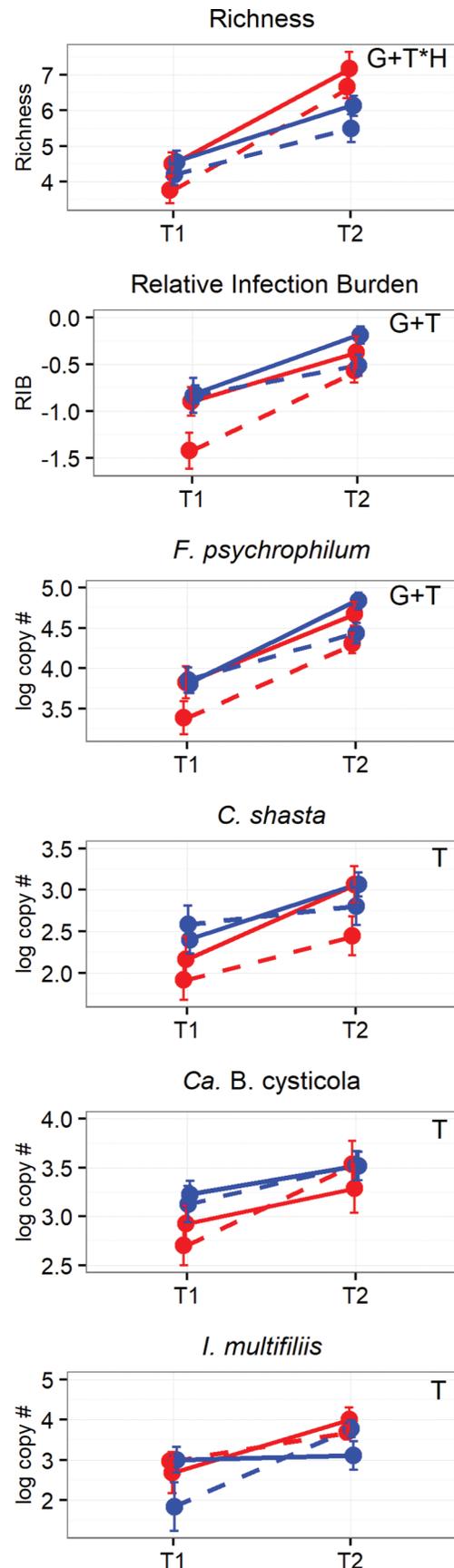
Note: T1: study start; T2: after 4 days.

Blood properties and gene expression measured at the termination of the study (T2) showed similar grouping of variables in PCAs to those identified at T1 (Fig. 6). Blood properties (PC1: 46%, PC2: 20%) and immune gene expression (PC1: 21%, PC2: 20%) were both adequately described by two components ($P < 0.001$); note that haematocrit was not calculated at death and so was not included in the T2 analysis. RIB in gill was negatively associated with PC2 of gill gene expression data ($\beta = -2.01 \pm 0.80$; $P = 0.017$; adjusted $r^2 = 0.18$) with similar gene associations as observed at T1 and no significant effect of gillnetting or temperature. Gillnet treatment and high temperature were negatively associated with PC2 of plasma data (gill net: $\beta = -0.97 \pm 0.44$, $P = 0.037$; temperature: $\beta = -2.11 \pm 0.58$, $P = 0.001$; adjusted $r^2 = 0.35$), but no interaction or association with RIB was detected. Testosterone, estradiol, potassium, and stress metabolites loaded negatively on PC2, showing association of these characteristics with fish held in warm water and (or) exposed to gillnet treatment.

Telemetry results

A total of 118 fish were tagged and released for the telemetry component of our study, equally divided between gillnet treatment and control groups (Table 1B). Two of the control and one gillnet-treated fish entered and remained in Sweltzer Creek (Fig. 1) and were therefore removed from analyses. A single control fish exited the Chilliwack River and swam up the Fraser River to the Harrison River and was also removed. Seven gillnet-treated and two control fish were captured and reported by anglers. Female fish were detected at lower proportions at the receivers upstream of the release location compared with males; only one female arrived at the spawning habitat and none returned to the hatchery (Fig. 7). Fifty-five percent of released Chinook salmon moved downstream after tagging (fell back), and 68% of these fish were subsequently detected upstream. Gillnet-treated and control fish moved downstream at similar proportions (55% and 56%, respectively), but a greater percentage of control fish were detected at

Fig. 4. Pathogen richness, relative infection burden (RIB), and loads (log RNA copy number) of prevalent pathogen measures in the gill of male adult fall run Chilliwack Chinook salmon on two occasions (T1: study start; T2: 4 days later). Colour indicates temperature during holding (9 °C = blue; 14 °C = red), and treatment is indicated by line type (20 s gillnet entanglement and 1 min air exposure = solid, control = dashed). Significant ($P < 0.05$) effects of time (T), gillnet treatment (G), and high temperature (H) on each metric are indicated in the upper right corner of each plot with additive effects (+) or interactions between terms (*). [Colour online.]



the start of the spawning grounds and hatchery (48% control, 31% gillnet-treated), with this difference more pronounced among males (males only: 76% control, 38% gillnet-treated).

Including censored individuals (39% of population), the median postrelease longevity for the population ($n = 97$) was 6.18 days (5.57–7.30 days, 95% CI; Fig. 3B). AIC_c comparison of models including different indices of infectious agents identified a model with the bacteria *F. psychrophilum* as the best fit of the longevity data (Table 3). For each log unit increase in copy number of *F. psychrophilum*, longevity decreased by a factor of 0.16 (0.04–0.29, 95% CI; Table 4; Fig. 8). Neither treatment nor sex were associated with longevity in any models (Table 4). There was not a single GLM that stood out from the others in terms of AIC_c (Table 3) when determining the factors associated with the probability of male Chinook salmon ($n = 67$) arriving on the spawning grounds. However, in all models, treatment was a significant predictor of success, with control fish having 1.4 (0.29–2.50, 95% CI) times greater odds of arriving at the spawning grounds than gillnetted fish (Table 4). The time required for male Chinook salmon ($n = 67$, 26% censored) to arrive at spawning grounds (median = 2.92 days, 2.49–3.41 days, 95% CI) was best fit by a model including RIB, although a competing model including infectious agent richness instead was within 1.5 AIC_c (Table 3). In the top model, both elevated RIB and the control treatment were associated with more rapid migration (Table 4). For each log unit increase in RIB, migration time decreased by a factor of 0.09 (0.02–0.17, 95% CI), and control fish required a factor of 0.31 (0.07–0.56, 95% CI) less time to migrate (Fig. 9).

Discussion

Our paired study design used laboratory holding and telemetry to demonstrate impacts of multiple stressors on infection development and host physiology and linked infection burdens to longevity, migration rate, and migration success of adult Chinook salmon in fresh water. Thermal stress was associated with early mortality of held fish, while simulated gillnet capture resulted in slower migration rates of tagged fish and reduced distance traveled upriver. Both stressors elicited physiological changes in blood properties soon after application. Correlations between physiological stress parameters and infectious disease agents support previous findings (Bass et al. 2017) but were also predictive of survival and stressor-dependent. Among held males, early mortality was characterized by higher composite scores of multiple infection intensities (RIB), infectious agent richness, and individual agent loads. We will argue in the discussion that these results support a role of infectious disease development in the migratory behaviour and early mortality of adult Chinook salmon in fresh water that is dependent on thermal and fishery stressors as well as host physiological responses.

We detected distinct immune responses correlated with RIB that were largely driven by load and prevalence of the bacteria *F. psychrophilum*. Similar patterns have been described in sockeye salmon (*O. nerka*) exposed to capture stress (Teffer et al. 2017), with upregulation of genes associated with tissue damage and bacterial defense. RIB and infection intensity of *F. psychrophilum* increased at a faster rate in fish exposed to experimental gillnetting, and, when measured at release, *F. psychrophilum* load was negatively

Fig. 5. Principal components analysis of (A) blood properties and (B) immune gene expression in gill of adult male Chilliwack River Chinook salmon collected soon after river entry and held in freshwater tanks. Blood and gill tissue sampling and treatment took place ~4 days after arrival at the holding facility (T1). Point colour corresponds to water temperature during holding (blue: 9 °C, n = 37; red: 14 °C, n = 27), and shape designates treatment group (triangles = 20 s gillnet entanglement and 1 min air exposure, n = 39; circles = control, n = 25). Relationships with early mortality and relative infection burden (RIB) in gill are shown by vectors. [Colour online.]

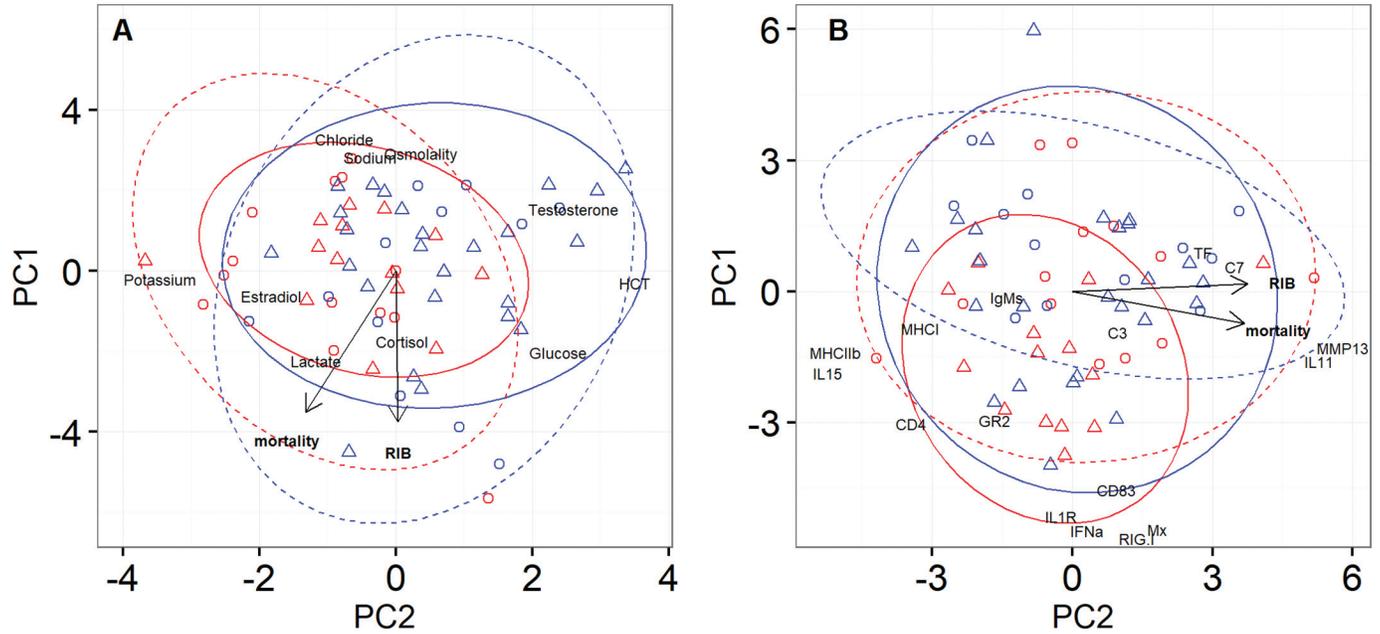
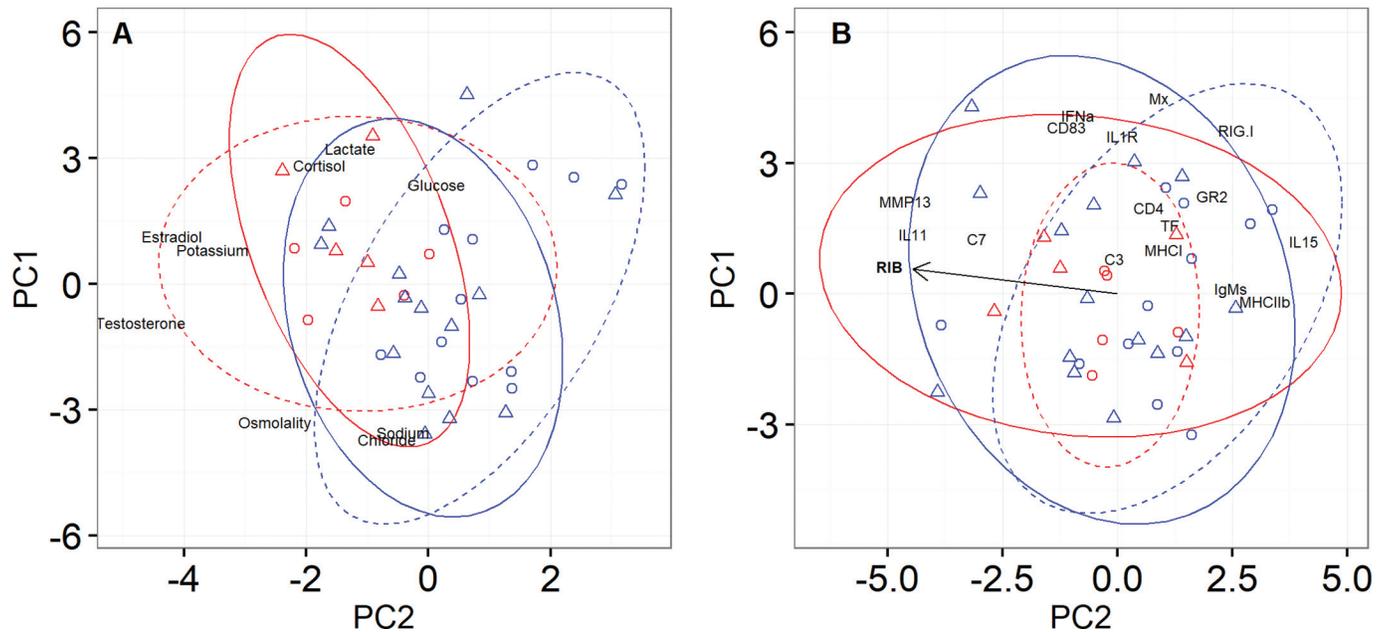


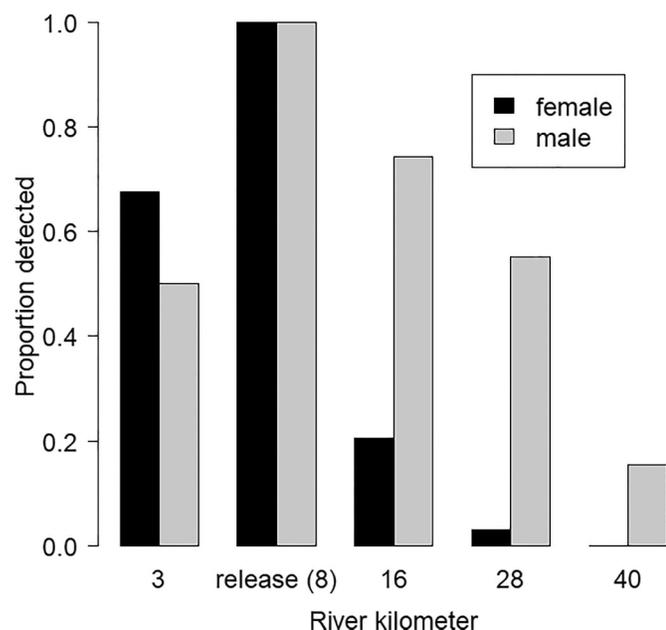
Fig. 6. Principal components analysis of (A) blood properties and (B) immune gene expression in gill of surviving adult male Chilliwack River Chinook salmon 4 days after treatment and holding in freshwater tanks (T2). Point colour corresponds to water temperature during holding (blue: 9 °C, n = 37; red: 14 °C, n = 27), and shape designates treatment group (triangles = 20 s gillnet entanglement and 1 min air exposure, n = 39; circles = control, n = 25). Ellipses show 95% confidence intervals of each treatment and temperature group (dashed = control, solid = gill net). Relationships with relative infection burden (RIB) in gill are shown by a black vector. [Colour online.]



associated with longevity of tagged fish in the river, while RIB was associated with faster migrations. Increased agent richness indicated enhanced transmission among stressed fish, especially after 4 days of holding, suggesting that less prevalent agents were introduced to new hosts more frequently under stressful conditions (gillnetting and high temperature). This increase in agent diversity would be expected to influence host responses and increase

immune surveillance. The correlation of RIB with immune activity in surviving males after 4 days of holding could suggest that fish were responding to new infections and (or) addressing current ones (e.g., *F. psychrophilum*). Both *F. psychrophilum* and agent richness featured prominently in our results, suggesting that multiple infections as well as prevalent pathogens modulate host responses to influence survival and migration behaviour.

Fig. 7. Proportions of radio-tagged female (dark bars) and male (light bars) Chinook salmon detected at fixed receiver stations along the Chilliwack River, British Columbia.



Telemetry data demonstrated lower proportions of females compared with males successfully arriving at the hatchery. Females and males were observed to be close to or fully mature at the time of release. Because tagged fish were transported downstream prior to release, the choice to repeat this migration or find suitable spawning habitat in other portions of the river may favor the latter for females investing finite energy stores into egg development, nest digging, and defense (Crossin et al. 2004; Kiessling et al. 2004; Esteve 2005), but cannot specifically be defined as migration failure. Females carry higher loads of several infectious agents (Bass et al. 2017) and have demonstrated greater susceptibility to mortality during stressful migrations (Martins et al. 2012; Donaldson et al. 2014), possibly due to sex-specific responses to stressors (e.g., Kubokawa et al. 2001; Jeffries et al. 2012a; Donaldson et al. 2014) that influence infection development (Teffer et al. 2017). These sex-specific differences in infectious loads and migration behaviours warrant further investigation before conclusions can be drawn as to their relationship.

Although survival of held males showed no effect of gillnet treatment, gillnet-treated males in the river were less likely to return to the hatchery and took longer to do so. These results suggest that at current autumn temperatures, acute fisheries stress can still slow migration rates and reduce migration success of adult male Chinook salmon but is unlikely to significantly impact longevity. The inherent stressors of river migration, such as hydraulic challenges and predator or fishery avoidance, would not have been captured by the holding component of our study but likely influenced migration behaviour in the river, possibly compounding gillnet stress (Miller et al. 2014). Slower migrations and augmented *F. psychrophilum* infection development following gillnet capture and release could increase bacterial loads at spawning grounds and indirectly amplify other infections by extending freshwater residence (Hinch et al. 2012; Benda et al. 2015; Chiaramonte et al. 2016). Richness, RIB, and *F. psychrophilum* loads were significantly increased by gillnet treatment in held fish measured 4 days after treatment, coinciding with when tagged fish would be arriving at the hatchery. Dermal abrasions, scale and mucus loss from the net, and handling likely enhance opportunistic infections (Svendsen and Bøgwald 1997; Baker and

Schindler 2009; Teffer et al. 2017); even minor injuries have been shown to reduce migration success of adult Chinook salmon in the Willamette River basin and are hypothesized to be the result of secondary infections (Keefer et al. 2017). *Flavobacterium psychrophilum* infection has also been associated with suppressed humoral immunity (Siwicki et al. 2004), which may facilitate infections by other organisms to enhance richness. These factors may contribute a higher likelihood of prespawn mortality (after arrival at spawning grounds) and warrant further study with a longer time course of observation, beginning shortly after freshwater entry and continuing throughout the spawning period. Efforts to reduce handling and injury during gillnet capture or use of alternative gears such as tangle nets may help to reduce impacts on released fish (Vander Haegen et al. 2004), especially during periods of high temperature.

All infectious agents and composite metrics measured in held fish increased with time. Immune suppression due to high cortisol levels associated with senescence and maturation (Maule et al. 1996; Dolan et al. 2016) as well as confinement stress (Donaldson et al. 2011) likely reduce defenses of adult salmon against these agents, in addition to experimentally applied stressors. Among held fish, initially distinct and sparse infectious agent communities in gill became more similar with greater richness over time, likely via transmission. Although holding enhances the likelihood of transmission, possibly contributing to these temporal increases, conditions experienced at the hatchery where returning fish crowd into raceways as they mature are similar and perhaps more intimate than those in our holding experiment. Behaviour also likely factors into infection development, as held males demonstrated frequent dominance displays (e.g., chasing, biting), causing injuries that likely altered hormone levels (Hruska et al. 2010) and influenced immune competence (Slater and Schreck 1993). Such behaviours are known to occur in the wild (Hruska et al. 2010) and were observed at the hatchery, adding another dimension to the disease dynamics of Pacific salmon.

In the river, higher RIB was associated with faster return of males to the hatchery. Time spent in fresh water correlates with decreased immune defenses (Dolan et al. 2016), increased infection intensity (Teffer et al. 2017), and advanced maturity (Fitzpatrick et al. 1986); hence, well-developed infections are likely characteristic of mature fish, which have been shown to migrate more quickly through the river (Crossin et al. 2008). Damage to osmoregulatory function caused by pathogens like *F. psychrophilum* (Barnes and Brown 2011), *Parvicapsula minibicornis* (Bradford et al. 2010), and *I. multifiliis* (Ewing et al. 1994) could signal other physiological changes that influence migration rate. For example, (Donaldson et al. 2010) found a negative correlation between osmolality and migration rate of Adams-Shuswap sockeye salmon (though not for Chilko sockeye). Low osmolality was also characteristic of summer run sockeye salmon held in a net pen prior to release and associated with a temporary increase in the migration rate and decreased overall survival and migration success (Donaldson et al. 2011). Those authors point to stress as a potential cause, but Cook et al. (2014) observed no influence of acute stress (elevated cortisol) on migration rate. It is possible that these relationships observed by Donaldson and colleagues are related to infectious disease processes, whereby damage to epithelial tissues caused by fishing gear and handling facilitate infections by agents like *Saprolegnia* fungus, *F. psychrophilum*, and others (Barnes and Brown 2011; Keefer et al. 2017; Teffer et al. 2017), causing a decrease in osmolality (Bradford et al. 2010). From a transcriptome perspective, a genomic signature indicative of poor health has also been linked to faster migrations and reduced migration success of sockeye salmon tagged and biopsied in the marine environment (Miller et al. 2011). Migration distance and difficulty as well as infection severity likely dictate the extent to which heavy pathogen loads and poor health are associated with faster migrations, but further evaluation of this relationship is warranted.

Table 3. Model results based on radio-tagged Chinook salmon released in the Chilliwack River, British Columbia, following experimental manipulation and biopsy.

Response	Common variables	Infectious agent variable	No. of parameters	AIC _c	ΔAIC _c	AIC _c weight
Longevity	Sex + treatment	<i>F. psychrophilum</i>	5	366.1	0.0	0.8
		RIB	5	371.2	5.1	0.1
		Richness	5	372.3	6.2	0.04
		“ <i>Ca. B. cysticola</i> ”	5	372.5	6.4	0.03
		<i>C. shasta</i>	5	372.6	6.5	0.03
Migratory success	Treatment + body size	Richness	4	92.7	0.0	0.3
		<i>C. shasta</i>	4	93.4	0.6	0.2
		“ <i>Ca. B. cysticola</i> ”	4	93.4	0.7	0.2
		<i>F. psychrophilum</i>	4	93.5	0.7	0.2
		RIB	4	93.5	0.8	0.2
Time to arrival at spawning	Treatment + body size	RIB	4	135.3	0.0	0.6
		Richness	4	136.7	1.5	0.3
		<i>C. shasta</i>	4	139.4	4.1	0.1
		“ <i>Ca. B. cysticola</i> ”	4	140.8	5.6	0.04
		<i>F. psychrophilum</i>	4	141.2	6.0	0.03

Note: AIC comparison of candidate models is presented for each response variable including longevity (modeled using accelerated failure time, AFT), migratory success (general linear models), and time to arrival at spawning (AFT). RIB, relative infection burden.

Table 4. Model statistics for the top model for each model comparison.

Response	Method	Distribution	Parameter	β	SE	Z	P
Longevity	Accelerated failure time	Weibull	Log <i>F. psychrophilum</i>	-0.16	0.06	-2.53	0.01
			Treatment (gill net)	0.25	0.19	1.29	0.20
			Sex (male)	0.21	0.21	1.02	0.31
Migratory success	Generalized linear model	Binomial	Richness	0.18	0.21	0.90	0.37
			Treatment (control)	1.35	0.56	2.41	0.02
			Fork length (cm)	-0.04	0.03	-1.43	0.15
Time to arrival at spawning	Accelerated failure time	Log-logistic	Log RIB	-0.09	0.04	-2.44	0.01
			Treatment (control)	-0.31	0.13	-2.48	0.01
			Fork length (cm)	0.001	0.01	0.20	0.84

Note: Variables significant at $P < 0.05$ are indicated in bold.

Our holding study findings support previous work demonstrating the negative impact of warm river temperatures on the physiology and survival of adult salmon (e.g., Crossin et al. 2008; Keefer et al. 2008; Martins et al. 2011; Jeffries et al. 2012b), with an associated 20% increased risk of mortality for fish held in warm water for 4 days. From a host physiological and aerobic standpoint, thermal tolerance varies among species and populations according to the historical conditions of migrations (Pörtner and Knust 2007; Eliason et al. 2011). Current and projected temperatures for the Fraser River watershed and other salmon-bearing rivers demonstrate clear impacts of climate change and a need to comprehend how alterations to the historical thermal experiences of adult spawners will impact population productivities (Patterson et al. 2007; Ferrari et al. 2007; Macdonald et al. 2010; McDaniels et al. 2010; Reed et al. 2011). Our results support previous findings from thermal stress studies on pink (*Oncorhynchus gorbuscha*) and sockeye salmon (Jeffries et al. 2012a), but also show increased infectious agent richness in individuals exposed to thermal stress for only a few days. The interaction between high temperature and time for increased richness suggests delayed impacts of chronic thermal stress that likely accelerated transmission among hosts. High temperature can enhance the development of many infections, including *I. multifiliis*, *P. minibicornis*, and *C. shasta* (Ewing et al. 1986; Crossin et al. 2008; Ray et al. 2012), and can also externally influence infection severity by accelerating the life cycle of parasites like *C. shasta*, increasing infective spore densities released by intermediate hosts into the migration corridor (Stocking et al. 2006). Environmental effects such as this would not be captured by our holding study where fish were held in a relatively pathogen-free environment (source water is UV-sterilized and filtered) but may be more prominent for tagged fish migrating in the wild. Although *F. psychrophilum* infections were

Fig. 8. Kaplan–Meier curves for longevity of Chinook salmon based on stationary radio receivers in the Chilliwack River, British Columbia. The raw data used to generate the curves were split at the median value for *F. psychrophilum* copy number in the population (>80 000 = red; <80 000 = black). The predicted curves overlaid (dashed lines) are based on the top accelerated failure time (AFT) model for longevity (longevity $\sim \log(F. psychrophilum) + \text{sex} + \text{treatment}$). Survival was predicted based on the mean *F. psychrophilum* copy number for each group in the plot. [Colour online.]

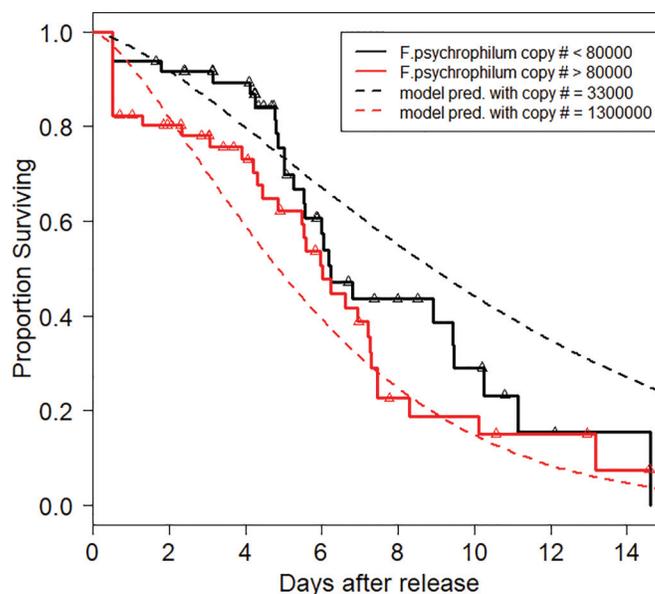
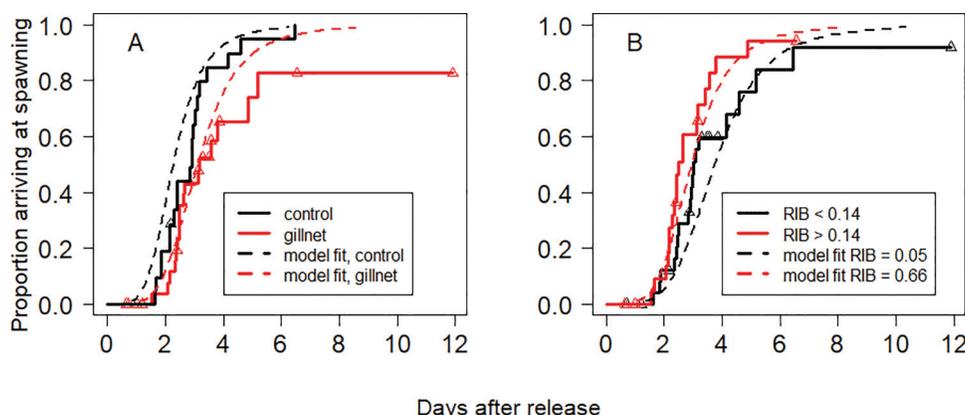


Fig. 9. Kaplan–Meier curves of migration time from release to spawning grounds for Chinook salmon released into the Chilliwack River, British Columbia. Both (A) treatment and (B) RIB were significant predictors of migration time in the model (time to arrival at spawning \sim RIB + treatment + fork length). In panel A, colour designates treatment (red = 20 s gillnet entanglement and 1 min air exposure, black = control); in panel B, colour represents infection burden (red = high RIB, black = low RIB). The predicted curves overlaid (dashed lines) show the model fit for each group plotted. The median value for RIB (panel B) was 0.14, and predicted curves were created for the mean RIB value for each group. [Colour online.]



not enhanced by high temperature, host physiology was affected, likely reducing the host's capacity to manage tissue damage caused by the bacteria. Despite its designation as the agent of bacterial cold-water disease, *F. psychrophilum* can cause severe disease and mortality of salmonids within the range of temperatures we evaluated (Nematollahi et al. 2003; Barnes and Brown 2011). The physiological limits of both salmon and infectious agents, including impacts of thermal stress on host immune capacity (Jeffries et al. 2012b; Hori et al. 2013) and cumulative effects of high temperature and infections on swimming stamina (Kocan et al. 2009), will dictate the resilience of salmon populations to climate-driven changes in river temperatures (Crozier et al. 2008; Altizer et al. 2013).

Our predictive analysis of held fish demonstrated that in addition to high temperature exposure, heavier infection intensities and extracellular immune responses in gill as well as stress metabolites and indices of osmoregulatory impairment in blood were characteristic of fish that would die within 4 days. Whether fate-associated differences in blood properties at the start of the holding study indicated advanced senescence (Hruska et al. 2010; Jeffries et al. 2011) or stress accrued prior to collection (Wendelaar Bonga 1997) is unknown. Because stronger physiological responses (cortisol and estradiol regulation) to stressors were observed in survivors, the initial condition of fish likely affected their capacity to respond to stressors and their likelihood of survival. Recent studies of Chinook (Bass et al. 2017) and sockeye salmon (Teffer et al. 2017) have demonstrated similar associations between infection status and physiological indices. Relationships among infection burdens, blood properties, and immune gene expression point to disease-associated interactions occurring locally and systemically that influence longevity and migration behaviour of Chinook salmon in fresh water. The fitness consequences of early mortality are therefore tied to infectious agents, host condition, and stress and immune responses.

Major sources of uncertainty in current and historical Chinook salmon productivity estimates (Riddell et al. 2013) emphasize the importance of understanding the mechanisms of adult mortality to provide reliable data for predictive modeling of en route losses. Here, we provide information to be used toward this purpose and add another dimension of data describing the disease ecology of Chinook salmon in southwestern British Columbia. Collectively, our results support an influence of infectious disease development on early mortality and migratory behaviour of adult Chinook salmon during freshwater residence. This role is modulated

by thermal and fishery stressors as well as host physiology during and after exposure. Our findings add to growing knowledge of the disease dynamics of wild Pacific salmon and improve our predictive capability regarding how multiple stressors can reduce migration success and longevity in the river.

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