Project Title: Understanding the mechanisms of population depression for endangered Cultus Lake Sockeye Salmon to inform fisheries management: Addressing a key exploitation constraint on bilateral Fraser River mixed-stock Sockeye Salmon fisheries

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SUMMARY

This report was prepared for the Pacific Salmon Commission to understand the underlying causes of population depression for endangered Cultus Lake Sockeye Salmon to inform fisheries management. Water quality degradation is known to contribute to the risk of extinction for endangered Cultus Lake sockeye salmon, with eutrophication having the potential to disrupt physical, biological, and chemical aspects of its freshwater habitat. We used a genomic salmon tool (FitChip) to identify specific stressors including thermal, hypoxia, hydrogen sulfide (H2S), and infectious agents. Using the specific gene expression biomarkers, we found that majority of Sockeye Salmon sampled in Cultus Lake showed physiological signals of exposure to hypoxia and thermal stress during summer and fall seasons. Moreover, we detected the signal of the presence of pathogen *I. moltifilis* in the gill tissue of fry and juvenile Sockeye Salmon both Cultus and Chilliwack Lakes. It is likely that Sockeye Salmon fry are exposed to both low oxygen water in benthic habitats and higher temperature in surface water during their dial migration in the Cultus Lake in summer and fall when the Cultus Lake is thermally stratified.The stress caused by hypoxia and high temperature in warmer seasons together with positive signals of *I. multifiliis* might contribute to increased mortality of Sockeye Salmon in Cultus Lake. It is likely that eutrophication and hypolimnetic oxygen depletion would increase the risk of extinction for Sockeye Salmon in Cultus Lake which require profundal and benthic habitats.

1. INTRODUCTION

The Fraser River watershed produces the most abundant bilateral (US and Canada) Sockeye Salmon (*Oncorhynchus nerka*) fisheries opportunities of any drainage in British Columbia, contributing significantly to commercial, recreational, and First Nations' interests. Within the watershed, variations amongst populations in run-timing, abundance trends (i.e. cyclical dominance), and other life history traits, evoke a very complex fishery management framework. As Fraser River Sockeye Salmon harvests are managed in a mixed-stock fisheries context (common migration run-timings), less abundant conservation units (CU) can have disproportionate influences on aggregate fishery exploitation potentials.

The Cultus Lake sockeye salmon stock, one of the most intensively studied populations of salmon in British Columbia (B.C.), has seen a dramatic decline in the abundance of returning adults over the past 40-50 years (Schubert et al. 2002; COSEWIC 2003). Several factors including harvest and predation, poor ocean survival, diseases and parasites, and exposure to warmer temperatures have been identified as contributing to the collapse of the Cultus Lake sockeye stock (Schubert et al. 2002; COSEWIC 2003). In response, Cultus Lake sockeye were listed as endangered by The Committee on the Status of Endangered Wildlife in Canada in 2003 (COSEWIC 2003). Cultus Lake sockeye are classified as part of the Late-run timing group of Fraser sockeye, which have historically migrated up river in the fall after holding in the Straight of Georgia for up to six weeks (Burgner 1991).

The Cultus Lake Sockeye Salmon CU is a critically-endangered Fraser River population exhibiting extremely depressed abundances (COSEWIC 2003; DFO Stock Assessment data, unpublished). Cultus Lake Sockeye Salmon are part of the Fraser River Late Run timing group, co-migrating with abundant CU's, such as the Shuswap Complex CU, which has experienced record escapements in recent years (dominant line). The conservation status of Cultus Lake Sockeye Salmon has necessitated significant reductions in exploitation rates on Late Run Sockeye Salmon in the Fraser River throughout the late 1990's and 2000's, from a historical dominant cycle average exploitation of 70% (1954-1994) to 32% (1998-2011), despite significant increases in Late Shuswap stock production, which likely explain recent dominant line density-dependent survival feedbacks in freshwater (i.e. rearing capacity limitation; Grant et al. 2011), eroding future production and fisheries opportunities.

Incidental harvest on the Cultus Lake Sockeye Salmon CU has begun to increase once again, despite persistently low returns and smolt outputs, directly pitting conservation objectives against current and future fisheries opportunities. Conservation hatchery efforts have likely staved off extinction of this population to date (Bradford et al. 2010), but overexploitation in the mixed-stock fishery is a primary driver of its endangerment (COSEWIC 2003). Recent limnological and fisheries investigations on Cultus Lake by DFO's Lakes Research Program have revealed substantial in-lake mortality of juveniles, linked to climate variations and lake eutrophication (artificial nutrient enrichment; Putt et al. 2019). Freshwater survival is strongly related to deep dissolved oxygen (DO) concentrations ($r2 = 0.93$, $P < 0.001$), which are depleted under warming-induced enhanced lake stability, intensifying lake productivity, and aerobic decomposition of excess organic matter (Shortreed, 2007; Putt et al. 2019). This new linkage is the first demonstration of a lake habitat effect on Cultus Sockeye Salmon survival, and owing to the direct linkages to eutrophication, likely reversible with appropriate watershed nutrient management (inputs already modeled for the watershed; Putt, 2014). However, several oxygen-mediated pathways of effect on juvenile Sockeye Salmon survival exist, which could be the ultimate drivers

of in-lake mortality, including direct hypoxic stress, increased susceptibility to diseases or pathogens, and exposure to internal loading of contaminants (e.g. ammonia, metals) from lake sediments. Determination of the ultimate mechanism(s) of influence on survival is critically important to engage informed habitat and fisheries management that succeeds in rebuilding this stock, and alleviating constraints on targeting other Late-Run CUs in the Fraser River mixed stock fishery.

Measuring DO concentrations in aquatic ecosystems is one aspect of monitoring hypoxic stress. However, frequent dissolved oxygen measurements over large regions and long periods of time are impractical. Furthermore, such measurements do not directly address whether hypoxic stress was experienced by fish. Therefore, the use of biomarkers for the response to stressors is an important approach (Zhang et al. 2012) and a more integrative technique (Froehlich et al. 2015). The ideal biomarker should be specific to the stressor of interest, be easy to assay, and be relatively unaffected by sampling procedures (Zhang et al. 2012). One major advantage of a biomarker is the ability to detect sub-lethal impacts at low levels of stressor intensity. Moreover, the inferred consequences of sub-lethal exposure can be made more robust because the biomarkers can provide detailed physiological information of an organism (Froehlich et al. 2015). Molecular ecologists have become interested in studying early genetic responses of various organisms to stressors such as hypoxia (Nikinmaa and Rees, 2005; Boswell et al. 2009).

A genomic tool called the 'salmon FitChip' has been recently developed by the DFO Molecular Genetics Laboratory to identify specific stressors (e.g. thermal, hypoxia, osmotic and general stress), and diseases (infectious agents, viral disease, inflammation, immune stimulation) in Pacific Salmon (*Oncorhynchus* spp.) that is based on targeted host response profiling of gill tissue (Akbarzadeh et al. 2018; Miller et al. 2017; Houde et al. 2019). Gill is an ideal tissue to monitor environmental responses due to its direct contact with water. The FitChip is a microfluidics quantitative (qRT)-PCR chip that can simultaneously assess the activity of 96 gene assays in 96 samples at once. The chip is populated with biomarker panels that are predictive of the presence of specific stressors, which have been validated in a series of control challenge studies. To predict the presence of a specific stressor requires only 6-12 co-expressed biomarkers (Miller et al. 2017). This is the first tool of its kind to enable the simultaneous assessment of multiple stressor and disease influences on salmonids, or any other species for that matter. We paired this new genomic tool with our habitat and fish condition information in Cultus Lake (and Chilliwack Lake as a reference) to assess fish stress responses to these potential drivers of poor freshwater survival. Identifying the underlying causes of this early life-stage mortality in nursery habitats will actively inform habitat and fisheries management on remedial actions to improve Cultus Sockeye survival, with the goal of alleviating future constraints on the broader Fraser late-run Sockeye fisheries.

2. MATERIAL AND METHODS

2.1. FISH COLLECTION

Juvenile Sockeye Salmon were collected during regular acoustic-trawl surveys of Cultus and Chilliwack Lakes (MacLellan and Hume 2010) in British Columbia, Canada. The location of Cultus (49.0679° N, 121.9762° W) and Chilliwack (49.0576° N, 121.4143° W) Lakes is presented in Fig. 1. Optimal trawling depth for Sockeye fry was determined from echogram data collected with a Biosonics model DT-X echosounder with a split beam transducer (208 kHz). As fish

densities were relatively low, the trawl was set to a depth that maximized fishing while maintaining a safe operating distance from the bottom of the lake. All work was conducted at night as juvenile Sockeye exhibit strong diel migration behavior (Levy, 1989), and are only within range of the hydroacoustic system and trawl at this time (Burczynski and Johnson 1986).

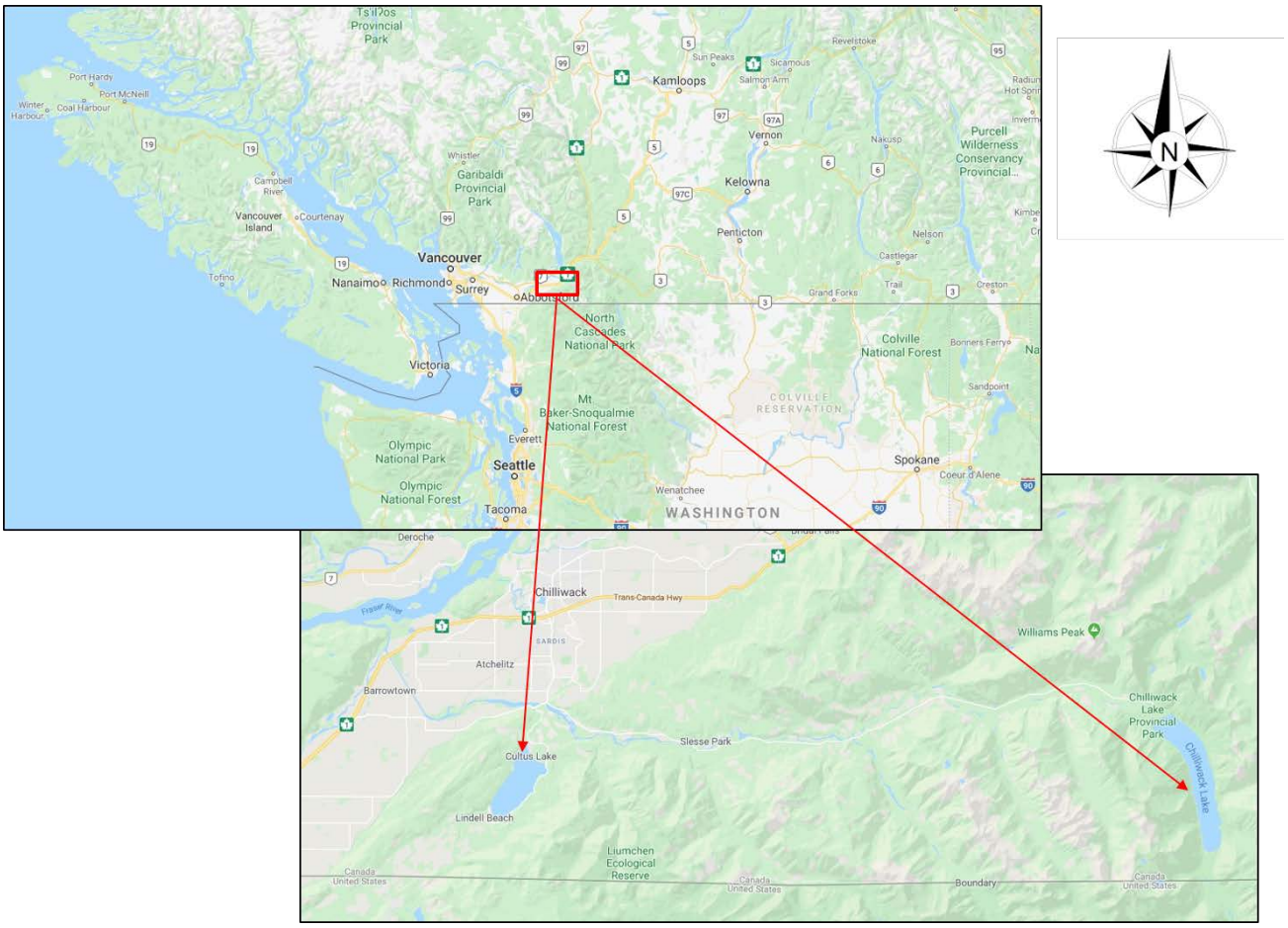

Figure 1. Map of Cultus Lake and Chilliwack Lake in BC, Canada.

Trawls were conducted from a 7 m vessel equipped with a hydraulic crane that allowed controlled setting and retrieval of the net. An 18 m long midwater trawl with an opening measuring 3m x 7m was used to collect fish from the lake. The net was equipped with a PVC codend with a screw cap that allowed for rapid fish retrieval from the net, and minimizes the impact of turbulent flows on fish caught in the net. Trawl duration and depth are summarized in table 1.

Table 1. Depth and duration of all trawls conducted in Cultus and Chilliwack Lakes, and the fish number and size (mean ± standard deviation)

Sample interval	Lake	Date	Trawl ID	Duration (min)	Depth (m)	Number of fish	Fork Length (mm)
1	Cultus	2018-07-10	20180201	35	16	24	52.3 ± 18.2
2	Chilliwack	2018-08-14	20180301	40	18	11	61.3 ± 12.1
2	Chilliwack	2018-08-14	20180302	40	19	4	66.6 ± 11.8
3	Cultus	2018-09-04	20180401	40	18	16	63.6 ± 4.1
3	Cultus	2018-09-04	20180402	40	18	14	59.8 ± 6.5
4	Cultus	2018-10-04	20180801	39	25	30	69.3 ± 5.9
5	Chilliwack	2018-11-01	20181001	40	21	8	63.0 ± 7.9
5	Chilliwack	2018-11-01	20181002	40	21	8	67.0 ± 7.2
6	Cultus	2018-11-06	20181101	40	26	19	73.6 ± 5.0
7	Cultus	2019-03-06	20190101	30	28	22	94.1 ± 10.7
8	Cultus	2019-04-04	20190201	28	29	8	106.0 ± 9.5
8	Cultus	2019-04-04	20190202	40	29	7	96.6 ± 14.5

Once the catch was brought on board, fish were quickly transferred from the codend of the trawl net to a 20 L bucket containing fresh lake water. Juvenile Sockeye were then netted out of the bucket and placed in a solution of MS-222 (tricane methanesulfonate) at a lethal concentration of 250 ppm, and buffered with sodium bicarbonate. Fish were observed to ensure euthanasia took place in a rapid manner. The whole fish were measured onboard, then immediately frozen in liquid nitrogen, shipped to the Molecular Genetics Laboratory (MGL), Pacific Biological Station, Nanaimo, BC, and stored in a -80°C freezer until used for RNA extraction. In the laboratory, tissues including gill, muscle, liver, heart, kidney, and spleen were dissected from each frozen fish. Tools were disinfected between samples using 3–5 min of 10% bleach and immersion in 95% ethanol and flame, with tools being allowed to cool before use on the next juvenile. 50-100 mg of dissected RNA was used for RNA extraction.

2.2. WATER MEASURMENT

Water temperature and dissolved oxygen (DO) concentration from the surface to the depth was measured with a YSI ProODO meter (Yellow Springs, OH, USA) on each sampling month before the acoustic-trawl survey.

2.3. GENE EXPRESSION

In this study to identify any signals of thermal stress and hypoxia, 10 thermal stress biomarkers (Houde et al. 2019), 30 discovered hypoxia biomarkers (Akbarzadeh et al. 2020), 24 general hypoxia biomarkers (Houde et al. 2019) were tested in Sockeye Salmon samples obtained from Cultus and Chilliwack lakes. Furthermore, 9 pathogen biomarkers including (*Myxobolus arcticus*, *Ichthyophthirius multifiliis*, *Loma salmonae*, *Ceratomyxa Shasta*, Pacific salmon orthoreovirus, *Parvicapsula minibicornis*, Sch, and Cov that are commonly reported in Sockeye Salmon in BC lakes were tested. Owing to some occasional personal reports of smelling the hydrogen sulfide (H2S) in Cultus Lake, 10 potential hydrogen sulfide biomarkers according to literature (Tobler et al. 2014; Kelley et al. 2016) including sulfidequinone oxidoreductase mitochondrial 1 and 2 (sqrdl_1 and sqrdl_1), cystathionine gamma-lyase (CTH), cysteine dioxygenase, type I (CDO1), cytochrome c oxidase assembly protein COX15 homolog (COX15), glutathione S-transferase Mu 3 (GSTM3), glutathione-disulfide reductase (gsr), 3-mercaptopyruvate sulfurtransferase (3MST), mitochondrial dicarboxylate carrier SLC25A10 (SLC25A10), persulfide dioxygenase mitochondrial (ETHE1) were also tested to discover any possible H2S exposure signals during hypoxic months.

A total of 157 individuals were examined for gill gene expression. This number includes 23, 27, 24, 19, 22, and 12 individuals caught in Cultus Lake in July (2018), September (2018), October (2018), November (2018), March (2019), and April (2019), respectively, and 14, and 15 specimens caught in Chilliwack Lake in August (2018) and November (2018), respectively. The methods used for gill tissue homogenization, RNA extraction and quantification, and cDNA synthesis were previously described (Akbarzadeh et al. 2018; Houde et al. 2019).

To test the efficiency of the examined biomarkers, cDNA from RNA extractions of pooled gill tissues from all individuals were serially diluted from 1/5 to 1/625 in five dilutions. Specific target amplification (STA), was performed to enrich targeted sequences within the pools using 3.76 µL 1X TaqMan PreAmp master mix (Applied Biosystems), 0.2 µM of each of the primers, and 1.24 µL of cDNA , as previously described (Akbarzadeh et al. 2018; Houde et al. 2019). Samples were run on a 14 cycle PCR program, with excess primers removed with EXO-SAP-IT (Affymetrix), and diluted 1/5 in DNA suspension buffer. The diluted samples and assays were run in singleton following the Fluidigm platform instructions. For sample reactions, 3.0 µL 2X TaqMan mastermix (Life Technologies), 0.3 µL 20X GE sample loading reagent, and 2.7 µL STA product were used. For assay reactions, 3.3 µL 2X assay loading reagent, 0.7 µL DNA suspension buffer, 1.08 µL forward and reverse primers (50 uM), and 1.2 µL probe (10 uM) were used. The PCR was 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and then 60°C for 1 min. Data were extracted using the Real-Time PCR Analysis Software (Fluidigm) using Ct thresholds set manually for each assay. PCR efficiencies for each assay were calculated using $(10^{1/\text{slope}} - 1) \times 100$, where the slope was estimated by plotting the Ct over the serial dilutions of cDNA.

The 96.96 gene expression dynamic array (Fluidigm Corporation, CA, USA) was applied and generally followed Miller et al. (2016). qRT-PCR data were analysed with Real-Time PCR Analysis 3 Software (Fluidigm Corporation, CA, USA). The expression of the target genes were normalized to the expression of the housekeeping gene, S100 calcium binding protein (78d16.1), which was found to be the most suitable housekeeping gene in NormFinder analysis as previously described (Houde et al. 2019). Sample gene expression was normalized with the ΔΔCt method (Livak and Schmittgen, 2001) using the inter-array calibrator sample. Gene expression was then log transformed: $\log_2(2^{-\Delta\Delta Ct})$.

Analyses were performed using R 3.4.4 (R Core Team, 2018) at a significance level of $\alpha = 0.05$. As the expression of hypoxia genes were influenced by water salinity and smolt stages, further analyses were separately carried out for each group. The dataset for each group was divided into a two-thirds training set and a one-third testing set. The training set was subjected to a Shrunken Centroid method (Tibshirani et al. 2002) to find out the most contributing genes for the classification of hypoxia vs. normoxia fish for each group. This method uses an internal cross-validation to select the 'best' threshold and returned a reduced list of genes. Then, the identified genes were subjected to a PCA analysis for the training set. This PCA was then applied to the testing set for visualization of unsupervised group separation within each group using the *fviz_pca* function of the *factoextra* R package (Kassambara and Mundt, 2017). This function provided 95% confidence ellipses for groups of the training set. The classification ability of the groups was also examined by subjecting the identified biomarkers to linear discriminant analysis (LDA) using the training set, followed by determining classification performance on the testing set.

3. RESULTS

3.1. PHYSICS

Cultus Lake was thermally stratified from July to November. In our study, surface temperatures were the highest in July, September and October (>15 ºC). Water surface did not exceed ~12ºC in November. The temperature of water column was relatively stable in March and April (Fig. 2&3). Chilliwack Lake was also thermally stratified in August and November. Water surface exceeded 20 ºC in August, but it was below 11 ºC in November (Fig. 4).

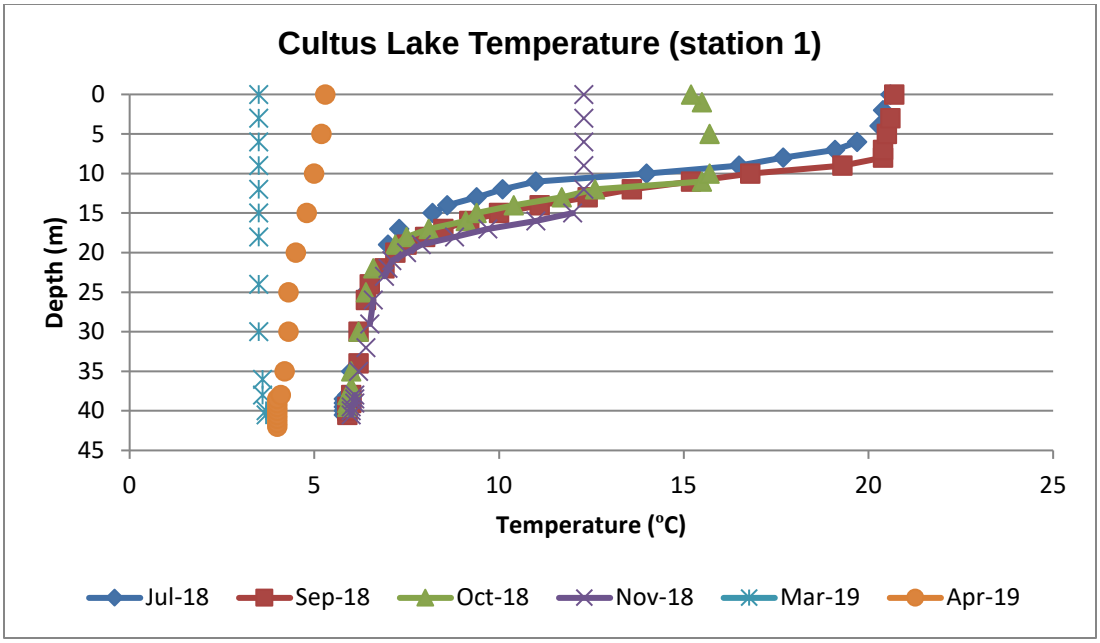

Figure 2. Vertical temperature profiles in Cultus Lake (station 1) in July, November, October, November 2018, March and April 2019.

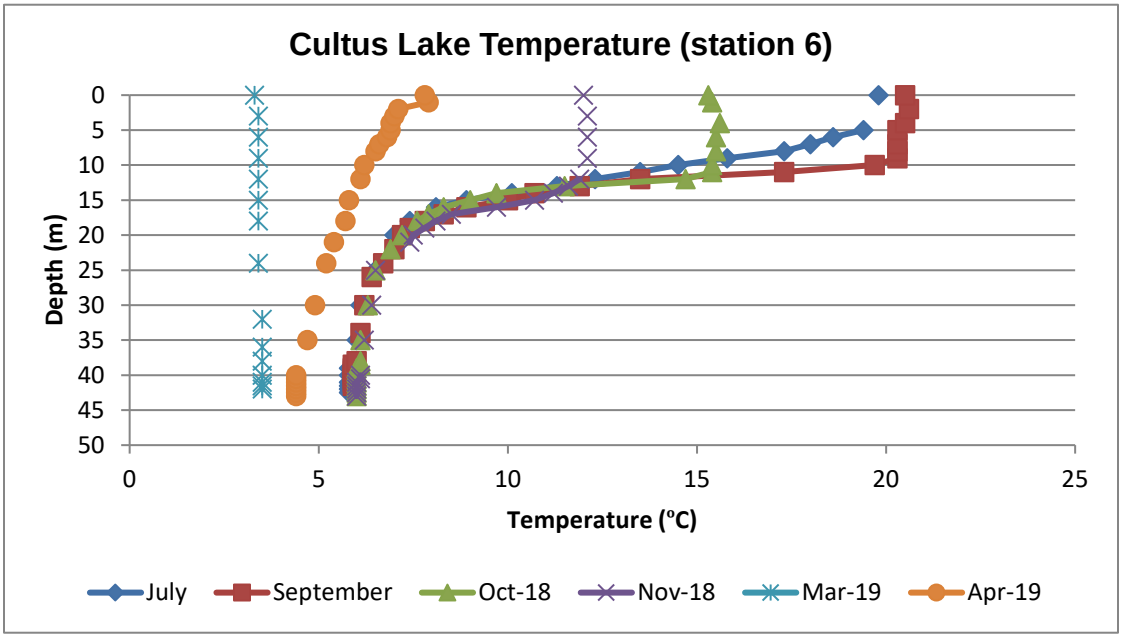

Figure 3. Vertical temperature profiles in Cultus Lake (station 6) in July, November, October, November 2018, March and April 2019.

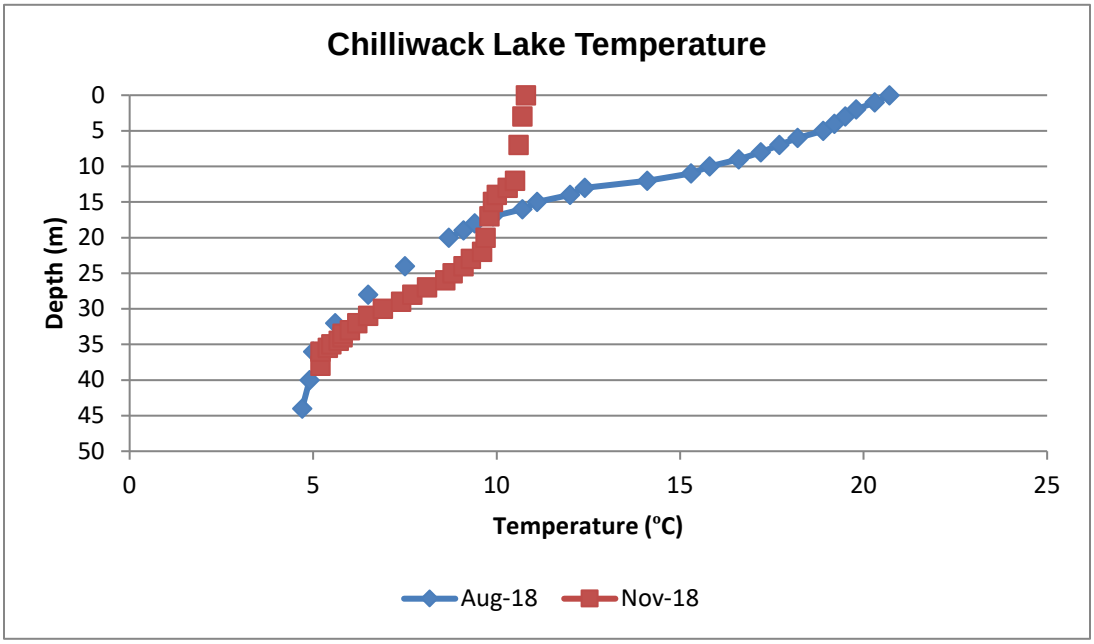

Figure 4. Vertical temperature profiles in Chilliwack Lake in August and November.

Dissolved oxygen (DO) concentrations were dramatically declined in the depth of Cultus Lake to hypoxic levels in July, September, October, and November. Hypolimnetic DO dropped to below 5 mg/L in depths 35 to 43 m during these months. In March and April, epilimnetic and hypolimnetic DO concentrations were similar owing to overturn (Figs. 5&6).

Contrary to Cultus Lake, DO concentrations were above 9 mg/L from surface to the bottom of the lake in both summer (August) and fall (November) (Fig. 7).

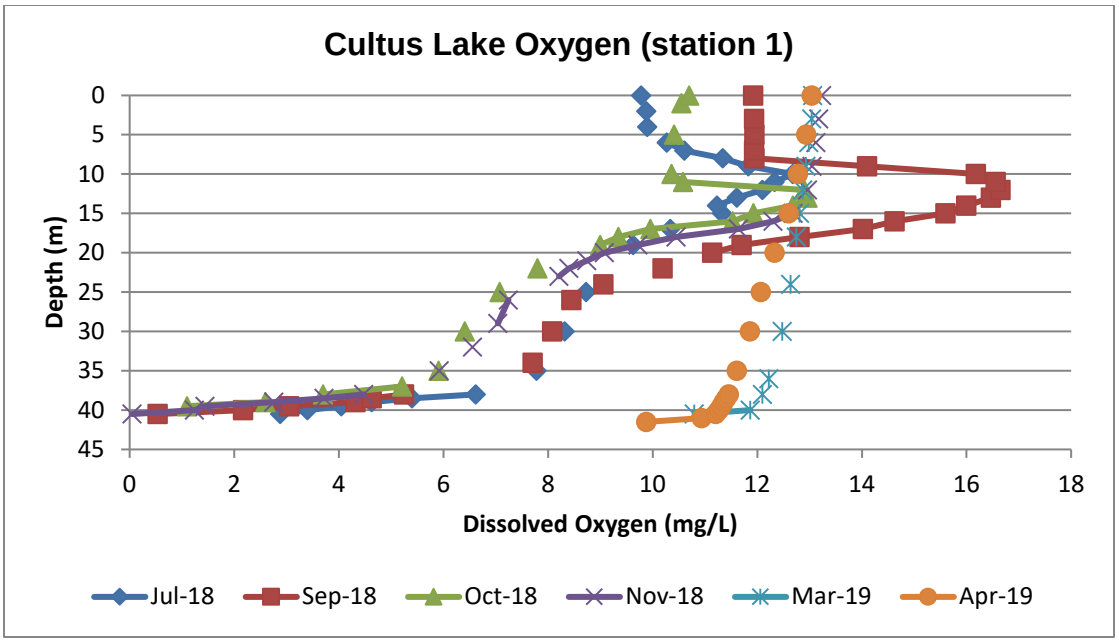

Figure 5. Vertical dissolved oxygen (DO) profiles in Cultus Lake (station 1) in July, November, October, November 2018, March and April 2019.

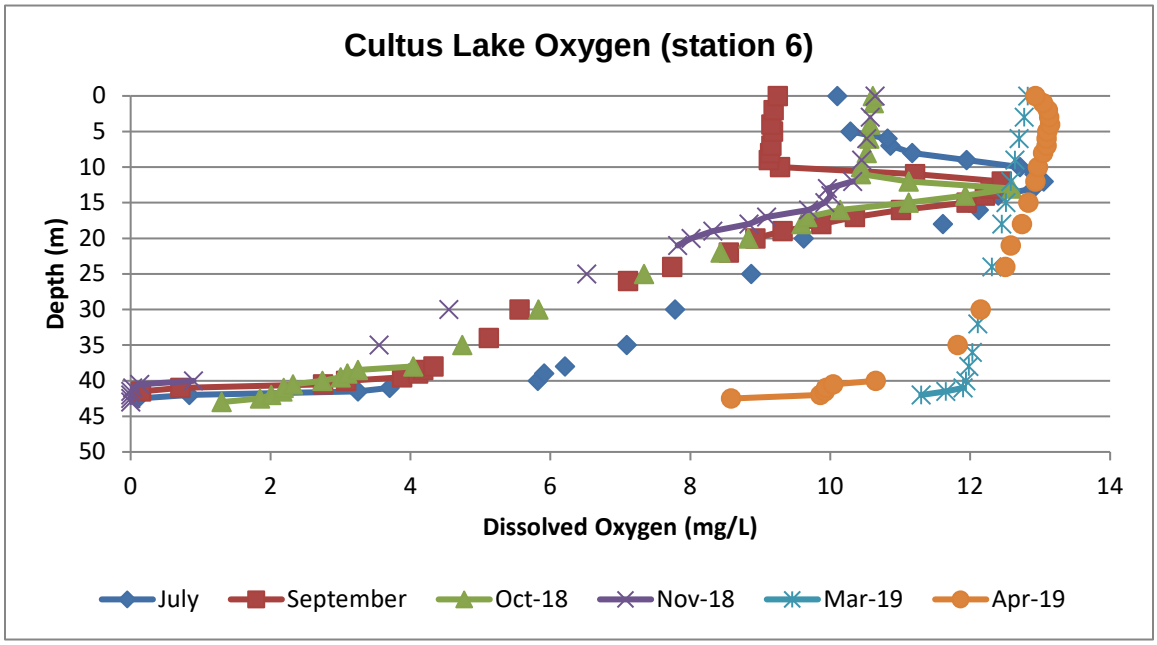

Figure 6. Vertical dissolved oxygen (DO) profiles in Cultus Lake (station 6) in July, November, October, November 2018, March and April 2019.

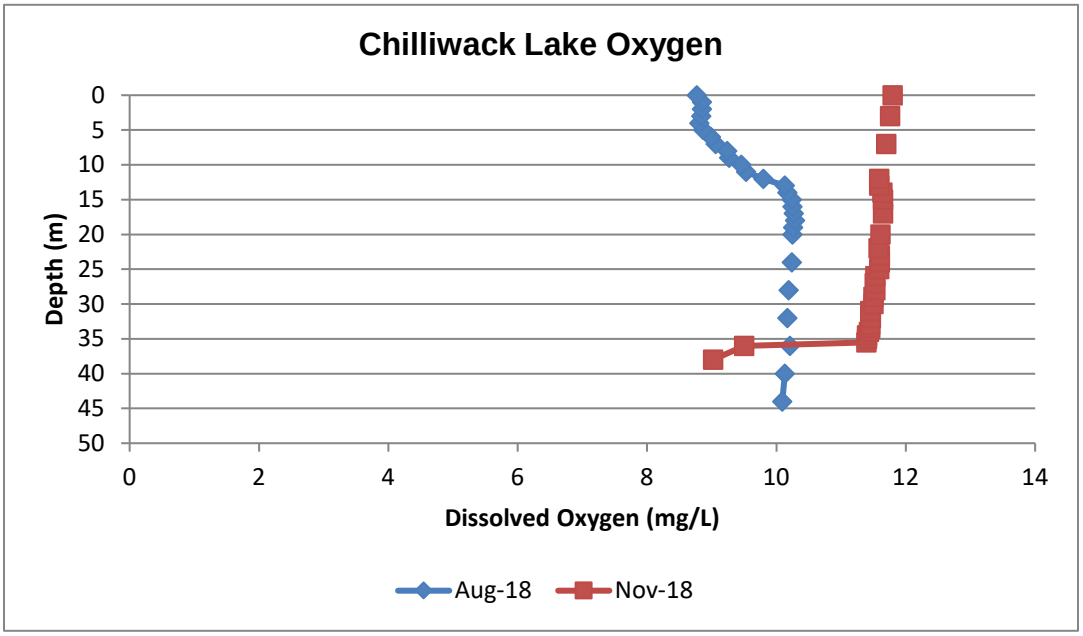

Figure 7. Vertical dissolved oxygen (DO) profiles in Chilliwack Lake in August and November.

3.2. GENE EXPRESSION RESULTS

Using the Shrunken centroid method 16 general and discovered hypoxia biomarkers including DNA excision repair protein ERCC-6-like (ERCC6L), rab9 effector protein with kelch motifs (rab9), anillin-like (anillin), aurora kinase B (AURKB), ribonucleoside-diphosphate reductase subunit M2 (RRM2), chromosome-associated kinesin KIF4 (kif4), structural maintenance of chromosomes protein 4 (SMC4), citron Rho-interacting kinase (CIT), NDC80, kinetochore complex component (ndc80), claspin-like (claspin), non-SMC condensin II complex, subunit D3 (ncapd3), proliferating cell nuclear antigen (PCNA), fructose-bisphosphate aldolase A4 (ALD_4._v1), neuroglobin 1 (Ngb1_2), hemoglobin A (HemA1_1), glycogen phosphorylase (GlPh_1) were known as the most contributing genes to separate hypoxia and normoxia samples. Therefore, these 16 biomarkers were selected for further PCA and LDA analyses. The gene expression profile and the results of ANOVA and Tukey tests for the 16 hypoxia, 10 thermal stress, and 10 H2S biomarkers using all 157 fish caught from Cultus and Chilliwack Lakes from July 2018 to April 2019 are presented in Figures 8-10, respectively.

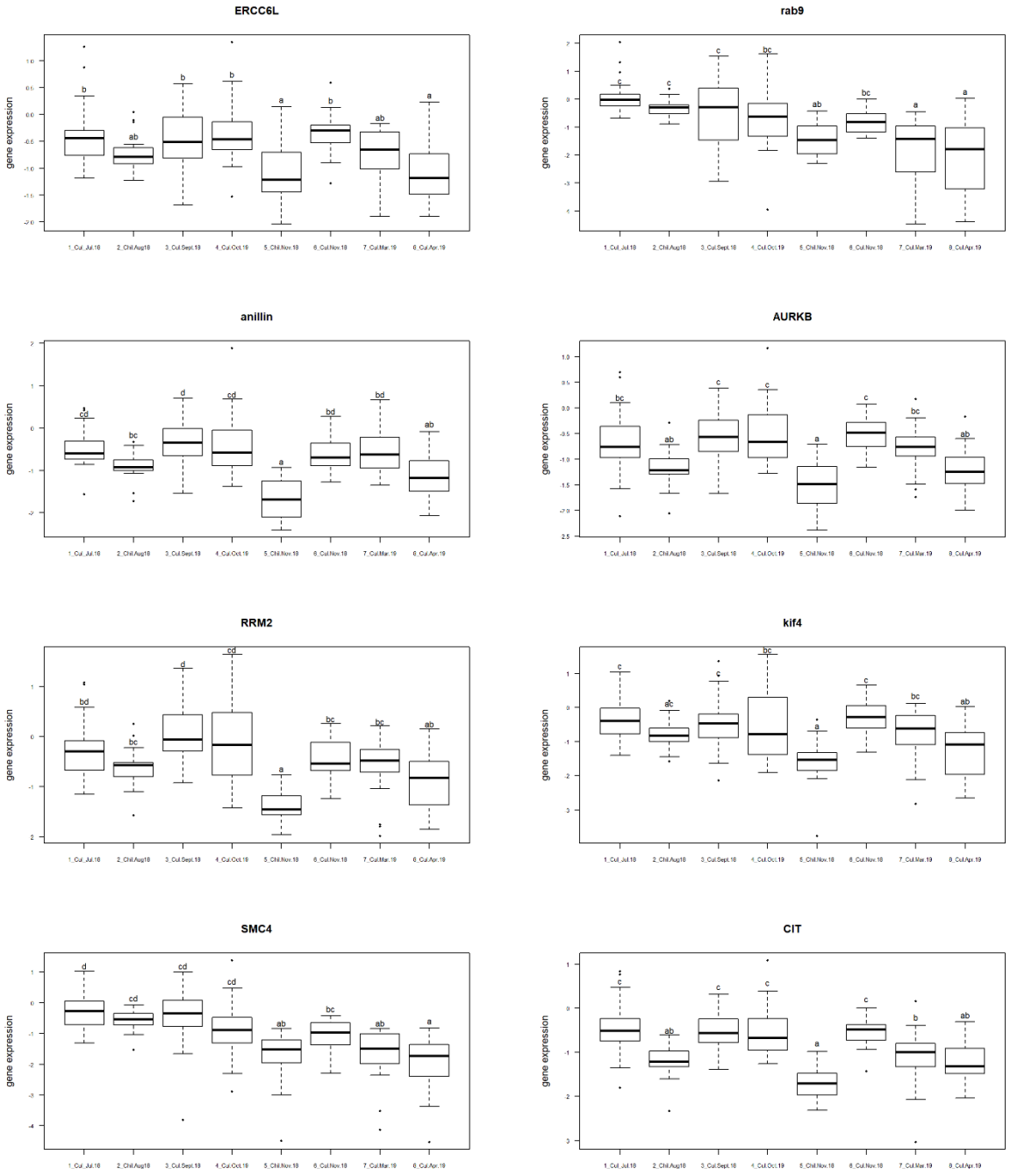

Figure 8. Gene expression box plots of hypoxia biomarkers for Sockeye Salmon in Cultus and Chilliwack lakes from July 2018 to April 2019. Bars with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$).

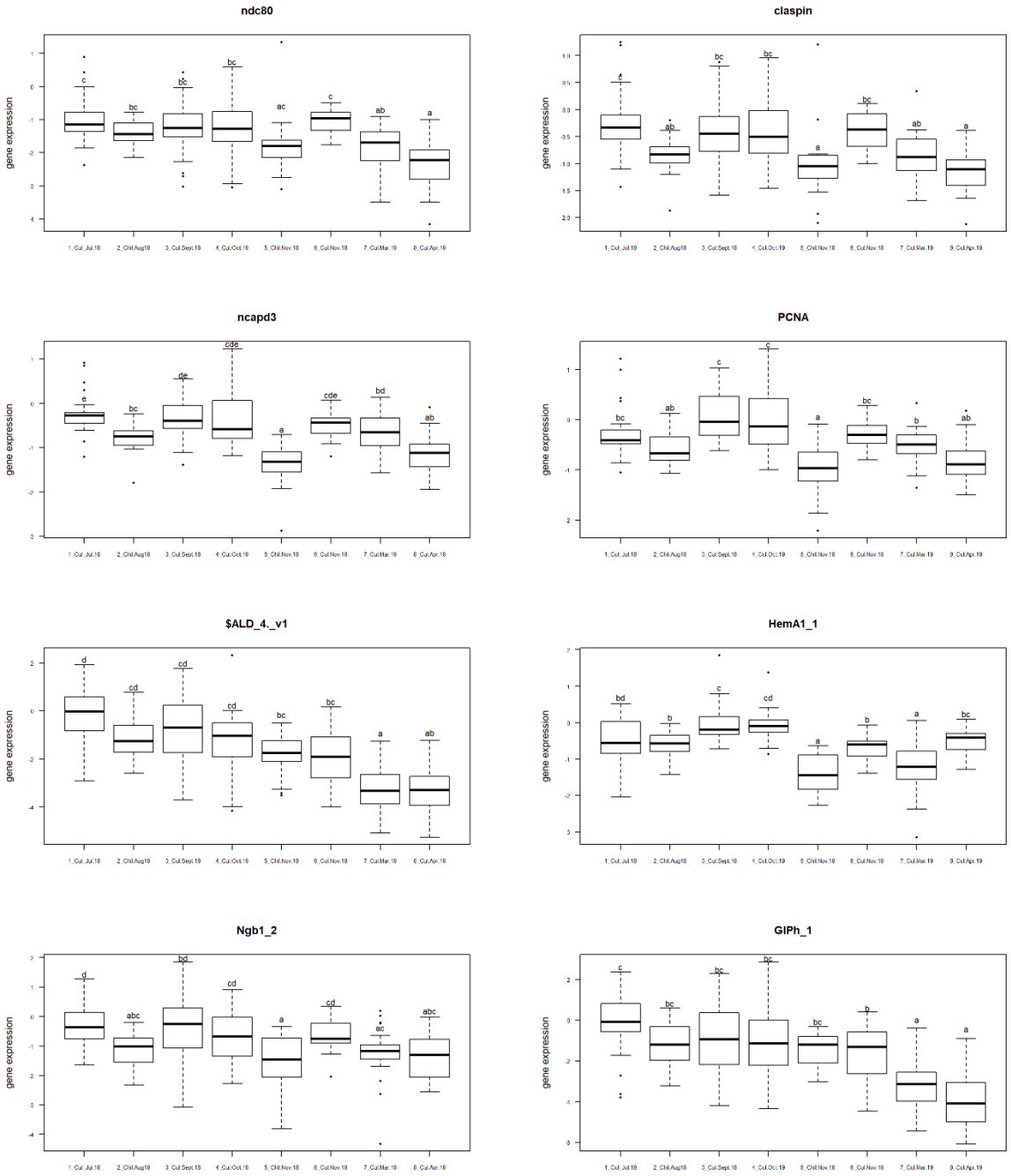

Figure 8 continued.

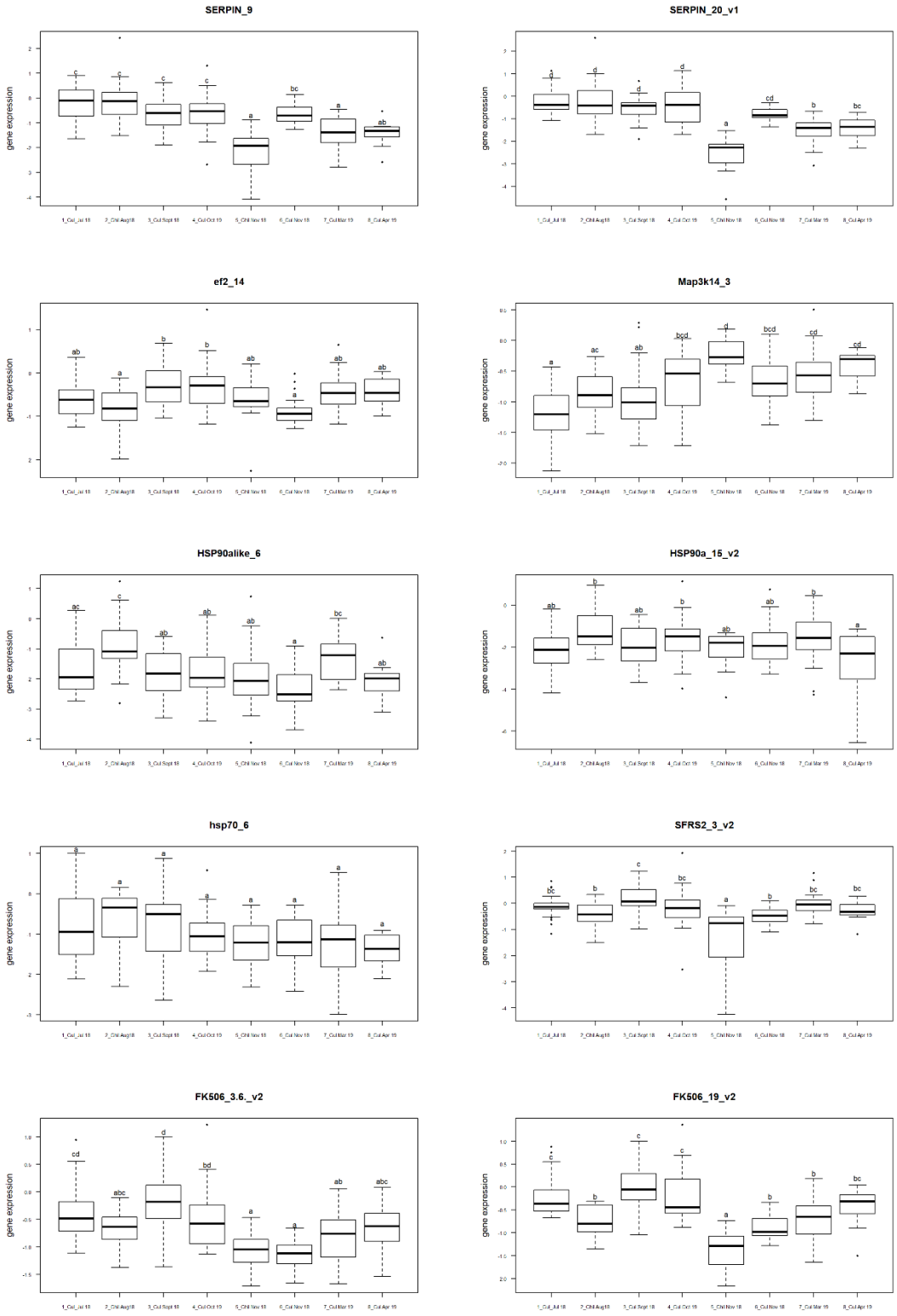

Figure 9. Gene expression box plots of thermal stress biomarkers for Sockeye Salmon in Cultus and Chilliwack lakes from July 2018 to April 2019. Bars with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$).

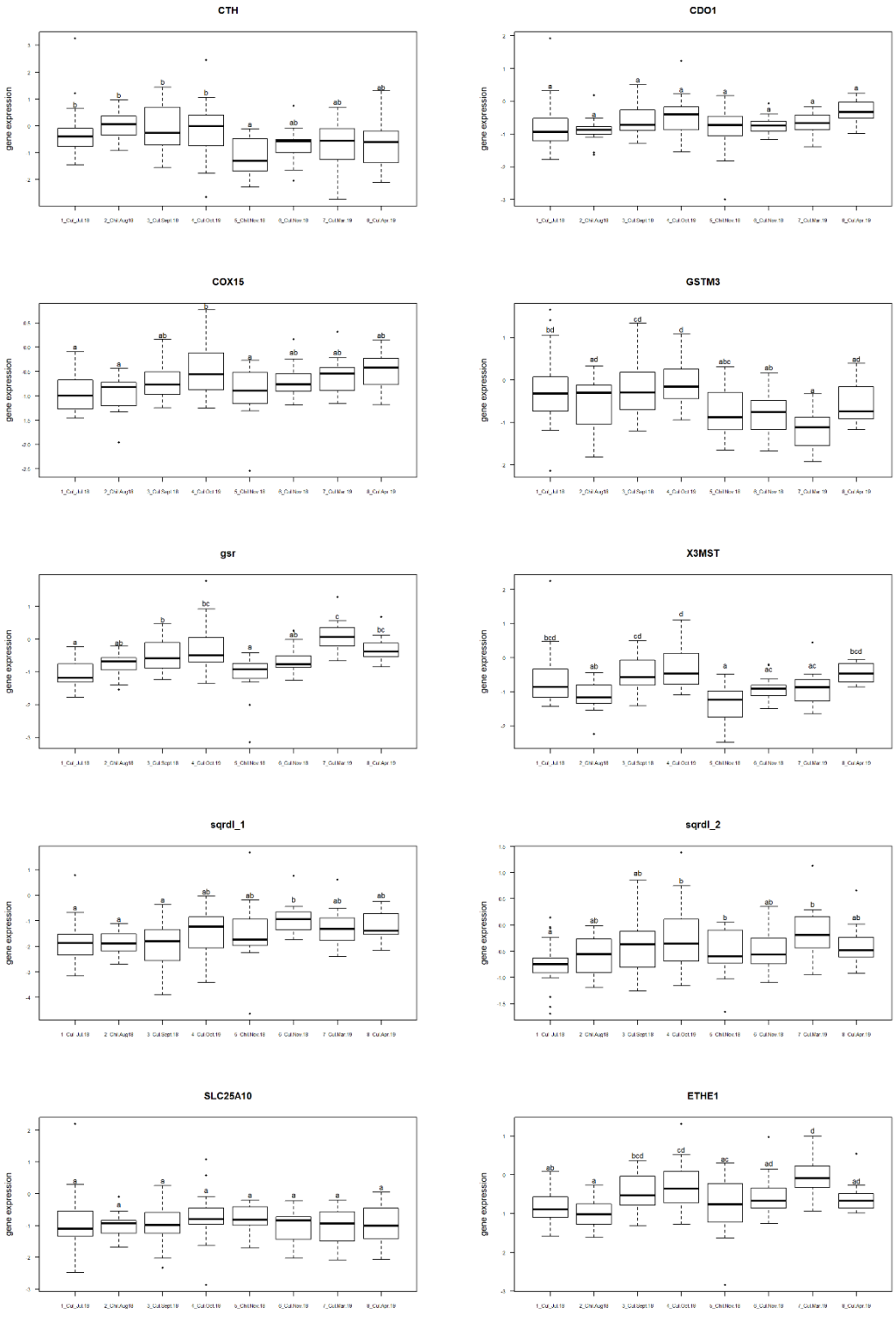

Figure 10. Gene expression box plots of hydrogen sulfide (H2S) biomarkers for Sockeye Salmon in Cultus and Chilliwack lakes from July 2018 to April 2019. Bars with different letters are significantly different among treatments according to ANOVA test ($p < 0.05$).

3.2.1. HYPOXIA BIOMARKERS

The results of PCA analysis and LDA scores for the two groups of fish caught in hypoxia months (July, September, October, and November) in Cultus Lake, and normoxia months in both Cultus (March and April) and Chilliwack lakes (August and November) using the 16 hypoxia biomarkers including are presented in Fig. 7. The PCA analysis showed a strong separation of normoxia and hypoxia groups for both training and testing groups along PC1 (Fig. 11). From 60 fish assigned as hypoxia group in training set of data, 37 (61.7%) fish were located in hypoxia group, and 38.3 % of individuals showed overlap with fish assigned as normoxia group. For testing data set, 20 (60.6%) of 33 fish were located in hypoxia group, and 39.4 % of individuals showed overlap with fish assigned as normoxia group. The separation accuracy for training and testing sets using LDA were 91.7 and 86.4% respectively.

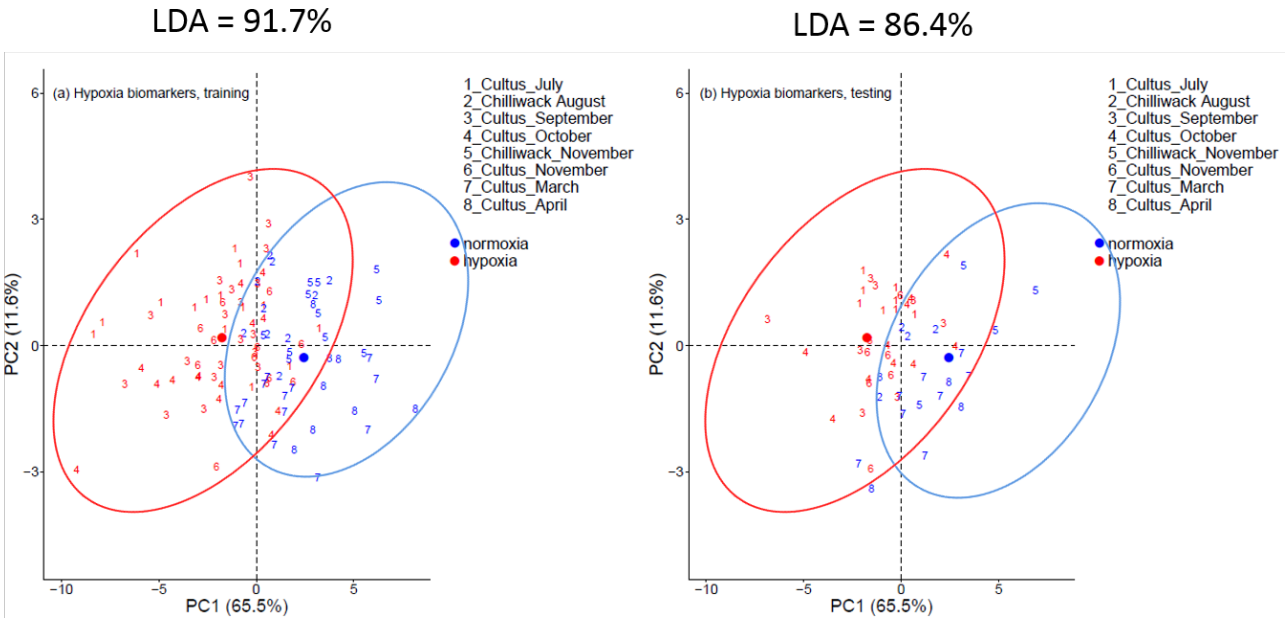

Figure 11. Canonical plots of the first two principal components of the hypoxia biomarkers for Sockeye Salmon in Cultus and Chilliwack lakes from July 2018 to April 2019. Principal component analysis was performed on the two-thirds (training set) of the entire dataset (left panel) and then applied to the remaining one-third testing set (right panel). Centroids are represented by the largest point of the same colour. Arrows represent loading vectors of the biomarkers using the training set. Classification ability of the groups were determined using linear discriminant analysis (LDA).

3.2.2. THERMAL BIOMARKERS

The results of PCA analysis and LDA scores for the two groups of fish caught in warm months (July, August, September, and October) and cold months (November, March, and April) in both Cultus and Chilliwack lakes, using the 10 thermal biomarkers including heat shock 70 kDa protein (hsp70_6), two FK506-binding protein 10 precursor genes (FK506_19_v2, and FK506_3.6._v2), two serpin H1 precursor genes SERPIN_9, and SERPIN_20_v1), mitogen-activated protein kinase kinase kinase 14 (Map3k14_3), two heat shock protein 90 alpha genes (hsp90a_15_v2), elongation factor 2 (ef2_14), and splicing factor, arginine/serine-rich 2 (SFRS2_3_v2) are presented in Figure 8. The PCA analysis showed a strong separation of normoxia and hypoxia groups for both training and testing groups along PC1 (Fig. 12). From 58 fish assigned as thermal stress group in training set of data, 41 (70.7%) fish were located in thermal stress group, and 29.3 % of individuals showed overlap with fish assigned as normal temperature group. For testing data set, 19 (61.3%) of 31 fish were located in thermal stress group, and 38.7 % of individuals showed overlap with fish assigned as normal temperature group. The separation accuracy for training and testing sets using LDA were 86.5 and 88.0% respectively.

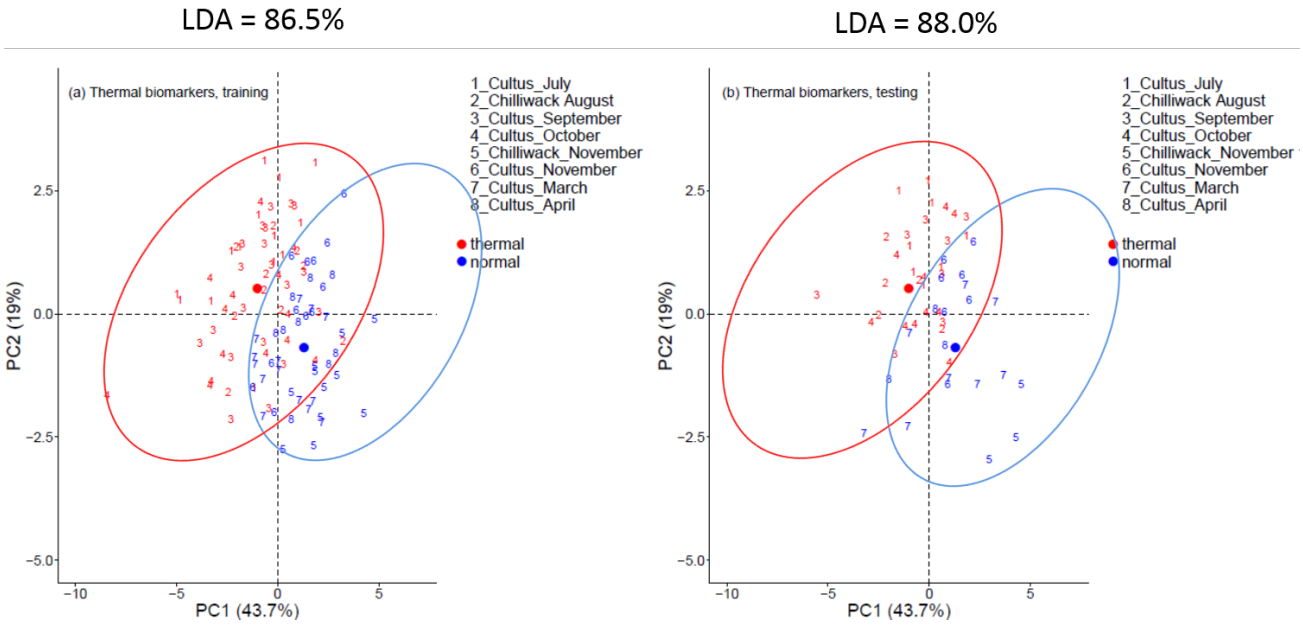

Figure 12. Canonical plots of the first two principal components of the thermal biomarkers in Cultus and Chilliwack lakes from July 2018 to April 2019. Principal component analysis was performed on the two-thirds (training set) of the entire dataset (left panel) and then applied to the remaining one-third testing set (right panel). Centroids are represented by the largest point of the same colour. Arrows represent loading vectors of the biomarkers using the training set. Classification ability of the groups were determined using linear discriminant analysis (LDA).

3.2.3. H2S BIOMARKERS

The results of PCA analysis using 10 potential H2S biomarkers showed no H2S exposure signals during hypoxia months. No obvious separation of samples between hypoxia and normoxia months was observed along PC1 and PC2 (Fig. 13).

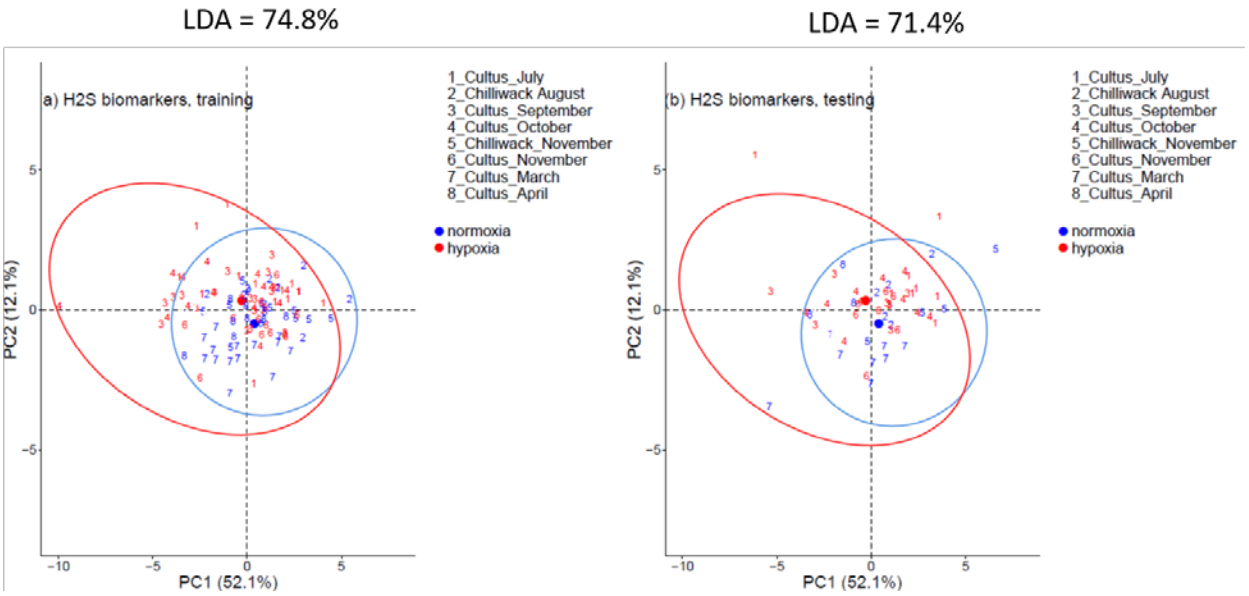

Figure 13. Canonical plots of the first two principal components of the potential hydrogen sulfide (H2S) biomarkers in Cultus and Chilliwack lakes from July 2018 to April 2019. Principal component analysis was performed on the two-thirds (training set) of the entire dataset (left panel) and then applied to the remaining one-third testing set (right panel). Centroids are represented by the largest point of the same colour. Arrows represent loading vectors of the biomarkers using the training set. Classification ability of the groups were determined using linear discriminant analysis (LDA).

3.2.4. PATHOGEN BIOMARKERS

Among nine pathogen biomarkers tested, only *I. moltifilis* was detected in individuals caught from both Cultus and Chilliwack lakes. In Cultus Lake, *I. multifiliis* was detected in 89 of 127 fish (70.1 %). In Chilliwack Lake *I. moltifilis* was detected in 10 of 31 individuals (32.3%) All other pathogen biomarkers did not show any signals in Sockoye salmon in both Cultus and Chilliwack lakes. The percentage of positive fish for *I. moltifilis* in Cultus Lake in July, September, October, November, March and April was 13.6, 93.0, 76.0, 100.0, 54.5, and 91.7%, respectively. The percentage of positive fish for *I. moltifilis* in Chilliwack Lake in August and November, was 60.0, and 6.7%, respectively.

4. DISCUSSION

Cultus Lake serves as critical habitat for the endangered Cultus Lake sockeye salmon which uses the lake for adult spawning and juvenile rearing. Water quality degradation is known to contribute to the risk of extinction for this species, with eutrophication having the potential to disrupt physical, biological, and chemical aspects of its freshwater habitat (Putt et al. 2019). The hypolimnetic oxygen depletion related to eutrophication has at least doubled in Cultus Lake since the 1920's -1930's (Shortreed, 2007). To complete its critical life functions, Sockeye Salmon fry rely significantly upon high levels of DO in profundal and benthic habitats where most of the fish spend a considerable amount of time. Therefore, hypoxic water in benthic habitats of Sockeye Salmon in Cultus Lake together with lake surface warming, increased susceptibility to diseases or pathogens, and exposure to internal loading of contaminants (e.g. ammonia, metals) from lake sediments might be the most important reasons of in-lake mortality of juvenile Sockeye Salmon in Cultus Lake. In this project, using the specific molecular biomarkers for hypoxia thermal stress, and infectious agents, the majority of Sockeye Salmon sampled in Cultus Lake showed signals of exposure to hypoxia and thermal stress during summer and fall months. The hypoxia and thermal stress signals observed in Sockeye Salmon were associated with the low DO in benthic habitat and high temperature in surface water in summer and fall when there was a thermal stratification in Cultus Lake. Moreover, a high positive signal of pathogen *I. moltifilis* was observed in Cultus Lake.

The results of the expression of both hypoxia and thermal stress biomarkers showed strong signal of exposure to hypoxia and higher temperature in a large number of fish caught during hypoxic months in Cultus Lake compared to fish sampled in Chilliwack Lake, and normoxic months of Cultus Lake. In PCA classification, more than 60% of fish caught in hypoxic seasons showed signals of hypoxia. Moreover, more than 65 % of Sockeye salmon fry sampled summer and fall when the epilimnion water was warm shown signals of thermal stress. It is likely that Sockeye Salmon fry are exposed to both low oxygen water in benthic habitats and higher temperature in surface water during their dial migration in the Cultus Lake in summer and fall when the Cultus Lake is thermally stratified. Sockeye salmon presumably migrate into warmer water in lakes to take advantage of available prey, and then migrate into cooler waters to take advantage of better food conversion efficiency and reducing predation risk (Scheuerell and Schindler, 2003). Indeed, hypoxia could potentially be a strong stress in Cultus Sockeye Salmon fry, particularly when the fish inhabits dark hypolimnon water below the lake thermocline during the day in summer and fall. Moreover, when fish move up the surface water to feed at night time, they also enter uncomfortably warmer water, so they move from one stress to the other. We did

not observe hypoxia and thermal stress signals across all individuals, probably because of differences in behaviour and possibly susceptibility of individual fish, that if there was a signal of temperature or hypoxia or both, that it would not be equally felt across all individuals within a sample, however, some portion of fish sampled during a period of higher hypoxia or temperature conditions would carry the signal.

Our findings revealed that a majority of examined fish were physiologically responsive to hypoxia and thermal stress. In general, waters having temperatures above 20 ºC and dissolved oxygen concentrations lower than 5 mg/L create a sub-optimal habitat conditions for lake salmon. Therefore, it is likely that poor lake conditions affecting freshwater survival of Sockeye Salmon in Cultus Lake, so that the habitat for fry and juvenile of Sockeye Salmon is not optimum in terms of DO in line with higher surface temperature during summer and fall that might lead to lower survival of this fish. Temperature increase and hypoxia have been considered to have synergistic effects, meaning that a small change in one stressor could cause a large change in the capacity of animals to respond to either of the stressors, when the animals are simultaneously exposed to the two stressors (McBryan et al. 2013). The probability of synergistic effects of hypoxia and temperature on whole-animal physiology follows from observations that the maximum oxygen consumption of tissues increases with temperature more than the capacity of the circulatory system to supply oxygen to tissues, leading to anaerobic energy production at high temperatures (Anttila et al. 2015). Living under sub-optimum DO can reduce temperature-dependent metabolic efficiencies, prey capture efficiency, and growth, and therefore impacting fish fitness. Avoidance of hypoxic bottom water can reduce or eliminate low-temperature thermal refuges for organisms and increase energy demands and respiration rates, and potentially reduce overall fitness if alternative habitats are sub-optimal (Roman et al. 2019). It has been known that hypoxia-reared eggs and fry salmon can reduce survival and growth, which can have larger population-level effects (Del Rio et al. 2019). Our results suggest that both warming and hypoxia are important factors to address in conservation strategies for Sockeye Salmon in Cultus Lake.

Shortreed (2007) compared limnological data taken in the 1920's-1930's with those from 2001-2003 and found that both biological productivity and surface water temperatures had increased through the 20th century, highlighting anthropogenic nutrient loading (i.e. cultural eutrophication) and climate change as the most parsimonious forcings. Eutrophication of Cultus Lake is likely to be exacerbated by climate change. Warmer air temperatures and reduced summer precipitation is likely yield stronger and protracted lake stratification, enhancing the effects of septic leaching and possibly gull guano loading on water quality and algal production. Lake surface warming and enhanced hypolimnetic oxygen depletion could create a "temperature-oxygen squeeze" whereby increasingly hypoxic hypolimnetic waters and thermally sub-lethal to lethal epilimnetic waters encroach upon one another, degrading and reducing available habitat for Cultus Lake Sockeye Salmon (Putt et al. 2019). Therefore, it is likely that eutrophication and hypolimnetic oxygen depletion would increase the risk of extinction for Sockeye Salmon in Cultus Lake which require profundal and benthic habitats.

In this project we only detected the signal of the presence of pathogen *I. moltifilis* in both Cultus and Chilliwack Lakes. The parasitic ciliate *I. moltifilis* has infected salmonids in hatcheries causing serious outbreaks of disease. The disease is commonly known as ''ich'' or ''white spot disease'' because of the visible raised white cysts in the epithelium of infected fish. Severe infections of the parasite in the gill epithelium result in the loss of the respiratory, excretory, and osmoregulatory functions of this organ, eventually leading to death of the host (Traxler et al. 1998).

Epizootics of *I. multifiliis* occurred in adult prespawning and spawning sockeye Salmon during the 1994 and 1995 spawning seasons in the Skeena River watershed in northern British Columbia, Canada (Traxler et al. 1998). Heavy infections of *I. multifiliis* can certainly cause high mortality in salmonids, including wild stocks of sockeye salmon (Kent, 2011). The rate of development and maturation of *I. multifiliis* is temperature-dependent with the development rate increasing with increasing temperature. In Cultus Lake, a high percentage of fish showed positive signals to *I. multifiliis* in spring, summer, and fall, but in Chilliwack Lake the rate of *I. multifiliis* positive fish was dramatically declined in fall when temperature dropped. The sampled fish did not show the signal of other tested pathogen biomarkers. Histological studies have shown that the myxosporean parasite *P. minibicornis* was a prevalent pathogen in Sockeye Salmon spawners in Cultus Lake (Bradford et al. 2010. However, we did not see any signals of this parasite in fry inhabiting both Cultus and Chilliwack Lakes.

In summary**,** using the specific molecular biomarkers, we found that majority of Sockeye Salmon sampled in Cultus Lake showed physiological signals of exposure to hypoxia and thermal stress during summer and fall months. The stress caused by hypoxia and high temperature in warmer seasons together with positive signals of *I. multifiliis* might contribute to increased mortality of Sockeye Salmon in Cultus Lake. It is likely that eutrophication and hypolimnetic oxygen depletion would increase the risk of extinction for Sockeye Salmon in Cultus Lake which require profundal and benthic habitats.

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