NRC Research Press

ARTICLE

Transcriptome patterns and blood physiology associated with homing success of sockeye salmon during their final stage of marine migration[1]

S. Matthew Drenner, Scott G. Hinch, Nathan B. Furey, Timothy D. Clark, Shaorong Li, Tobi Ming, Ken M. Jeffries, David A. Patterson, Steven J. Cooke, David Robichaud, David W. Welch, Anthony P. Farrell, and Kristina M. Miller

Abstract: To better understand the mechanisms that lead to marine mortality of homing adult sockeye salmon (*Oncorhynchus nerka*), gill and blood biopsies were used in combination with biotelemetry to demonstrate how survival to freshwater entry is related to gene expression and physiological indices of stress. Microarray analysis of gene expression indicated multiple biological processes, including immune and stress responses, protein biosynthesis, and metabolism. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analysis indicated fish with upregulation of genes related to stress and infection had higher marine survival compared with fish without this genomic signature. We proposed that higher marine survival of potentially stressed and immune compromised fish can be explained by stressed and infected fish being highly motivated to enter fresh water, leading to enhanced marine survival. However, once in a river, stressed and immune compromised fish could suffer higher mortality because of premature river entry. Overall, this study supports the idea that infection and stress are important biological processes influencing behaviour and fate of sockeye salmon during homing migrations.

Résumé : Pour mieux comprendre les mécanismes dont découle la mortalité en mer de saumons rouges (*Oncorhynchus nerka*) adultes en migration de retour, des biopsies de branchies et de sang ont été utilisées de concert avec la biotélémétrie pour établir comment la survie jusqu'à l'entrée en eau douce est reliée à l'expression génique et à des indices physiologiques du stress. L'analyse de l'expression génique à l'aide de biopuces fait ressortir plusieurs processus biologiques, dont des réactions immunitaires et de stress, la biosynthèse de protéines et le métabolisme. L'analyse qRT-PCR indique que les poissons présentant une régulation positive des gènes associés au stress et à l'infection ont un taux de survie en mer plus élevé que les poissons ne présentant pas cette signature génomique. Nous proposons que la plus grande survie en mer de poissons potentiellement stressés et immunocompromis peut s'expliquer par le fait que les poissons stressés et infectés seraient plus motivés à entrer en eau douce, ce qui se traduirait par un taux de survie en mer plus grand. Une fois en rivière, cependant, les poissons stressés et immunocompromis pourraient avoir une plus forte mortalité en raison de leur entrée prématurée en rivière. Globalement, les résultats de l'étude appuient la notion voulant que l'infection et le stress soient d processus biologiques qui influencent le comportement et le destin des saumons rouges durant leurs migrations de retour. [Traduit par la Rédaction]

Received 14 September 2017. Accepted 23 November 2017.

S.M. Drenner,* **S.G. Hinch,§** **N.B. Furey, and T.D. Clark.**† Department of Forest and Conservation Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
S. Li, T. Ming, and K.M. Miller. Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada.
K.M. Jeffries.‡ Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California at Davis, 160 Haring Hall, Davis, CA 95616, USA.
D.A. Patterson. Fisheries and Oceans Canada, Science Branch, Pacific Region, Cooperative Resource Management Institute, School of Resource and Environmental Management, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.
S.J. Cooke. Fish Ecology and Conservation Physiology Laboratory, Institute of Environmental Science and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada.
D. Robichaud. LGL Limited, 9768 Second Street, Sidney, BC V8L 3Y8, Canada.
D.W. Welch. Kintama Research Services Ltd., 4737 Vista View Cr., Nanaimo, BC V9V 1N8, Canada.
A.P. Farrell. Department of Zoology and Faculty of Land and Food Systems, The University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada.

Corresponding author: Stephen Matthew Drenner (email: smdrenner@gmail.com).

*Present address: Cramer Fish Sciences, 600 NW Fariss Rd., Gresham, OR 97030, USA.

†Present address: Deakin University, School of Life and Environmental Sciences, Geelong, Victoria, Australia 3216.

‡Present address: Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, MB R3T 2N2, Canada.

§Scott G. Hinch currently serves as a Guest Editor for this special issue; peer review and editorial decisions regarding this manuscript were handled by Joanna Mills.

[1]This article is being published as part of the special issue "The Ocean Tracking Network: Advancing aquatic research and management".

Introduction

Anadromous salmonids (from *Salmo*, *Oncorhynchus*, and *Salvelinus* genera) migrate from ocean feeding grounds to fresh water to reproduce, during which migrants undergo extensive physiological shifts associated with reproductive maturation and cope with complex environmental challenges, including freshwater acclimation (Groot et al. 1995; Healey 2000; Hinch et al. 2006). Elevated mortality rates during return migrations for some salmonid populations have recently reduced population sizes to below replacement rates, warranting conservation and management actions for these ecologically, economically, and culturally valuable species (Nehlsen et al. 1991; Bradford 1995; Parrish et al. 1998; Boisclair 2004). In response, biotelemetry studies have focused on characterizing patterns of mortality and identifying factors correlated with in-river mortality, such as water temperature (Thorstad et al. 2008; Martins et al. 2012), stress (Cooke et al. 2006*a*; Thorstad et al. 2008), energetic state (Crossin et al. 2009*a*), and disease (Miller et al. 2011). However, studies examining marine survival have lagged, in part due to greater logistical and financial challenges of collecting and tracking adult salmonids in a marine environment.

Previous studies on adult sockeye salmon (*Oncorhynchus nerka*) from the Fraser River, British Columbia (B.C.), Canada, combined telemetry with nonlethal biopsies of blood and gill tissues to link physiological state of fish to marine and freshwater survival (Cooke et al. 2006*a*, 2006*b*, 2008; Crossin et al. 2007, 2009*a*, 2009*b*). Gross somatic energy, stress hormones, reproductive status, and osmoregulation (i.e., plasma ions and osmolality) were all identified as important. More recently, immune responses to pathogens (e.g., parasites, bacteria, viruses, fungi) have been shown to be associated with mortality during reproductive migrations in fresh water (Miller et al. 2014; Teffer et al. 2017).

Application of transcriptomics techniques, such as microarray, RNA-seq, and quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR), can provide further insights into the physiological mechanisms of homing sockeye salmon (Shrimpton et al. 2005; Miller et al. 2009, 2011; Evans et al. 2011; Flores et al. 2012; Jeffries et al. 2012, 2014*a*). In particular, a 16K gene salmonid cDNA microarray identified a mortality-related signature (MRS) that showed signs of infection, stress, and osmoregulatory perturbations in homing sockeye salmon sampled in marine and freshwater environments (Miller et al. 2011). Notably, fish with the MRS had a 13.5-fold greater chance of dying in fresh water (Miller et al. 2011).

For the present study, we combined telemetry with nonlethal biopsies to investigate the physiological mechanisms associated with successful homing of sockeye salmon through the final phase of the marine environment into the Fraser River, B.C., Canada. First, a 4 × 44K gene salmonid oligonucleotide microarray (Jantzen et al. 2011) and high throughput microfluidics qRT-PCR was used on gill samples to determine if gene expression could predict migration success in the final stages of marine migration to fresh water. Second, we related marine migration success to physiological parameters measured from blood biopsies (stress hormones, ions, and reproductive hormones) and other factors including energetic state, sex, fish size, population, fisheries capture method, and tissue biopsy. This study represents the first attempt to relate gene expression patterns of wild-migrating homing salmonids to fate in the marine environment.

Methods

All experiments were conducted with the approval of the Animal Care Committee of The University of British Columbia, in accordance with the Canadian Council on Animal Care.

Fish capture, biopsy, and telemetry

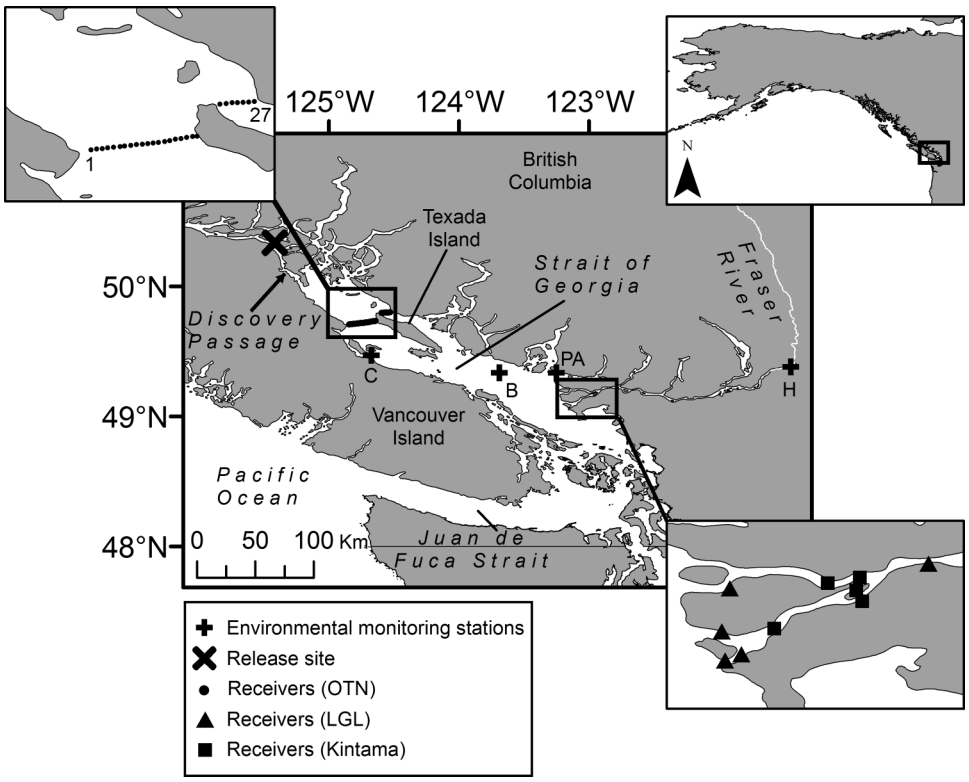
Adult sockeye salmon (n = 400) were captured in August 2010 by either troll (i.e., multiple lines with hooks drawn through the water from a boat; n = 375) or purse seine (i.e., fish surrounded by a large net that is collected alongside the boat; n = 25) commercial vessels in northern Discovery Passage, ~215 km north of the Fraser River during their migration (Fig. 1). After capture, individual fish were either transferred directly into a holding tank on board the vessel (troll captured fish) or netted from a purse seine vessel and transferred to a holding tank on board a separate vessel (purse seine captured fish). The holding tank was constantly flushed with free-flowing ambient salt water. Fish were held for <15 min for troll captured fish or <30 min for purse seine captured fish before undergoing one of two treatments. The biopsy treatment (n = 285; ~78% of fish tagged and released) followed established procedures for handling and biopsy of unanaesthetized sockeye salmon in a nonlethal manner (Cooke et al. 2005). Each individual fish was moved from the holding tank to a foam-padded, v-shaped trough with the gills irrigated with ambient saltwater. A 3 mL blood sample was quickly (<1 min) taken from the caudal vasculature and stored in an ice slurry until processing at the end of the workday, when blood was centrifuged and plasma was transferred into liquid N_2 for subsequent laboratory analysis (Farrell et al. 2001). The plasma was used to assess concentrations of stress parameters (glucose, cortisol, lactate), sex hormones (testosterone, 17β-oestradiol), and osmoregulatory state (concentrations of Na^+ and Cl^-, as well as plasma osmolality). We acknowledge that the storage of blood samples prior to centrifugation may have altered Cl^- and glucose concentrations to some degree (Clark et al. 2011); this was unavoidable to process fish expeditiously and did not influence the main objectives of the study.

Following blood sampling, a small (<4 mm, 0.03 g) gill biopsy was taken from gill filament tips for gene expression analysis. Gill samples were placed in RNA*later* solution (Qiagen, Maryland, USA) before being transferred to liquid N_2. An adipose fin sample (<0.5 g) was stored in 95% ethanol prior to analysis for DNA stock (i.e., aggregates of populations in a spawning region) identification (Beacham et al. 2004). Fork length (FL) was measured to the nearest centimetre, and gross somatic energy was determined with a hand-held microwave radio emitter (Distell Fish FatMeter FM 692, Distell Inc., West Lothian, Scotland, UK; Crossin and Hinch 2005). Finally, an individually coded acoustic transmitter (Vemco V16-3x, 16 mm diameter and <70 mm length, Vemco, Nova Scotia, Canada) was gastrically inserted with a plastic applicator, and a spaghetti tag (Floy Tag & Mfg. Inc., Seattle, Washington, USA) was applied anterior to the dorsal fin through the dorsal musculature to assist visual identification after release. The nonbiopsy treatment group (n = 80; ~22% of fish released) was included to test for tissue biopsy effects on subsequent migration success by only implanting the acoustic transmitter and taking an adipose fin sample (i.e., no blood biopsy, gill biopsy, gross somatic energy sample, or spaghetti tag). Total handling time per fish was <3 min for the nonbiopsy group and <5 min for the biopsy group.

All fish were immediately released overboard following treatment. The release site was deliberately at the lower boundary for the main commercial fishery to minimize possible recapture of tagged fish in fisheries that occur between Southern Discovery passage and the first acoustic receivers (Vemco VR3-UWM, 69 kHz, Vemco, Nova Scotia, Canada) in the lower Fraser River (see Fig. 1). Two tagged fish were, however, recaptured by commercial fishing vessels close to the release site and were excluded from the analysis.

Individual fish were detected at fixed acoustic telemetry arrays along the migration route (see Heupel et al. (2006) for details on the technology; Fig. 1), from which a survival estimate was determined. Each detection registered on arrays included an individual tag ID and an associated timestamp. The Northern Strait of Georgia (NSOG) acoustic telemetry array (Fig. 1) provided the first point of detection, ~84 km southward from the release site (a former

Fig. 1. Map of study area. Upper left inset shows Northern Strait of Georgia (NSOG) acoustic telemetry array, and lower right inset shows acoustic receivers within the mouth of the Fraser River. C = Chrome Island, B = Buoy 46146, PA = Point Atkinson, H = Hope. Kintama-maintained receivers were paired at each location in the lower Fraser River (n = 10), whereas all other receiver points represent a single receiver at each location (i.e., 27 Ocean Tracking Network receivers, 5 LGL receivers).



Pacific Ocean Shelf Tracking array that is currently maintained by the Ocean Tracking Network Canada). The NSOG array consisted of 27 receivers spanning ~25 km across the Strait of Georgia, from Vancouver Island to the B.C. mainland, crossing the northern tip of Texada Island (Fig. 1). A series of acoustic receivers (maintained by Kintama Research, Nanaimo, B.C., Canada, or LGL Ltd., Victoria, B.C., Canada) within the estuarine arms of the Fraser River were the next detection locations, ~131 km southeast of the NSOG array (Fig. 1) and extending ~85 km upriver of the river mouth (Fig. 1). A successful river entry was represented by at least two detections on any of these lower Fraser River receivers. Potential false detections were removed from the data set and were defined as any individual tag that was detected only once within 24 h across either the NSOG array or any Fraser River receiver.

To investigate any potential for short-term mortality or tag expulsion following the sampling and tagging procedures, an additional group of fish (n = 35) were captured at the same location, biopsied, tagged, and transferred to a 5 m × 1.5 m × 1.5 m metal holding cage fixed to the side of a dock (<3 km from the normal release site). This group was compared with control fish (n = 30) that were placed in this cage without tagging and biopsy to compare survival between tagged and untagged fish. Any detrimental effects due to capture, handling, and tissue biopsy were expected to be short-lived (Donaldson et al. 2012) and therefore would be detected during the 32 h holding period, which approximates the time for a fish to swim to the first telemetry array (Crossin et al. 2009*a*). All tagged (n = 35) and control (n = 30) sockeye salmon survived the 32 h holding study in the metal net pen, and there was no tag regurgitation. All fish from the holding study were released and were not included in survival estimates or analyses described below.

RNA and microarray preparation

Total RNA isolation

Total RNA was purified from gill tissue using Magmax-96 for Microarrays Kits (Ambion Inc., Austin, Texas, USA) with a Biomek NXP (Beckman-Coulter, Mississauga, Ontario, Canada) automated liquid-handling instrument. Samples were homogenized with stainless steel beads in TRI reagent (Ambion Inc., Austin, Texas, USA) on a MM301 mixer mill (Retsch Inc., Newtown, Pennsylvania, USA). Aliquots (100 mL) of the aqueous layer of the homogenate were pipetted into 96-well plates, and extractions were carried out according to the manufacturer's instructions using the "No-Spin Procedure" for tissues, on the Biomek NXP. RNA yield was determined by measuring the absorbance at 260 nm (A_{260}) of the eluate. Purity was assessed by measuring the A_{260}/A_{280} ratio of the eluate. Solutions of RNA were stored at −80 °C.

cRNA labeling

Amplification and labeling steps were performed on 96-well plates all at once and with the same kit batches to minimize technical artifacts. A 1 μg sample of total RNA was amplified using the Amino Allyl MessageAmp II-96 kit (Ambion, Texas, USA) according to manufacturer's instructions. A dye coupling reaction was performed using 5 μg of amino-allyl aaRNA (cRNA) and Alexa dyes (Invitrogen, Carlsbad, California). During dye labeling, samples were processed individually by adding dimethyl sulfoxide (DMSO) to the Alexa dye tube and coupling buffer to the appropriate cRNA. The dye mix was then combined with the sample mix and incubated for 1 h at room temperature. For microarray experiments, a pooled tissue reference composed of the RNA from all of the fish used in the experiment was used against each individual sample. All individual (experimental) samples were

fluorescently tagged with Alexa 555 (Invitrogen) and the reference tagged with Alexa 647 (Invitrogen). Samples and references were purified using the Amino Allyl MessageAmp II-96 kit (Ambion) according to manufacturer's instructions and eluted in 35 μL of elution buffer. A fragmentation mix containing 825 ng of Alexa 555 labeled cRNA sample, 825 ng of the Alexa 647 labeled reference cRNA pool, 10× blocking agent (Agilent Technologies) and 25× fragmentation buffer (Agilent Technologies) was made and incubated at 60 °C for 30 min. Equal volumes of the fragmented cRNA were added to GEx Hybridization buffer HI-RPM (Agilent Technologies) and spun down at room temperature for 1 min to reduce bubbles. These were immediately frozen and stored in the dark at –20 °C until use.

Salmon microarrays

The cGRASP (http://web.uvic.ca/grasp/) 44K Salmonid Oligo Array (Agilent Technologies) was used in this study. In each of the technical steps leading to slide hybridization, samples were either randomized (RNA extraction, hybridization) or ran all at once (amplification, labeling); as a result, extensive biological replication was chosen over technical replication, as the approach ensured that technical variation would not be confounded by biological variables of interest. Hybridizations were performed in batches of up to 48 samples and conducted by a single technician over a 10-day period to minimize technical variance. A 55 μL sample was loaded onto an array and hybridization occurred using the quad hybridization chambers in the Tecan-HS4800 Pro Hybridization Station (Tecan Trading AG, Switzerland). Slide processing steps within the Tecan HS4800 were conducted as follows: one 1 min slide wash step (aCGH prehybridization buffer) at 65 °C, sample injection at 63 °C with agitation, hybridization for 17 h at 63 °C with high viscosity mode agitation, two 1 min washes (GE wash 1 with 0.005% Triton-X102) at 23 °C with a 1 min soak time, two 1 min washes (GE wash 2 with 0.005% Triton-X102 and 0.01% surfactant) at 37 °C with a 1 min soak time, followed by slide drying at 30 °C for 2 min.

Slides were scanned using the Tecan LS Reloaded scanner (TecanTrading AG, Switzerland) and the Array-Pro Analyzer software according to manufacturer's instructions. Images were quantified using Imagene (BioDiscovery, El Segundo, California; www.biodiscovery.com) and spots with poor quality or no signal (<2 standard deviations from background) at both wavelengths were flagged. Raw microarray intensity data were normalized in GeneSight (version 4.1, BioDiscovery, Inc. El Segundo, California; www.biodiscovery.com) using the local intensity-dependent loess normalization to remove intensity-dependent dye bias (Yang et al. 2002). All data were $\log_2$-transformed and an intensity ratio was computed by taking the differences in log-transformed intensities between the sample and reference control. These log-transformed intensity ratios were used in all further analyses.

Features with observations missing for greater than 50% of the samples were eliminated from the analysis, leaving ~26K features upon which analyses were based. For hierarchical clustering and principal component analysis (PCA), the remaining features with missing values had their intensity ratios imputed using the *K*-nearest neighbour method, but were unmodified for ANOVAs.

Microarray data were deposited in the NCBI Feature Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with the accession number GPL11299.

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR was conducted using TaqMan assays run on the Fluidigm BioMark platform (Fluidigm Corp., San Francisco, California; technical details can be found in supporting information for Miller et al. (2014)). Briefly, total RNA was used to synthesize cDNA (SuperScript VILO MasterMix; Life Technologies) following manufacturer's instructions. Fifty-eight qRT-PCR assays (see Table 5) plus three reference genes were examined using the qRT-PCR assays from Miller et al. (2014) and which were run separately on the Biomark. Full details of the qRT-PCR protocol are described in supporting information for Miller et al. (2014). Briefly, a pre-amplification step is required; therefore, 1.25 μL of cDNA from each sample was pre-amplified with primer pairs corresponding to all assays in a 5 μL reaction volume using TaqMan Preamp Master Mix (Life Technologies) according to the BioMark protocol. Unincorporated primers were removed using ExoSAP-IT High-Throughput PCR Product Clean Up (MJS BioLynx Inc., Brockville, Ontario), and samples were diluted 1:5 in DNA Suspension Buffer (TEKnova, Hollister, California). A 10× assay mix was prepared containing 10 $\mu mol \cdot L^{-1}$ primers and 3 $\mu mol \cdot L^{-1}$ probes for the TaqMan assays. The amplified cDNA was diluted 1:2 with 2× Assay Loading Reagent (Fluidigm). A 5 μL reaction mix was prepared (2× TaqMan Mastermix (Life Technologies), 20× GE Sample Loading Reagent, nuclease-free water, and 2.7 μL of amplified cDNA) and added to each assay inlet of the array following manufacturer's recommendations. After loading the assays and samples into the chip by an IFC controller HX (Fluidigm), PCR was performed with the following conditions: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Gene expression data were preprocessed using GenEx (www.multid.se), normalized to two housekeeping genes, 78D16.1 and Coil-P84-2, and relative gene expression was assessed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). These assays were developed based on the MRS signature as defined on the GRASP 16K salmon microarray (from the Miller et al. 2011 study). Because the mapping of genes across the 16K microarray and the cGRASP 44K Agilent array was very poor, to compare results with the earlier study (i.e., Miller et al. 2011), the biomarkers of the MRS signature had previously been validated to predict the MRS microarray signature (K.M. Miller, unpublished data).

Blood plasma laboratory analysis

Plasma osmolality, ions (Na^+, Cl^-), glucose, and lactate were measured using procedures outlined in Farrell et al. (2001). Plasma cortisol, testosterone, and 17β-oestradiol were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Neogen). Testosterone and 17β-oestradiol samples were extracted in ethyl ether in accordance with the manufacturer's protocols. Cortisol, testosterone, and 17β-oestradiol samples were all run in duplicate at appropriate dilutions. Additional details on assays are provided in Farrell et al. (2001).

Data analysis

DNA analysis revealed that four Fraser River sockeye salmon stocks (Chilko, Early Shuswap, Harrison, Late Shuswap) accounted for the majority of the captured fish (313 out of 400), which allowed for a focused analysis of these stocks and a comparison with previous work on the marine survival of these stocks (Cooke et al. 2006*b*; Crossin et al. 2009*a*). Differences in survival among stocks to the NSOG array and the lower Fraser River receivers were examined with a test of equal proportions. Variables most associated with marine survival were identified with generalized linear models (GLMs; family = binomial, link = logit), using marine survival (i.e., a successful river entry) as the response variable. Sample size was maximized by separately running three submodels, each with a set of related explanatory variables, which allows for comparison among models and for complementary rather than competing models (Planque et al. 2011) while preventing over-fitting and reducing colinearity.

The primary study objective was addressed by the survival ~ transcriptome submodel, which tested the gene expression features that were related to marine survival (n = 145 fish for which microarrays were run). We used an unsupervised PCA approach to summarize gene expression and contrast survivorship along PC axes rather than a supervised approach comparing survivors and

Table 1. Numbers of sockeye salmon tagged and released and detected at the Northern Strait of Georgia (NSOG) and lower Fraser River acoustic receiver locations from four stocks (i.e., populations) used in the study.

Stock	Released	Detected at NSOG	Detected at Fraser River
Chilko	68	48 (71)	36 (53)
Early Shuswap	71	53 (75)	47 (66)
Harrison	5	4 (80)	3 (60)
Late Shuswap	169	130 (77)	102 (60)

Note: Numbers in parentheses indicate the cumulative percent success to the respective receiver location.

non-survivors because there are numerous potential factors related to survival that may not be represented in the gene expression patterns. PCA was used to construct PC axes that captured variability in gene expression across all fish (i.e., ~26K genes after filtering; see Salmon microarrays section). The PC scores for each of the first five PC axes of each fish were used as explanatory variables. To investigate further the broad classes of genes related to marine survival, a functional analysis was performed on all genes from any PC axis from microarray results found to be significantly associated with marine survival. Using PathWay Studio (Nikitin et al. 2003), gene-set enrichment analysis was performed to identify processes that are over-represented in the gene expression profiles. Redundancy reduction was performed using REVIGO (Supek et al. 2011) on significant ($p < 0.01$) gene ontology terms resulting from the functional analysis. Finally, to test for similarities between the present study, which attempted to identify transcriptional signatures associated with marine migration survival, and the study of Miller et al. (2011), which identified a transcriptional signature (dubbed the MRS) associated with freshwater migration survival, *t* tests were used to relate MRS biomarker gene expression, generated through qRT-PCR, to any PC axes that were significantly related to marine survival.

The second study objective was addressed by the survival ~ blood physiology submodel, which identified blood plasma variables related to marine survival ($n = 250$ biopsied fish). Explanatory variables included cortisol, lactate, glucose, sodium, chloride, testosterone, osmolality, sex, and gross somatic energy. MANOVA comparisons were used to compare plasma variables and gross somatic energy among stocks (Cooke et al. 2006*a*; Crossin et al. 2009*a*). Variables with significant differences among stocks based on MANOVA results were added as interactions with stock in the model. In addition, data exploration revealed potential differences in plasma variables between troll and purse seine capture methods, and therefore only troll captured fish ($n = 225$ versus $n = 25$ for seine caught fish) were used in the survival ~ blood physiology submodel. Visual inspection of multipanel scatterplots indicated possible colinearity between some plasma variable pairs. Based on a Pearson correlation coefficient > 0.8 and variance inflation factor > 3 (Zuur et al. 2010), osmolality, which was identified as colinear with lactate, cortisol, Na^+, and Cl^-, was excluded from subsequent analyses. Lactate, cortisol, Na^+, and Cl^- were not found to be colinear.

The second study objective was also addressed by the survival ~ nonphysiology submodel, which pooled biopsied and nonbiopsied fish ($n = 313$) to identify factors beyond blood plasma and genomics that were related to marine survival. Explanatory variables entered in this model included capture method (troll versus purse seine), biopsy treatment, stock, day of release, and FL. This model also assessed the null hypothesis that neither tissue biopsy nor capture method had an effect on marine survival.

Within each submodel, all continuous explanatory variables were standardized (i.e., subtracting global mean from each value and dividing by two times the standard deviation; Gelman 2008), which allowed for the estimation and comparison of effect sizes among predictor variables. To identify potentially important variables within each submodel, an all-subsets regression was conducted, and models were ranked based on AIC corrected for small sample sizes (AIC_c; Burnham and Anderson 2002), ΔAIC_c, and AIC_c weights (w_i) using the "MuMIn" package (Bartoń 2013) in R (R Core Team 2017). Model averaging was applied to a 95% confidence set of models (all models with a cumulative summed $w_i \geq 0.95$) to incorporate model uncertainty (Burnham and Anderson 2002). Model fit was assessed using adjusted-R^2. The relative support for individual predictor variables in the models was evaluated based on whether the 95% confidence intervals for estimates of effect size intersected zero (i.e., if the 95% confidence intervals did not intersect zero, there is greater support for the variable being associated with marine survival). Diagnostics for heteroscedasticity, normality, and independence of residuals were visually inspected in all models. Model predictions were made based on all variables present in the 95% confidence set of models and using the median value for continuous variables and selecting a level of a categorical variable that was predicted to have the least effect on survival. All data analyses were performed using R version 3.4.1 (R Core Team 2017).

To better understand factors influencing gene expression and to contrast results between microarray and blood plasma data, further GLMs were constructed for any PC axis from microarray data significantly related to marine survival. For these GLMs, the PC scores of the PC axis was the response, and predictor variables included fish stock, capture method, sex, blood plasma variables, gross somatic energy, and FL. Otherwise, model diagnostics, model construction, selection, and assessment followed the procedure described above (i.e., all subsets regression, ranking via AIC_c, model averaging, and investigation of effect sizes).

Table 2. Summary of the first five principal components (PCs) generated from PCA analysis of microarray data.

Principal components	SD	Proportion of variance	Cumulative proportion
PC1	73.39	0.19	0.19
PC2	55.54	0.11	0.30
PC3	36.08	0.05	0.34
PC4	33.34	0.04	0.38
PC5	29.18	0.03	0.41

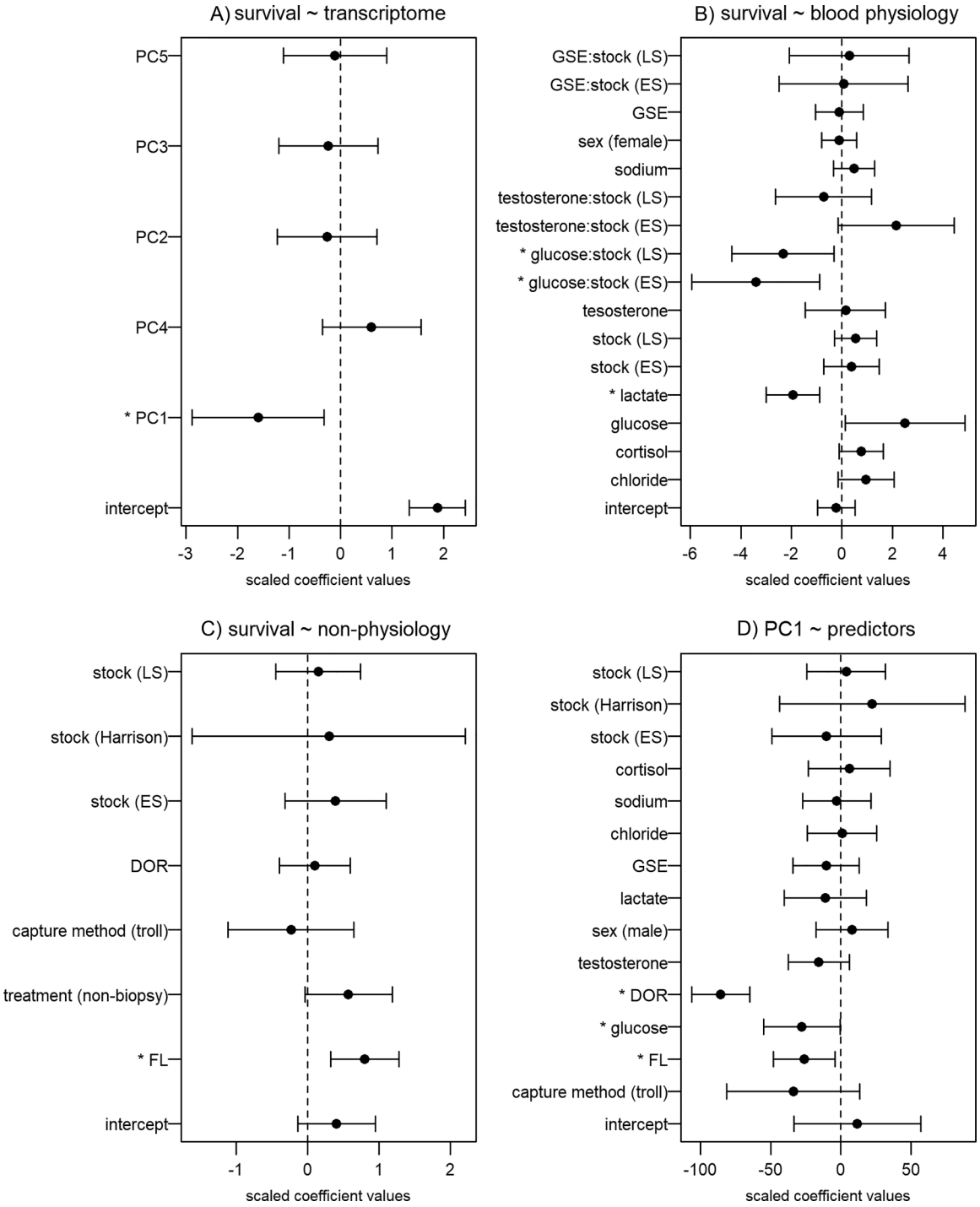
Results

Of the 313 sockeye salmon from the four fish stocks (i.e., aggregates of populations in a spawning region) examined in this study, 71%–80% of tagged fish survived to the first detection point (e.g., the NSOG array) and 53%–66% to the lower Fraser River array (Table 1). However, no significant differences in survival existed among stocks to either the NSOG array (df = 3; $p = 0.76$) or the lower Fraser River array (df = 3; $p = 0.52$).

PC axis 1 (PC1) of the PCA on gene expression from the microarray captured 19% of the variability, and cumulatively the first five PC axes contained 41% of the total variability (Table 2). Of these five PC axes, the survival ~ transcriptome submodel selected only PC1 as being related to marine survival (Fig. 2A), such that fish with more positive PC1 scores had a lower probability of survival to the lower Fraser River (Fig. 3A). The top survival ~ transcriptome submodel that contained only PC1 explained a small proportion of the data variability (adjusted-$R^2 = 0.10$; Table 3).

The biological processes associated with PC1 of microarray data, and therefore marine survival, were explored further in a functional analysis. Survival of fish in the 15% ends of PC1 was contrasted separately to identify groups of fish with the strongest differences in survival. Proportional survival was significantly different between fish in the 15% most PC1-positive grouping, which had a proportional marine survival of 68.2% (15 out of 22 fish), and fish in the 15% most PC1-negative grouping, which had a propor-

Fig. 2. Model-averaged, scaled parameter estimates (circles) with 95% confidence intervals (lines) for three models examining the factors related to marine survival: (A) survival ~ transcriptome, (B) survival ~ blood physiology, (C) survival ~ nonphysiology; and one model examining the factors related to PC1 of gene expression from microarray data: (D) PC1 ~ predictors. An asterisk preceding the variable names signifies that the 95% confidence intervals for the scaled parameter estimate do not intersect zero. Abbreviations are given for Early Shuswap (ES), Late Shuswap (LS), gross somatic energy (GSE), day of release (DOR), and fork length (FL).



tional marine survival of 95.5% (21 out of 22 fish) (two-sided Fisher's exact test; $p = 0.046$ and an odds ratio of 9.35 (95% confidence interval = 1.03–461.0)). Based on significant differences between the proportions of fish surviving in the 15% PC1 groupings, hereinafter fish in the 15% most PC1-positive and 15% most PC1-negative groups will be referred to as the "lower survival" and "higher survival" PC1 groupings, respectively. To identify the strongest genomic signal in the functional analysis, the PC scores of individual fish ($n = 145$) were ranked along the first PC axis, and fish in the lower and higher survival PC1 groupings were selected ($n = 44$). Fold-change was then calculated by averaging intensity ratios across individuals within a given PC1 grouping, then calculating the difference in averaged ratios between PC1 groupings as [mean(higher survival) – mean(lower survival)]. The resulting value (x) was converted to fold-change (if $x > 1$, then fold-change = $2^{(x)}$; if $x \leq 1$, then fold-change = $(-1)2^{(x)}$). Thus, a positive fold-change

Fig. 3. Model-averaged predictions for the probability of marine survival based on variables selected as being associated with marine survival in three submodels: survival ~ transcriptome (A); survival ~ blood physiology (B); survival ~ nonphysiology (C). The upper *x* axis is the actual value of the variable, and the lower *x* axis is the standardized variable (in standard deviation units). Abbreviations are given for fork length (FL), Early Shuswap (ES), and Late Shuswap (LS).

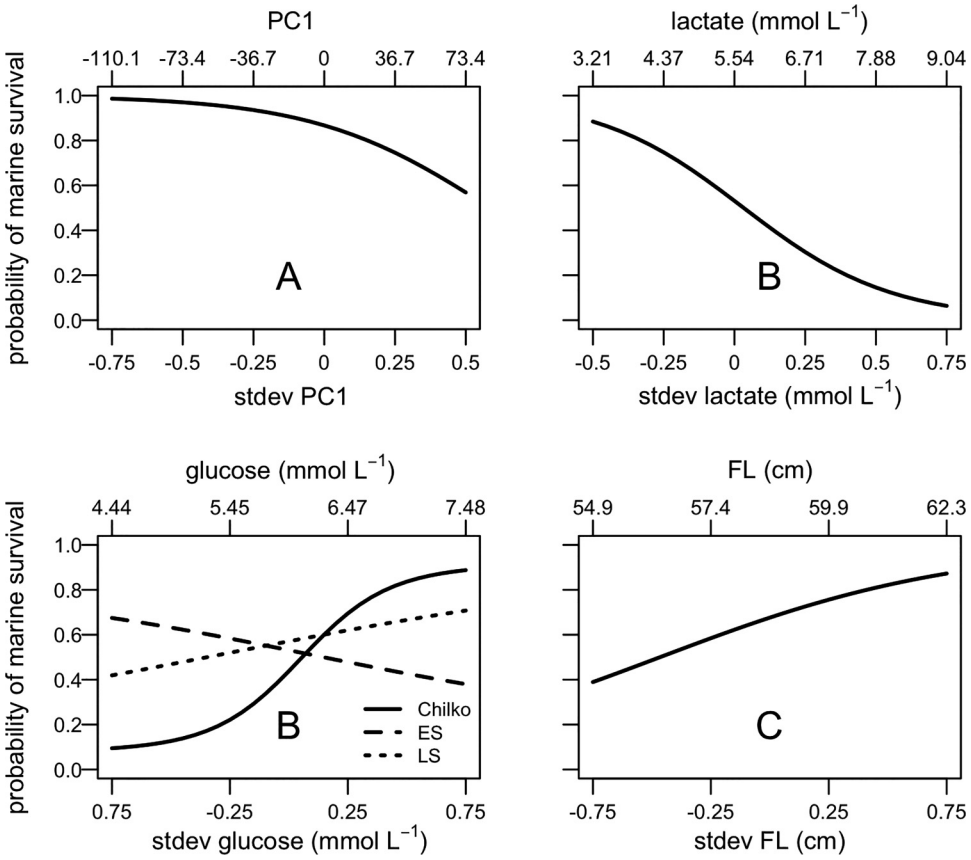


Table 3. Model selection results based on AIC_c showing top models ($\Delta AIC_c < 2$), to a maximum of five models, from a 95% confidence set of models.

Model type	Model	*K*	ΔAIC_c	w_i	Adjusted-R^2
Survival ~ transcriptome	PC1	1	0.00	0.20	0.10
	PC1, PC4	2	0.46	0.16	
	PC1, PC2	2	1.76	0.08	
	PC1, PC3	2	1.86	0.08	
Survival ~ blood physiology	Lactate, glucose, stock × glucose, stock × testosterone, Cl^-, cortisol	10	0.00	0.10	0.25
	Lactate, glucose, stock × glucose, stock × testosterone, Cl^-	9	0.30	0.08	
	Lactate, glucose, stock × glucose, stock × testosterone, cortisol, Na^+	10	1.51	0.05	
	Lactate, glucose, stock × glucose, stock × testosterone, Cl^-, cortisol, Na^+	11	1.67	0.04	
	Lactate, glucose, stock × glucose, stock × testosterone, Cl^-, Na^+	10	1.79	0.04	
Survival ~ nonphysiology	FL, treatment	2	0.00	0.31	0.06
	FL	1	1.38	0.15	
	FL, treatment, capture method	3	1.69	0.13	
	FL, treatment, DOR	3	1.76	0.13	
PC1 ~ predictors	DOR, FL, glucose, capture method, testosterone	5	0.00	0.02	0.39
	DOR, FL, glucose, capture method	4	0.22	0.02	
	DOR, FL, glucose, testosterone	4	0.38	0.02	
	DOR, FL, glucose	3	0.79	0.02	
	DOR, FL, glucose, capture method, sex	5	1.28	0.01	

Note: Model results are shown for "survival ~ transcriptome", "survival ~ blood physiology", "survival ~ nonphysiology", and "PC1 ~ predictors" models. DOR, day of release; FL, fork length.

value reflects upregulation of a gene in the higher survival PC1 grouping. The 50 most upregulated and 50 most downregulated genes (based on fold-change values) included genes involved in metabolic processes, cell proliferation, apoptosis, protein synthesis, stress, immune response, protein binding, structural proteins, and osmoregulation (Table S1[2]). In agreement, the functional analysis indicated protein biosynthesis, cellular metabolism, apoptosis, stress and immune defense, protein binding, oxidative

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2017-0391.

Table 4. Functional groupings of genes from gene set enrichment analysis based on fold-change values calculated between fish from the 15% most PC1-positive and 15% most PC1-negative groups of fish.

GO ID	Name	No. of measured entities	Median fold-change	*p* value
Apoptosis				
0043066	Negative regulation of apoptosis	177	1.059	<0.001
Cell proliferation				
0007090	S phase of mitotic cell cycle	106	1.030	<0.001
0000278	Mitotic cell cycle	248	–1.024	<0.001
0001938	Positive regulation of endothelial cell proliferation	29	–1.085	<0.001
0015074	DNA integration	5	1.288	0.001
0008283	Cell proliferation	231	1.044	0.004
0007568	Aging	115	–1.008	<0.001
0031018	Endocrine pancreas development	90	1.116	<0.001
Immune response				
0019083	Viral transcription	75	1.133	<0.001
0002474	Antigen processing and presentation of peptide antigen via MHC class I	6	1.279	0.002
0044130	Negative regulation of growth of symbiont in host	8	1.159	0.004
0051918	Negative regulation of fibrinolysis	9	1.167	0.002
Metabolic processes				
0006200	ATP catabolic process	195	–1.036	<0.001
0006006	Glucose metabolic process	87	–1.039	<0.001
0015991	ATP hydrolysis coupled proton transport	22	–1.003	<0.001
0045471	Response to ethanol	79	1.043	0.004
0006600	Creatine metabolic process	7	–1.175	0.005
0010888	Negative regulation of lipid storage	7	1.066	0.008
0006521	Regulation of cellular amino acid metabolic process	44	1.062	<0.001
0046688	Response to copper ion	23	1.060	0.003
Oxidative phosphorylation				
0022904	Respiratory electron transport chain	85	1.104	<0.001
0051881	Regulation of mitochondrial membrane potential	10	1.116	0.006
Oxidative stress				
0050665	Hydrogen peroxide biosynthetic process	5	–1.207	0.002
Protein binding and metabolism				
0051437	Positive regulation of ubiquitin–protein ligase activity involved in mitotic cell cycle	70	1.080	<0.001
0031145	Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	78	1.072	<0.001
0050821	Protein stabilization	40	1.001	0.008
0042026	Protein refolding	7	–1.077	0.006
0006898	Receptor-mediated endocytosis	38	–1.060	0.007
Protein synthesis				
0010467	Gene expression	348	1.026	<0.001
0008380	RNA splicing	251	1.064	<0.001
0006415	Translational termination	80	1.133	<0.001
0006412	Translation	308	1.054	<0.001
0016070	RNA metabolic process	141	1.049	<0.001
0016071	mRNA metabolic process	103	1.035	<0.001
0006413	Translational initiation	63	1.078	0.001
0051028	mRNA transport	59	1.027	0.002
0006396	RNA processing	83	1.090	0.003
0006366	Transcription from RNA polymerase II promoter	213	1.040	0.004
0046939	Nucleotide phosphorylation	9	1.281	0.006
Structural component				
0030198	Extracellular matrix organization	65	–1.069	0.001
0097435	Fibril organization	7	1.213	0.002
0034501	Protein localization to kinetochore	6	1.344	0.002
0006928	Cellular component movement	77	1.034	0.004
0007155	Cell adhesion	325	–1.044	0.005
0031529	Ruffle organization	12	1.153	0.002

Note: Redundancy reduction was performed on significant ($p < 0.01$) functional groupings. GO, gene ontology.

phosphorylation, and structural processes were biological processes significantly overrepresented among genes most differentially regulated between the PC1 groupings (Table 4).

To further explore biological differences between PC1 groupings, we used *t* tests to compare migration rates of fish with the NSOG detection array between higher and lower survival PC1 fish groupings. This post hoc comparison showed significant (df = 1, $p = 0.03$) differences in migration rate between PC1 groupings, the relationship indicating fish in the higher survival PC1 grouping migrated faster to the NSOG line after release compared with fish in the lower survival PC1 grouping.

We compared transcriptional signatures associated with survival from the present study with that of Miller et al. (2011) by running qRT-PCR of biomarkers developed from the MRS genomic signature identified in Miller et al. (2011) on fish in this study. Based on *t* tests relating these biomarkers to PC scores of fish in the higher and lower survival PC1 groupings (15% ends), 40 out of 58 (70%) biomarkers were significant at $p < 0.05$, 30 out of 58 (52%) biomarkers were significant at $p < 0.01$, and 22 out of 58 (38%) biomarkers were significant at $p < 0.001$ (Table 5). Comparisons of biomarker fold-change between the PC1 groupings indicated the majority (33 out of 40; ~83%) of significant biomarkers were upregulated in the higher survival PC1 grouping (Table 5). Notably, significant immune-, stress-, and osmoregulatory-related biomarkers that were upregulated in the higher survival PC1 grouping included genes involved in antigen presentation via the proteasome (PSMB4), proteolysis or inflammation (MMP25), apoptosis (PRF1, RALB), osmotic stress (CIRP), interferon immune response (IFNA2, IRF1, MX, RIG-1, MCSF), immune response through activation of the complement cascade (C4B), viral release (SGTA), osmoregulation (NKAA1B, NKAA3), and other immune-related responses (CD4, NKA_B1, ZAP7; Table 5). In contrast, significant immune-, stress-, and osmoregulatory-related biomarkers that were downregulated in the higher survival PC1 grouping included genes involved in interferon immune response (STAT1), oxidative stress (WDR16, SHOP21), and osmoregulation (NKAA1C; Table 5).

MANOVA comparisons indicated significant differences among stocks for plasma testosterone (df = 3; $p < 0.001$) and glucose (df = 3; $p = 0.03$), as well as gross somatic energy (df = 3; $p < 0.001$) (Table S2[2]). Therefore, a stock interaction term was included in the survival ~ blood physiology submodel. The Harrison stock was excluded because of a low sample size ($n = 5$). The survival ~ blood physiology submodel selected lactate and interactions between glucose and the Early Shuswap and Late Shuswap stocks as variables associated with marine survival (Fig. 2B). The survival ~ blood physiology submodel predicted a higher probability of marine survival for fish with lower plasma lactate (Fig. 3B; upper right), and the probability of marine survival was negatively related to plasma glucose for Early Shuswap fish but positively related for Late Shuswap fish (Fig. 3B; lower left). The top survival ~ blood physiology submodel explained a quarter of the data variability (adjusted-$R^2 = 0.25$; Table 3).

The survival ~ nonphysiology submodel selected only FL as being associated with marine survival, with no support for an effect of capture method, biopsy treatment, or stock (Fig. 2C). The survival ~ nonphysiology submodel predicted a higher probability of survival to river entry for fish with a greater FL (Fig. 3C). The top survival ~ nonphysiology submodel explained a small proportion of the data variability (adjusted-$R^2 = 0.06$; Table 3).

Beyond the relationship to marine survival, PC1 from microarray results was negatively correlated with day of release, FL, and plasma glucose, but not related to stock, sex, cortisol, lactate, Cl^-, Na^+, testosterone, gross somatic energy, or capture method (Fig. 2D). Model-averaged and scaled parameter estimates indicated that fish that had more negative PC1 scores were released on a later date, had a higher plasma glucose concentration, and had a greater FL (Fig. 2D). The top model predicting PC1, which included day of release, FL, and glucose, explained over one-third of the data variability (adjusted-$R^2 = 0.39$; Table 3).

Discussion

This study is the first to link gene expression and blood characteristics to survival of homing sockeye salmon during final stages of marine migration prior to river entry. Migration typically took 40 h from the tagging and release site to the first telemetry line (e.g., ~84 km to the NSOG array), during which 20%–29% of the tagged fish (population-dependent) disappeared (presumably died) unlike 100% survival of cage-held fish for approximately the same time period. A further 11%–25% died during the next period of migration to river entry (~131 km in ~270 h). Moreover, gene expression differed substantially between those fish that showed the best survival (96%) and those that showed 68% survival. Marine survival was specifically related to multiple physiological processes identified from gene expression and blood physiology including stress, immune response, metabolic processes, and osmoregulation.

Telemetry studies such as ours assume that survival estimates are not biased by imperfect receiver detection efficiencies, tag collisions, tagging- or handing-induced mortality, tag regurgitation, and unreported fish capture. Detection efficiencies of our acoustic telemetry arrays were estimated as 100% (i.e., no fish were detected on receivers further along migration pathway after not being detected on prior receivers), which corresponds to previous estimates (Crossin et al. 2009*a*). Tag collisions were reduced by releasing fish individually over a 2-week period, and the acoustic tags were programmed to emit a random signal every 40–120 s. There were no differences in migration success between biopsied and nonbiopsied fish tracked using telemetry, as well as there was no tag regurgitation observed nor differences in short-term (32 h) survival between biopsied–tagged and control fish in the holding study. Fish were deliberately released at the southern boundary for the commercial fishery to reduce tag recapture, with commercial fisheries aware of the tagging activities. Finally, our survival estimates fall within ranges reported previously for homing sockeye salmon that were sampled, tagged, and released north of the Strait of Georgia and tracked to the Fraser River (i.e., 52–75%; Cooke et al. 2006*a*, 2006*b*; Crossin et al. 2009*a*).

Our primary objective was to test whether gene expression could predict marine survival. Both microarray and biomarker data indicated marine survival was related to multiple biological processes, including responses to infection, stress, protein biosynthesis, metabolism, and osmoregulation. Many of these cellular processes we identified were similar to those identified by Miller et al. (2011), despite using two different microarray platforms (i.e., 16K cDNA microarray versus 44K oligonucleotide microarray). Among the 43 biomarkers specifically identified from a stress- and infection-related genomic signature (i.e., the MRS) in Miller et al. (2011), 67% were also significantly related to PC1 from the microarray results in this study, which was associated with marine survival. Furthermore, genes involved in osmoregulatory function (i.e., CIRP, SHOP21, NKAA1B, NKAA1C, NKAA3), a key biological process identified in the genomic signature reported by Miller et al. (2011), were significantly related to PC1 from the microarray results in the present study.

Identification of these biological processes (e.g., stress, infection, metabolism, protein biosynthesis, and osmoregulation) from gene expression may not be surprising given homing salmon in the ocean are preparing for reproduction and freshwater entry, both of which are associated with elevated levels of circulating stress hormones and are energetically costly (Hinch et al. 2006). Interestingly, there was an upregulation of the majority (33 out of 40; ~83%) of significant immune and stress biomarkers in the higher survival PC1 grouping. Upregulation of immune and stress genes in fish with higher marine survival was contrary to

Table 5. MRS biomarker genes selected for qRT-PCR analysis and resulting *p* values from *t* tests relating biomarker load to PC1 scores of fish from the 15% most PC1-negative and 15% most PC1-positive groupings.

Gene name	Gene symbol	*p* value	Fold-change	General function
Complement C4-B precursor	C4B	0.014*	0.983	Complement cascade; immune
Complement component C7 precursor	C7	0.459	0.036	Complement component C7 precursor
C-X-C chemokine receptor type 4	CXC4	0.444	0.200	
Elongation factor 1-alpha, oocyte form	EEF1AO	0.029*	–0.384	
BTB–POZ domain-containing protein KCTD10	KCTD1	0.003**	–1.327	Potassium ion transport, immune response
Krueppel-like factor 2	KIF2	0.081	0.613	Positive regulation of transcription
Oncorhynchus mykiss mRNA for macrophage colony-stimulating factor (csf1 gene)	MCSF	0.006**	0.783	Macrophage colony stimulating factor
Matrix metalloproteinase-25 precursor	MMP25	<0.001***	1.132	Proteolysis, inflammatory response
Oncorhynchus mykiss G-protein (P-ras) mRNA, complete cds	PRAS	<0.001***	1.917	
Ras-related protein Ral-B precursor	RALB	0.002**	0.482	Apoptosis
SUMO-activating enzyme subunit 2	SAE2	0.486	0.077	Protein modification, ubiquitin cycle
Secretogranin II (*Ctenopharyngodon idella*)	SCG	<0.001***	1.109	
Small glutamine-rich tetratricopeptide repeat-containing protein A	SGTA	<0.001***	0.508	Viral release
Signal transducer and activator of transcription 1-alpha and beta	STAT1	0.023*	–0.760	Transcription activation, viral response
Transmembrane protein 18	TMEM	0.074	–0.706	
Unknown	UK8	0.120	0.293	
Unknown	UKBC15	0.113	0.240	
WD repeat protein 16	WDR16	<0.001***	–2.990	Cell proliferation, tumor, oxidative stress
Tyrosine–protein kinase ZAP-70	ZAP7	0.039*	0.590	Immune, T-cell
Actin, alpha skeletal muscle	ACA	0.050*	0.773	Structural protein
ADP-ribosylation factor 6	ARF6	0.059	0.650	
ADP-ribosylation factor-like protein 8B	ARL8B	<0.001***	0.516	Cell proliferation, metabolism
Unknown	CA054698	0.020*	0.600	
Unknown	CA055640	<0.001***	1.030	
Unknown	CA063814	0.011*	0.537	
Tropomyosin-1 alpha chain	CB486176	0.019*	0.638	
86791 pfam05110, AF-4, AF-4 proto-oncoprotein	CB511853	0.001**	1.059	
Unknown	CB512538	0.812	0.136	
C-type lectin domain family 4 member M	CLC	0.356	–0.157	
COMM domain-containing protein 7	COMMD7	<0.001***	0.616	Protein synthesis
FYN-binding protein	FYB	<0.001***	0.980	T-cell signal cascade
Heterogeneous nuclear ribonucleoprotein A1	HNR1	0.002**	0.532	
Histone acetyltransferase HTATIP	HTA	<0.001***	0.843	
Keratin, type II cytoskeletal 8	KRT8	<0.001***	–0.880	Cell structure
Platelet-activating factor acetylhydrolase precursor	PLA	0.008**	0.487	
Peptidyl-prolyl cis–trans isomerase A	PPIA	0.095	0.283	Pathogen virulence factor
Perforin-1 precursor	PRF1	0.016*	0.778	Apoptosis, lysis of viral infected cells
Proteasome subunit beta type 4 precursor	PSMB4	<0.001***	0.435	Gamma interferon-inducible proteasomal genes with roles in antigen presentation
60 S ribosomal protein L6	RPL6	0.004**	1.282	
SAM domain-containing protein SAMSN-1	SAMSN	0.848	0.096	
ADP–ATP translocase 2	SLC1	<0.001***	0.468	Metabolism
Transcription initiation factor TFIID subunit 11	TAF11	0.275	–0.267	Protein synthesis
Tropomyosin-1 alpha chain	TPM1	0.760	–0.200	
Complement factor C3	C3	0.507	–0.357	Indiction complement system
CD4	CD4_ONMY	<0.001***	0.836	T-cell activity–B-cell activity
IFN-alpha	IFNA2	<0.001***	1.215	Interferon responses typical of antiviral activity
Interferon regulatory factor 1	IRF1	<0.001***	1.524	Antiviral response; interferon activity
MHC I	MHC1	0.407	–0.815	Cellular immune responses typical of responses to intracellular pathogens
Mx	MX_ONTS	<0.001***	1.415	Interferon responses typical of antiviral activity
NKA_B1	NKA_B1	<0.001***	1.403	Immune response
CRP–SAP like pentraxin	PTX_ONMY	0.066	0.738	
Retinoid-inducible gene	RIG-I	<0.001***	0.742	Interferon responses typical of antiviral activity
Serum amyloid protein a (SAA)	SAA_ONMY	0.092	0.891	
Hyperosmotic protein 21	SHOP21	0.008**	–0.532	Part of ubiquitin–ligase complex inducible upon exposure to thermal and osmotic stress
Cold inducile RNA binding protein	CIRP	<0.001***	1.512	Stress; osmoregulation

Table 5 (*concluded*).

Gene name	Gene symbol	*p* value	Fold-change	General function
Na^+/K^+-ATPase alpha 1b	NKAA1B	<0.001***	0.820	Osmoregulation
Na^+/K^+-ATPase alpha 1c	NKAA1 C	<0.001***	–0.468	Osmoregulation
Na^+/K^+-ATPase alpha 3	NKAA3	0.033*	0.724	Osmoregulation

Note: Positive and negative fold-change values indicate genes were up- and downregulated, respectively, in the 15% most PC1-negative grouping that had a higher probability of marine survival relative to the 15% most PC1-positive grouping that had a lower probability of marine survival. When possible, individual biomarker general functions were inferred using RefSeq (O'Leary et al. 2016) and Ingenuity Target Explorer (Sosinsky et al. 2003). Information on probe sequences can be found in Miller et al. (2011).

our prediction that stressed and immune compromised fish would have lower survival to river entry and was also contrary to Miller et al. (2011) that found lower freshwater survival of fish that showed upregulation of many of the same infection- and stress-related genes. Below, we offer a hypothesis to explain these apparently contradictory results.

We propose that increased survival in the marine environment of potentially stressed and immune compromised fish (as we observed) was a result of these fish migrating faster through the marine environment, thereby reducing predation by pinniped predators, assuming predation risk is time-dependent. Supporting this hypothesis, there was a significant relationship between PC1 groupings and migration rate in the marine environment, and this relationship was in the direction we would have predicted; fish in the higher survival PC1 grouping that showed upregulation of stress and immune genes migrated faster to the first detection point (i.e., the NSOG line) after release compared with fish in the lower survival PC1 grouping.

While faster migration of potentially compromised individuals seems counterintuitive, it is possible that signals associated with enhanced stress and immunity may accelerate their drive to move towards spawning grounds before they die. Osmotic disturbance may have also encouraged fish to be more direct in their migration into fresh water. Expression of many osmoregulatory genes was significantly different between higher and lower survival PC1 groupings. For example, the higher survival PC1 group of fish, which showed signs of an infection and stress, had elevated expression of NKAA1b, a gill Na^+/K^+-ATPase (NKA) isoform associated with saltwater acclimation (Richards et al. 2003; Shrimpton et al. 2005) that increases upon entering fresh water (Miller et al. 2011). Blood plasma ions (Na^+ and Cl^-) were not related to survival or PC1 and thus did not provide additional evidence for an osmoregulatory influence on marine survival. Overall, our hypothesis that higher survival of fish showing signs of stress, infection, and osmotic dysfunction was related to faster migration rates is further supported by findings from Miller et al. (2011), who showed fish that were more freshwater acclimated and had signs of stress and infection (i.e., fish with the MRS genomic signature) migrated faster in both marine and freshwater environments.

Although potentially stressed and immune compromised fish had higher survival in the marine environment, it is possible that these intracellular signals may also indicate these fish were less capable of meeting long-term migratory demands. For example, stressed and infected fish migrated faster into fresh water, improving their short-term survival in the marine environment. However, once in fresh water, survival of stressed and infected fish may diminish as they become further compromised due to advancement of a disease-state (as observed in Miller et al. 2011), which could result from exposure to elevated river temperatures (Wedemeyer 1996; Jeffries et al. 2012) or stressful flow conditions in-river (Costa et al. 2017). Indeed, previous research showed portions of a sockeye salmon population that entered the Fraser River before historical timing (a phenomenon referred to as "early-entry" behaviour) encountered warmer temperatures and experienced reduced in-river survival (Hinch 2009). The factors contributing to this early-entry behaviour have remained largely unresolved, but this phenomenon could be related to an infectious state that motivates fish to enter fresh water prematurely. As a result, these fish could experience reduced in-river survival due to advancement of a disease-state after encountering warm river temperatures that occur earlier in the season. Unfortunately, we were unable to evaluate freshwater fate of fish from this study due to limited telemetry infrastructure in-river.

It is worth noting that gene expression explained a relatively small amount (10%) of the variability in survival. In addition, greater than 50% of fish in the "lower survival" PC1 grouping still survived to freshwater entry. These results highlight the complexity of factors influencing migratory fate (i.e., there is no single factor or genomic signature that explains all variability in survival). In our study, PCA was used to summarize variability across tens of thousands of genes being expressed in gill tissue. Thus, we were only measuring aspects of the physiology that were most strongly associated with the first PC axis (PC1 accounted for ~19% of the variance across all gene expression) and that are possible based on the function of gill tissue. In other words, our results identified a genomic signature that made fish more likely to survive in the marine environment, but we were not able to account for everything that influenced survival.

The second objective of our study was to relate blood plasma variables and other nonphysiological variables (e.g., sex, FL, stock (i.e., population), tissue biopsy) to marine survival. We found that glucose (and the interaction between stock and glucose), lactate, and FL were related to marine survival. Plasma glucose is mobilized in response to stress and exercise in fish (Pagnotta and Milligan 1991), and the general trend between glucose and survival indicated elevated glucose in fish with higher marine survival, which provides support for the gene expression results that indicated more highly stressed fish had higher survival. However, we also found that plasma glucose interacted with stock, indicating population-level differences underlying metabolic and physiological processes. Population-level differences in glucose have been found in previous studies on sockeye salmon (Cooke et al. 2006*a*; Donaldson et al. 2010), and these differences could be associated with physiological and metabolic adaptations to population-specific migratory challenges (e.g., distance, elevation; see Crossin et al. 2004) and historical conditions (e.g., temperature and flow; see Eliason et al. 2011).

Results from this study also showed elevated plasma lactate was related to lower marine survival. In teleosts, lactate is released into the blood following exhaustive exercise that requires anaerobic metabolism and peaks after 1–2 h (Wydoski et al. 1976; Wood et al. 1983; Milligan 1996; Farrell et al. 2001; Clark et al. 2012). Therefore, the elevated plasma lactate in the present study could be a result of sampling fish after exhaustive exercise during the fisheries capture event. Interestingly, plasma lactate was not related to PC1 from microarray results, suggesting that this response was not captured in at least the first PC axis from microarray results. It is worth noting that gene expression in gill tissue (examined herein) may differ from muscle tissue, where we might expect the presence of a signature related to exercise. Similar relationships between plasma lactate and marine survival have also been found in previous studies on homing sockeye

salmon (Cooke et al. 2006*a*, 2006*b*; Crossin et al. 2009*a*), suggesting plasma lactate as perhaps the most consistent blood plasma variable related to marine survival among studies. Cortisol, a stress hormone, was not related to survival in this study nor to gene expression. Cortisol is involved in numerous physiological processes occurring during this stage of migration, including reproductive development, osmoregulation, and metabolic processes (Wendelaar Bonga 1997), and therefore cortisol levels may already be elevated in migrating fish, reducing our ability to detect a response of this hormone to an acute stressor such as a fishery encounter.

The model relating nonphysiological variables to marine survival indicated that larger fish had higher survival. Size-selective survival in the ocean is common in salmon, with larger fish typically having higher survival (Healey 1982; Ewing and Ewing 2002; Saloniemi et al. 2004). However, a previous study found sockeye salmon with smaller bodies had higher survival to spawning grounds (Cooke et al. 2006*a*), which the authors attributed to smaller fish potentially avoiding gill net fisheries in the lower Fraser River. During the present study, there were no in-river commercial fisheries that could have captured fish prior to reaching detection sites in the lower river. Therefore, once the potential for a fishery-imposed size bias was removed, larger fish may have had a selective advantage over smaller fish given the assumption that fish size is related to overall condition (Tomaro et al. 2012) and thus the ability to fight infection (Arkoosh et al. 2006) and evade predators (Mesa et al. 1994). Interestingly, the relationship between fish size (i.e., FL) and PC1 from the microarray results also suggests that larger fish were more likely to carry the MRS, which could negatively influence survival once fish enter the river (Miller et al. 2011). Notably, smaller fish size could also be beneficial when river temperatures are high, as smaller individuals can be more thermally tolerant (see Clark et al. (2012) and references within).

We also examined whether factors other than survival (e.g., blood physiology, sex, stock, day of release, FL) were related to PC1 from the microarray results. Model results indicated three variables (plasma glucose, FL, and day of release) were related to PC1. Notably, two variables (plasma glucose, FL) were related both to PC1 from the microarray results and to marine survival in their respective models. These variables influenced survival in the same direction between analyses, increasing our confidence in the relationships between these variables and marine survival. However, as mentioned previously, the relationship between glucose and marine survival was not consistent among stocks. Interestingly, we did not find associations between stock and gene expression, but this could be due to reduced statistical power in models with lower samples sizes (i.e., sample size in the gene expression model was approximately two-thirds that of the model relating blood plasma variables to marine survival). Overall, our study results suggest that the relationship between marine survival and glucose is complex, likely population-specific, and warrants additional study.

Because some processes such as reproductive maturation and osmoregulatory preparedness for fresh water changes temporally over the migration, Julian date of sampling often is a strong predictor of physiological state (Crossin et al. 2009*a*). In our study, day of release was associated with PC1 from the microarray results; fish with a higher probability of marine survival (i.e., fish with the immune and stress related genomic signature) arrived later at the tagging site. This finding was somewhat surprising given sampling took place over a relatively short time period (2 weeks), and day of release was not related to survival in the model with the largest sample sizes (e.g., the "survival ~ fish-related" submodel). Ultimately, fish that arrived later at the tagging site were more likely to have the immune- and stress-related genomic signature, suggesting that these individuals were responding to pathogen infection(s). Stock-specific responses to the MRS were detected in Miller et al. (2011); however, their samples were collected over a longer time period. In our study, stock was not related to PC1 from microarray results, with the three stocks being equally distributed throughout the relatively narrow period of sampling.

In conclusion, transcriptomic and blood plasma variables related to immune responses, stress, osmoregulation, and metabolism predicted survival of homing sockeye salmon in the marine environment, providing further evidence of the importance of these biological processes during the homing stage of salmon migrations. Immune and stress responses are tightly linked, and there is mounting evidence that these processes, coupled with environmental conditions, are responsible for migration success of salmon in both marine and freshwater environments (Miller et al. 2011, 2014; Jeffries et al. 2014*b*; Teffer et al. 2017). In this study, we did not observe physical or behavioural impairments in fish that would suggest disease presence. Some sea lice were present on the majority of fish sampled, but we did not attempt to quantify their abundance among individual fish. A next step could be screening migrating salmon in the ocean for specific disease-associated microbes (e.g., Miller et al. 2014; Teffer et al. 2017) and relating their presence to expression of host immune, stress, and osmoregulatory biomarkers, as well as migratory fate. By pairing tissue biopsy and molecular approaches with biotelemetry, future studies can gain important insights in the mechanisms underlying fish migration success.

Acknowledgements

This study was funded through the Ocean Tracking Network Canada via a Natural Sciences and Engineering Research Council of Canada (NSERC) and Canada Foundation for Innovation grant to Dalhousie University, and the Genome British Columbia "FishManOmics" project co-funded by Fisheries and Oceans Canada (DFO). Cooke and Farrell are supported by the Canada Research Chairs Program. Support for Furey was provided by the Vanier Graduate Scholarship program, the Mitacs Elevate program, and the Pacific Salmon Foundation. Special thanks to Jayme Hills, Charlotte Whitney, Jenn Burt, Andrea Haas, members of the DFO Environmental Watch crew, and the Pacific Salmon Conservation and Physiology laboratory for laboratory and field assistance. Additional thanks to Captain Michael and Penny Griswold.

References

Arkoosh, M.R., Kagley, A.N., Anulacion, B.F., Boylen, D.A., Sandford, B.P., Loge, F.J., Johnson, L.L., and Collier, T.K. 2006. Disease susceptibility of hatchery Snake River spring–summer Chinook salmon with different juvenile migration histories in the Columbia River. J. Aquat. Anim. Health. **18**(4): 223–231. doi:10.1577/H05-051.1. PMID:26599158.

Bartoń, K. 2013. MuMIN: Multimodel inference [online]. R package version 1.9.0. Available from http://CRAN.R-project.org/package=MuMIn.

Beacham, T.D., Lapointe, M., Candy, J.R., McIntosh, B., MacConnachie, C., Tabata, A., Kaukinen, K., Deng, L., Miller, K.M., and Withler, R.E. 2004. Stock identification of Fraser River sockeye salmon using microsatellites and major histocompatibility complex variation. Trans. Am. Fish. Soc. **133**(5): 1117–1137. doi:10.1577/T04-001.1.

Boisclair, D. 2004. The status of Atlantic salmon (*Salmo salar*): populations and habitats. Can. J. Fish. Aquat. Sci. **61**(12): 2267–2270. doi:10.1139/f05-028.

Bradford, M.J. 1995. Comparative review of Pacific salmon survival rates. Can. J. Fish. Aquat. Sci. **52**(6): 1327–1338. doi:10.1139/f95-129.

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information-theoretical approach. Springer-Verlag, New York.

Clark, T.D., Donaldson, M.R., Drenner, S.M., Hinch, S.G., Patterson, D.A., Hills, J., Ives, V., Carter, J.J., Cooke, S.J., and Farrell, A.P. 2011. The efficacy of field techniques for obtaining and storing blood samples from fishes. J. Fish Biol. **79**(5): 1322–1333. doi:10.1111/j.1095-8649.2011.03118.x. PMID:22026608.

Clark, T.D., Donaldson, M.R., Pieperhoff, S., Drenner, S.M., Lotto, A., Cooke, S.J., Hinch, S.G., Patterson, D.A., and Farrell, A.P. 2012. Physiological benefits of being small in a changing world: responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. PLoS ONE, **7**(6): e39079. doi:10.1371/journal.pone.0039079. PMID:22720035.

Cooke, S.J., Crossin, G.T., Patterson, D.A., English, K.K., Hinch, S.G., Young, J.L., Alexander, R.F., Healey, M.C., Van der Kraak, G., and Farrell, A.P. 2005. Coupling non-invasive physiological assessments with telemetry to understand inter-individual variation in behaviour and survivorship of sockeye salmon:

development and validation of a technique. J. Fish Biol. **67**(5): 1342–1358. doi:10.1111/j.1095-8649.2005.00830.x.

Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Healey, M.C., Shrimpton, J.M., Van Der Kraak, G., and Farrell, A.P. 2006*a*. Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology, **87**: 1575–1586. doi:10.1890/0012-9658(2006)87[1575:MBOIMI]2.0.CO;2. PMID:16869433.

Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Shrimpton, J.M., Van der Kraak, G., and Farrell, A.P. 2006*b*. Physiology of individual late-run Fraser River sockeye salmon (*Oncorhynchus nerka*) sampled in the ocean correlates with fate during spawning migration. Can. J. Fish. Aquat. Sci. **63**(7): 1469–1480. doi:10.1139/f06-042.

Cooke, S.J., Hinch, S.G., Crossin, G.T., Patterson, D.A., English, K.K., Healey, M.C., Macdonald, J.S., Shrimpton, J.M., Young, J.L., Lister, A., Van Der Kraak, G., and Farrell, A.P. 2008. Physiological correlates of coastal arrival and river entry timing in late summer Fraser River sockeye salmon (*Oncorhynchus nerka*). Behav. Ecol. **19**(4): 747–758. doi:10.1093/beheco/arn006.

Costa, M.J., Lennox, R.J., Katopodis, C., and Cooke, S.J. 2017. Is there evidence for flow variability as an organism-level stressor in fluvial fish? J. Ecohydraul. **2**(1): 68–83. doi:10.1080/24705357.2017.1287531.

Crossin, G.T., and Hinch, S.G. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult pacific salmon. Trans. Am. Fish. Soc. **134**(1): 184–191. doi:10.1577/FT04-076.1.

Crossin, G.T., Hinch, S.G., Farrell, A.P., Higgs, D.A., Lotto, A.G., Oakes, J.D., and Healey, M.C. 2004. Energetics and morphology of sockeye salmon: effects of upriver migratory distance and elevation. J. Fish Biol. **65**(3): 788–810. doi:10.1111/j.0022-1112.2004.00486.x.

Crossin, G.T., Hinch, S.G., Cooke, S.J., Welch, D.W., Batten, S.D., Patterson, D.A., Van Der Kraak, G., Shrimpton, J.M., and Farrell, A.P. 2007. Behaviour and physiology of sockeye salmon homing through coastal waters to a natal river. Mar. Biol. **152**(4): 905–918. doi:10.1007/s00227-007-0741-x.

Crossin, G.T., Hinch, S.G., Cooke, S.J., Cooperman, M.S., Patterson, D.A., Welch, D.W., Hanson, K.C., Olsson, I., English, K.K., and Farrell, A.P. 2009*a*. Mechanisms influencing the timing and success of reproductive migration in a capital breeding semelparous fish species, the sockeye salmon. Physiol. Biochem. Zool. **82**(6): 635–652. doi:10.1086/605878. PMID:19780650.

Crossin, G.T., Hinch, S.G., Welch, D.W., Cooke, S.J., Patterson, D.A., Hills, J.A., Zohar, Y., Klenke, U., Jacobs, M.C., Pon, L.B., Winchell, P.M., and Farrell, A.P. 2009*b*. Physiological profiles of sockeye salmon in the Northeastern Pacific Ocean and the effects of exogenous GnRH and testosterone on rates of homeward migration. Mar. Freshw. Behav. Physiol. **42**(2): 89–108. doi:10.1080/10236240902846770.

Donaldson, M.R., Hruska, K.A., Hinch, S.G., Patterson, D.A., Farrell, A.P., Shrimpton, J.M., Miller-Saunders, K.M., Robichaud, D., Hanson, K.C., English, K.K., and Cooke, S.J. 2010. Physiological condition differentially affects the behaviour and survival of two populations of sockeye salmon during their freshwater spawning migration. Physiol. Biochem. Zool. **83**(3): 446–458. doi:10.1086/649627. PMID:20367319.

Donaldson, M.R., Hinch, S.G., Raby, G.D., Patterson, D.A., Farrell, A.P., and Cooke, S.J. 2012. Population-specific consequences of fisheries-related stressors on adult sockeye salmon. Physiol. Biochem. Zool. **85**(6): 729–739. doi:10.1086/664931. PMID:23099469.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G., and Farrell, A.P. 2011. Differences in thermal tolerance among sockeye salmon populations. Science, **332**: 109–112. doi:10.1126/science.1199158. PMID:21454790.

Evans, T.G., Hammill, E., Kaukinen, K., Schulze, A.D., Patterson, D.A., English, K.K., Curtis, J.M.R., and Miller, K.M. 2011. Transcriptomics of environmental acclimatization and survival in wild adult Pacific sockeye salmon (*Oncorhynchus nerka*) during spawning migration. Mol. Ecol. **20**(21): 4472–4489. doi:10.1111/j.1365-294X.2011.05276.x. PMID:21951593.

Ewing, R.D., and Ewing, G.S. 2002. Bimodal length distributions of cultured Chinook salmon and the relationship of length modes to adult survival. Aquaculture, **209**(1): 139–155. doi:10.1016/S0044-8486(01)00755-4.

Farrell, A.P., Gallaugher, P.E., Fraser, J., Pike, D., Bowering, P., Hadwin, A.K.M., Parkhouse, W., and Routledge, R. 2001. Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. Can. J. Fish. Aquat. Sci. **58**(10): 1932–1946. doi:10.1139/f01-136.

Flores, A.M., Shrimpton, J.M., Patterson, D.A., Hills, J.A., Cooke, S.J., Yada, T., Moriyama, S., Hinch, S.G., and Farrell, A.P. 2012. Physiological and molecular endocrine changes in maturing wild sockeye salmon, *Oncorhynchus nerka*, during ocean and river migration. J. Comp. Physiol. B, **182**(1): 77–90. doi:10.1007/s00360-011-0600-4. PMID:21735221.

Gelman, A. 2008. Scaling regression inputs by dividing by two standard deviations. Stat. Med. **27**(15): 2865–2873. doi:10.1002/sim.3107. PMID:17960576.

Groot, C., Margolis, L., and Clarke, W.C. 1995. Physiological ecology of Pacific salmon. UBC Press, Vancouver, B.C.

Healey, M.C. 1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. **39**(7): 952–957. doi:10.1139/f82-130.

Healey, M.C. 2000. Pacific salmon migrations in a dynamic ocean. *In* Fisheries oceanography: an integrative approach to fisheries ecology and management. *Edited by* P.J. Harrison and T.R. Parsons. Blackwell Science, Oxford. pp. 29–54.

Heupel, M.R., Semmens, J.M., and Hobday, A.J. 2006. Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. Mar. Freshw. Res. **57**(1): 1–13. doi:10.1071/MF05091.

Hinch, S.G. 2009. Overview and synthesis: early migration and premature mortality in Fraser River late-run sockeye salmon. *In* Conference on Early Migration and Premature Mortality in Fraser River Late-Run Sockeye Salmon: Proceedings, Vancouver, B.C. *Edited by* S.G. Hinch and J. Gardner. Published by Pacific Fisheries Resource Conservation Council, Vancouver, B.C. pp. 8–14.

Hinch, S.G., Cooke, S.J., Healey, M.C., and Farrell, A.P. 2006. Behavioral physiology of fish migrations: salmon as a model approach. *In* Fish physiology. Vol. 24: Behaviour and physiology of fish. *Edited by* K. Sloman, S. Balshine, and R. Wilson. Elsevier, San Diego, Calif. pp. 239–295.

Jantzen, S.G., Sanderson, D.S., von Schalburg, K.R., Yasuike, M., Marass, F., and Koop, B.F. 2011. A 44K microarray dataset of the changing transcriptome in developing Atlantic salmon (*Salmo salar* L.). BMC Res. Notes, **4**(1): 88. doi:10.1186/1756-0500-4-88. PMID:21447175.

Jeffries, K.M., Hinch, S.G., Sierocinski, T., Clark, T.D., Eliason, E.J., Donaldson, M.R., Li, S., Pavlidis, P., and Miller, K.M. 2012. Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). Ecol. Evol. **2**(7): 1747–1764. doi:10.1002/ece3.274. PMID:22957178.

Jeffries, K.M., Hinch, S.G., Sierocinski, T., Pavlidis, P., and Miller, K.M. 2014*a*. Transcriptomic responses to high water temperature in two species of Pacific salmon. Evol. Appl. **7**(2): 286–300. doi:10.1111/eva.12119. PMID:24567748.

Jeffries, K.M., Hinch, S.G., Gale, M.K., Clark, T.D., Lotto, A.G., Casselman, M.T., Li, S., Rechisky, E.L., Porter, A.D., Welch, D.W., and Miller, K.M. 2014*b*. Immune response genes and pathogen presence predict migration survival in wild salmon smolts. Mol. Ecol. **23**(23): 5803–5815. doi:10.1111/mec.12980. PMID:25354752.

Livak, K.J., and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, **25**(4): 402–408. doi:10.1006/meth.2001.1262. PMID:11846609.

Martins, E.G., Hinch, S.G., Patterson, D.A., Hague, M.J., Cooke, S.J., Miller, K.M., Robichaud, D., English, K.K., and Farrell, A.P. 2012. High river temperature reduces survival of sockeye salmon (*Oncorhynchus nerka*) approaching spawning grounds and exacerbates female mortality. Can. J. Fish. Aquat. Sci. **69**(2): 330–342. doi:10.1139/f2011-154.

Mesa, M.G., Poe, T.P., Gadomski, D.M., and Petersen, J.H. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. J. Fish Biol. **45**: 81–96. doi:10.1111/j.1095-8649.1994.tb01085.x.

Miller, K.M., Schulze, A.D., Ginther, N., Li, S.R., Patterson, D.A., Farrell, A.P., and Hinch, S.G. 2009. Salmon spawning migration: metabolic shifts and environmental triggers. Comp. Biochem. Physiol. Part D Genomics Proteomics, **4**(2): 75–89. doi:10.1016/j.cbd.2008.11.002. PMID:20403740.

Miller, K.M., Li, S., Kaukinen, K.H., Ginther, N., Hammill, E., Curtis, J.M.R., Patterson, D.A., Sierocinski, T., Donnison, L., Pavlidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., and Farrell, A.P. 2011. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science, **331**(6014): 214–217. doi:10.1126/science.1196901. PMID:21233388.

Miller, K.M., Teffer, A., Tucker, S., Li, S., Schulze, A.D., Trudel, M., Juanes, F., Tabata, A., Kaukinen, K.H., Ginther, N.G., Ming, T.J., Cooke, S.J., Hipfner, J.M., Patterson, D.A., and Hinch, S.G. 2014. Infectious disease, shifting climates, and opportunistic predators: cumulative factors potentially impacting wild salmon declines. Evol. Appl. **7**(7): 812–855. doi:10.1111/eva.12164. PMID:25469162.

Milligan, C.L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **113**(1): 51–60. doi:10.1016/0300-9629(95)02060-8.

Nehlsen, W., Williams, J.E., and Lichatowich, J.A. 1991. Pacific salmon at the crossroads — stocks at risk from California, Oregon, Idaho, and Washington. Fisheries, **16**(2): 4–21. doi:10.1577/1548-8446(1991)016<0004:PSATCS>2.0.CO;2.

Nikitin, A., Egorov, S., Daraselia, N., and Mazo, I. 2003. Pathway studio — the analysis and navigation of molecular networks. Bioinformatics, **19**(16): 2155–2157. doi:10.1093/bioinformatics/btg290. PMID:14594725.

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C.M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O'Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkeda, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A.R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D., and Pruitt, K.D. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. **44**(D1): D733–D745. doi:10.1093/nar/gkv1189. PMID:26553804.

Pagnotta, A., and Milligan, C.L. 1991. The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (*Oncorhynchus mykiss*) and winter flounder (*Pseudopleuronectes americanus*). J. Exp. Biol. **161**: 489–508. PMID:1757776.

Parrish, D.L., Behnke, R.J., Gephard, S.R., McCormick, S.D., and Reeves, G.H. 1998. Why aren't there more Atlantic salmon (*Salmo salar*)? Can. J. Fish. Aquat. Sci. **55**(S1): 281–287. doi:10.1139/d98-012.

Planque, B., Loots, C., Petitgas, P., Lindstrøm, U., and Vaz, S. 2011. Understanding what controls the spatial distribution of fish populations using a multi-model approach. Fish. Oceanogr. **20**(1): 1–17. doi:10.1111/j.1365-2419.2010.00546.x.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richards, J.G., Semple, J.W., Bystriansky, J.S., and Schulte, P.M. 2003. Na^+/K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J. Exp. Biol. **206**(24): 4475–4486. doi:10.1242/jeb.00701. PMID:14610032.

Saloniemi, I., Jokikokko, E., Kallio-Nyberg, I., Jutila, E., and Pasanen, P. 2004. Survival of reared and wild Atlantic salmon smolts: size matters more in bad years. ICES J. Mar. Sci. **61**(5): 782–787. doi:10.1016/j.icesjms.2004.03.032.

Shrimpton, J.M., Patterson, D.A., Richards, J.G., Cooke, S.J., Schulte, P.M., Hinch, S.G., and Farrell, A.P. 2005. Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. J. Exp. Biol. **208**(21): 4069–4078. doi:10.1242/jeb.01871. PMID:16244166.

Sosinsky, A., Bonin, C.P., Mann, R.S., and Honig, B. 2003. Target Explorer: an automated tool for the identification of new target genes for a specified set of transcription factors. Nucleic Acids Res. **31**(13): 3589–3592. doi:10.1093/nar/gkg544. PMID:12824372.

Supek, F., Bošnjak, M., Škunca, N., and Šmuc, T. 2011. REVIGO summarizes and visualies long lists of Gene Ontology terms. PLoS ONE, **6**(7): e21800. doi:10.1371/journal.pone.0021800. PMID:21789182.

Teffer, A.K., Hinch, S.G., Miller, K.M., Patterson, D.A., Farrell, A.P., Cooke, S.J., Bass, A.L., Szekeres, P., and Juanes, F. 2017. Capture severity, infectious disease processes and sex influence post-release mortality of sockeye salmon bycatch. Conserv. Physiol. **5**(1): cox017. doi:10.1093/conphys/cox017. PMID:28852514.

Thorstad, E.B., Okland, F., Aaerestrup, K., and Heggberget, T.G. 2008. Factors affecting the within-river spawning migration of Atlantic salmon, with emphasis on human impacts. Rev. Fish. Biol. Fish. **18**(4): 345–371. doi:10.1007/s11160-007-9076-4.

Tomaro, L.M., Teel, D.J., Peterson, W.T., and Miller, J.A. 2012. When is bigger better? Early marine residence of middle and upper Columbia River spring Chinook salmon. Mar. Ecol. Prog. Ser. **452**: 237–252. doi:10.3354/meps09620.

Wedemeyer, G.A. 1996. Physiology of fish in intensive culture systems. Chapman and Hall, New York.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiol. Rev. **77**: 591–625. PMID:9234959.

Wood, C.M., Turner, J.D., and Graham, M.S. 1983. Why do fish die after severe exercise? J. Fish Biol. **22**(2): 189–201. doi:10.1111/j.1095-8649.1983.tb04739.x.

Wydoski, R.S., Wedemeyer, G.A., and Nelson, N.C. 1976. Physiological response to hooking stress in hatchery and wild rainbow trout (*Salmo gairdneri*). Trans. Am. Fish. Soc. **105**(5): 601–606. doi:10.1577/1548-8659(1976)105<601:PRTHSI>2.0.CO;2.

Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J., and Speed, T.P. 2002. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res. **30**(4): e15. doi:10.1093/nar/30.4.e15. PMID:11842121.

Zuur, A.F., Ieno, E.N., and Elphick, C.S. 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. **1**(1): 3–14. doi:10.1111/j.2041-210X.2009.00001.x.