1 **Title:** Host-pathogen-environment interactions determine survival outcomes of adult sockeye salmon

2 (*Oncorhynchus nerka*) released from fisheries

3 **Running title**: Pathogens, temperature, and salmon survival

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23 **Abstract:** Incorporating host-pathogen(s)-environment axes into management and conservation planning

24 is critical to preserving species in a warming climate. However, the role pathogens play in host stress

25 resilience remains largely unexplored in wild animal populations. We experimentally characterized how

26 independent and cumulative stressors (fisheries handling, high water temperature) and natural infections

27 affected the health and longevity of released wild adult sockeye salmon (*Oncorhynchus nerka*) in British

28 Columbia, Canada. Returning adults were collected before and after entering the Fraser River, yielding

29 marine- and river-collected groups, respectively. Fish were exposed to a mild (seine) or severe (gill net)

30 fishery treatment at collection, and then held in circulating freshwater tanks for up to four weeks at

31 historical (14°C) or projected migration temperatures (18°C). Using weekly nonlethal gill biopsies and

32 high-throughput qPCR, we quantified loads of up to 46 pathogens with host stress and immune gene

33 expression. Marine-captured fish had less severe infections than river-captured fish, a short migration

34 distance (100 km, 5-7 d) that produced profound infection differences. At 14°C, river-collected fish

35 survived 1-2 weeks less than marine-collected fish. All fish held at 18°C died within 4 weeks unless they

36 experienced minimal handling. Gene expression correlated with infections in river-collected fish, while

37 marine-collected fish were more stressor-responsive. Cumulative stressors were detrimental regardless of

38 infections or collection location, likely due to extreme physiological disturbance. Because river-derived

39 infections correlated with single stressor responses, river entry likely decreases stressor resilience of adult

40 salmon by altering both physiological status and pathogen burdens, which redirect host responses toward

41 disease resistance.

42

43 **Introduction**

44 Infectious agents are integral components of wild animal ecology and commonly occur as coinfections

45 with variable impacts on host health (Johnson *et al.* 2015; Sofonea *et al.* 2017). In a traditional

46 framework, disease occurs at the intersection of the host, pathogen, and environment, but when studying

47 wild animal disease dynamics, an array of host-pathogen, pathogen-environment, and host-environment

48 relationships must be considered (Alizon, de Roode, & Michalakis, 2013; Engering, Hogerwerf, &

49 Slingenbergh, 2013; S. E. Mitchell, Rogers, Little, & Read, 2005). For example, migration is a common

50 life history trait of wild animals that can influence disease development at individual and population

51 scales (Altizer, Bartel, & Han, 2011). As the environment around the host changes, so does the nature of

52   host-pathogen relationships, thereby affecting pathogen species composition, host immune responses, and

53   whole animal performance (Altizer et al., 2011; Evans et al., 2011). Migration can also amplify the effects

54   of stressors experienced by wild animals (Lennox et al., 2016), with disease outcomes that presumably

55   depend on host responses and recovery as well as pathogen community dynamics (Altizer, Ostfeld,

56   Johnson, Kutz, & Harvell, 2013; S. E. Mitchell et al., 2005). Our knowledge of how multiple infections

57   and cumulative stressors affect wild animal population dynamics is limited, especially within the context

58   of migratory species.

59   Environmental and anthropogenic stressors are increasingly experienced by wild animals, with

60   potentially cumulative effects (Crain, Kroeker, & Halpern, 2008). Stressors can influence infection

61   development through mechanisms like immunological suppression (Tort, 2011), but whether infected

62   wild animals are less resilient to individual and cumulative stressors in the wild has proved logistically

63   difficult to test. Effectively characterizing pathogen influences on stressor resilience of wild animals

64   requires the measurement of stress responses under "natural" co-infection conditions (i.e., co-infections of

65   endemic agents; Paterson 2013). Most data describing the pathogenicity of infectious agents are from

66   studies that isolate a single pathogen. The virulence of a single infectious agent, however, may be

67   diminished or enhanced by the presence of another, so collective pathogen community structure is more

68   relevant to proximal virulence (e.g., current host survival and reproductive success) as well as the

69   evolution of virulence factors (Sofonea, Alizon, & Michalakis, 2015; Sofonea et al., 2017). Predictions of

70   population resilience to individual and cumulative stressors can be improved by identifying how pathogen

71   diversity and cumulative infection intensity are associated with host survival outcomes.

72   Pacific salmon (*Oncorhynchus* spp) are ideal model species for such an investigation given their

73   migratory life histories, multiple infections, and cumulative stressors affecting their survival (Groot &

74   Margolis, 1991; Miller et al., 2014; Teffer et al., 2017). Pacific salmon begin their lives in fresh water as

75   eggs, then migrate as juveniles to the marine environment to feed and grow, and finally return to natal

76   freshwater spawning grounds to spawn and then die (Groot & Margolis, 1991). Pre-spawning adult

77   mortality can have population-level impacts with economic, ecological, and cultural repercussions, in

78   addition to lost individual fitness and reduced spawning biomass (Hinch et al., 2012; Jacob, McDaniels,

79   & Hinch, 2010; Spromberg & Scholz, 2011; Willson & Halupka, 1995). Adult Pacific salmon cease

80   feeding prior to river entry, using endogenous energy reserves to fuel migration, maturation, and other

81   biological processes like immunity (Miller et al., 2009; Rand et al., 2006). Recent work has demonstrated

82   correlations between infection development, reduced immune defenses, and early mortality of adult

83   Pacific salmon in fresh water (e.g., Miller et al. 2014; Dolan et al. 2016; Teffer et al. 2017; Teffer et al.

84   2018), but causal linkages have yet to be established, especially considering multiple stressors and

85   spatially variant coinfection dynamics (marine versus freshwater).

86   Pacific salmon physiology and disease ecology have been well studied in the Fraser River watershed,

87   British Columbia (BC), Canada. Surveys have captured snapshots of infectious agent communities in

88   adult salmon that shift throughout the spawning migration (Bass, 2018; Bass, Hinch, Teffer, Patterson, &

89   Miller, 2017). Pathogen richness and loads generally increase after river entry where adult salmon are

90   exposed to an array of freshwater pathogens (e.g., myxozoan parasites; Atkinson et al. 2011;

91   Bartholomew et al. 1997; Bass 2018; Bass et al. 2017). Infection intensities of bacterial and parasitic

92   agents generally continue to increase with time spent and distance traveled in rivers (Bass et al., 2017;

93   Miller et al., 2014; Teffer et al., 2018, 2017). The array of infectious agents carried by migrating salmon

94   can have detrimental impacts on hosts if the environment becomes more stressful. High temperatures, for

95   example, alter infection development and host physiology (Bettge, Wahli, Segner, & Schmidt-Posthaus,

96   2009; Bruneaux et al., 2016; Farrell et al., 2008) and can compound the effects of additional stressors like

97   fishery capture and release (Gale, Hinch, & Donaldson, 2013). The Fraser River has experienced climate-

98   driven warming in recent decades (Patterson et al., 2007) and is Canada's largest salmon producer.

99   Several Pacific salmon species co-migrate during fishery openings, so non-target species are frequently

100   caught and released, which can have harmful delayed impacts on health, maturity, and survival, especially

101   if rivers are warm (Baker, Swanson, & Young, 2013; Patterson et al., 2017; Raby et al., 2015; Teffer et

102   al., 2017).

103   To identify the role of pathogens in the resilience of adult sockeye salmon (*Oncorhynchus nerka*) to

104   multiple stressors during spawning migration, fish were collected prior to or after river entry and

105   experimentally treated with thermal and (or) fishery treatments. Marine- and river-captured fish were

106   matched in maturation trajectories by timing collections with projected migration rates for the dominant

107   stock (Adams-Shuswap) and genetically confirming stock identity. We compared host responses to mild

108   (seine) or severe (gillnet) fishery bycatch simulation followed by holding at optimal (historic; 14°C) or

109   projected (18°C) water temperatures.

110   We hypothesized that infection burdens would be lower and survival rates higher in marine-captured

111   fish relative to river-captured fish and that survival regardless of collection location would be reduced

112   under independent and cumulative stressor treatments relative to treatment controls. The objectives of this

113   study were to identify differences in survival rates of adult sockeye salmon based on internal (infection

114   profiles, genomic responses) and external factors (thermal and capture stress, collection environment),

115   and characterize the interplay between infection profiles and genomic responses over time relative to

116   collection context, thermal experience, and longevity.

117

118   **Materials and Methods**

119   *Fish collection and treatment*

120   We focused fishing effort during the "Late run" sockeye salmon migration in the Fraser River, which

121   was dominated by the Adams-Shuswap stock complex during our collection period, confirmed by DNA

122   analysis (Beacham et al., 2004). On September 11-12, 2014, 153 sockeye salmon were collected by a

123   commercial purse seiner in the Strait of Georgia (15°C; 49.232 N, 123.271 W; Fig. 1). It is unlikely that

124   marine-captured fish entered and exited the river prior to collection given previously documented high

125   mortality of adult Pacific salmon exposed to seawater following freshwater exposure (Cooperman et al.,

126   2010; Hinch, Cooperman, Crossin, & Olsson, 2008). Fish were transported in live-wells filled with

127   seawater to a dock at the Fisheries and Oceans Canada (DFO) West Vancouver Laboratory, West

128   Vancouver, BC (40 min transport), where they were transferred using dipnets to truck-mounted tanks

129   filled with cold (~10°C), filtered, UV-treated water for transport to the DFO Cultus Lake Salmon

130   Research Laboratory, Cultus Lake, BC (1.25 h transport). Transport tanks were fitted with air stones and

131   continually monitored for temperature and dissolved oxygen.

132   At the Cultus Lake Laboratory, fish were sequentially distributed among 12 holding tanks filled with

133   sand-filtered, UV-treated water from the neighboring Cultus Lake at equal temperature to the lower

134   Fraser River during collection (14°C). Densities within holding tanks depended on tank size, which

135   included large (8000-10000 L), medium (4000 L) and small (1400 L) tanks; large tanks held ≤22 fish,

136   while medium tanks held ≤13 fish, and small tanks ≤5 fish. All tanks were covered and fitted with air

137   stones and a submersible pump (large tanks only) that produced a slow current around the tank periphery,

138   encouraging fish to swim in place during holding (approximately 1 body length sec^{-1}). The velocity of

139   water entering small tanks produced a similar current with no pump needed. Tank replicates included one

140   large and one small or medium tank per temperature-treatment group. Fish were left undisturbed for one

141   week to allow recovery from transport and to simulate the approximate migration time from the collection

142   location to the lower Fraser River. No marine-captured fish died during the first week of holding.

143   Beginning on September 17, the temperature was incrementally increased over 48 h from 14°C to 18°C in

144   half (six) of the tanks, producing two temperature groups with either a cool (14°C) or warm (18°C)

145   thermal experience; both temperatures are ecologically relevant: 14°C is the historical average

146   temperature that Late run Fraser River sockeye experienced during up-river migrations and 18°C is a

147   thermal extreme that late run salmon are encountering with increasing frequency resulting from climate

148   change and recent changes in river migration entry timing (Morrison, Quick, & Foreman, 2002; Patterson

149   et al., 2007).

150   One week after collection (19 Sep), one third of the marine-captured fish from each temperature group

151   was exposed to a fishery treatment that simulated capture and release from a gillnet. Following

152   methodologies applied successfully in previous studies (Teffer et al., 2018, 2019, 2017), the treatment

153   proceeded as follows: a fish was removed from its holding tank using a dipnet and immediately

154   submerged in a small (1400 L) treatment tank within the dipnet. The opening of the dipnet faced a taught

155   monofilament gillnet (mesh size: 5.25-inch, 13.3 cm) mounted in a wide frame. Upon exiting the dipnet,

156   the fish was "caught" in the gillnet and entanglement was maintained for 20 s. If the fish escaped, the

157   timer was stopped until entanglement had been achieved. After 20 s of sustained entanglement, the fish

158   and gillnet were pulled from the water and placed into a dipnet for 1 min of air exposure while the fish

159   was detangled from the gillnet (simulating bycatch release by fishers). The fish was then submerged in a

160   foam-lined, flow-through sampling trough (water flowing over gills and body) where a small amount of

161   gill tissue (2-3 filament tips, ~0.5 mg) was taken using sterile end clippers (sample preservation details

162   below), 2 mL of blood was extracted from the caudal vasculature (21-gauge needle with lithium

163   heparinized Vacutainer®, Becton-Dickson, NJ; data not shown), a Floy® "spaghetti" style tag (Seattle,

164   WA) was secured in the dorsal musculature, and a brief assessment of external injuries and condition was

165   recorded. The fish was then placed into a recovery tank (3000 L) for up to 30 min before being returned

166   to its holding tank. Water temperature throughout the treatment, biopsy, and recovery were consistent

167   with that of the fish's holding tank. The remaining marine-captured fish were divided into two control

168   groups: one biopsied and one left undisturbed until the termination of the study. Biopsied controls

169   followed the same tissue and blood sampling protocol described for gillnet-treated fish but proceeded

170   directly from holding tanks to the sampling trough (no gillnet or air treatment). The biopsy procedure

171   took <2 min overall and included <10 s of total air exposure.

172   During 24-26 Sep, 183 sockeye salmon were collected from the lower Fraser River near Fort Langley

173   (15-17°C), approximately 50 river kilometers (rkm) from the mouth of the Fraser River. River-captured

174   fish were not gillnet-treated in the laboratory but instead collected with either a gillnet (treatment; N=125)

175   or a beach seine (control; N=58) to reduce experimental handling. Beach seines have been previously

176   demonstrated as a minimally invasive fishing gear, associated with high survival of released catch relative

177   to other gear types (Bass, Hinch, Patterson, Cooke, & Farrell, 2018; Donaldson et al., 2012; Raby et al.,

178   2015). Disparity in sample sizes between gear types was unavoidable due to river conditions at the time of

179   collection that were more favorable to gillnet capture. Beach seines were deployed from shore, encircling

180   and corralling fish into shallow (0.5-1 m depth) water without beaching them. Gillnets were deployed in

181   deeper water near the middle of the river for <20 min sets. Gillnet- and seine-collected fish were removed

182   from nets following best fishery practices (e.g., quick removal of fish from gillnets by fishers "picking"

183   fish from the gill net by boat, dip-net removal of fish from the seine) and placed into net pens anchored in

184   the river until biopsy and (or) transfer to truck-mounted tanks.

185   Subsets of gillnet-collected (N=70) and seine-collected (N=25) fish were biopsied riverside for gill

186   tissue and blood and tagged following the same protocols described for marine-captured fish (sampling

187   trough supplied with fresh river water) prior to transport to the Cultus Lake Laboratory. The remaining

188   fish were not biopsied, serving as non-handled controls; however, to identify "treatment" (gear type), the

189   adipose fin was clipped from gillnet-collected controls using scissors within a cylindrical recovery bag

190   submerged in water (duration ≤10 s, no air exposure). Truck-transport conditions were identical to those

191   described for marine-collected fish, but transit time was approximately 40 min. Upon arrival at the lab,

192   fish were sequentially distributed among 12 holding tanks of equal temperature to the river during

193   collection (14°C), separate from marine-captured fish. Transport mortalities (N=16) were immediately

194   biopsied for gill and blood, examined for gross pathology (lesions, organ discoloration, macroparasites),

195   and morphometrics recorded including length (post-orbital hypural, ±1.0 cm), total weight (±1.0 g) and

196   organ weights as well as tag ID if applicable and gear type. An operculum biopsy punch was preserved in

197   90% EtOH for stock identification using microsatellite analysis (Beacham et al., 2004).

198   Tank temperatures were held relatively constant, allowing for some diurnal variation (±1.5°C).

199   However, we did incorporate behavioral thermoregulation of adult Pacific salmon during freshwater

200   migration into the thermal experience of held fish. In the wild, individuals temporarily reside near the

201   thermocline of corridor lakes (Newell & Quinn, 2005). Therefore, beginning approximately 10 d after

202   treatment (30 Sep – 1 Oct for marine-sourced, 5–6 Oct for river-sourced), all tank temperatures were

203   decreased to 10°C for 48 h and then increased back to experimental temperatures (14°C or 18°C) and

204   maintained for the remainder of the holding period.

205   Biopsy of all fish was repeated weekly until study termination on 16-18 Oct, resulting in four weeks of

206   gill biopsies for marine-captured fish and 3 weeks for river-captured fish, plus a terminal gill biopsy at

207   death for all fish. Sampling troughs and recovery tanks were sanitized after all fish in each tank had been

208   processed to prevent transmission of infectious agents among tanks. Throughout the experiment, tanks

209   were monitored for water quality, temperature, and fish morbidity at ≤4 h intervals from 0800–2400 h.

210   Fish that became moribund (gulping, loss of equilibrium) during the study, and all surviving fish at the

211   termination of the study (16–18 Oct, marking beginning of the spawning period for the Adams-Shuswap

212   stock complex), were sacrificed using cerebral concussion and cervical dislocation and gill biopsied. Gill

213   samples were immediately stored in 1.5 mL RNA*later*® solution (Ambion, Inc., Austin, TX, USA) and

214   stored at 4°C for 24 h, then -20°C for up to two months, and then -80°C for three months until analysis.

215

216   *Laboratory analyses*

217   Gill samples were processed at the DFO Pacific Biological Station in Nanaimo, BC using high-

218   throughput quantitative polymerase chain reaction (HT-qPCR) on the Fluidigm BioMark Dynamic Array

219   microfluidics platform™ (Fluidigm, San Francisco, CA, USA). This technology allows for the

220   simultaneous quantification of 96 molecular assays (i.e., targeting either host or infectious agent genes)

221   on 96 tissue samples; the platform has been analytically validated against traditional qPCR for its use in

222   infectious agent screening (Miller et al., 2016), applied in multiple field surveys of wild salmon

223   populations (Bass et al., 2017; Nekouei et al., 2018; Thakur et al., 2018; Tucker, Li, Kaukinen, Patterson,

224   & Miller, 2018), and paired with evaluations of host gene expression (Jeffries, Hinch, Gale, et al., 2014;

225   Miller et al., 2017, 2014; Teffer et al., 2019, 2017). Here, we used this tool to characterize the

226   development of multiple infections in gill during a ≥5-week period simultaneously with the expression of

227   a suite of host stress and immune genes to describe how differences in initial infection burdens, infection

228   development, and host responses in gill contribute to the early mortality of Pacific salmon. A suite of 17

229   infectious agents were evaluated in gills based on a survey of Late run Adams sockeye salmon conducted

230   in the same year as the present study (Bass, 2018). The survey screened for 45 infectious agents,

231   including viruses, bacteria and various parasites, in multi-tissue pools of wild sockeye salmon throughout

232   their migration to spawning grounds. Agents detected by the survey, including high and low prevalence

233   and potentially pathogenic organisms, were included in our analysis (Table 1). We evaluated biomarkers

234   of host stress and immunity (N=27 genes) that comprised aspects of osmotic stress, heat shock, innate and

235   adaptive immunity, tissue repair and others, evaluated simultaneously with two host reference genes and

236   17 infectious agents (Table 1). Our infectious agent screening approach quantifies RNA rather than DNA

237   of infectious agents to measure variation in 'productivity' (e.g., RNA transcription and maintenance) of

238   active infections based on the expression of each target gene. As target gene types differed among assays

239   depending on the infectious agent (i.e. surface protein, ribosomal, etc.; see Miller et al. 2016), relative

240   loads can only be compared within agent species, not across.

241   Tissue samples were trimmed in the lab for size uniformity and then homogenized in sterile microtubes

242   with stainless steel beads using 600 µL TRI-reagent^{TM} 148 (Ambion Inc., Austin, TX, USA), 75 µL 1-

243   bromo-3-chloropropane and a MM301 mixer mill (Restch Inc., Newtown, PA, USA). Centrifugation (6.5

244   min) separated the aqueous phase, which was aliquoted into 96-well plates for RNA purification. The

245   "spin method" for Magmax™-96 for Microarrays Kits (Ambion Inc.) was used to purify RNA following

246   manufacturer's instructions, using a Biomek FXP liquid handler (Beckman-Coulter, Indianapolis, IN,

247   USA) and including a DNase treatment after the first wash. RNA quality and quantity were assessed

248   using spectrophotometry (A_{260}, $A_{260/280}$) and samples were normalized to 1 µg RNA prior to cDNA

249   synthesis. Samples with low RNA yield (<62.5 ng/ul) were removed from analyses. Invitrogen™

250   SuperScript™ VILO™ (Carlsbad, CA, USA) cDNA Synthesis Kit synthesized cDNA under cycling

251   conditions 25°C for 10 min, 42°C for 60 min and 85°C for 5 min. As per manufacturer's

252   recommendations (BioMark™), pre-amplification of cDNA was completed in a multiplex PCR including

253   all primers to be evaluated by qPCR (200 nM primer mix, TaqMan Preamp Master Mix, Applied

254   Biosystems, Foster City, CA, USA). Due to the nanofluidic properties of the Fluidigm BioMark™, pre-

255   amplification is necessary to achieve adequate sensitivity. Cycling conditions for the pre-amplification

256   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

257   ExoSap-it® Product Clean-up (Affymetrix Inc., Santa Clara, CA, USA) cycled at 37°C for 15 min then

258   80°C for 15 min, and then a 5-fold dilution (TEKnova suspension buffer, Hollister, CA, USA). A pool of

259   gill samples from N=20 fish sacrificed riverside during the collection of river-sourced fish was included

260   on all chips as a positive control prior to and following pre-amplification (e.g. cDNA positive control,

261   pre-amplification control); negative controls were also included at each step in the protocol. A serial

262   dilution of artificial positive constructs (APC clones) matching the primer-probe sequence for each

263   infectious agent under evaluation was added to the dynamic array just prior to qPCR and tagged with a

264   secondary probe (NED™ reporter dye) to identify potential contamination of samples. Samples (TaqMan

265   Universal Master-Mix, Life Technologies; GE Sample Loading Reagent, Fluidigm, pre-amplified cDNA)

266   and assays (in duplicate; 10 μM primers, 3μM probes for Taqman assays) were loaded onto dynamic

267   arrays using the integrated fluidics controller HX (Fluidigm) and qPCR was completed following 50°C

268   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

269   sample combinations were analyzed in independent 7 nl wells on the dynamic arrays.

270   The BioMark Real-Time PCR analysis software was used to manually score output, following

271   protocols described in Miller et al. (2016). Infectious agents that were not positive in duplicate were failed

272   and quantification cycles (Cq) for duplicates were averaged for host genes and infectious agents. Host

273   biomarkers were normalized to the average of the two reference genes and are reported as relative

274   expression following the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Infectious agent Cq was subtracted

275 from 40 (maximum Cq), producing what is referred to herein as "relative load," and is therefore a

276 representation of RNA expression. Note that relative loads are platform-specific, as the range of Cq

277 values differ between the BioMark™ and traditional qPCR platforms. Further information regarding the

278 protocols described above can be found in Teffer et al. (2017) and details regarding the BioMark™

279 platform's applications in infectious agent screening and validation are described in Miller et al. (2016).

280

281 *Statistical analyses*

282 　　Longevity was calculated as the total days surviving after treatment (marine-captured: lab treatment;

283 river-captured: collection); note that the holding period was shorter for river-captured fish due to the lag

284 between collection dates. Sex-specific differences in infection development (Bass et al., 2017; Teffer et

285 al., 2017) and migration success (Martins et al., 2012) under sub-optimal conditions prompted the

286 investigation of sex as a cofactor. Differences in survival between capture locations (source), sexes,

287 treatments (gillnet-treated/captured, biopsied controls, non-biopsied controls), and temperatures were

288 characterized using survival analysis (Cox proportional hazards, *survival* package) and linear models

289 (LM) in the R statistical software (R Core Team, 2015; Therneau, 2014). For Cox regression analyses that

290 included both sources, days surviving was censored at 21 d post-treatment to avoid the bias of extended

291 holding time for low temperature marine-sourced fish (i.e., earlier capture and longer survival), while

292 models including only one source group and Kaplan-Meier curves used all days surviving. For marine-

293 sourced fish, survival analyses were performed relative to non-handled and handled (biopsied) controls to

294 identify potential impacts of experimental handling and biopsy on survival. For river-sourced fish, non-

295 handled controls and biopsied fish were included among both gillnet- and seine-captured groups, which

296 allowed "experimental handling and biopsy" to be included as a cofactor in the survival analyses of river-

297 sourced fish.

298 　　Overall effects of sex were tested in a model that stratified source, treatment, and temperature to

299 determine if sex should be included in subsequent models. Sex was not identified as significantly

300   affecting survival, so sexes were pooled for subsequent analyses (see results). Source effects were then

301   evaluated, stratifying all data by treatment and temperature. Treatment, temperature, and biopsy

302   (experimental handling) effects were then evaluated within each source group. For Cox proportional

303   hazards analyses, hazard ratios (exponents of coefficients; e^{β}) for significant effects are presented, which

304   correspond to the daily hazard of mortality, as well as model r^2 and likelihood ratio tests for model

305   significance. Where assumptions of the Cox regressions could not be met, coefficients ($\beta \pm$ standard

306   error) for significant parameters and interactions are presented from linear models.

307   To assess the relative influences of source, temperature, gillnetting, sex, and time on infection metrics,

308   we used linear mixed effects (LME) models with a random intercept that accounted for re-sampling of

309   individuals over time (i.e., fish ID as a random effect for repeated measures). Interactions of time (weeks

310   post-treatment) with source, temperature, and gillnetting tested for differences in infection development

311   depending on each factor. Interactions of source with temperature and treatment elucidated source-

312   dependent differences in infections. A top-down approach for model selection was used to identify

313   significant interactions and factors associated with relative infections burden (RIB, a composite metric of

314   infectious loads and richness, see Bass, Hinch, Teffer, Patterson, & Miller, 2019), richness, and infectious

315   loads of highly prevalent agents (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). A P<0.05 *a priori* cut-

316   off was applied for likelihood ratio tests comparing models including and excluding each variable and

317   interaction term, starting with a full model that included all possible factors and interactions and then

318   removing those with low t-values and high P-values in a stepwise fashion. Significance and coefficients

319   therefore pertain to the role of each variable or interaction in describing the infection metric data within

320   the final model. The final model included only significant interaction terms and factors, as well as main

321   effects that served as components of significant interactions.

322   Survival analysis with time-dependent covariates tested whether enhanced RIB or individual infections

323   increased daily host mortality risk. Monitored temporal changes in RIB and individual infectious agent

324   presence and loads in gill were incorporated into a survival analysis with Cox proportional hazards. All

325   models included treatment and sex as constant co-factors to account for their associated variance. Model

326   assumptions were evaluated: influential observations were removed and models with proportional hazard

327   violations were stratified by treatment or sex or complemented by an interaction of the offending factor

328   with time. Models were constructed separately for marine- and river-collected fish and within temperature

329   groups due to the strong influence of these factors on survival. Separate models for each infection metric

330   were performed because each is measured on a unique scale (i.e., qPCR assays designed to different

331   genes with varying functions). Infection data varied over time at the resolution of experimental day: non-

332   lethal gill samples were generally taken at ~7-d increments, but gill data from dying fish in the interim

333   between sampling events were also incorporated, with the lowest temporal increment set at 1 d.

334   Experimental start was the same as that described above for the general survival analysis. Time-

335   dependent covariates included RIB (log-transformed), presence/absence (1=positive detection, 0=no

336   detection), and high loads (1=greater than the mean load of positive detections, 0=less than the mean load

337   or negative detection). Sample sizes of positive detections across all individuals and repeated samples (N)

338   and exponents of coefficients (e^{β}, daily hazard of mortality) for significant (P<0.05) infection metrics are

339   reported for significant models (likelihood ratio test, P<0.05). Infectious agent community composition

340   among biopsied fish was qualitatively evaluated by normalizing the loads of each positive detection to the

341   maximum for each species (i.e., the first step in the RIB calculation). Normalized loads were then

342   summed for each agent in each source-temperature-treatment group and totals plotted as a function of

343   time (i.e., relative contributions to total normalized loads of all agents in each temperature-treatment

344   group at each weekly interval).

345   Permutational multivariate analysis of variance (PERMANOVA) was used to assess contributions of

346   source, sex, RIB, temperature, and gillnetting (including a temperature-gillnet interaction term) to overall

347   variation in gene expression data (i.e., multivariate response). Twenty-two biomarkers of stress and

348   immunity comprised aspects of heat shock responses, osmotic imbalance, innate and adaptive immunity

349   and tissue repair (Table 1). Relative expression of all biomarkers was used as the response matrix for the

350   PERMANOVA during each week of the study. Data from samples taken at morbidity were included in

351   the analysis within the week that the animal died. Non-biopsied marine controls and seine-collected river

352   fish were excluded from the PERMANOVA as the longevity of these groups was biased toward study

353   termination, producing unbalanced sample sizes with disparate response profiles. Samples taken at death

354   from non-biopsied gillnet-collected fish were included in the analysis because they showed similar

355   survival patterns to biopsied fish, which improved our power to detect thermal and sex-specific

356   differences. Unsupervised principal component analysis (PCA) was used to relate individual biomarker

357   expression to each factor using linear models with component axes (PC) as response variables and factors

358   used in PERMANOVAs as predictors, including a temperature-treatment interaction where sample sizes

359   permitted. PCs were included that explained >10% of the variance (i.e., eigenvalues) and contributed to

360   significant (P<0.05) linear models. Significant factors in linear models are discussed with respect to

361   biomarkers most positively and negatively loaded (i.e., eigenvectors) on the corresponding PC axis. Only

362   fish identified as part of the Adams-Shuswap stock complex were included in analyses of survival,

363   infection metrics, and gene expression to avoid potential stock biases.

364

365   **Results**

366   *Survival*

367   Percentages of fish surviving to the spawning period of this population are presented first, followed by

368   the results of the survival analyses. Survival was highest at 14°C for both marine and freshwater collected

369   groups. Marine-captured controls (with and without biopsy) and gillnet-treated males survived 100% at

370   14°C, while gillnet-treated females survived 92% (Fig. 2, Table 2). Low sample sizes for several groups

371   warrant caution in sex-specific comparisons of survival for river-captured fish (see Table 2). Among

372   river-captured seine-collected fish held at 14°C, males and females survived 100% if not biopsied, while

373   biopsy reduced survival of females to 0% (N=1) and males to 75% (N=4). Among river-captured gillnet-

374   collected fish held at 14°C, both sexes survived better if not biopsied (F=55%, M=82%) than if biopsied

375   (F=44%, M=50%). At 18°C, gillnetting and biopsy reduced the survival of marine-captured males and

376   females to 0%, while non-biopsied controls survived relatively well (F=73%, M=100%). Similarly, 0% of

377   river-captured fish held at 18°C survived if biopsied (regardless of gear type) and few non-biopsied

378   gillnetted (F=8%, M=0%) or seine-collected fish (F=8%, M=17%) survived to study termination.

379   Survival analysis identified a significant effect of collection location, where river-captured fish were

380   3.3 times (P<0.001) more likely to die (each day) than marine-captured fish (model r^2=0.14, P<0.001). No

381   significant effect of sex on survival was identified overall (P=0.255) or within source groups (P>0.400),

382   so sexes were pooled in subsequent survival models. Among river-captured fish, high temperature

383   (e^β=47.98, P<0.001) and gillnetting (e^β=5.30, P=0.04) significantly decreased the likelihood of survival,

384   with no significant interaction (P=0.098). However, the effect of biopsy (e^β=5.16, P=0.012) was similar to

385   that of gillnetting, which we interpret as no effect beyond experimental handling and biopsy.

386   Furthermore, a significant interaction between temperature and biopsy (e^β=0.26, P=0.015) suggested that

387   the negative biopsy effect on survival was reduced (or masked) at high temperature (model r^2=0.48,

388   P<0.001).

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

390   Cox model assumptions and prompted the use of simple linear models for survival analysis. Qualitatively,

391   survival among marine-captured treatments was similar at 14°C (only one female held at 14°C died prior

392   to study termination). A linear model including both temperature groups of marine-captured fish

393   identified significant interactions of temperature with gillnetting (β=-11.99±1.15, P<0.001) and biopsy

394   (β=-13.39±1.23, P<0.001), supporting decreased resilience of marine-captured fish to handling stress at

395   high temperature only. Longevity of marine-captured fish was also independently reduced by high

396   temperature (β=-1.75±0.84, P=0.041). Among fish held at 14°C, no impact of either biopsy or gillnetting

397   was apparent (P>0.05), while at 18°C, biopsy (β=-12.54±1.07, P<0.001) and gillnetting (β=-12.05±1.03,

398   P<0.001) similarly reduced survival relative to non-handled controls. Gillnetting, however, had no effect

399   beyond experimental handling (significance of gillnet effect relative to biopsy: P=0.587).

400

Infection metrics in gill

402   Prevalence was highest overall for '*Ca*. B. cysticola', *I. multifiliis*, *F. psychrophilum*, and RLO (Table

403   S1). *C. shasta* and *P. minibicornis* were prevalent only in river-captured fish, especially in later weeks. *P.*

404   *theridion*, *L. salmonae*, *P. pseudobranchicola* (primarily cool water)*, A. hydrophila* and *A. salmonicida*

405   (river) were moderately prevalent, whereas *D. salmonis* (primarily first biopsy, river), *M. arcticus* and *S.*

406   *destruens* were minimally prevalent. Overall prevalence by treatment and source can be found in Table

407   S2.

408   Richness, *F. psychrophilum*, *I. multifiliis*, and '*Ca.* B. cysticola' loads increased at a faster rate in river-

409   captured fish (i.e., significant interaction of source with time; Fig. 3, model coefficients in Table 3). RIB

410   and '*Ca.* B. cysticola' increased at a faster rate in warm water, whereas *I. multifiliis* and RLO were

411   consistently higher in warm water (no interaction). '*Ca.* B. cysticola' increased at a faster rate in

412   gillnetted fish from both marine and river locales, but positive effects of gillnetting on RIB were only

413   evident in river-captured fish. *F. psychrophilum* was higher in females than males. *C. shasta* and *P.*

414   *minibicornis* loads were only increased by time (evaluated in river-sourced fish only). Low intraclass

415   correlation coefficients were apparent for *I. multifiliis*, RLO, *C. shasta* and *P. minibicornis*, suggesting

416   high temporal variability within individuals.

417   Survival analysis with time-dependent infection covariates demonstrated associations of various

418   infectious agents and RIB with early mortality (Table 4). This analysis could not be completed for

419   marine-captured cool water fish due to extremely low mortality (insufficient data). For marine-captured

420   fish held in warm water, only *A. hydrophila* was associated with a higher risk of mortality (presence

421   effect decreased with time), whereas the presence of *F. psychrophilum* was associated with slightly

422   decreased mortality risk. For river-captured fish in cool water, the presence of *L. salmonae* (stratified by

423   sex to satisfy proportional hazards), high loads of *F. psychrophilum*, and the presence and high loads of *P.*

424   *minibicornis*, *I. multifiliis* and RLO, and high RIB all increased mortality risk. For river-captured fish in

425   warm water, the presence of *A. hydrophila*, *L. salmonae*, and *P. minibicornis*, high loads of *I. multifiliis*

426   and the presence and high loads of *A. salmonicida*, RLO and *C. shasta* and high RIB were associated with

427   increased mortality risk.

428   Infectious agent community composition was dynamic over time, but with few drastic shifts in

429   composition and a high degree of similarity between source-temperature-treatment groups (Fig. 4).

430   Several compositional changes were temperature- and source-dependent, with less distinction between

431   gear types. Greater species richness in river-captured fish allowed for more variation in composition with

432   time, demonstrating losses and decreased loads of several agents within the first two weeks and

433   subsequent increases in the representation of myxozoan and other parasites as well as some bacteria (*A.*

434   *salmonicida*, RLO). In cool water, representation of *F. psychrophilum* was consistently reduced in later

435   weeks among marine-sourced fish, due to both mortality of infected fish and apparent clearance of the

436   bacteria in survivors (Fig S1), but was well-represented throughout time among river-captured fish. In

437   warm water, however, losses of *F. psychrophilum* representation at later sampling dates were evident and

438   concurrent with enhanced representation of *A. salmonicida* (river) and *A. hydrophila* (marine).

439

440   *Host stress and immune responses*

441   There was a consistent relationship between gene expression and temperature regardless of capture

442   location, though among river fish, RIB was also a consistent driver of gene expression and some specific

443   gene responses were shared between RIB and thermal stress. Intracellular immunity genes showed

444   decreasing relevance to stressor responses in later weeks in both marine- and river-captured fish.

445   For marine-captured fish, high temperature was the primary factor contributing to variation in gene

446   expression during weeks 0-3 (r^2 range: 0.24-0.42), increasing in importance over time (Table 5, Fig. 5).

447   The effect of gillnet treatment was weakly temperature-dependent during weeks 0-1 (interactions

448     $P \leq 0.022$), but after only cool temperature fish remained (weeks 3-4), its independent effect increased

449   (r^2=0.08-0.19). RIB was only significantly associated with gene expression of marine-captured fish during

450   week 2 (r^2=0.17, P<0.001).

451   The temperature-gillnet interaction term at week 0 was negatively associated with PC1 for marine-

452   captured fish, while high temperature negatively associated with PC2, demonstrating cellular and osmotic

453   stress (HSP90, GR2, NKA_a1b), iron regulation (TF) and tissue repair (MMP13) in thermally stressed

454   fish. Thermally stressed gillnetted fish also associated with the expression of JUN, HSC70, and all other

455   biomarkers, which loaded negatively on PC1. Gillnetting was positively associated with PC3 along with

456   stress indices, intracellular immunity, and antiviral activity, whereas females were negatively associated

457   with PC3, correlating with the expression of cytokines, immune receptors (CD83), and antiviral genes

458   (IFNa, Mx). By the end of week 1, thermally stressed fish were negatively associated with PC1 and

459   positively associated with PC2, which corresponded to the expression of HSP90, MMP13 and C7,

460   whereas cool temperature fish were more strongly associated with antiviral indicators, cellular receptors

461   and cellular energy generation. At week 2, thermally stressed fish were positively associated with PC1

462   and PC2, corresponding to gene loadings indicating tissue repair, cellular stress, cytokine and chemokine

463   activity, iron regulation (TF) and complement (C7). RIB was also positively associated with PC1, but

464   negatively with PC2, suggesting greater energy needs and osmotic stress (NKA_a1b) in addition to the

465   associations described for thermally stressed fish. Weeks 3 and 4 included only cool temperature fish due

466   to mortality at high temperature (i.e., low sample sizes due to fewer surviving fish), and week 4 included

467   survivors sacrificed at the start of the spawning period (study termination). Gillnetted fish were positively

468   loaded on PC2 at week 3 indicating tissue repair and inflammation (IL8, MMP13), iron regulation,

469   complement, and increased cellular stress and energy needs. At week 4, gillnetted fish loaded negatively

470   on PC1 with indices of inflammation (IL8), cellular stress (JUN, HSC70) and some cellular receptors,

471   which loaded opposite to the expression of Mx and other antiviral components.

472   For river-captured fish, temperature was not evaluated at week 0 (no thermal application at capture) but

473   gained importance in weeks 1 and 2 (r^2=0.06 & 0.22, respectively). Gillnetting was marginally associated

474   with gene expression ($r^2 \leq 0.06$) only at week 0 and 1 and with no interaction with temperature. RIB was

475   the primary factor associated with gene expression of river-collected fish, increasing the amount of

476   variation explained with time (r^2 range: 0.04-0.51).

477   For river-captured fish at collection (treatment), gillnetted fish were negatively associated with PC2

478   and PC3, indicating expression profiles consistent with cellular stress (GR2, JUN, HSC70), osmotic

479   imbalance (NKA_a1b), iron regulation (TF), antiviral activity (RIG1) and extracellular receptor (CD4)

480   genes. During week 1, RIB was positively associated with PC1, indicating inflammation (IL11, IL8,

481   CXCR4), iron regulation, tissue repair (MMP13) and complement (C7) as characteristics of fish with high

482   RIB, while adaptive immunity (b2m, MHCIIb), RIG1 and protein repair (HSC70) were associated with

483   low RIB. At week 2, RIB was again strongly positively associated with PC1, demonstrating a similar

484   profile to that described for week 1. Thermally stressed fish and females were both positively associated

485   with PC2, suggesting that these fish were recruiting aspects of cellular stress response, inflammation,

486   tissue repair, and iron regulation, while neglecting most cellular immune aspects. In the final week of

487   holding, including only cool water fish and survivors sacrificed at study termination, RIB was strongly

488   negatively associated with PC1, reflecting the same gene set correlations as in previous weeks.

489

490   **Discussion**

491   This study experimentally quantified how environmental conditions, pathogen burden, and host

492   genomic responses collectively influence the longevity of wild adult Pacific salmon during their once-in-

493   a-lifetime spawning migration. Pathogen community dynamics and host genomic responses to

494   experimentally applied cumulative stressors differed between fish collected from marine and freshwater

495   locations. Our results provide evidence to implicate river-derived infections as causal factors of stressor-

496   mediated early mortality of sockeye salmon during spawning migration. As predicted and previously

497   shown (Bass, 2018; Bass et al., 2017), fish collected from the lower Fraser River carried heavier gill

498   infections than fish collected from the Strait of Georgia. This result is striking in that such a short

499   migration distance (approx. 100 km, 5-7 d lag in sampling) could produce these profound differences in

500   infection burden, primarily due to the accumulation of river-derived infections (e.g., myxozoan parasites)

501   upon freshwater entry. Also consistent with our initial hypothesis, marine-collected fish that bypassed the

502   lower river had higher survival in cool water than river-exposed fish, which survived poorly (~50%) and

503   died sooner unless they were collected under the most benign conditions (seine) with no additional

504   handling. Handling effects are emphasized in our results because survival of fish exposed to gillnetting

505   and air exposure showed no difference from biopsied controls, highlighting the detrimental effects of any

506   level of handling.

507   Supporting our predictions about stressor tolerance, river-captured fish survived more poorly than

508   marine-captured fish when exposed to a single stressor (handling or high temperature). Thermal stress

509   tolerance of adult sockeye salmon was drastically reduced in river-collected fish as only non-handled

510   marine-collected fish survived well at high temperature (~80%). Relative to seine-collected controls,

511   gillnetting and repeated handling (biopsy) reduced the survival of river-captured fish, though sample sizes

512   were low. Furthermore, river-captured fish demonstrated stronger relationships between infection

513   profiles, host gene expression, and early mortality than marine-captured fish. These results support our

514   prediction that enhanced pathogen burdens due to river exposure would reduce host stressor resilience,

515   but only in the context of single stressors. Conversely, we found no difference in survival rates between

516   marine- and river-collected groups when exposed to cumulative stressors (combined thermal and

517   fisheries). Host physiological and immune data from this and previous studies (Teffer et al., 2018, 2019,

518   2017) suggest that the mechanisms of adult Pacific salmon mortality under cumulative stressors are likely

519   associated with physiological impairment and a decreased threshold for multiple infections. Most notably,

520   nearly all handled fish held in warm water died prior to the spawning period for this population,

521   regardless of capture locale. These results are highly relevant as climate change continues to warm

522   salmon bearing rivers across their range.

523

524   *Fishery and thermal stressor effects on fish survival*

525   Our survival results align with those described by Martins *et al.* (2011), in that model-averaged survival

526   for Adams sockeye (the same stock complex that is studied in the present study) at 14°C was 90-100%

527   (standard error range) in seawater and 60-100% in the river. At 18°C, however, Martins et al. (2011)

528   estimated 80-90% survival in seawater and 15-25% in the river, which more closely resembles survival

529   rates of non-biopsied controls in the present study. This discrepancy suggests that experimental holding

530   may compound handling effects at high temperature. Regarding improved survival of fish released in the

531   ocean versus the river, differences in salinity and temperature of the recovery environment, predation,

532   repeat capture probability, and gear type (Raby et al., 2015), as well as infection burdens (present study),

533   collectively influence survival likelihood and will vary by capture locations. Far more fish died in the first

534   24 h following collection from the river than from the marine environment in the present study. This

535   finding points to proximal causes of mortality in a subset of river-exposed fish, such as cardiac collapse or

536   anaerobiosis (Eliason et al., 2011; Fenkes, Shiels, Fitzpatrick, & Nudds, 2016; Raby et al., 2015).

537   Linkages between infections and aerobic or osmotic capacity should be assessed in future studies as these

538   responses could be due to compromised osmoregulatory or aerobic capacity from previous and current

539   infections (i.e., disease effects on aerobic resiliency to fishery stress; Ewing et al. 1994; Nematollahi et al.

540   2003; Bradford et al. 2010).

541

542   *Stressor effects on infection trajectories and host responses*

543   Temperature has been coined the "master" factor (Fry, 1971) due to its strong influence on fish

544   physiology and behavior (Pacific salmon e.g., Jain and Farrell 2003, MacNutt *et al.* 2004, Kocan *et al.*

545   2009, Jeffries *et al.* 2012, 2014b), including the potential to exacerbate fishery impacts (Gale et al., 2013).

546   Elevated river temperatures similar to those applied in this study are already impacting sockeye salmon

547   populations during freshwater migrations (Patterson et al., 2007). Regardless of collection location (and

548   hence initial infection status), handled fish held at an ecologically relevant 18°C did not survive to the

549   spawning period of their population (≥4 weeks). This result has drastic implications for the fate of

550   released sockeye bycatch in the lower Fraser River when temperatures are high (even with minimal

551   handling) and supports the closure of fisheries when waters are warm to improve post-release survival

552   (Martins et al., 2012). Additionally, given that river-captured fish survived poorly at high temperature

553   even in the absence of handling, river-derived infections are likely causal factors of natural mortality at

554   high temperature as well.

555   High temperatures have been shown here and previously to accelerate infection development

556   (Bradford, Lovy, & Patterson, 2010; Miller et al., 2014; Teffer et al., 2018; Wagner et al., 2005),

557   modulate immune gene expression (Jeffries et al., 2012; Teffer et al., 2018), and occur concurrently with

558   increased mortality of wild adult Pacific salmon during freshwater migration (Hinch et al., 2012; Keefer,

559   Peery, & Heinrich, 2008; Martins et al., 2012). Thermal stress enhanced bacterial and parasitic infections

560   that either maintained high loads over time or showed accelerated infection development (i.e., higher

561   loads at later time points). Enhanced infection development is a common response of infectious agents to

562   increased temperature (e.g. Mitchell *et al.* 2005, Bettge *et al.* 2009, Kocan *et al.* 2009) in combination

563   with host cellular stress responses (Jeffries, Hinch, Sierocinski, et al., 2014; Kassahn, Crozier, Pörtner, &

564   Caley, 2009). Thermally driven amplification of RIB did not differ between marine- and river-captured

565   fish, suggesting that collective infection development is independent of initial infection status. RIB was

566   initially low for fish from both capture locales at high temperature, followed by a sharp increase

567   especially among gillnetted fish and early mortalities. Infectious agent community composition, richness,

568   and structure did vary between marine- and river-captured fish, and thermal impacts on loads differed

569   among agent species. The mechanisms of mortality therefore depend at least partially on capture

570   locations, as well as immune impairment (Dittmar, Janssen, Kuske, Kurtz, & Scharsack, 2014) and

571   variable direct thermal impacts on pathogens (e.g., Udey *et al.* 1975; Groberg *et al.* 1978; Aihua and

572   Buchmann 2001).

573   Thermally stressed fish showed greater expression of stress and immune genes such as complement,

574   iron metabolism, and inflammatory responses rather than adaptive immunity and antiviral responses. Our

575   survival results suggest that these responses are inadequate to prevent mortality at high temperature, given

576   that nearly all thermally stressed fish died early. Chronic stress is known to be immunosuppressive,

577   including negative impacts of cortisol on antibody production and inflammatory responses through

578   glucocorticoid receptor suppression (Tort, 2011; Zwollo, 2018). Indeed, thermally stressed marine-

579   captured fish showed an initial positive association with GR2 expression in gill but then little correlation

580   in following weeks when inflammatory biomarkers were more prominently featured. An acute stress

581   response, as would be expressed follow handling, can divert immunity toward innate responses (Demers

582   & Bayne, 1997; Zwollo, 2018). Regarding the mechanisms of cumulative stressor effects on the survival

583   of marine-captured fish, acute stress responses may be maladaptive in thermally stressed salmon that are

584   already immune-compromised (Jeffries et al., 2012) and heavily infected (present study).

585   River-derived infections may reduce the ability of hosts to maintain low infection burdens following

586   acute fishery stress given that infection development following gillnetting (at optimal temperature) was

587   accelerated among river-exposed fish only. Our results showed that river-captured fish demonstrated

588   divergence from marine-captured fish in stress and immune gene expression, with profiles associating

589   with infections more than stressors. Conversely, gene expression profiles of marine-captured fish were

590   more strongly influenced by temperature and showed divergent responses to fishery stress. The survival

591   and host responses of marine-captured fish support additive effects of thermal and capture stress

592   previously documented in sockeye and coho (*Oncorhynchus kisutch*) salmon (Gale, Hinch, Eliason,

593   Cooke, & Patterson, 2011; Teffer et al., 2019), but this effect may only occur in a low infection scenario.

594   Overall, a divergence in expression profiles depending on river-exposure suggests that alternate host

595   response tactics to capture and thermal stressors are contingent on capture location (marine or

596   freshwater), with river entry associated with enhanced infection burdens and decreased longevity.

597   Heightened demands on the immune system following river entry are evidenced by the strong

598   correlation between infection burden and immune gene expression in river-exposed fish. Up to eight

599   different bacterial and parasitic agents were associated with early mortality of river-exposed fish, with a

600   greater influence of parasitic agents (*P. minibicornis, I. multifiliis, L. salmonae*) in cool water and

601   bacterial agents (*A. hydrophila, A. salmonicida*) in warm water. Each of these parasitic agents have been

602   associated with migratory failure, premature mortality, and (or) thermal stress in Pacific salmon

603   populations (Bradford, Lovy, Patterson, et al., 2010; Crossin et al., 2008; Miller et al., 2014; Traxler,

604   Richard, & Mcdonald, 1998). Mortality of other *Oncorhynchus* species experimentally challenged with *A.*

605   *hydrophila* and *A. salmonicida* was previously demonstrated at high temperature (Groberg et al., 1978).

606   Interestingly, relationships with mortality do not necessarily align with positive temperature-load

607   correlations, as thermal stress also increased the loads of several agents that were not associated with host

608   mortality. Therefore, although the loads of some agents, including bacteria, may be useful virulence

609   indicators at high temperature, other agents may have pathogenic properties at low infection intensities.

610   For example, the tolerance thresholds for infection development for some agents may be decreased in

611   hosts at high temperature due to physiological impairment (Alcorn, Murra, & Pascho, 2002) or an

612   inhospitable host environment (Thomas & Blanford, 2003). Interactions between pathogens may also be

613   altered by thermal stress through changes in the production of public goods or cross-reactive immune

614   responses to agents with differing thermal tolerances (Alizon et al., 2013). Our data are limited to changes

615   in qPCR load levels and not metabolites, so the nature of potential inter-pathogen relationships is beyond

616   the scope of this study. We can only comment on changes in community composition that preceded

617   mortality, such as the influx of *Aeromonas* species among thermally stressed fish concurrently with

618   elimination of *F. psychrophilum* and high levels of host mortality.

619   Early mortality of marine-sourced fish was associated with *A. hydrophila* but only in warm water. This

620   Gram-negative bacteria causes hemorrhagic septicemia in fish and gastroenteritis in humans

621   (Harikrishnan & Balasundaram, 2005; Janda & Abbott, 2010). Among marine-captured fish, *A.*

622   *hydrophila* only occurred at high temperature and only in fish that tested negative for *F. psychrophilum*, a

623   negative correlation that partially explains the positive effect of *F. psychrophilum* on marine fish survival

624   in warm water. Competitive exclusion (Sofonea et al., 2015), however, is unlikely since co-infections of

625   these bacteria occurred in river-exposed fish at both temperatures, and frequently at high *F.*

626   *psychrophilum* loads. *A. salmonicida* was the more prevalent *Aeromonas* species in the gills of river-

627   exposed fish and was associated with early mortality at high temperature; *A. salmonicida* showed no clear

628   load relationship with *F. psychrophilum*. Our results also demonstrate mortality of river-captured fish in

629   association with RLO, the bacterial agent of Strawberry Disease and an endosymbiont of *I. multifillis* that

630   is rarely directly linked to salmon mortality (Sonja J Lloyd et al., 2008; Sun et al., 2009). Further research

631   should be directed toward describing the relationship between bacterial and other agents in the context of

632   temperature in freshwater, especially given warming trajectories in salmon-bearing rivers (Ferrari, Miller,

633   & Russell, 2007; Petersen & Kitchell, 2001) that may alter pathogen community structure in migrating

634   adults.

635   The life history of an infectious agent plays a major role in disease dynamics. As has been observed

636   previously (Bass, 2018; Bass et al., 2017), the freshwater myxozoans *C. shasta* and *P. minibicornis* were

637   highly prevalent in fish collected in the river, but not entirely absent in marine-collected fish. Resilience

638   of spores in the Fraser River plume in the Strait of Georgia or retention of myxozoan infections from

639   juvenile life stages may explain marine detections (likely the former, see Mahony, Johnson, Neville,

640   Thiess, & Jones, 2017; Thakur et al., 2018; Tucker et al., 2018). These agents require an intermediate

641   freshwater polychaete host, which releases infective myxozoan spores into the river during salmon

642   migrations (Bartholomew, Atkinson, & Hallett, 2006; Bartholomew et al., 1997). Greater prevalence of

643   these myxozoans in river-exposed fish contributed to heavier overall infection burdens and richness;

644   continued river exposure if fish were not transported to the laboratory would further increase infective

645   dosage (Benda, Naughton, Caudill, Kent, & Schreck, 2015; Ray, Holt, & Bartholomew, 2012). New

646   infectious agents accumulated during river entry may elicit host responses that were not activated for

647   carrier-state infections, and this enhanced response may be deleterious due to cross-reactive immune

648   responses (Alizon et al., 2013; Alvarez-Pellitero, 2008). The demands of osmoregulatory transition and

649   new infections likely reduce the capacity of adult salmon to effectively respond to thermal, fishery, and

650   other stressors, especially given limited endogenous resources (Kiessling, Lindahl-kiessling, & Kiessling,

651   2004).

652

653   *Broader implications and future directions*

654   Future work should attempt to overcome the methodological constraints of our study. Our results

655   characterize differences in post-release survival between marine or freshwater environments depending

656   on gear type, but also demonstrate differences between naturally accumulated low and heavy infection

657   groups. We chose to allow river-captured fish to complete early river migration and acquire a realistic

658   "dose" of river-derived infections to improve the ecological context of our findings. The experience of

659   early river migration requires simultaneous osmoregulatory transition and predator and fishery avoidance

660   with associated energetic costs, which may have contributed to the physiological differences observed

661   between collection groups. The experience of river-captured fish in this study accurately simulated

662   capture and release in the lower river, which was crucial to ascertaining the role of natural infections in

663   host responses to cumulative stressors. Seine nets are a preferred gear type for fishers in the marine

664   environment; working with commercial fishers for this study meant that marine-collected fish needed to

665   be experimentally gillnetted in the lab for our comparison. River-captured fish were also gillnetted in

666   relatively cool river water and then held in warm or cool water, whereas treatment temperatures for

667   marine-captured fish matched holding temperatures. Our results demonstrate substantially elevated

668   infectious loads in the gills of an adult population of sockeye salmon following river entry despite these

669   logistical constraints, which was associated with altered genomic responses to stressors and pathogen

670   community trajectories.

671   Several key findings can be more broadly applied in the context of disease ecology. Thermal stress was

672   again identified as a "master factor" influencing host resilience (Fry, 1971) but our data add multiple

673   infection burdens and altered pathogen community composition to the array of known thermal impacts on

674   wild exothermic host species. Projected temperature increases in freshwater, marine, and terrestrial

675   habitats (Isaak, Wollrab, Horan, & Chandler, 2012; Poloczanska et al., 2013; Walther et al., 2002) can

676   therefore be expected to alter host-pathogen relationships, not only within hosts as demonstrated by this

677   study, but at regional spatial scales if host or geographic ranges are subsequently shifted (Altizer et al.,

678   2013; Engering et al., 2013). The dependence of genomic stress responses on infection status is intriguing

679   and alludes to the allocation of host resources to responses that maximize host survival odds in the

680   absence of infections (e.g., protein stabilization and repair) versus under heavy infection burdens (e.g.,

681   immune modulation, inflammation). Hosts are therefore modulating genomic responses to external

682   conditions based on internal factors – specifically immune activity and pathogen dynamics – thereby

683   supporting a basic host-pathogen(s)-environment framework. However, the plurality of "pathogen" adds

684   complexity to this triangle as host-pathogen relationships are the product of coevolution, and may be

685   unbalanced by shifts in both pathogen diversity (species, genotypes) and temperature (Mitchell et al.,

686   2005; Sofonea et al., 2017). Our results provide a foundation of data that can be used to test specific

687   hypotheses regarding inter-pathogen dynamics and the mechanisms of host resource allocation to manage

688   stress under different infection levels.

689   Infections are a natural component of ecosystems and can drive the evolutionary basis of wild animal

690   migrations. However, anthropogenic changes to these conditions may alter the effectiveness of life history

691   strategies (Altizer et al., 2011). As climate change continues to impact freshwater and marine thermal

692   regimes, pathogen virulence will also change, challenging our ability to manage wild salmon productivity

693   and maintain indigenous, commercial, and recreational fisheries (Altizer et al., 2013; Jacob et al., 2010;

694   McDaniels, Wilmot, Healey, & Hinch, 2010; Reed et al., 2011). Our study offers insight into the

695   mechanisms of premature mortality of adult sockeye salmon. We identified significant differences in the

696 survival and infection burdens of sockeye salmon based on river exposure. Multiple infections responded

697 to thermal stress with increases in the loads of most pathogens evaluated, whereas gillnetting only

698 increased infections among river-exposed fish. Subtle differences in community composition over time

699 and between capture locations, temperatures, and gear types suggested that the infectious agent

700 communities in surviving fish are responsive to stressors, increase with time, but were stabilized at the

701 population level by host mortality under heavy infections (Bass et al., 2017; Dolan et al., 2016; Teffer et

702 al., 2018, 2017). Host resilience to single stressors was reduced after river entry and likely pathogen-

703 mediated, whereas cumulative stressors are detrimental regardless of river exposure and initial pathogen

704 loads. Importantly, nearly all handled fish held at an ecologically relevant elevated water temperature died

705 prior to the spawning period, even with behavioral thermoregulation (a temporary decrease in

706 temperature). Given these results, fisheries managers should continue to reduce or cease fishing pressure

707 when rivers are warm and focus fishing effort in the marine environment using minimally invasive gear

708 types. Subject to the socioeconomic constraints of fisheries, including cost, culture, and logistics, strategic

709 fishing prior to river entry or only when rivers are cool will likely reduce *en route* losses of wild sockeye

710 salmon and more effectively preserve populations in a warming future.

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References

Aihua, B. L., & Buchmann, K. (2001). Temperature- and salinity-dependent development of a Nordic strain of Ichthyophthirius multifiliis from rainbow trout. *Journal of Applied Ichthyology*, *17*, 273–276.

Alcorn, S. W., Murra, A. L., & Pascho, R. J. (2002). Effects of rearing temperature on immune functions in sockeye salmon (Oncorhynchus nerka). *Fish & Shellfish Immunology*, *12*, 303–334. doi: 10.1006/fsim.2001.0373

Alizon, S., de Roode, J. C., & Michalakis, Y. (2013). Multiple infections and the evolution of virulence. *Ecology Letters*, *16*(4), 556–567. doi: 10.1111/ele.12076

Altizer, S., Bartel, R., & Han, B. A. (2011). Animal migration and infectious disease risk. *Science*, *331*(6015), 296–302. doi: 10.1126/science.1194694

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: from evidence to a predictive framework. *Science*, *341*(6145), 514–519. doi: 10.1126/science.1239401

Alvarez-Pellitero, P. (2008). Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. *Veterinary Immunology and Immunopathology*, *126*(3–4), 171–198. doi: 10.1016/j.vetimm.2008.07.013

Atkinson, S. D., Jones, S. R. M., Adlard, R. D., & Bartholomew, J. L. (2011). Geographical and host distribution patterns of *Parvicapsula minibicornis* (Myxozoa) small subunit ribosomal RNA genetic types. *Parasitology*, *138*(8), 969–977. doi: 10.1017/S0031182011000734

Baker, M. R., Swanson, P., & Young, G. (2013). Injuries from non-retention in gillnet fisheries suppress reproductive maturation in escaped fish. *PloS One*, *8*(7), e69615. doi: 10.1371/journal.pone.0069615

Bartholomew, J. L., Atkinson, S. D., & Hallett, S. L. (2006). Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. *The Journal of Parasitology*, *92*(4), 742–748.

Bartholomew, J. L., Whipple, M. J., Stevens, D. G., & Fryer, J. L. (1997). The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *The Journal of Parasitology*, *83*(5), 859–868. doi: 10.2307/3284281

Bass, A. L. (2018). *Fisheries gear and biological context drive fishing-related incidental mortality in Pacific salmon spawning migrations*. University of British Columbia.

Bass, A. L., Hinch, S. G., Patterson, D. A., Cooke, S. J., & Farrell, A. P. (2018). Location-specific consequences of beach seine and gillnet capture on upriver-migrating sockeye salmon migration behavior and fate. *Canadian Journal of Fisheries and Aquatic Sciences*. doi: 10.1139/cjfas-2017-0474

Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2017). A survey of microparasites present in adult migrating Chinook salmon (*Oncorhynchus tshawytscha*) in south-western British Columbia determined by high-throughput quantitative polymerase chain reaction. *Journal of Fish Diseases*, *40*(4), 453–477. doi: 10.1111/jfd.12607

Bass, A. L., Hinch, S. G., Teffer, A. K., Patterson, D. A., & Miller, K. M. (2019). Fisheries capture and infectious agents were associated with travel rate and survival of Chinook salmon during spawning migration through a natal river. *Fisheries Research*, *209*, 156–166. doi: 10.1016/j.fishres.2018.09.009

Beacham, T. D., Lapointe, M. F., Candy, J. R., Mcintosh, B., Macconnachie, C., Tabata, A., … Withler, R. E. (2004). Stock identification of Fraser River sockeye salmon using microsatellites and major histocompatibility complex variation. *Transactions of the American Fisheries Society*, *133*, 1117–1137. doi: 10.1577/T04-001.1

Benda, S. E., Naughton, G. P., Caudill, C. C., Kent, M. L., & Schreck, C. B. (2015). Cool, pathogen-free refuge lowers pathogen-associated prespawn mortality of Willamette River Chinook salmon. *Transactions of the American Fisheries Society*, *144*(6), 1159–1172. doi: 10.1080/00028487.2015.1073621

Bettge, K., Wahli, T., Segner, H., & Schmidt-Posthaus, H. (2009). Proliferative kidney disease in rainbow trout: time- and temperature-related renal pathology and parasite distribution. *Diseases of Aquatic Organisms*, *83*(1), 67–76. doi: 10.3354/dao01989

Bradford, M. J., Lovy, J., & Patterson, D. A. (2010). Infection of gill and kidney of Fraser River sockeye salmon, *Oncorhynchus nerka* (Walbaum), by *Parvicapsula minibicornis* and its effect on host physiology. *Journal of Fish Diseases*, *33*(9), 769–779. doi: 10.1111/j.1365-2761.2010.01178.x

Bradford, M. J., Lovy, J., Patterson, D. A., Speare, D. J., Bennett, W. R., Stobbart, A. R., & Tovey, C. P. (2010). *Parvicapsula minibicornis* infections in gill and kidney and the premature mortality of adult sockeye salmon (*Oncorhynchus nerka*) from Cultus Lake, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(4), 673–683. doi: 10.1139/F10-017

Bruneaux, M., Visse, M., Gross, R., Pukk, L., Saks, L., & Vasemägi, A. (2016). Parasite infection and decreased thermal tolerance: Impact of proliferative kidney disease on a wild salmonid fish in the context of climate change. *Functional Ecology*. doi: 10.1111/1365-2435.12701

Cooperman, M. S., Hinch, S. G., Crossin, G. T., Cooke, S. J., Patterson, D. A., Olsson, I., … Farrell, A. P. (2010). Effects of experimental manipulations of salinity and maturation status on the physiological condition and mortality of homing adult sockeye salmon held in a laboratory. *Physiological and Biochemical Zoology*, *83*(3), 459–472. doi: 10.1086/650473

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, *11*(12), 1304–1315. doi: 10.1111/j.1461-0248.2008.01253.x

Crossin, G. T., Hinch, S. G., Cooke, S. J., Welch, D. W., Patterson, D. A., Jones, S. R. M., … Farrell, A. P. (2008). Exposure to high temperature influences the behaviour, physiology, and survival of sockeye salmon during spawning migration. *Canadian Journal of Zoology*, *86*(2), 127–140. doi: 10.1139/Z07-122

Demers, N. E., & Bayne, C. J. (1997). The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Developmental and Comparative Immunology*, *21*(4), 363–373. doi: S0145-305X(97)00009-8 [pii]

Dittmar, J., Janssen, H., Kuske, A., Kurtz, J., & Scharsack, J. P. (2014). Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology*, *83*(4), 744–757. doi: 10.1111/1365-2656.12175

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

Donaldson, M. R., Hinch, S. G., Raby, G. D., Patterson, D. A., Farrell, A. P., & Cooke, S. J. (2012). Population-specific consequences of fisheries-related stressors on adult sockeye salmon. *Physiological and Biochemical Zoology*, *85*(6), 729–739. doi: 10.1086/664931

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

Eder, K. J., Leutenegger, C. M., Köhler, H. R., & Werner, I. (2009). Effects of neurotoxic insecticides on heat-shock proteins and cytokine transcription in Chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicology and Environmental Safety*, *72*(1), 182–190. doi: 10.1016/j.ecoenv.2008.04.020

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., … Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science (New York, N.Y.)*, *332*(6025), 109–112. doi: 10.1126/science.1199158

Engering, A., Hogerwerf, L., & Slingenbergh, J. (2013). Pathogen-host-environment interplay and disease emergence. *Emerging Microbes & Infections*, *2*(2), e5. doi: 10.1038/emi.2013.5

Evans, T. G., Hammill, E., Kaukinen, K. H., Schulze, A. D., Patterson, D. A., English, K. K., … Miller, K. M. (2011). Transcriptomics of environmental acclimatization and survival in wild adult Pacific

sockeye salmon (*Oncorhynchus nerka*) during spawning migration. *Molecular Ecology*, *20*(21), 4472–4489. doi: 10.1111/j.1365-294X.2011.05276.x

Ewing, M. S., Black, M. C., Blazer, V. S., & Kocan, K. M. (1994). Plasma chloride and gill epithelial response of channel catfish to infection with *Ichthyophthirius multifiliis*. *Journal of Aquatic Animal Health*, *6*, 187–196. doi: 10.1577/1548-8667(1994)006<0187:PCAGER>2.3.CO;2

Farrell, A. P., Hinch, S. G., Cooke, S. J., Patterson, D. A., Crossin, G. T., Lapointe, M. F., & Mathes, M. T. (2008). Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological and Biochemical Zoology*, *81*(6), 697–708. doi: 10.1086/592057

Fenkes, M., Shiels, H. A., Fitzpatrick, J. L., & Nudds, R. L. (2016). The potential impacts of migratory difficulty, including warmer waters and altered flow conditions, on the reproductive success of salmonid fishes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *193*, 11–21. doi: 10.1016/j.cbpa.2015.11.012

Ferrari, M. R., Miller, J. R., & Russell, G. L. (2007). Modeling changes in summer temperature of the Fraser River during the next century. *Journal of Hydrology*, *342*(3–4), 336–346. doi: 10.1016/j.jhydrol.2007.06.002

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In W. Hoar & D. Randall (Eds.), *Fish Physiology: Environmental Relations and Behaviour* (Vol. 6, pp. 1–98). doi: 10.1016/S1546-5098(08)60146-6

Gale, M. K., Hinch, S. G., & Donaldson, M. R. (2013). The role of temperature in the capture and release of fish. *Fish and Fisheries*, *14*(1), 1–33. doi: 10.1111/j.1467-2979.2011.00441.x

Gale, M. K., Hinch, S. G., Eliason, E. J., Cooke, S. J., & Patterson, D. A. (2011). Physiological impairment of adult sockeye salmon in fresh water after simulated capture-and-release across a range of temperatures. *Fisheries Research*, *112*(1–2), 85–95. doi: 10.1016/j.fishres.2011.08.014

Groberg, W. J. J., McCoy, R. H., Pilcher, K. S., & Fryer, J. L. (1978). Relation of Water Temperature to Infections of Coho Salmon (*Oncorhynchus kisutch*), Chinook Salmon (*O. tshawytscha*), and Steelhead Trout (*Salmo gairdneri*) with *Aeromonas salmonicida* and *A. hydrophila*. *Journal of the Fisheries Research Board of Canada*, *35*, 1–7.

Groot, C., & Margolis, L. (1991). Pacific Salmon Life Histories. Vancouver, BC, Canada: University of British Columbia Press.

Hallett, S. L., & Bartholomew, J. L. (2006). Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in river water samples. *Diseases of Aquatic Organisms*, *71*(2), 109–118. doi: 10.3354/dao071109

Hallett, S. L., & Bartholomew, J. L. (2009). Development and application of a duplex QPCR for river water samples to monitor the myxozoan parasite *Parvicapsula minibicornis*. *Diseases of Aquatic Organisms*, *86*(1), 39–50. doi: 10.3354/dao02104

Harikrishnan, R., & Balasundaram, C. (2005). Modern trends in *Aeromonas hydrophila* disease management with fish. *Reviews in Fisheries Science*, *13*(4), 281–320. doi: 10.1080/10641260500320845

Haugland, O., Torgersen, J., Syed, M., & Evensen, O. (2005). Expression profiles of inflammatory and immune-related genes in Atlantic salmon (*Salmo salar* L.) at early time post vaccination. *Vaccine*, *23*(48–49), 5488–5499. doi: 10.1016/j.vaccine.2005.07.034

Hinch, S. G., Cooke, S. J., Farrell, A. P., Miller, K. M., Lapointe, M. F., & Patterson, D. A. (2012). Dead fish swimming: a review of research on the early migration and high premature mortality in adult Fraser River sockeye salmon *Oncorhynchus nerka*. *Journal of Fish Biology*, *81*(2), 576–599. doi: 10.1111/j.1095-8649.2012.03360.x

Hinch, S. G., Cooperman, M. S., Crossin, G. T., & Olsson, I. (2008). *Investigations to determine the cause of early migration behaviour and magnitude of in-river survival and losses above Mission for adult Late Run Fraser River sockeye. Report to the Pacific Salmon Commission, Southern Boundary Restoration and Enhancement.* Vancouver, BC.

860   Ingerslev, H.-C., Rønneseth, A., Pettersen, E. F., & Wergeland, H. I. (2009). Differential expression of
861   immune genes in Atlantic salmon (*Salmo salar* L.) challenged intraperitoneally or by cohabitation
862   with IPNV. *Scandinavian Journal of Immunology*, *69*(2), 90–98. doi: 10.1111/j.1365-
863   3083.2008.02201.x

864   Isaak, D. J., Wollrab, S., Horan, D., & Chandler, G. (2012). Climate change effects on stream and river
865   temperatures across the Northwest U.S. from 1980-2009 and implications for salmonid fishes.
866   *Climatic Change*, *113*(2), 499–524. doi: 10.1007/s10584-011-0326-z

867   Jacob, C., McDaniels, T., & Hinch, S. (2010). Indigenous culture and adaptation to climate change:
868   Sockeye salmon and the St'át'imc people. *Mitigation and Adaptation Strategies for Global Change*,
869   *15*(8), 859–876. doi: 10.1007/s11027-010-9244-z

870   Jain, K. E., & Farrell, A. P. (2003). Influence of seasonal temperature on the repeat swimming
871   performance of rainbow trout *Oncorhynchus mykiss*. *Journal of Experimental Biology*, *206*(20),
872   3569–3579. doi: 10.1242/jeb.00588

873   Janda, J. M., & Abbott, S. L. (2010). The genus Aeromonas: Taxonomy, pathogenicity, and infection.
874   *Clinical Microbiology Reviews*, *23*, 35–73. doi: 10.1128/CMR.00039-09

875   Jeffries, K. M., Hinch, S. G., Gale, M. K., Clark, T. D., Lotto, A. G., Casselman, M. T., … Miller, K. M.
876   (2014). Immune response genes and pathogen presence predict migration survival in wild salmon
877   smolts. *Molecular Ecology*, *23*(23), 5803–5815. doi: 10.1111/mec.12980

878   Jeffries, K. M., Hinch, S. G., Sierocinski, T., Clark, T. D., Eliason, E. J., Donaldson, M. R., … Miller, K.
879   M. (2012). Consequences of high temperatures and premature mortality on the transcriptome and
880   blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). *Ecology and Evolution*, *2*(7),
881   1747–1764. doi: 10.1002/ece3.274

882   Jeffries, K. M., Hinch, S. G., Sierocinski, T., Pavlidis, P., & Miller, K. M. (2014). Transcriptomic
883   responses to high water temperature in two species of Pacific salmon. *Evolutionary Applications*,
884   *7*(2), 286–300. doi: 10.1111/eva.12119

885   Johnson, P. T. J., de Roode, J. C., & Fenton, A. (2015). Why infectious disease research needs
886   community ecology. *Science (New York, N.Y.)*, *349*, 1259504. doi: 10.1126/science.1259504

887   Jørgensen, A., Nylund, A., Nikolaisen, V., Alexandersen, S., & Karlsbakk, E. (2011). Real-time PCR
888   detection of *Parvicapsula pseudobranchicola* (Myxozoa: Myxosporea) in wild salmonids in
889   Norway. *Journal of Fish Diseases*, *34*(5), 365–371. doi: 10.1111/j.1365-2761.2011.01248.x

890   Kassahn, K. S., Crozier, R. H., Pörtner, H. O., & Caley, M. J. (2009). Animal performance and stress:
891   responses and tolerance limits at different levels of biological organisation. *Biological Reviews of
892   the Cambridge Philosophical Society*, *84*(2), 277–292. doi: 10.1111/j.1469-185X.2008.00073.x

893   Keefer, M. L., Peery, C. a., & Heinrich, M. J. (2008). Temperature-mediated en route migration mortality
894   and travel rates of endangered Snake River sockeye salmon. *Ecology of Freshwater Fish*, *17*(1),
895   136–145. doi: 10.1111/j.1600-0633.2007.00267.x

896   Keeling, S. E., Brosnahan, C. L., Johnston, C., Wallis, R., Gudkovs, N., & McDonald, W. L. (2013).
897   Development and validation of a real-time PCR assay for the detection of *Aeromonas salmonicida*.
898   *Journal of Fish Diseases*, *36*(5), 495–503. doi: 10.1111/jfd.12014

899   Kiessling, A., Lindahl-kiessling, K., & Kiessling, K. (2004). Energy utilization and metabolism in
900   spawning migrating Early Stuart sockeye salmon (*Oncorhynchus nerka*): the migratory paradox.
901   *Canadian Journal of Fisheries and Aquatic Sciences*, *61*, 452–465. doi: 10.1139/F04-006

902   Kocan, R., Hershberger, P. K., Sanders, G., & Winton, J. R. (2009). Effects of temperature on disease
903   progression and swimming stamina in *Ichthyophonus*-infected rainbow trout, *Oncorhynchus mykiss*
904   (Walbaum). *Journal of Fish Diseases*, *32*(10), 835–843. doi: 10.1111/j.1365-2761.2009.01059.x

905   Larsen, H. A. S., Austbø, L., Mørkøre, T., Thorsen, J., Hordvik, I., Fischer, U., … Koppang, E. O. (2012).
906   Pigment-producing granulomatous myopathy in Atlantic salmon: a novel inflammatory response.
907   *Fish and Shellfish Immunology*, *33*(2), 277–285. doi: 10.1016/j.fsi.2012.05.012

908   Lee, D.-Y., Shannon, K., & Beaudette, L. A. (2006). Detection of bacterial pathogens in municipal
909   wastewater using an oligonucleotide microarray and real-time quantitative PCR. *Journal of*

910   *Microbiological Methods*, *65*(3), 453–467. doi: 10.1016/j.mimet.2005.09.008
911 Lennox, R. J., Chapman, J. M., Souliere, C. M., Tudorache, C., Wikelski, M., Metcalfe, J. D., & Cooke,
912   S. J. (2016). Conservation physiology of animal migration. *Conservation Physiology*, *4*(1), 1–15.
913   doi: 10.1093/conphys/cov072
914 Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time
915   quantitative PCR and. *Methods*, *25*, 402–408. doi: 10.1006/meth.2001.1262
916 Lloyd, S J, LaPatra, S. E., Snekvik, K. R., Cain, K. D., & Call, D. R. (2011). Quantitative PCR
917   demonstrates a positive correlation between a *Rickettsia*-like organism and severity of strawberry
918   disease lesions in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, *34*(9),
919   701–709. doi: 10.1111/j.1365-2761.2011.01285.x
920 Lloyd, Sonja J, LaPatra, S. E., Snekvik, K. R., St-Hilaire, S., Cain, K. D., & Call, D. R. (2008).
921   Strawberry disease lesions in rainbow trout from southern Idaho are associated with DNA from a
922   *Rickettsia*-like organism. *Diseases of Aquatic Organisms*, *82*(2), 111–118. doi: 10.3354/dao01969
923 MacNutt, M. J., Hinch, S. G., Farrell, A. P., & Topp, S. (2004). The effect of temperature and acclimation
924   period on repeat swimming performance in cutthroat trout. *Journal of Fish Biology*, *65*, 342–353.
925   doi: 10.1111/j.1095-8649.2004.00453.x
926 Mahony, A. M., Johnson, S. C., Neville, C. M., Thiess, M. E., & Jones, S. R. M. (2017). *Myxobolus*
927   *arcticus* and *Parvicapsula minibicornis* infections in sockeye salmon *Oncorhynchus nerka*
928   following downstream migration in British Columbia. *Diseases of Aquatic Organisms*, *126*, 89–98.
929   doi: 10.3354/dao03158
930 Martins, E. G., Hinch, S. G., Patterson, D. A., Hague, M. J., Cooke, S. J., Miller, K. M., … Farrell, A. P.
931   (2012). High river temperature reduces survival of sockeye salmon (*Oncorhynchus nerka*)
932   approaching spawning grounds and exacerbates female mortality. *Canadian Journal of Fisheries*
933   *and Aquatic Sciences*, *69*(2), 330–342. doi: 10.1139/F2011-154
934 McDaniels, T., Wilmot, S., Healey, M., & Hinch, S. G. (2010). Vulnerability of Fraser River sockeye
935   salmon to climate change: a life cycle perspective using expert judgments. *Journal of Environmental*
936   *Management*, *91*(12), 2771–2780. doi: 10.1016/j.jenvman.2010.08.004
937 Miller, K. M., Gardner, I. A., Vanderstichel, R., Burnley, T., Angela, D., Li, S., … Ginther, N. G. (2016).
938   *Report on the performance evaluation of the Fluidigm BioMark platform for high-throughput*
939   *microbe monitoring in salmon. DFO Canadian Science Advisory Secretariat Research Document*
940   *2016/038*. Ottawa, ON, Canada.
941 Miller, K. M., Günther, O. P., Li, S., Kaukinen, K. H., & Ming, T. J. (2017). Molecular indices of viral
942   disease development in wild migrating salmon. *Conservation Physiology*, *5*, cox036. doi:
943   10.1093/conphys/cox036
944 Miller, K. M., Schulze, A. D., Ginther, N. G., Li, S., Patterson, D. A., Farrell, A. P., & Hinch, S. G.
945   (2009). Salmon spawning migration: metabolic shifts and environmental triggers. *Comparative*
946   *Biochemistry and Physiology - Part D: Genomics and Proteomics*, *4*(2), 75–89. doi:
947   10.1016/j.cbd.2008.11.002
948 Miller, K. M., Teffer, A. K., Tucker, S., Li, S., Schulze, A. D., Trudel, M., … Hinch, S. G. (2014).
949   Infectious disease, shifting climates, and opportunistic predators: Cumulative factors potentially
950   impacting wild salmon declines. *Evolutionary Applications*, *7*(7), 812–855. doi: 10.1111/eva.12164
951 Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-
952   environment interactions: temperature modifies potential for selection by a sterilizing pathogen.
953   *Evolution; International Journal of Organic Evolution*, *59*(1), 70–80. Retrieved from
954   http://www.ncbi.nlm.nih.gov/pubmed/15792228
955 Mitchell, S. O., Steinum, T. M., Toenshoff, E. R., Kvellestad, A., Falk, K., Horn, M., & Colquhoun, D. J.
956   (2013). *Candidatus* Branchiomonas cysticola is a common agent of epitheliocysts in seawater-
957   farmed Atlantic salmon (*Salmo salar*) in Norway and Ireland. *Diseases of Aquatic Organisms*,
958   *103*(1), 35–43. doi: 10.3354/dao02563
959 Mordecai, G. J., Di Cicco, E., Günther, O. P., Schulze, A. D., Kaukinen, K. H., Li, S., … Miller, K. M.

(2020). Emerging viruses in British Columbia salmon discovered via a viral immune response biomarker panel and metatranscriptomic sequencing. *BioRxiv*, 2020.02.13.948026. doi: 10.1101/2020.02.13.948026

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

Morrison, J., Quick, M. C., & Foreman, M. G. G. (2002). Climate change in the Fraser River watershed: flow and temperature projections. *Journal of Hydrology*, *263*(1–4), 230–244.

Nekouei, O., Vanderstichel, R., Ming, T., Kaukinen, K. H., Thakur, K., Tabata, A., … Miller, K. M. (2018). Distribution of infectious agents in juvenile Fraser River Sockeye salmon, Canada, in 2012 and 2013. *Diseases of Aquatic Organisms*, *9*(December), 1–32. doi: 10.3389/fmicb.2018.03221

Nematollahi, A., Decostere, A., Pasmans, F., & Haesebrouck, F. (2003). *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases*, *26*(10), 563–574. doi: 10.1046/j.1365-2761.2003.00488.x

Newell, J. C., & Quinn, T. P. (2005). Behavioral thermoregulation by maturing adult sockeye salmon (*Oncorhynchus nerka*) in a stratified lake prior to spawning. *Canadian Journal of Zoology*, *83*, 1232–1239. doi: 10.1139/Z05-113

Nilsen, T. O., Ebbesson, L. O. E., Madsen, S. S., McCormick, S. D., Andersson, E., Björnsson, B. T., … Stefansson, S. O. (2007). Differential expression of gill Na+,K+-ATPase alpha- and beta-subunits, Na+,K+,2Cl- cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon Salmo salar. *The Journal of Experimental Biology*, *210*(Pt 16), 2885–2896. doi: 10.1242/jeb.002873

Nylund, S., Nylund, A., Watanabe, K., Arnesen, C. E., & Karlsbakk, E. (2010). *Paranucleospora theridion* (Microsporidia, Enterocytozoonidae) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*). *Journal of Eukaryotic Microbiology*, *57*(2), 95–114. doi: 10.1111/j.1550-7408.2009.00451.x

Paterson, S. (2013). The immunology and ecology of co-infection. *Molecular Ecology*, *22*(10), 2603–2604. doi: 10.1111/mec.12253

Patterson, D. A., Macdonald, J. S., Skibo, K. M., Barnes, D. P., Guthrie, I., & Hills, J. (2007). *Reconstructing the summer thermal history for the lower Fraser River, 1941 to 2006, and implications for adult sockeye salmon (Oncorhynchus nerka) spawning migration. Can. Tech. Rep. Fish. Aq. Sci. 2724*.

Patterson, D. A., Robinson, K. A., Lennox, R. J., Nettles, T. L., Donaldson, L. A., Eliason, E. J., … Hinch, S. G. (2017). *Review and evaluation of fishing-related incidental mortality for Pacific salmon. DFO Canadian Science Advisory Secretariat Research Document 2017/010. ix + 155p.* Retrieved from http://www.dfo-mpo.gc.ca/csas-sccs/

Petersen, J. H., & Kitchell, J. F. (2001). Climate regimes and water temperature changes in the Columbia River: bioenergetic implications for predators of juvenile salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*, 1831–1841. doi: 10.1139/cjfas-58-8-1831

Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., … Richardson, A. J. (2013). Global imprint of climate change on marine life. *Nature Climate Change*, *3*(10), 919–925. doi: 10.1038/nclimate1958

Purcell, M. K., Pearman-Gillman, S., Thompson, R. L., Gregg, J. L., Hart, L. M., Winton, J. R., … Hershberger, P. K. (2016). Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of quantitative real-time PCR assays for quantification of ENV DNA. *Journal of Veterinary Diagnostic Investigation*, *28*(4), 382–391. doi: 10.1177/1040638716646411

R Core Team. (2015). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.* doi: ISBN 3-900051-07-0

Raby, G. D., Donaldson, M. R., Hinch, S. G., Clark, T. D., Eliason, E. J., Jeffries, K. M., … Cooke, S. J.

1010   (2015). Fishing for effective conservation: Context and biotic variation are keys to understanding
1011   the survival of Pacific salmon after catch-and-release. *Integrative and Comparative Biology*, *55*(4),
1012   554–576. doi: 10.1093/icb/icv088
1013 Raida, M. K., & Buchmann, K. (2008). Development of adaptive immunity in rainbow trout,
1014   *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. *Fish & Shellfish
1015   Immunology*, *25*(5), 533–541. doi: 10.1016/j.fsi.2008.07.008
1016 Raida, M. K., & Buchmann, K. (2009). Innate immune response in rainbow trout (*Oncorhynchus mykiss*)
1017   against primary and secondary infections with *Yersinia ruckeri*. *Developmental and Comparative
1018   Immunology*, *33*(1), 35–45. doi: 10.1016/j.dci.2008.07.001
1019 Raida, M. K., Holten-Andersen, L., & Buchmann, K. (2011). Association between *Yersinia ruckeri*
1020   infection, cytokine expression and survival in rainbow trout (*Oncorhynchus mykiss*). *Fish and
1021   Shellfish Immunology*, *30*(6), 1257–1264. doi: 10.1016/j.fsi.2011.03.022
1022 Rand, P. S., Hinch, S. G., Morrison, J., Foreman, M. G. G., MacNutt, M. J., Macdonald, J. S., … Higgs,
1023   D. A. (2006). Effects of river discharge, temperature, and future climates on energetics and mortality
1024   of adult migrating Fraser River sockeye salmon. *Transactions of the American Fisheries Society*,
1025   *135*(3), 655–667. doi: 10.1577/T05-023.1
1026 Ray, R. A., Holt, R. A., & Bartholomew, J. L. (2012). Relationship between temperature and *Ceratomyxa
1027   shasta*-induced mortality in Klamath River salmonids. *The Journal of Parasitology*, *98*(3), 520–526.
1028   doi: 10.1645/JP-GE-2737.1
1029 Reed, T. E., Schindler, D. E., Hague, M. J., Patterson, D. A., Meir, E., Waples, R. S., & Hinch, S. G.
1030   (2011). Time to evolve? Potential evolutionary responses of Fraser River sockeye salmon to climate
1031   change and effects on persistence. *PLoS ONE*, *6*(6), e20380. doi: 10.1371/journal.pone.0020380
1032 Sofonea, M. T., Alizon, S., & Michalakis, Y. (2015). From within-host interactions to epidemiological
1033   competition: a general model for multiple infections. *Philosophical Transactions of the Royal
1034   Society B: Biological Sciences*, *370*(1675), 20140303. doi: 10.1098/rstb.2014.0303
1035 Sofonea, M. T., Alizon, S., & Michalakis, Y. (2017). Exposing the diversity of multiple infection
1036   patterns. *Journal of Theoretical Biology*, *419*, 278–289. doi: 10.1016/j.jtbi.2017.02.011
1037 Spromberg, J. A., & Scholz, N. L. (2011). Estimating the Future Decline of Wild Coho Salmon
1038   Populations Resulting from Early Spawner Die-Offs in Urbanizing Watersheds of the Pacific
1039   Northwest , USA. *Integrated Environmental Assessment and Management 9999*, *7*, 648–656. doi:
1040   10.1002/ieam.219
1041 Sun, H. Y., Noe, J. G., Barber, J., Coyne, R. S., Cassidy-Hanley, D., Clark, T. G., … Dickerson, H. W.
1042   (2009). Endosymbiotic bacteria in the parasitic ciliate *Ichthyophthirius multifiliis*. *Applied and
1043   Environmental Microbiology*, *75*(23), 7445–7452. doi: 10.1128/AEM.00850-09
1044 Tadiso, T. M., Krasnov, A., Skugor, S., Afanasyev, S., Hordvik, I., & Nilsen, F. (2011). Gene expression
1045   analyses of immune responses in Atlantic salmon during early stages of infection by salmon louse
1046   (*Lepeophtheirus salmonis*) revealed bi-phasic responses coinciding with the copepod-chalimus
1047   transition. *BMC Genomics*, *12*, 141. doi: 10.1186/1471-2164-12-141
1048 Teffer, A. K., Bass, A. L., Miller, K. M., Patterson, D. A., Juanes, F., & Hinch, S. G. (2018). Infections,
1049   fisheries capture, temperature and host responses: Multi-stressor influences on survival and
1050   behaviour of adult Chinook salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*, 2069–
1051   2083. doi: 10.1139/cjfas-2017-0491
1052 Teffer, A. K., Hinch, S. G., Miller, K. M., Jeffries, K. M., Patterson, D. A., Cooke, S. J., … Juanes, F.
1053   (2019). Cumulative effects of thermal and fisheries stressors reveal sex-specific effects on infection
1054   development and early mortality of adult coho salmon (Oncorhynchus kisutch). *Physiological and
1055   Biochemical Zoology*, *92*(5), 502–529.
1056 Teffer, A. K., Hinch, S. G., Miller, K. M., Patterson, D. A., Farrell, A. P., Cooke, S. J., … Juanes, F.
1057   (2017). Capture severity, infectious disease processes and sex influence post-release mortality of
1058   sockeye salmon bycatch. *Conservation Physiology*, *5*(1), cox017. doi: 10.1093/conphys/cox017
1059 Thakur, K. K., Vanderstichel, R., Li, S., Laurin, E., Tucker, S., Neville, C., … Miller, K. M. (2018). A

1060   comparison of infectious agents between hatchery-enhanced and wild out-migrating juvenile
1061   chinook salmon (*Oncorhynchus tshawytscha*) from Cowichan River, British Columbia. *Facets*, *3*(1),
1062   695–721. doi: 10.1139/facets-2017-0113

1063 Therneau, T. M. (2014). *survival: A Package for Survival Analysis in R. R package version 2.37.7*.

1064 Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology*
1065   *& Evolution*, *18*(7), 344–350. doi: 10.1016/S0169-5347(03)00069-7

1066 Tort, L. (2011). Stress and immune modulation in fish. *Developmental and Comparative Immunology*,
1067   *35*(12), 1366–1375. doi: 10.1016/j.dci.2011.07.002

1068 Traxler, G. S., Richard, J., & Mcdonald, T. E. (1998). *Ichthyophthirius multifiliis* (Ich) epizootics in
1069   spawning sockeye salmon in British Columbia, Canada. *Journal of Aquatic Animal Health*, *10*(2),
1070   143–151. doi: 10.1577/1548-8667(1998)010<0143

1071 Tucker, S., Li, S., Kaukinen, K. H., Patterson, D. A., & Miller, K. M. (2018). Distinct seasonal infectious
1072   agent profiles in life-history variants of juvenile Fraser River Chinook salmon: An application of
1073   high-throughput genomic screening. *PLoS One*, *13*(4), e0195472. doi:
1074   https://doi.org/10.1371/journal. pone.0195472 Editor:

1075 Udey, L. R., Fryer, J. L., & Pilcher, K. S. (1975). Relation of water temperature to Ceratomyxosis in
1076   Rainbow Trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). *Journal of the*
1077   *Fisheries Research Board of Canada*, *32*, 1545–1551.

1078 Wagner, G. N., Hinch, S. G., Kuchel, L. J., Lotto, A. G., Jones, S. R. M., Patterson, D. A., … Farrell, A.
1079   P. (2005). Metabolic rates and swimming performance of adult Fraser River sockeye salmon
1080   (Oncorhynchus nerka) after a controlled infection with *Parvicapsula minibicornis*. *Canadian*
1081   *Journal of Fisheries and Aquatic Sciences*, *62*, 2124–2133. doi: 10.1139/F05-126

1082 Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., … Bairlein, F. (2002).
1083   Ecological responses to recent climate change. *Nature*, *416*(6879), 389–395. doi: 10.1038/416389a

1084 Willson, M. F., & Halupka, K. C. (1995). Anadromous fish as keystone species in vertebrate
1085   communities. *Conservation Biology*, *9*(3), 489–497. Retrieved from
1086   http://doi.wiley.com/10.1046/j.1523-1739.1995.09030489.x

1087 Yada, T., Azuma, T., Hyodo, S., Hirano, T., Grau, E. G., & Schreck, C. B. (2007). Differential expression
1088   of corticosteroid receptor genes in rainbow trout (*Oncorhynchus mykiss*) immune system in response
1089   to acute stress. *Canadian Journal of Fisheries and Aquatic Sciences*, *64*(10), 1382–1389. doi:
1090   10.1139/f07-110

1091 Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and*
1092   *extensions in ecology with R*. doi: 10.1007/978-0-387-87458-6

1093 Zwollo, P. (2018). The humoral immune system of anadromous fish. *Developmental and Comparative*
1094   *Immunologydevelo*, *80*, 24–33. doi: 10.1016/j.dci.2016.12.008

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

Assay name	Assay type	Gene information	EST/Accession#	Forward primer	Reverse primer	Probe	Source
b2m	Acquired immunity	Cell receptor	AF180490	F - TTTACAGCGCGGTGGAGTC	R - TGCCAGGGGTTACGGCTGTAC	P - AAAGAATCTCCCCCCAAGGTGCAGG	(Haugland *et al.* 2005)
CD83	Acquired immunity	Cell receptor	AY263794	F - GATGCACCCCTTGAGAAGAA	R - GAACCCTGTCTCGACCAGTT	P - AATGTTGATTTACACTCTGGGGCCA	(Raida *et al.*, 2011)
MHCIIb	Acquired immunity	Major histocompatibility complex IIβ	AF115533	F - TGCCATGCTGATGTGCAG	R - GTCCCTCAGCCAGGTCACT	P - CGCCTATGACTTCTACCCCAAACAAAT	(Raida and Buchmann, 2008)
CD4	Acquired immunity	Cell receptor	AY973028	F - CATTAGCCTGGGTGGTCAAT	R - CCCTTTCTTTGACAGGGAGA	P - CAGAAGAGAGAGCTGGATGTCTCCG	(Raida and Buchmann, 2008)
Mx	Antiviral	Antiviral protein		F - AGATGATGCTGCACCTCAAGTC	R - CTGCAGCTGGGAAGCAAAC	P - ATTCCCATGGTGATCCGCTACCTGG	(Eder *et al.*, 2009)
RIGI	Antiviral	Retinoic acid inducible gene I	NM_001163699	F - ACAGCTGTTACACAGACGACATCA	R - TTTAGGGTGAGGTTCTGTCCGA	P - TCGTGTTGGACCCCACTCTGTTCTCTC	(Larsen *et al.*, 2012)
ATP5G3C	Cellular energy	ATP synthase lipid-binding protein	CB493164	F - GGAACGCCACCATGAGACA	R - CGCCATCCTGGGCTTTG	P - AGCCCCATTGCCTC	(Miller *et al.*, 2016)
CXCR4	Immune regulation	chemokine receptor	CA054133	F - GGAGATCACATTGAGCAACATCA	R - GCTGCTGGCTGCCATACTG	P - TCCACGAAGATCCCCA	In house
IFNa	Immune regulation	Interferon-α	AY216595	F - CGTCATCTGCAAAGATTGGA	R - GGGGTAGCTTCTGAAATGA	P – TGCAGCACAGATGTACTGATCATCCA	(Ingerslev *et al.*, 2009)
IL11	Immune regulation	Cytokine	AJ535687	F - GCAATCTCTTGCCTCCACTC	R - TTGTCACGTGCTCCAGTTTC	P - TCGCGGAGTGTGAAAGGCAGA	(Raida and Buchmann, 2008)
IL15	Immune regulation	Cytokine	AJ555868.1	F - TTGGATTTTGCCCTAACTGC	R - CTGCGCTCCAATAAACGAAT	P - CGAACAACGCTGATGACAGGTTTTT	(Raida *et al.*, 2011)
IL1R	Immune regulation	Cytokine	AJ295296	F - ATCATCCTGTCAGCCCAGAG	R - TCTGGTGCAGTGGTAACTGG	P - TGCATCCCCTCTACACCCCAAA	(Raida *et al.*, 2011)
IL8	Immune regulation	Cytokine	AJ279069	F - AGAATGTCAGCCAGCCTTGT	R - TCTCAGACTCATCCCCTCAGT	P - TTGTGCTCCTGGCCCTCCTGA	(Raida and Buchmann 2008)
C7	Innate immunity	Complement factor	CA052045	F - ACCTCTGTCCAGCTCTGTGTC	R - GATGCTGACCACATCAAACTGC	P - AACTACCAGACAGTGCTG	In house
IgMs	Innate immunity	Immunoglobulin	S63348, AB044939	F - CTTGGCTTGTTGACGATGAG	R - GGCTAGTGGTGTTGAATTGG	P - TGGAGAGAACGAGCAGTTCAGCA	(Raida *et al.*, 2011)
NKA_a1b	Ion regulation	Sodium potassium ATPase subunit	CK879688	F - GCTACATCTCAACCAACAACATTACAC	R - TGCAGCTGAGTGCACCAT	P - ACCATTACATCCAATGAACACT	(Nilson et al. 2007)
TF	Iron regulation	Transferrin	D89083	F - TTCACTGCTGGAAAATGTGG	R - GCTGCACTGAACTGCATCAT	P - TGGTCCCTGTCATGGTGGAGCA	(Raida and Buchmann, 2009)
GR-2	Stress	Glucocorticoid receptor		F - TCCAGCAGCTATGCCAGTTCT	R - TTGCCCTGGGTTGTACATGA	P - AAGCTTGGTGGTGGCGCTG	(Yada *et al.*, 2007)
HSC70	Stress	Heat shock cognate 70	CA052185	F - GGGTCACACAGAAGCCAAAG	R - GCGCTCTATAGCGTTGATTGGT	P - AGACCAAGCCTAAACTA	In house
HSP90	Stress	Heat shock protein 90	CB493960, CB503707	F - TGGGCTACATGGCTCCAAG	R - TCCAAGGTGAACCCAGAGGAC	P - AGCACCTGGAGATCAA	In house
JUN	Stress	Transcription factor	CA056351	F - TTGTTGCTGGTGAGAAAACTCAGT	R - CCTGTTGCCCTATGAATTGTCTAGT	P - AGACTTGGGCTATTTAC	In house
MMP13	Wound healing	Matrix metalloproteinase	213514499	F - GCCAGCGGAGCAGGAA	R - AGTCACCTGGAGGCCAAAGA	P - TCAGCGAGATGCAAAG	(Tadiso *et al.*, 2011)
78d16.1	Reference gene		CA056739	F - GTCAAGACTGGAGGCTCAGAG	R - GATCAAGCCCCAGAAGTGTTTG	P - AAGGTGATTCCCTCGCCGTCCGA	In house
COIL-P84-2	Reference gene		CA053789	F - GCTCATTTGAGGAAGGAGGGATG	R - CTGGCGATGCTGTTCCTGAG	P - TTATCAAGCACGAAGCC	In house
ae_hyd	Bacterium	*Aeromonas hydrophila*		F - ACCGCTGCTCATTACTCTGATG	R - CCAACCCAGACGGGAAGAA	P - TGATGGTGAGCTGGTTG	(Lee *et al.*, 2006)
ae_sal	Bacterium	*Aeromonas salmonicida*		F - TAAAGCACTGTCTGTTACC	R - GCTACTTCACCCTGATTGG	P - ACATCAGCAGGCTTCAGAGTCACTG	(Keeling *et al.*, 2013)
c_b_cys	Bacterium	*Candidatus Branchiomonas cysticola*		F - AATACATCGGAACGTGTCTAGTG	R - GCCATCAGCCGCTCATGTG	P - CTCGGTCCCAGGCTTTCCTCTCCCA	(Mitchell *et al.*, 2013)
fl_psy	Bacterium	*Flavobacterium psychrophilum*		F - GATCCTTATTCTCACAGTACCGTCAA	R - TGTAAACTGCTTTTGCACAGGAA	P - AAACACTCGGTCGTGACC	(Duesund *et al.*, 2010)
rlo	Bacterium	*Rickettsia-like organism*		F - GGCTCAACCCAAGAACTGCTT	R - GTGCAACAGCGTCAGTGACT	P - CCCAGATAACCGCCTTCGCCTCCG	(Lloyd *et al.*, 2011)
ce_sha	Parasite	*Ceratonova shasta*		F - CCAGCTTGAGATTAGCTCGGTAA	R - CCCCGGAACCCGAAAG	P - CGAGCCAAGTTGGTCTCTCCGTGAAAAC	(Hallett and Bartholomew, 2006)
cr_sal	Parasite	*Cryptobia salmositica*		F - TCAGTGCCTTTCAGGACATC	R - GAGGCATCCACTCCAATAGAC	P - AGGAGGACATGGCAGCCTTTGTAT	(Miller *et al.*, 2016)
de_sal	Parasite	*Dermocystidium salmonis*		F - CAGCCAATCCTTTCGCTTCT	R - GACGGACGCACACCACAGT	P - AAGCGGCGTGTGCC	(Miller *et al.*, 2016)
ic_mul	Parasite	*Ichthyophthirius multifiliis*		F - AAATGGGCATACGTTTGCAAA	R - AACCTGCCTGAAACACTCTAATTTTT	P - ACTCGGCCTTCACTGGTTCGACTTGG	(Miller *et al.*, 2016)
lo_sal	Parasite	*Loma salmonae*		F - GGAGTCGCAGCGAAGATAGC	R - CTTTTCCTCCCTTTACTCATATGCTT	P - TGCCTGAAATCACGAGAGTGAGACTACCC	(Miller *et al.*, 2016)
my_arc	Parasite	*Myxobolus arcticus*		F - TGGTAGATACTGAATATCCGGGTTT	R - AACTGCGCGGTCAAAGTTG	P - CGTTGATTGTGAGGTTGG	(Miller *et al.*, 2016)
pa_min	Parasite	*Parvicapsula minibicornis*		F - AATAGTTGTTTGTCGTGCACTCTGT	R - CCGATAGGCTATCCAGTACCTAGTAAG	P - TGTCCACCTAGTAAGGC	(Hallett and Bartholomew, 2009)
pa_pse	Parasite	*Parvicapsula pseudobranchicola*		F - CAGCTCCAGTAGTGTATTTCA	R - TTGAGCACTCTGCTTTATTCAA	P - CGTATTGCTGTCTTTGACATGCAGT	(Jørgensen *et al.*, 2011)
pa_ther	Parasite	*Paranucleospora theridion*		F - CGGACAGGGAGCATGGTATAG	R - GGTCCAGGTGGGTCTTGAG	P - TTGGCGAAGAATGAAA	(Nylund *et al.*, 2010)
sp_des	Parasite	*Sphaerothecum destruens*		F - GCGCGAGGTGTTTGC	R - CTCGACGCACACTCAATTAAGC	P - CGAGGGGTATCCTTCCTCTCGAAATTGGC	(Miller *et al.*, 2016)
pspv	Virus	Pacific salmon parvovirus		F - CCCTCAGGCTCCGATTTTTAT	R - CGAACGCAACATGGAGGTGACA	P - CAATTGGAGGCAACTGTA	(Miller *et al.*, 2016)
ven	Virus	Viral erythrocytic necrosis virus		F - CGTAGGGCCCCAATAGTTTCT	R - GGAGGAAATGCAGACAAGATTTG	P - TCTTGCCGTTATTTCCAGCACCCG	(Purcell *et al.* 2016)

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. Gillnet treatment included entanglement and air exposure in the lab (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 7d reduced sample sizes to 16, 1, 9, and 10 for river-collected females from cool gillnet, cool seine, warm gillnet and warm seine groups.

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	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	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	8.9±4.1	49.3±1.9	0
			M	9	13.0±2.4	50.3±2.1	0
		No biopsy	F	12	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

Table 3. Parameters (β ± s.e.m.) of significant (P<0.05) factors associated with infection metrics measured in adult sockeye salmon during five weeks of freshwater residence. 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.

Metric	R	R:T	R:G	H	H:T	G	G:T	S	T	ΔAICc	ICC
RIB*	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 ‡									1.43±0.53, P=0.007	3.57	<0.001
P. minibicornis ‡									2.70±0.44, P=0.001	3.65	<0.001

*log transformed
‡ 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. 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<0.05) parameters from significant models (log-likelihood P<0.05) are shown. Sample sizes (*N*) describe the total positive detections in all fish within the group over time for each agent or metric.

Infectious agent or metric	Marine: warm† N	Presence e^β	Presence P	Load e^β	Load P	River: cool N	Presence e^β	Presence P	Load e^β	Load P	River: warm N	Presence e^β	Presence P	Load e^β	Load P
A. hydrophila	12	7.0	<0.001*	8.2	<0.001	2					2	27.4	0.009		
A. salmonicida	0					3					6	17.5	<0.001	14.2	<0.001
F. psychrophilum	45	0.2	0.008			41			19.7	0.022	31				
Rickettsia-like organism	68					24	7.6	0.038	16.1	0.002	26	6.2	0.018	4.6	0.004
C. shasta	4					42					35	3.8	0.005	6.7	<0.001
P. minibicornis	2					28	16.7	0.010	34.4	0.004	35	3.4	0.034		
I. multifiliis	85					34	16.2	0.040	28	0.008	38			5.1	0.008
L. salmonae	13					7	19.1‡	0.017			8	6.3	0.001		
Relative Infection Burden	95	NA	NA			51	NA	NA	42434	0.005	59	NA	NA	12.6	<0.001

†All marine warm models stratified by treatment

*effect decreased with time (P=0.038)

‡sex stratified

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. 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). Non-significant (P>0.05) models and factor parameters ($\beta\pm$s.e.m.) are not shown, or in grey if components of significant interactions in LMs.

A

	PerMANOVA			
	H	G	H:G	RIB
Marine				
Wk 0	r^2=0.24, P<0.001	r^2=0.07, P<0.001	r^2=0.06, P<0.001	
Wk 1	r^2=0.29, P<0.001	r^2=0.02, P=0.040	r^2=0.03, P=0.022	
Wk 2	r^2=0.42, P<0.001			r^2=0.17, P<0.001
Wk 3*	NA	r^2=0.19, P<0.001	NA	
Wk 4*‡	NA	r^2=0.08, P=0.028	NA	
River				
Wk 0	NA	r^2=0.06, P=0.003	NA	r^2=0.04, P=0.048
Wk 1	r^2=0.06, P=0.006	r^2=0.05, P=0.032		r^2=0.23, P<0.001
Wk 2	r^2=0.22, P<0.001			r^2=0.32, P<0.001
Wk 3*‡				r^2=0.51, P=0.001

B

	PC1						PC2						PC3			
	V	LM	H	G	H:G	RIB	V	LM	H	G	RIB	S	V	LM	G	S
Marine																
Wk 0	25	r^2=0.14, P=0.019	1.23±0.84, P=0.151	0.83±0.91, P=0.365	-3.14±1.15, P=0.009		14	r^2=0.70, P<0.001	-3.39±0.38, P<0.001				12	r^2=0.39, P<0.001	1.21±0.54, P=0.030	-1.17±0.36, P=0.002
Wk 1	35	r^2=0.44, P<0.001	-3.88±0.77, P<0.001	-1.62±0.76, P=0.037			17	r^2=0.11, P=0.037	1.73±0.67, P=0.012				10	r^2=0.06, P=0.132		
Wk 2	41	r^2=0.70, P<0.001	3.27±0.56, P<0.001		4.40±0.89, P<0.001		20	r^2=0.16, P=0.035	1.68±0.66, P=0.015		-2.21±1.04, P=0.040		10	r^2=0.01, P=0.362		
Wk 3*	28	r^2=0.04, P=0.287					17	r^2=0.30, P=0.017	NA	2.49±0.71, P=0.002			16	r^2=0.18, P=0.073		
Wk 4*‡	26	r^2=0.34, P=0.004	NA	-2.47±0.75, P=0.003	NA		15	r^2=0.10, P=0.131					13	r^2=0.00, P=0.952		
River																
Wk 0	26	r^2=0.09, P=0.062					15	r^2=0.18, P=0.008	NA	-1.82±0.53, P=0.002			10	r^2=0.21, P=0.003	-1.44±0.41, P=0.001	
Wk 1	31	r^2=0.59, P<0.001				5.14±0.82, P<0.001	22	r^2=0.07, P=0.145					11	r^2=0.02, P=0.328		
Wk 2	36	r^2=0.66, P<0.001				8.29±1.27, P<0.001	27	r^2=0.49, P<0.001	3.53±0.69, P<0.001		1.71±0.71, P=0.025		8	r^2=0.01, P=0.389		
Wk 3*‡	41	r^2=0.69, P<0.001				-10.46±1.85, P<0.001	21	r^2=0.09, P=0.252					15	r^2=0.05, P=0.322		

*cool water only

‡includes survivors
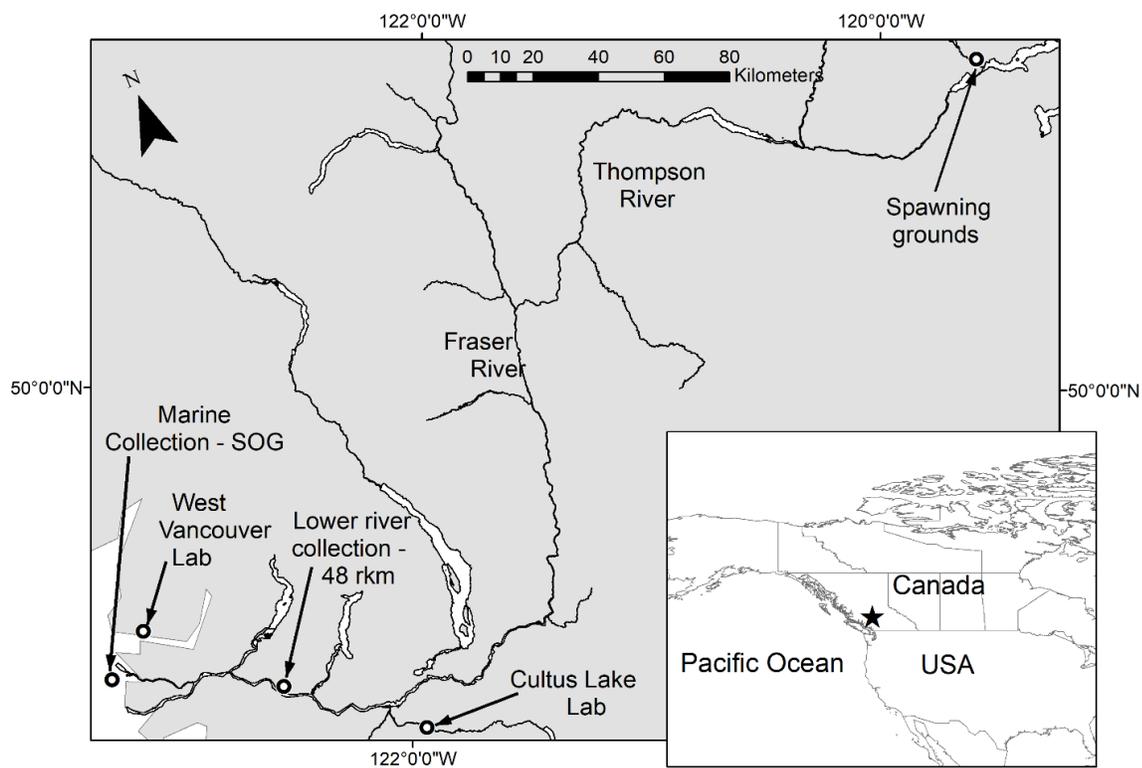
Fig. 1 The southern portion of the Fraser River watershed, BC, Canada, showing collection locations in the Pacific Ocean (Strait of Georgia – SOG), and lower Fraser River (48 river km), transfer location for marine-captured fish from boat to truck tanks (West Vancouver Lab), Cultus Lake Lab holding facility and spawning grounds for the Adams-Shuswap sockeye salmon population under study.
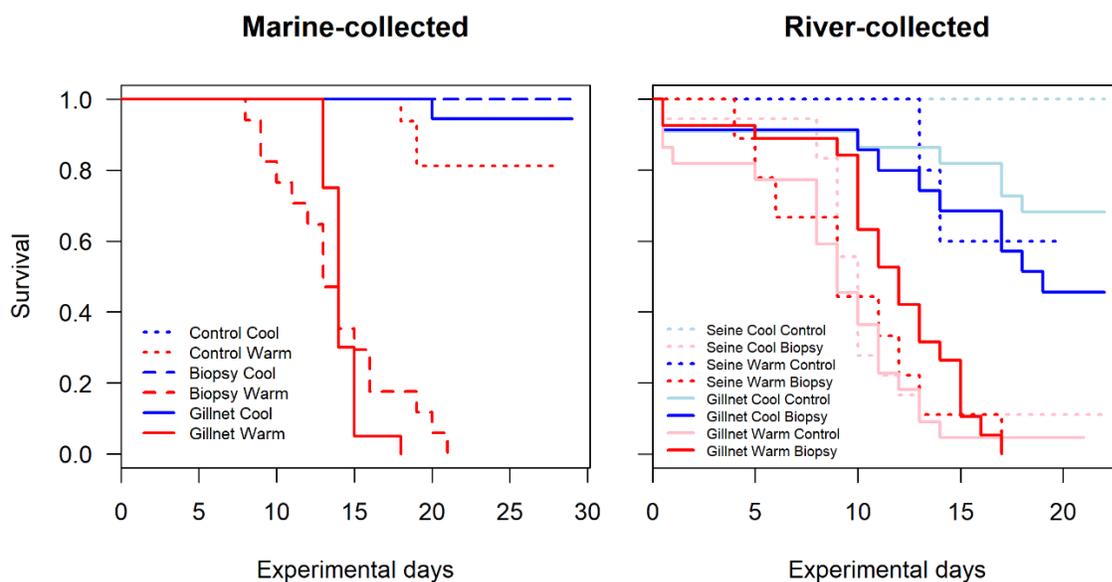
Fig. 2 Kaplan Meier curves describing the survival of adult sockeye salmon held in fresh water at 14°C (blue) or 18°C (red) for up to four weeks. The left panel shows survival of fish captured in the Strait of Georgia. The right panel shows survival of fish collected from the lower Fraser River. Line type denotes treatment (left plot: solid = gillnetted and air exposed, dashed = biopsied control, dotted = non-biopsied control; right plot: solid = gillnet-collected, dashed = seine-collected, lighter colors = non-biopsied, darker colors = biopsied). To simulate behavioural thermoregulation, tank temperatures were decreased to 10°C for 48 h on 30 Sep – 1 Oct for marine-captured and 5–6 Oct for river-captured fish, and then increased back to experimental temperatures.
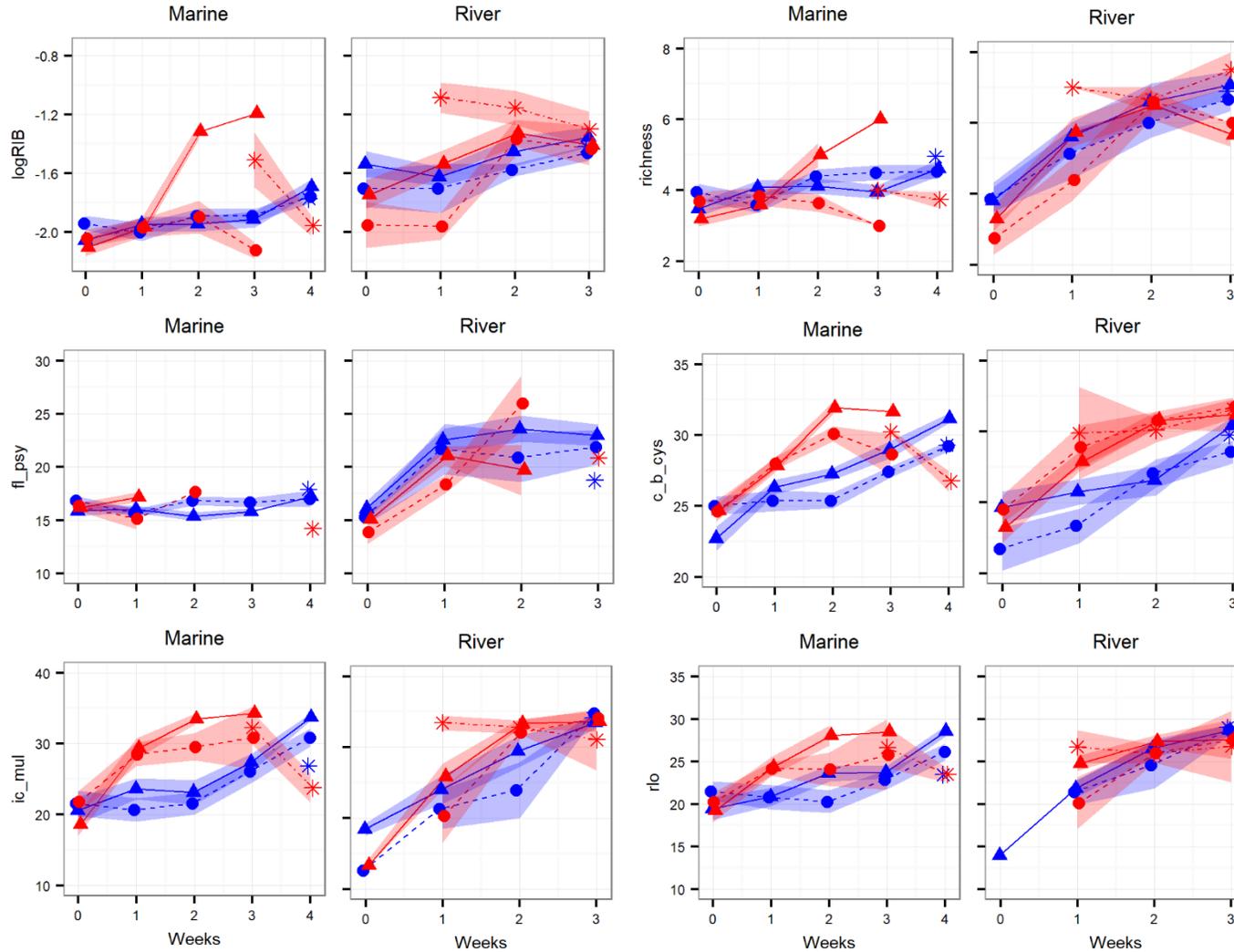
Fig. 3 Relative infection burden (RIB) and loads (40 - Cq) of prevalent agents in adult sockeye salmon gill during a 4-wk freshwater holding period following capture from marine or river environments. Colors indicate temperature (blue=14°C, red=18°C), lines and symbols indicate treatment (solid, triangle = gillnet; dashed, circle = seine). Ribbons describe loads from fish that were biopsied weekly while independent points represent controls that were not biopsied. Mean ± s.e.m. including zeros.
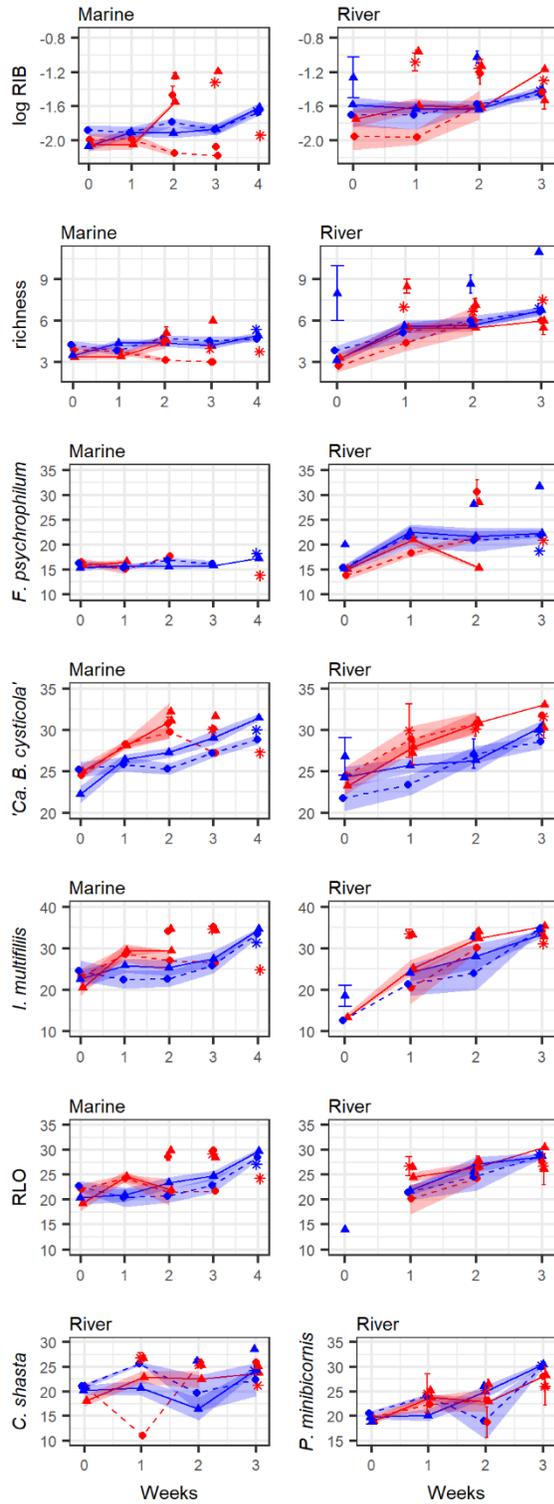
Fig.4 Temporal shifts in proportional load composition of infectious agents measured using HT-qPCR in repeated gill biopsies over 3-4 weeks. Proportional load composition was calculated by normalizing individual agent loads by the maximum load for each agent, summing normalized loads within agent species, and then plotting sums as a proportion of the total sum from all agents in each temperature-source-treatment group at each week.
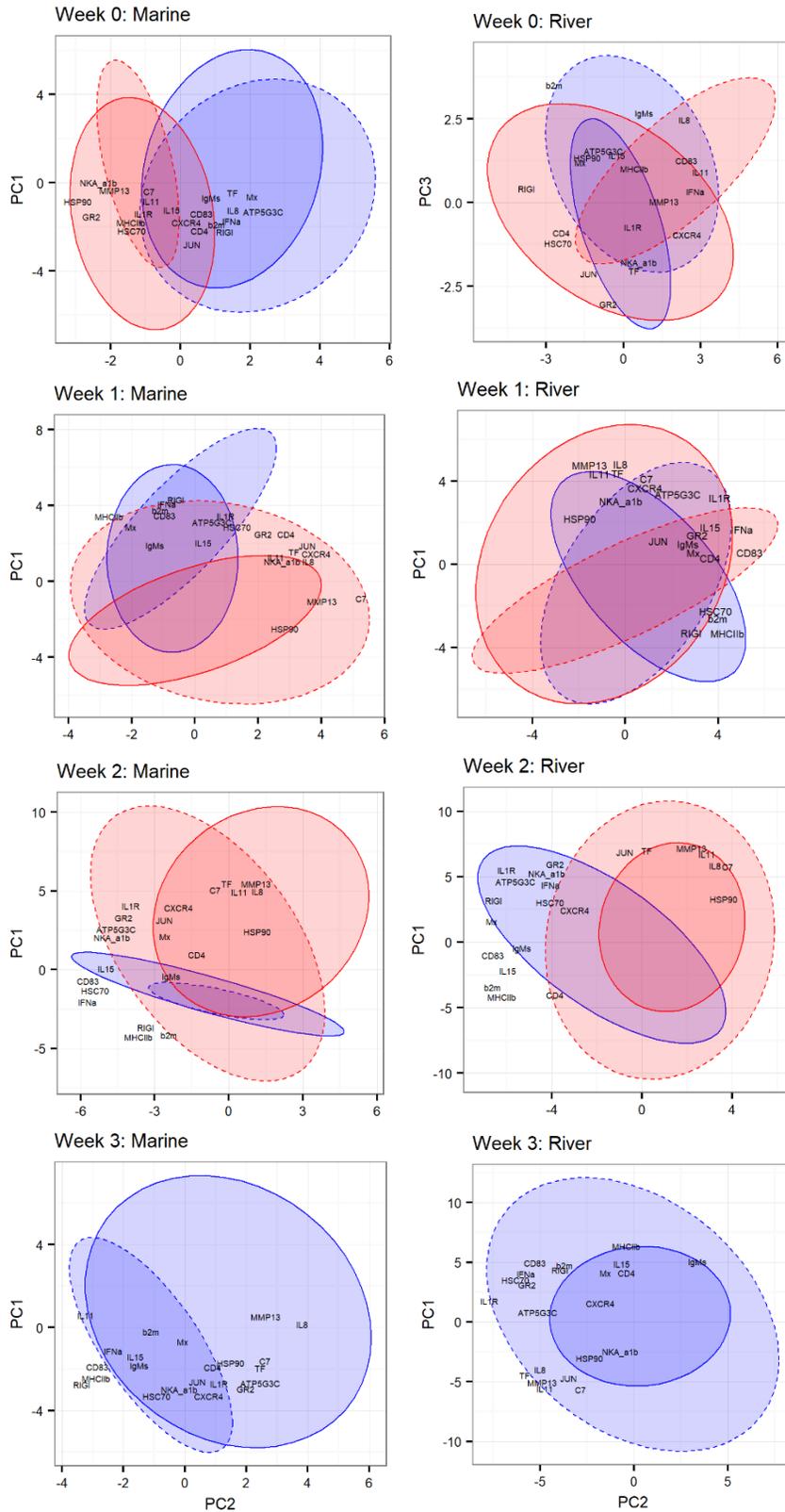
Fig. 5. Principal component analysis of stress and immune gene expression in adult sockeye salmon gill at 14°C (blue) and 18°C (red) from seined (dashed) or gillnetted (solid) fish (95% confidence intervals).
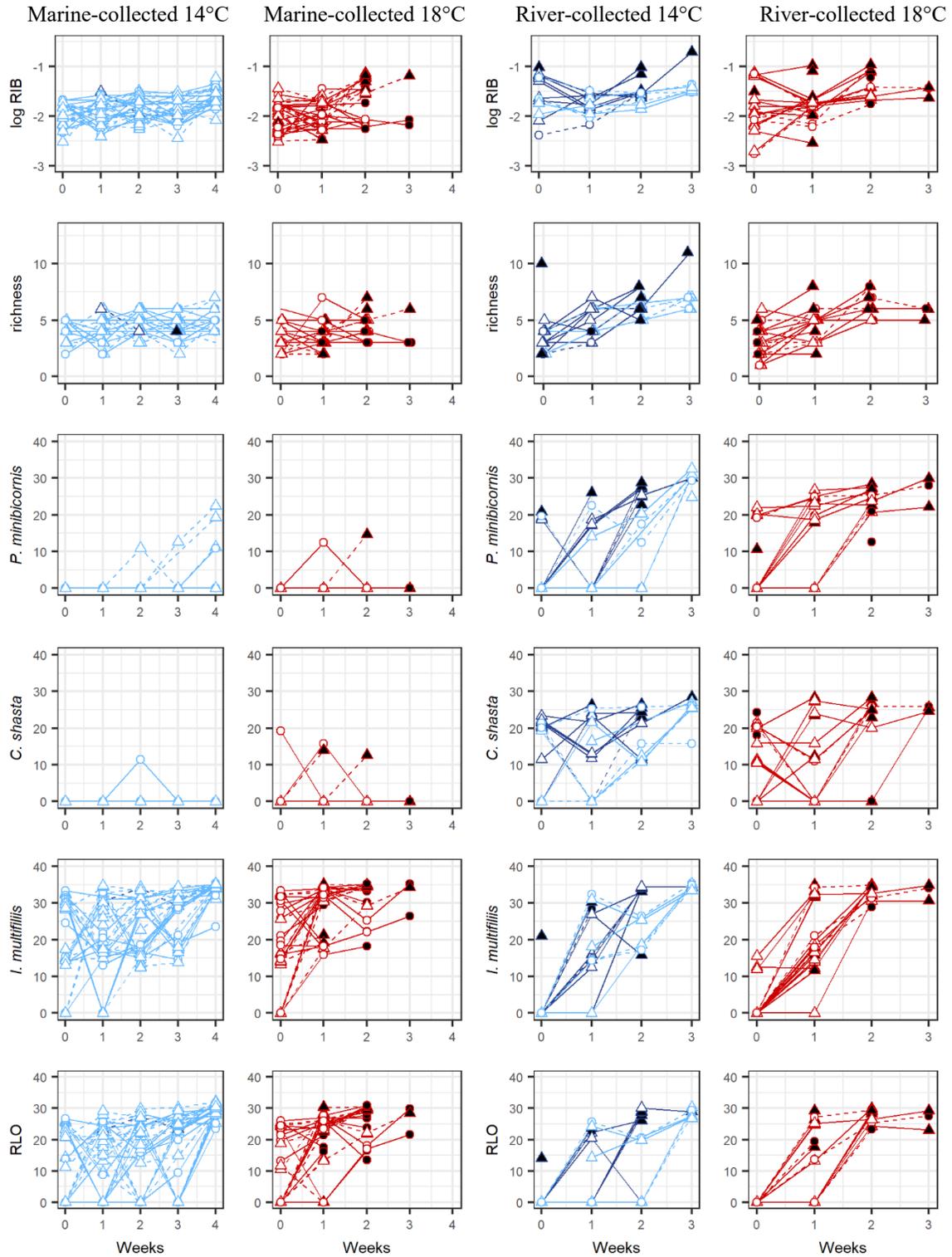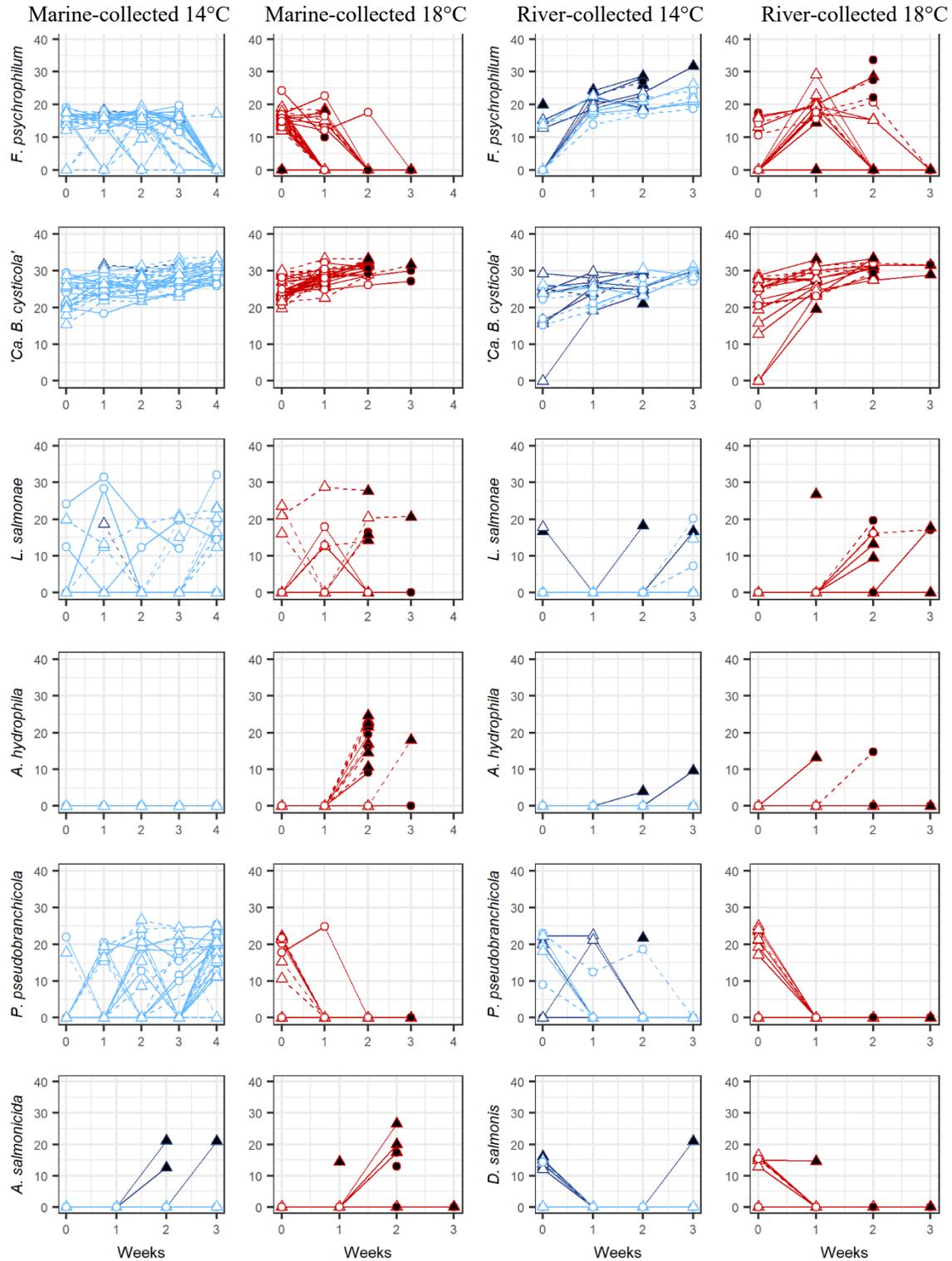
Fig S1. Individual load trajectories of each agent and relative infection burdens (RIB) per host. Dashed line = biopsy, solid line = gillnet, white fill = live biopsy, black fill = last biopsy before mortality.

Table S1. Gill biopsy totals (lethal and nonlethal) from adult female and male sockeye salmon (late run Adams stock only) over 4 weeks (0-4) of freshwater holding following capture from either marine or river environments. Experimental treatments included holding at 14°C or 18°C and gillnet entanglement with air exposure with biopsied and non-biopsied controls; biopsy refers to repeated biopsy (Biopsy) during holding, while non-biopsied groups (No biopsy) were only sampled at morbidity.

Source	Temperature	Treatment	Sex	0	1	2	3	4	Total
Marine	14°C	Gillnet (biopsy)	F	10	13	13	11	12	59
			M	4	5	5	4	4	22
		Control							
		Biopsy	F	7	7	7	7	7	35
			M	5	6	6	6	6	29
		No biopsy	F					9	9
			M					2	2
	18°C	Gillnet (biopsy)	F	13	14	6	1		34
			M	6	5	3			14
		Control							
		Biopsy	F	12	12	7	2		33
			M	5	5	4			14
		No biopsy	F				1	4	5
			M					5	5
River	14°C	Gillnet							
		Biopsy	F	13	12	8	4		37
			M	1	1	1	1		4
		No biopsy	F				3		3
			M	1		1	6		8
		Seine							
		Biopsy	F	3	3	1			7
			M	4	4	3	3		14
		No biopsy	F				8		8
			M				2		2
	18°C	Gillnet							
		Biopsy	F	12	13	4			29
			M	9	9	4	2		24
		No biopsy	F		1		1		2
			M			2			2
		Seine							
		Biopsy	F	5	3	3	1		12
			M	3	2	2			7
		No biopsy	F		1	1	1		3
			M		1	2	1		4

Table S2. Percent prevalence of infectious agents in the gill of adult sockeye salmon during 4 weeks of holding.

| | | No river exposure | | | | River-exposed | | | |
| | | Cool | | Warm | | Cool | | Warm | |
	week	Gillnet	Seine	Gillnet	Seine	Gillnet	Seine	Gillnet	Seine
N	0	12	14	17	19	14	7	20	8
	1	13	18	17	19	13	7	22	5
	2	13	18	11	9	9	4	8	5
	3	13	15	2	1	5	3	2	1
	4	13	16						
Ca. B. cysticola	0	100	100	100	100	86	100	90	88
	1	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100
	4	100	100						
I. multifiliis	0	86	92	58	88	7	14	15	
	1	94	85	100	100	85	100	95	100
	2	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100
	4	100	100						
F. psychrophilum	0	64	100	89	94	36	57	25	63
	1	83	85	21	41	100	100	64	100
	2	89	92		9	100	100	38	80
	3	47	100			100	100		
	4	6							
RLO	0	57	67	32	47	7			
	1	89	46	89	82	62	57	64	60
	2	78	77	100	100	78	50	100	100
	3	80	77	100	100	100	100	100	100
	4	100	100						
C. shasta	0				6	64	57	55	63
	1			5	6	92	43	68	20
	2		8	11		100	100	75	80
	3					100	100	100	100
P. minibicornis	0					21	29	45	25
	1				6	62	43	77	40
	2	6		11		78	75	100	100
	3	7				100	100	100	100
	4	19	8						
P. theridion	0	29	42	16	24	29	43	20	
	1	28	31	16	29	31	14	27	
	2	28	38	44	18	11	50	38	
	3	27	23	100		40			
	4	50	38						
L. salmonae	0	7	17	16		14	14		
	1	17	15	11	12		14	9	
	2	6	15	44	9	11		38	40
	3	20	15	100		40	67	50	100
	4	38	23						
P. pseudobranchicola	0	7	8	26	29	57	57	50	25
	1	28	23		6	23	14		
	2	33	46			11	25		
	3	33	38						
	4	75	100						
A. hydrophila	1					8	14	23	
	2			89	27	11			20
	3			100		20			
A. salmonicida	1						14	27	
	2					22		38	40
	3					20			
D. salmonis	0					43	14	30	13
	1							5	
	3					20			
	4	6							
M. arcticus	1								20
S. destruens	3	7							