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Way out there: Pathogens, health, and condition of overwintering salmon in the Gulf of Alaska

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Abstract

Salmon are keystone species across the North Pacific, supporting ecosystems, commercial opportunities, and cultural identity. Nevertheless, many wild salmon stocks have experienced significant declines. Salmon restoration efforts focus on fresh and coastal waters, but little is known about the open ocean environment. Here we use high throughput RT-qPCR tools to provide the first report on the health, condition, and infection profile of coho, chum, pink, and sockeye salmon in the Gulf of Alaska during the 2019 winter. We found lower infectious agent number, diversity, and burden compared to coastal British Columbia in all species except coho, which exhibited elevated stock-specific infection profiles. We identified *Loma* sp. and *Ichthyophonus hoferi* as key pathogens, suggesting transmission in the open ocean. Reduced prey availability, potentially linked to change in ocean conditions due to an El Niño event, correlated with energetic deficits and immunosuppression in salmon. Immunosuppressed individuals showed higher relative infection burden and higher prevalence of opportunistic pathogens. Together, we highlight the cumulative effects of infection and environmental stressors on overwintering salmon, establishing a baseline to document the impacts of a changing ocean on salmon.

Keywords: Pacific Salmon, Winter, Gulf of Alaska, Pathogens, Stressors

Plain language summary

Ecosystems on land and in the ocean around the North Pacific rely on abundant salmon. Similarly, salmon provide a sustainable source of nutrition, income, and cultural identity for communities. Unfortunately, many wild salmon stocks have been declining in numbers, despite efforts to halt and reverse this trend by many organizations and governments. Most of these efforts focus on easily accessible coastal waters and rivers, while the factors influencing salmon survival in the remote open ocean are poorly understood, specifically during the winter. Here we used large-scale molecular screening tools to detect infectious agents and shifts in gene expression of salmon overwintering in the open Pacific Ocean. We found that offshore salmon carry fewer pathogens compared to the coastal waters of Canada. Coho salmon were an exception in this trend and had elevated levels of infection of three pathogens which resulted in a higher overall infection burden compared to their coastal counterparts. Of these, two common parasites were identified as key pathogens of all salmon species, suggesting that they are transmitted in the open ocean. Salmon experiencing reduced prey availability, and in some cases warmer temperatures, in their environment showed signs of malnourishment and overall reduced activity of many genes, including ones relating to immunity, possibly to conserve energy. This pattern was predominantly observed in smaller or lower condition salmon. Such salmon had higher levels of infections and were more often infected with opportunistic pathogens that take advantage of weak individuals, emphasizing a critical period for the survival of salmon. Together, this study provides the first report on the health, condition, and infections of four Pacific salmon species in the Northeast Pacific during the winter. We also highlight the cumulative interplay between pathogens and environmental conditions linked to climate. This much-needed information fills a gap in understanding of factors important to survival of salmon in the ocean.

Introduction

The semelparous and anadromous life history of Pacific salmon makes them crucial to coastal and terrestrial ecosystems around the North Pacific by connecting oceanic and terrestrial food webs and nutrient cycles (Cederholm *et al.*, 1999; Radchenko, 2006). Similarly, salmon are highly valued around the northern Pacific Rim due to their significant contribution to commercial and recreational fisheries as well as their cultural importance, especially for indigenous peoples (Lichatowich and Lichatowich, 2001). Despite this significance, many wild Pacific salmon stocks have experienced population fluctuations and declines throughout their range, most notably on their southern distribution limits, due to a combination of compounding factors. Most prominently featured are overexploitation, habitat degradation, pathogens, predators, prey availability, and climate change (Rand, 2002; Ruckelshaus *et al.*, 2003; Miller *et al.*, 2014). A vivid display of these influences is the long-term fluctuation and decline of sockeye salmon returns to the Fraser River in British Columbia, Canada, which in 2019 and 2020 reached their lowest levels in recorded history (https://www.psc.org/publications/fraser-panel-in-season-information/).

Efforts to rebuild stocks include habitat restoration, stock enhancements through hatcheries, and stock monitoring through several assessment methods intended to inform targeted management strategies (Cooke *et al.*, 2012). These monitoring strategies include spawning escapement and smolt survival assessments as well as test fisheries in riverine and coastal waters (Woodey, 1987; Irvine and Akenhead, 2013; Zimmerman *et al.*, 2015; Kendall, Marston and Klungle, 2017). Recent advances in molecular methods have also allowed the health surveillance of individual salmon through the detection of infectious agents and use of host "biomarker panels" to assess health and condition using a high throughput nanofluidics quantitative polymerase chain reaction approach (Miller *et al.*, 2014, 2016; Houde, Günther, *et al.*, 2019). While these novel genetic tools have been applied on the coastal margins to identify infection-related factors associated with health and survival of juvenile and adult salmonids, the open ocean remains a key compartment of the life cycle of Pacific salmon where information is virtually absent due to insufficient sampling.

Salmon stocks and species vary considerably in the length of time they spend on the coastal margin after smoltification, but most Pacific salmon ultimately leave coastal waters and head out into the open ocean of the North Pacific. There, they spend one to six years gaining the majority of their body mass feeding on marine resources, but since these remote open-ocean habitats are not under the direct jurisdiction of nations, the factors influencing salmon productivity and survival are poorly understood, despite the observed large temporal shifts in marine survival over recent decades (Holtby, Andersen and Kadowaki, 1990; NAGASAWA and K, 2000; Radchenko, 2012; Naydenko, Temnykh and Figurkin, 2016; Shuntov, Temnykh and Naydenko, 2019). Pacific salmon stocks mix in the ocean, meaning that fish from home streams as distant as North America and Asia might be found in the same aggregation (Wood, Rutherford and McKinnell, 1989; Beacham *et al.*, 2009; Urawa *et al.*, 2009, 2016). The Northwestern and central North Pacific have been the subject of decade-long research efforts of Russian and Japanese researchers and are comparatively well understood, allowing Russian researches to predict returns of pink salmon with unparalleled precision (Startsev and Rassadnikov, 1997;

Shuntov and Temnykh, 2011; Beamish, 2018). Comprehensive surveys of the Northeastern Pacific on the other hand remain absent, with only a small number of spatially and temporally limited observations during long-line and drift net operations in the 1960s and 1990s, and a single trawl transect in 2006 (Welch, Chigirinsky and Ishida, 1995; UENO and Y, 1999; Fukuwaka, Sato and Takahashi, 2007; Beacham *et al.*, 2009). The winter months in particular, when open-ocean conditions might critically impact ocean survival of first ocean-winter juvenile and subadult salmon, are the least understood but could largely determine stock performance (Ishida *et al.*, 2000; NAGASAWA and K, 2000; Beamish and Mahnken, 2001; Naydenko, Temnykh and Figurkin, 2016; Shuntov, Temnykh and Ivanov, 2017). Despite progress on salmon marine ecology during the winter, questions regarding the health and survival of salmon during this period remain unanswered, specifically in the open ocean.

To address these knowledge gaps, we performed an end-of-winter survey in the Gulf of Alaska (GoA) in February and March of 2019. Under the banner of the International Year of the Salmon initiative, scientists from the five member nations of the North Pacific Anadromous Fish commission (NPAFC: Russia, Canada, USA, South Korea, and Japan) collaborated onboard the Russian research trawler *Prof. Kaganovskiy* to conduct oceanographic sampling and trawl surveys to provide the baseline data for future pan basin studies.

Here we present a comprehensive overview on the health and condition of 252 overwintering individuals, including coho, chum, pink, and sockeye salmon, sampled in the GoA. We survey the prevalence and load of 48 infectious agents--most well established or opportunistic salmon pathogens, for which Koch's postulates have been demonstrated (Miller et al. 2014), but including several newly discovered viruses with unknown pathogenic potential that are thus referred to as infectious agents (Mordecai et al. 2019, 2020)-by high throughput qPCR. We deploy Fit-Chips, a recently developed genomic technology to recognize specific stressors and disease states in salmon, to assess trends in the expression of 89 genes associated with a wide range of stressors and correlate these two measures of individual health and oceanographic observations. For selected agents, we also verify infection and assess potential for disease through histopathology. Finally, we contrast these findings with observations from the coastal margins and suggest mechanisms that govern infectious-agent burden in the open ocean that might influence marine survival.

Methods

Sampling

Samples of Pacific salmon were collected during the 2019 International Year of the Salmon Gulf of Alaska (GoA) expedition in February and March 2019 onboard the Russian research trawler *Prof. Kaganovskiy*. Sixty 1h trawls accompanied by oceanographic sampling were performed along a grid of stations separated by 1 degree of latitude or 1.5 degree longitude (approximately 110 km apart), and 422 salmon were captured over the course of the expedition (Sup. Fig. 1, Sup. Table 1). Subsampled salmon from all species were dissected in a clean environment within one hour of capture (Sup. Fig. 2). Notes on gross pathologies were collected during dissections. Presence of nematodes in organs or the peritoneum was noted on a non-species-specific level; no other macroscopic parasites were observed. Tissue samples were preserved in RNAlater (Thermo Fisher Scientific, MA, USA) for nucleic acid extraction as well as in 10% neutral buffered formalin for histopathology.

Genetic stock Identification

Genetic stock identification for coho and sockeye salmon was performed by the Department of Fisheries and Oceans Canada, Pacific Biological Station Molecular Genetics Laboratory as described by Beacham et al. (Beacham, McIntosh and Wallace, 2010; Beacham *et al.*, 2020).

Oceanographic data

Oceanographic data was collected at each station with a 24-position rosette equipped with CTDs as described by Pakhomov *et al.* (Pakhomov *et al.*, 2019). In short, turbidity, fluorescence, and oxygen saturation were measured, and water samples were collected for assessing salinity, chlorophyll, and macronutrients. To survey zooplankton communities two Juday nets as well as one Bongo net were deployed as described by Pakhomov *et al.* (Pakhomov *et al.*, 2019).

Calorimetry

Calorimetric data on the energy content of salmon individuals in this study was provided by the National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Auke Bay Laboratories in Juneau, Alaska, U.S.A. for 46 Sockeye. In brief, tissue samples were weighed, dried with their skin on at 135℃, homogenized, and analyzed using bomb calorimetry (kJ/g dry mass) (Siddon, Heintz and Mueter, 2013).

Nucleic acid extraction and processing

Tissue samples from gill, heart, kidney, and spleen were homogenized using TRI-reagent

(Ambion Inc., Austin, Texas) and 1-bromo-3-chloropropane was added to the homogenate. Total RNA was extracted from the aqueous phase using the Total RNA Isolation kits (Ambion Inc., Austin, Texas) on a Biomek FXP liquid handling instrument (Beckman-Coulter, Mississauga, Ontario, Canada) (Miller *et al.*, 2011; Jeffries, Hinch and Sierocinski, 2014). RNA quality was assessed after DNase treatment by spectrophotometry and RNA was normalized to 62.5 ng/µL so that 1 µg of RNA was used for cDNA synthesis (SuperScript VILO MasterMix, Life Technologies).

DNA was extracted from the organic/interphase of TRI-reagent using a high salt TNES-urea buffer (Asahida *et al.*, 1996) followed by the BioSprint 96 DNA extraction kit (Qiagen, MD). DNA quantity and quality were assessed by spectrophotometry on a Beckman Coulter DTX 880 Multimode Detector (Brea, CA, USA). Samples were normalized to 62.5 ng/µL.

For infectious agent monitoring, cDNA from all pooled organs was mixed with equal amounts of pooled DNA extract from all organs to 1.25 µL final volume. Samples were pre-amplified with primer pairs for all 48 infectious agent assays (Table 1) in a 5 µL reaction using TaqMan PreAmp Master Mix (Life Technologies), following the BioMark protocol, to increase sensitivity of the small assay volume (7 µL) on the dynamic arrays. Unincorporated primers were eliminated using ExoSAP-IT PCR Product Cleanup (MJS BioLynx Inc., Ontario, Canada). Samples were diluted 1:5 in DNA Suspension Buffer (TEKnova, Hollister, California). An assay mix containing 9 µM artificial positive control clones (labelled with fluorescent dye VIC) allowed for the detection of contamination. For host gene expression monitoring, an equivalent procedure was performed on cDNA from gill tissues only, targeting 89 host genes individually. A serial dilution of pooled gill cDNA was used to assess assay efficiency across runs.

High throughput qPCR infectious agent screening

The qPCR assays and individual samples were loaded onto 96.96 dynamic arrays (Fluidigm, San Francisco, CA, USA) and run on the BioMark™ HD platform. The same distribution of assays was used for each array and samples from different dates and locations were stratified among arrays. The Fluidigm 2× Assay Loading Reagent was mixed with primer pairs and probes to 5 µL per well. A 5 µL sample loading mix was prepared using 2× TaqMan Gene Expression Master Mix (Life Technologies), 20× GE Sample Loading Reagent, and 2.7 µL of pre-amplified cDNA. The reaction mixes were added to the assay and sample inlets of the dynamic array as per the manufacturer's protocols and loaded into the chip by an IFC controller HX (Fluidigm). PCR was performed under 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.

In addition to known pathogens, we incorporated assays for newly discovered putative viruses of unknown pathogenic potential. These viruses originate from an unpublished polyA amplified metatranscriptomic sequencing library from 20 Chinook salmon targeting unknown infectious agents. We screened these libraries using a translated blast search (see Mordecai et al. 2019 for methods) and found short contigs (Genbank accessions MW373508-MW373514, Sup. Table 2). These contigs showed protein homologies to hanta-like, rhabdo-like, picorna-like, and qin-like viruses, as well as contigs with high sequence homology to an unpublished viral contig

SalmovirusWFRC1 (NC_034441). Additionally, we included an assay for a sequence variant of Pacific salmon Nidovirus (PsNV; Mordecai et al. 2020) and a co-infected bafini-like virus (Sup. Table 2).

Infectious agent screen analysis

Cycle threshold (CT) for each assay was determined using the BioMark Real-Time PCR analysis software (Fluidigm). For each infectious agent assay, samples with detection in only one duplicate were treated as negatives and duplicate values were averaged. Samples contaminated by high load controls (indicated by VIC positives) were removed. Amplification curves of all assays were visually assessed for irregularities and consistency between replicates. R statistical software (R Core Team 2017) was used to calculate the efficiencies for each assay using the slope of a regression between CT values and serial dilutions of the APC standards. We removed values that were not within the linear relationship, often either the lowest or highest RNA concentrations, to improve accuracy of assay efficiency estimates and r^2 values. Only assays with an amplification factor of 1.80–2.20, an r^2 value of ≥0.98, and with typical shaped amplification curves were used in analyses. Minimum averaged CT values indicating infectious agent detection with high statistical certainty for each specific infectious agent assay (95% confidence limit of detection (LOD)), were defined by Miller et al. (Miller, Gardner et al. 2016). Infectious agent prevalence was calculated as the percentage of individuals testing positive for a given infectious agent. All infectious agents found within one host were summarized as a single variable termed 'Relative Infection Burden' that takes into account the infection load of all detected pathogens in an individual compared to the population average (Bass *et al.*, 2019). Infectious agent load is the number of copies of a given infectious agent in an individual testing positive. To examine differences between high-sea samples and coastal populations we compared the GoA data to baseline data from coastal British Columbia based on 11,790 wild or non-hatchery marked salmon of all species and age classes sampled between 2014 and 2019 between the Juan de Fuca Strait in the south and waters at the Alaskan border near Dixon Entrance. We calculated location (coast vs GoA) and species-specific RIB and Shannon Weaver diversity of infectious agents and compared prevalence of specific agents on a species-specific level using Fisher's exact test (Clarke and Warwick, 2001).

Fit-Chip screen of stressors

To determine the primary stressors experienced by salmon in the GoA, we deployed salmon Fit-Chips that utilize curated panels of 89 host genes (biomarkers) to detect transcriptional responses to stressors in gill tissue on the same nanofluidics qPCR platform described above (Miller *et al.*, 2011, 2017; Akbarzadeh *et al.*, 2018; Houde, Akbarzadeh, *et al.*, 2019; Houde, Günther, *et al.*, 2019). Physiological states are recognized based on co-expression of curated biomarker panels that have consistently segregated stress and disease states in challenge studies. For the GoA samples, we applied biomarker panels for hypoxic stress, thermal stress, osmotic stress, general stress, and viral disease development (genes expressed in response to active viral infection), as well as imminent mortality (over-expressed in salmon experiencing mortality within 72h), and mortality related (associated with poor long term survival) markers

(Miller *et al.*, 2011, 2017; Akbarzadeh *et al.*, 2018; Houde, Akbarzadeh, *et al.*, 2019; Houde, Günther, *et al.*, 2019). We also included biomarkers associated with different branches of immune stimulation (over-expressed in diseased individuals with known pathogens) and with inflammation (individuals showing pathological signs of inflammation; Table 2). All biomarkers have been assessed for efficiency of amplification across all salmon species, but development of the panels used Chinook and sockeye. Applications across four salmonid species herein offers our first glimpse into recurring patterns of stress- and disease-related gene expression patterns across species co-inhabiting offshore waters of the North Pacific.

Host genes assays were run singularly on cDNA from gill tissues and included three reference genes for normalization (Miller *et al.*, 2016; Teffer *et al.*, 2017). Host gene assay efficiencies were calculated using the serial dilution of pooled pre-amplified host cDNA run on each dynamic array (Miller *et al.*, 2016). Expression heat-maps were visually assessed for failed assays or samples; samples with low expression of reference genes were removed; failed assays were assigned the mean of the respective species. Samples with less than 55ng/ µL cDNA were excluded from the analysis. Salmon gene CTs were normalized between runs one species at a time using calibrator samples, converted to relative expression by normalizing against the average of the best two out of three reference genes as determined by normfinder and the relative fold gene expression was calculated using the ddCT method (Livak and Schmittgen, 2001; Jensen and Ørntoft, 2004). Assays that failed in more than 50% of individuals for the respective species were excluded from analysis.

Fit-Chip analysis

To gain an overview of gene expression and cluster individuals into groups, expression profiles were visualized as heatmaps using the package ComplexHeatmap in R (Gu, Eils and Schlesner, 2016). Heatmaps were augmented with pathogen and significantly co-varied metadata between gene expression clusters as determined by ANOVA and t-test analysis in R (base, stats). To determine the dominant stressors experienced at a population level, we compared the expression of all genes in all individuals of a given species using principal component analysis (PCA). For multidimensional data, PCA identifies the dominant axes (or dimensions) of variation, allowing quantitative interpretation of differential expression among individuals through “ordination” at reduced dimensionality. We deployed the prcomp function in R (base, stats). For visualization, we depict all individuals in the first four dimensions of the PCA, as well as showing the top 20% of genes responsible for ordination in the depicted dimensions as determined by the ordiselect function of the package goeveg in R (https://cran.r-project.org/web/packages/goeveg/). Three outlier individuals that had dissection comments suggesting severe damage during capture explaining the aberrant gene expression profiles were excluded from the analysis. To interrogate correlations of gene expression with infectious agent, physical, and oceanographic data at site of capture, this information was ordinated onto the PCA plots using the envfit function of the vegan package in R (https://cran.r-project.org/web/packages/vegan/index.html; Table 3). This function scores correlation of data with the given ordination dimensions and provides a quantitative directional vector depicting this correlation. For visualization, only significant vectors with a $p < 0.05$ after 999 permutations are

displayed with the metadata name indicating the tip of the scaled arrow segments in the supplementary figures. For the main figures, we summarized the vectors of all genes belonging to a specific biomarker. Next the correlation of this superimposed data with the genes driving the ordination was evaluated using ordiselect in R and the top 20% of genes showing significant correlation in expression with the data were also depicted in the figure.

In addition to PCA ordination of gene expression, we also deployed Non-metric MultiDimensional Scaling (NMDS) to describe the different pathogen profiles carried by individuals using the metaMDS function of the vegan package in R. This ordination approach is preferable to PCA for the pathogen data, as it produces an ordination based on abundance rank order, rather than absolute values, which is better able to deal with missing data (in this case absence of pathogen detections). To find the correlations of pathogen profiles with gene expression, physical, and oceanographic data, we used the same approach as described for PCA data above (Table 3).

Results

Salmon infectious agents in the Gulf of Alaska *show species-specific trends and lower prevalence than in coastal waters*

Infectious agent burden in the GoA is species dependent

All 252 salmon, consisting of 84 chum, 80 coho, 61 sockeye, and 27 pink salmon, were screened by qPCR for 48 microscopic infectious agents commonly observed in British Columbia coastal waters using high throughput qPCR (Table 1). Across all species surveyed, coho had the highest average number of infectious agents detected (3.13), followed by sockeye (2.48), chum (1.86), and pink salmon (1.89) (Sup. Fig. 3). Similarly, Shannon Weaver infectious agent diversity was highest in sockeye (0.32), followed by coho (0.27), chum (0.18), and pink salmon (0.11; Sup. Fig. 3).

Infectious agent profiles in the Gulf of Alaska show species-specific trends

Across all salmon species, 21 of the 48 assayed infectious agents were detected. Two were bacteria, thirteen eukaryotic parasites, and six viruses (Fig. 1, Sup. Table 3).

Of the two bacterial agents, both opportunistic pathogens, *Candidatus* Branchiomonas cysticola (c_b_cys) was detected in all species at high prevalence (56-89%), while *Candidatus* Syngnamydia salmonis (sch) showed modest prevalence in all tested species (4-11%, Fig. 1, Sup. Table 3).

Among the eukaryotic parasites, *Loma* sp. (lo_sal; 19-67%), *Ichthyophonus hoferi* (ic_hof; 29-59%), and *Parvicapsula pseudobranchicola* (pa_pse; 16-27%) were detected in moderate to high prevalence in all four salmon species (Fig. 1). *Ichthyobodo* sp. (ICD; 14-30%) was detected

at moderate prevalence in pink, chum and coho, but was rarely detected in sockeye (3%; Fig. 1). *Sphaerothecum destruens* (sp_des) was particularly prevalent in sockeye (25%), but rarely encountered in coho, pink, and chum (5-1%; Fig. 1, Sup. Table 3). The remaining saltwater transmitted parasites showed more specific species distributions, with *Myxobolus insidiosus* (my_ins; 1-4%) and *Parvicapsula kabatai* (pa_kab; 3% and 2%) detected only in chum and coho, *Kudoa thyrsites (* ku_thy; 4% and 1%) detected in pink and coho, and *Paranucleospora theridion (*pa_ther) detected only in coho salmon (6%; Fig. 1, Sup. Table 3). Freshwater transmitted parasites, *Parvicapsula minibicornis* (pa_min) and *Ichthyophthirius multifiliis* (ic_mul)*,* were detected only rarely in coho and sockeye (*1-9%*), while *Ceratanova shasta* (ce_sha) was detected only in chum and coho (10% and 4% prevalence respectively), and *Nanophyetus salmincola* (na_sal; 1%) only in coho (Fig. 1, Sup. Table 3). Notably, all coho with *M. insidiosus, I. multifiliis,* and the majority with *P. minibicornis* detections originated from southern stocks from the Columbia and Yaquina rivers, while all *P. kabatai* and *S. destruens* detections were in fish from Northern BC and Alaskan stocks.

Six viruses were detected in salmon from the Gulf of Alaska (GoA), although most only in a single species (Fig. 1, Sup. Table 3). The exception was Encephalopathy and retinopathy virus (VER), highly prevalent in coho (36%), but also detected in sockeye and chum (2% and 1%; Fig. 1). Sockeye salmon was the only species where Pacific salmon parvovirus (PSPV) (39%) and a Putative-Picorna virus (Picorna2) (2%) were found (Fig. 1). Three viruses were exclusively observed in coho salmon: SalmovirusWFRC1_virus (5%), Erythrocytic necrosis virus (ENV) (3%), and an uncharacterized Rhabdovirus (1%; Fig. 1). No viruses were detected in pink salmon (Fig. 1).

Infectious agent profiles of salmon in the Gulf of Alaska differ from Coastal waters

To determine how salmon infectious agents may shift between the coastal margin and the deeper offshore waters, we compared the prevalence of infectious agents in salmon in the GoA and Coastal British Columbia (Fig. 1).

Ichthyophonus hoferi was significantly more prevalent in the GoA in all four species, with pathogen loads in pink, coho, and chum higher than any observed on coastal waters (Fig. 1, Fig. 2a). Sockeye and coho with high *I. hoferi* loads showed systemic infection as seen by multiple granulomatous inflammatory foci in several organs (Sup. Fig. 4 a,b).

Similarly, *Loma* sp. was present at loads higher than typically seen in coastal waters for coho, chum, sockeye, and pink, with prevalence being significantly higher in the GoA for the latter three species (Fig. 1, Fig. 2a). High loads corresponded with abundant gill xenomas in coho and sockeye that were absent from individuals without Loma sp. detections (Sup. Fig. 4 c,d).

Other pathogens with significantly higher GoA prevalence in individual species were *S. destruens* in sockeye, *Ca.* B. cysticol*a* in pink, *C. shasta* in chum, and VER in coho (Fig. 1). The latter virus was also observed at unusually high loads in the GoA (Fig. 2a). *P. pseudobranchicola*, detected in GoA chum, has not been detected in chum in coastal waters,

but has been found in other Pacific salmon species.

There were numerous infectious agents and pathogens more prevalent in coastal salmon than GoA. Among marine transmitted parasites, *P. theridion* was significantly lower in prevalence in all species in the GoA and *P. kabatai* was lower in sockeye, pink, and chum (Fig. 1). Among freshwater transmitted parasites, *P. minibicornis* was observed at reduced prevalence in GoA sockeye and chum and was absent in GoA coho while *Myxobolus arcticus* was absent from all species, likely due to brains not being sampled in this screen. *Ca.* B. *cysticola* showed lower prevalence in GoA coho and sockeye (Fig. 1). Salmon pescarenavirus-2 showed lower prevalence in GoA chum and sockeye than in coastal regions (Fig. 1). Other pathogens with reduced prevalence in the GoA were ENV and *C. shasta* in sockeye, *P. pseudobranchicola, Tenacibaculum maritimum,* and *Tetracapsuloides bryosalmonae* in coho, and *Ca.* S. salmonis in chum (Fig. 1).

Three recently discovered viruses that have not yet been surveyed in salmon on the coastal margin were detected in salmon in the GoA, including a Putative-Rhabdovirus and Salmovirus WFRC1 in coho, and Putative-picorna virus in sockeye (Fig. 1).

Together, chum, pink, and sockeye showed lower Relative Infection Burden (RIB) in the GoA compared to coastal British Columbia, Canada (BC), with the difference being significant for chum salmon (Fig. 2b). In contrast, RIB in coho was significantly higher in the GoA than in coastal waters (Fig. 2b), although the number of infectious agents as well as their diversity within individual fish was significantly lower in the GoA for all species except sockeye (Fig. 2c,d). This suggests that the higher RIB in coho in the GoA is due to the higher loads of VER, *Loma* sp., and *I. hoferi.* Only sockeye showed no significant differences in infectious agent number or diversity between the coast and the GoA (Fig. 2c,d).

Differential gene expression provides clues on stressors experienced by salmon in the Gulf of Alaska

Prey availability, temperature related factors, and infectious agent profile correlate with differential gene expression of salmon in the Gulf of Alaska

To investigate stressors of salmon in the GoA we compared the expression of all genes from all biomarker panels across all individuals of the same species. First, we visualized gene expression using heatmaps, also displaying pathogen detections as well as co-varying metadata (Fig. 3). Hierarchical clustering of gene expression allowed us to identify clusters of salmon showing similar expression patterns (Fig. 3). In chum, clusters four and five showed markedly reduced overall gene expression that was associated with elevated Relative Infection Burden (RIB) and lower biomass of hydromedusa at capture location, the primary prey of chum salmon *(Somov et al., 2019)*, as well as lower levels of dissolved oxygen (Fig. 3, Sup. Fig. 5). Further, temperature at site of capture was also significantly associated with overall gene expression, with warmer temperatures correlating to higher gene expression (Fig. 3, Sup. Fig. 5). Similarly, in Sockeye, elevated temperature, and prey availability (e.g., small zooplankton)

was associated with a global increase in gene expression (Fig. 3, Sup. Fig. 6). Condition factor K was significantly covaried between clusters in sockeye (Fig. 3, Sup. Fig. 6). Coho salmon showed no large-scale changes in gene expression and clusters differed primarily in the response of individual biomarkers to RIB and prey availability (Fig. 3, Sup. Fig. 7). Pink salmon also showed large scale changes to gene expression associated with RIB, prey availability, and temperature, but interestingly high values of these factors were associated with reduced global gene expression rather than an increase as had been seen in chum and sockeye salmon (Fig. 3, Sup. Fig. 8).

Next, we performed a Principal Component Analysis (PCA) to ordinate gene expression profiles of individual salmon. We focused on the first four principal components to identify dominant biomarker panels driving differential gene expression. We then tested observational data on salmon health and condition as well as oceanographic data for correlations with principal components and plotted significantly correlated data scaled and directional on the ordination plots to depict the direction of correlation. In the last step, we queried what genes showed changes in expression correlated with the superimposed data by using a Euclidean distance-based approach or plotted a vector summarizing of all genes of a biomarker panel respectively (Sup. Fig. 9, Fig. 4). By visualizing the hierarchical clusters identified earlier, this allowed us to identify the environmental factors and pathogens associated with the differential gene expression providing a population scale overview of stressors of overwintering salmon (Sup. Fig. 9, Fig. 4).

Differential gene expression in chum salmon was primarily driven by variations in biomarkers for inflammation (MMP13, NAPEPLD2, TXN, GILT), immune stimulation (SAA, CD83, IFNa), mortality related (C7, P_RAS), VDD biomarker panels (viral disease development: HERC6, IFIT5, IFI44, VAR1), followed by imminent mortality and hypoxia (TAGLN3, CDKN1B; Fig. 4a, Sup. Fig. 9a). Along PC1, these factors explained 36% of the variation in gene expression. Chum clusters four and five showed lower gene expression across all biomarker panels and clustered on the positive end of PC1 (Fig. 3, Fig. 4a, Sup. Fig. 9a). Inflammation (MMP13, NAPEPLD2, TXN) and immune stimulation biomarkers (SAA) contributed the negatively to PC2 (10.8% explanatory power), while hypoxia and imminent mortality biomarkers (CDKN1B) contributed positively (Fig. 3, Fig. 4a, Sup. Fig. 9a). Relative Infection Burden (RIB) as well as nematode prevalence was correlated with lower overall gene expression in individuals of cluster four and five, but positively associated with inflammation (MMP13, NAPEPLD2), immune stimulation (SAA), and VDD (HERC6, IFIT5) markers on PC2. Conversely, pathogens *P. pseudobranchicola* (pa_pse) and *S. destruens* (sp_des) were negatively associated with these immune response markers along PC2 (Fig. 4a, Sup. Fig. 9a). Biomass of hydromedusae (Medu) and other the prey of chum (small zooplankton: Zoo_S) was positively correlated with global upregulated gene expression along PC1 and lower expression of the immune response markers associated with PC2 (Somov et al., 2019), while being directly opposed to RIB and nematode prevalence across PC1 and PC2 (Fig. 4a, Sup. Fig. 9a). Principal components three and four (explaining 10% and 6.2%) were driven by the same genes driving PC1 and PC2, however, inflammation and VDD markers were opposing each other along PC3, with inflammation driven by individuals of cluster four, that showed enlarged gall bladders (a sign of prolonged low stomach fullness) and larger size (Mass) and smaller individuals at higher

temperature associated with VDD expression (Fig. 4b, Sup. Fig. 9b). PC4 was driven by opposing trends of immune stimulation and hypoxia biomarkers, primarily associated with zooplankton (euphausiids and medium size zooplankton; Fig. 3, Fig. 4b, Sup. Fig. 9a).

Sockeye showed similar patterns to chum salmon with two clusters (one and four) showing reduced overall gene expression associated with the positive end of PC1 (43.8%). The primary drivers associated with these global expression changes were the biomarker panels immune stimulation (B2M, HEP, IGMs, CD83, SAA), inflammation (IL_17D, ES1), mortality related (SCG2, RPL6), VDD (HERC6, DEXH, MX, IFI), and a group of hypoxia genes (RRI, CLASPIN, KIF15, COX6B, RRM2)(Fig. 4c, Sup. Fig. 9c). These hypoxia genes were also major contributors to PC2 (13.8%) opposed by the general stress marker JUN_F3 (Fig. 4c, Sup. Fig. 9c). Globally lowered gene expression in sockeye clusters 1 and 4 was associated with lower abundance of small zooplankton (Zoo_S), pteropods (Ptero), and hydromedusae (Medu) along PC1, and to a lesser degree lower temperature at site of capture (TEM) (Fig. 4c, Sup. Fig. 9c). Euphausiids (Euphaus) that were identified as the primary prey of sockeye, were correlated with the positive end of PC2, opposed to temperature and showed increased expression of inflammation and immune stimulation markers, but showed weaker association with gene expression than other prey groups (Fig. 4c, Sup. Fig. 9c). The prevalence of the gill parasite *Loma spp.* (lo_sal) was associated with expression of inflammation and immune stimulation biomarkers along PC1 and PC2. Principal component three (7.7% exploratory power) saw a strong correlation of immune stimulation (SAA, IFNa, IGM) and inflammation biomarkers (IL_17D, MMP24, MMP13) with the parasites *I. hoferi* and *P. pseudobranchicola*, while inflammation (ES1, EPD) and imminent mortality markers (TAGLN3, RGS21) were associated with nematode prevalence (Fig. 4d, Sup. Fig. 9d). Fish with higher caloric content (CAL) and better condition factor (K) were also associated with lower temperatures at site of capture (TEM) and lower prevalence of pathogens (ic_hof, pa_pse) (Fig. 4d, Sup. Fig. 9d).

Differential gene expression in coho salmon showed a nuanced response of biomarker panels along the first two principal components where inflammation (MMP13, IL_11, NAPEPLD2, IL_17D), general stress (JUN_F3), immune stimulation (IL_1b, HEP, SAA, IFNa), and VDD (HERC6, GAL3) associated positively with RIB and fish of cluster four on the positive end of PC1 (21.2%; Fig. 4e, Sup. Fig. 9e). RIB was inversely related to the biomass of pteropods (ptero) that were the preferred prey of coho salmon in GoA in 2019 (Somov et al., 2019), with fish from cluster 4 experiencing the lowest pteropod biomass (Fig. 4e, Sup. Fig. 9e). Hypoxia biomarkers (COX6B, RRM2, CDKN1B) were correlated with the prevalence of the gill parasite *Loma* sp. (lo_sal) along PC1 and PC2 (17.5%) (Fig. 4e, Sup. Fig. 9e), specifically amongst individuals of clusters two, three and five. Principal components three and four (12.4% and 7.1% respectively) showed a global increase in expression that was associated with the size of fish (Mass) of individuals in cluster four, as well as an increased expression of VDD biomarkers related to *P. pseudobranchicola (pa_pse)* load (Fig. 4f, Sup. Fig. 9f).

Pink salmon of cluster one showed reduced global gene expression compared to other clusters, grouped along the negative spectrum of PC1 (64.6 %) and were associated with higher temperatures, higher RIB, and higher biomass of prey species (Fig. 4g, Sup. Fig. 9g). On the positive spectrum of PC1, cluster two, three and four was associates with increased expression

imminent mortality markers (CDKN1B, CBEBP, GPX), but were differentiated along PC2 (10.2%) with expression of hypoxia (COX_6B, RRI, GPX) and inflammation biomarkers (NAPEPLD2, IL_17D) associated with cluster four, while clusters two and three showed increase expression of VDD (TRIM, GAL3, MX, VAR1, IFI) and immune stimulation markers (SAA, IGMs, HEP, CD83) that were associated with increased RIB, number of infectious agents as well as the prevalence of the parasites *S. destruens* (sp_des) and *P. psudobranchiola* (pa_pse) (Fig. 4g, Sup. Fig. 9g). Principal components three (explaining 5.6%) showed elevated expression of VDD (GAL3, Mx, IFI, HERC6), immune stimulation (SAA, IL_15), and general stress genes (HSP90) along the positive end of PC3 which was correlated with larger individuals (Mass; Fig. 4h, Sup. Fig. 9h). Higher biomass of Euphausiids (Euphaus) along PC4 (4.1%) correlated with VDD expression and inflammation (EPD).

To highlight overlying trends of pathogens and environmental factors such as prey biomass and temperature, we plotted the biomass of primary prey species in relation to ocean temperature, and RIB across the first two principal components of gene expression (Fig. 5, Sup. Fig.10). Since global depression of immune response genes (immune stimulation, inflammation and viral disease development) effectively equals immunosuppression, we created the inverse vector of gene expression of said biomarker panels to depict this suppressed immune status. Indeed, immunosuppression showed an inverse relationship with the biomass of the primary prey species as well as a direct correlation with RIB in all species (Fig. 5). In chum and pink salmon this trend dominated gene expression along PC1 (Fig. 5). Coho showed a strong inverse correlation between primary prey biomass and RIB, but immunosuppression was only weakly associated with them along PC2, suggesting that large scale changes in gene expression resulting in immunosuppression are subordinate to other factors relating to RIB (Fig. 5). In sockeye, gene expression patterns were more strongly associated with small zooplankton, rather than the primary stomach content which was Euphausiids (Fig. 5). Accordingly, lower biomass of small zooplankton was associated with immunosuppression and elevated RIB in sockeye along PC1(Fig. 5). Coho and pink salmon that were primarily caught along the southern border of the distribution area and experienced the highest ocean temperatures showed a strong correlation of immunosuppression and RIB with increased temperature (Fig. 5).

Infectious agent profiles correlated with gene response to viral and gill infections and stock of origin in coho

To determine if infectious agent profiles were associated with environmental factors and gene expression, we visualized the latter data in rank order-based NMDS-ordinated pathogen profiles of individuals by species (Fig. 6).

Differences in chum infectious agent profiles were primarily driven by *C. shasta* (ce_sha) with minor opposing contributions of *P. pseudobranchicola* (pa_pse) and *S. destruens* (sp_des) along NMDS1 (Fig. 6a). *C. shasta* was only found in individuals of gene expression cluster one and was associated with the expression of a mortality-related biomarker (MARCH2) (Fig. 6a). NMDS2 differentiation was driven by *Loma* sp. (lo_sal) and *P. pseudobranchicola* (pa_pse) on the positive end of NMDS2 that were correlated with larger individuals (Mass; Fig. 6a). Smaller

individuals on the negative end of NMDS2 were associated with *Ca.* S. *salmonis* (sch) and Ca. B. *cysticola* (c_b_cys), as well as the expression of imminent mortality/hypoxia (GPX3) and inflammation (EPD) biomarkers.

Sockeye infectious agent profiles differed primarily by the opposing trends of *Loma* sp. (lo_sal) against PSPV and *I. hoferi* (ic_hof) along NMDS1 with mortality related (FYNTBP) and immune stimulation biomarkers (IL_15) associated with *Loma* sp. (Fig. 6b). Differences across NMDS2 were driven by Putative-picornavirus (Picorna2), *Ichthyobodo* sp. (IcD), and *P. minibicornis* (pa_min) (Fig. 6b).

Stock of origin was significantly associated with pathogen profile variation in coho salmon. Accordingly, the pathogen profiles were primarily differentiated by rare and stock-specific pathogens such as *C. shasta* (ce_sha), Salmovirus, *P. minibicornis* (pa_min), *P. theridion* (pa_ther), and *M. insidiosus* (my_ins) along the negative end of NMDS1, present in only a few individuals each; the latter three pathogens were only found in fish originating from within the contiguous United States (Fig. 6c). Correlating gene expression was seen in genes from biomarker panels imminent mortality and hypoxia (CDKN1B, TAGLN3, AURKB), inflammation (GILT, ES1), immune stimulation (CD83), mortality related signature (P_RAS), as well as the prevalence of medium sized and small zooplankton (Zoo_S/M) (Fig. 6c). Hypoxia gene expression (RAMP1) was correlated with large individuals along NMDS2, while small individuals were associated with *Ichthyobodo* sp. (IcD) (Fig. 6c)

Infectious agent profiles in pink salmon differed primarily in the presence of Ca. S. *salmonis* (sch), *S. destruens* (sp_des), and *Ichthyobodo* sp. (IcD) opposed by *I. hoferi* (ic_hof) and *K. thyrsites* (ku_thy) along NMDS1 (Fig. 6d). Immune stimulation (SAA), inflammation (TGFb, GILT), VDD (IFI, GAL3) and mortality related biomarkers (FYNTBP) were correlated with the gill pathogen *Loma* sp. (lo_sal) and *S. destruens* (sp_des) and at higher abundance of pteropods (Ptero) and lower sea surface temperature (SST; Fig. 6d).

Discussion

The Gulf of Alaska (GoA) is the main overwintering habitat for North American-origin Pacific salmon stocks as well as a significant proportion of Asian-origin chum salmon. To better understand factors that may contribute to changes in ocean survival, it is critical to monitor the health and condition of salmon in this environment, specifically during the winter months that are thought to be a critical time period for first-year fish (Beamish and Mahnken, 2001). Here we report the first comprehensive overview on the health and condition of Pacific salmon during the 2018/2019 winter period in the GoA, illustrating the linkages between food limitations, immunosuppression, and infective burdens in ocean-dwelling salmon.

Most high prevalence pathogens could be acquired by trophic transmission in the Gulf of Alaska

Relative Infection Burden of microparasites in the GoA was lower compared to coastal samples in all species except coho, which had a significantly higher relative infection burden due to high prevalence of the virus VER as well as the parasites *I. hoferi,* and *Loma* sp. These two parasites and the bacterium *Ca.* B. cysticol*a* were the highest prevalence pathogens in the Gulf of Alaska across all species.

Ichthyophonus hoferi was present at significantly higher prevalence and load in all salmon in the GoA compared to coastal areas. This common parasite causes systemic disease in marine fish and is thought to transmit trophically (Hershberger *et al.*, 2002; Bass *et al.*, 2017). This suggests that the GoA is a reservoir for this parasite and that piscivorous species acquire infection through their prey. *Ichthyophonus hoferi* detections in chum salmon, a species with low proportion of fish in its diet (1.8% in the study area) is surprising but suggests very high *I. hoferi* prevalence in prey species (Somov et al., 2019). Sockeye showed significant stimulation of immune and inflammatory genes associated with *I. hoferi* prevalence.

Similar to *I. hoferi,* the microsporidian parasite *Loma* sp. (most likely *Loma salmonae*) was present at significantly higher prevalence and load in all salmon in the GoA compared to coastal areas (Shaw *et al.*, 2000). This parasite can result in respiratory distress, impaired swimming, and reduced growth rates (Shaw *et al.*, 2000). Transmission is initiated by release of spores from ruptured gill xenomas and is completed by the spores infecting the pillar and endothelial cells of the gills of a new host (Shaw, Kent and Adamson, 1998). In the GoA, coho showed significant correlation of gene expression profiles with *I. hoferi* prevalence.

The bacterium *Ca.* B. cysticola causes epitheliocystis in gill tissue of salmonids and is associated with proliferative gill inflammation (PGI) (Toenshoff *et al.*, 2012; Mitchell *et al.*, 2013). This bacterium is commonly encountered in Pacific salmon and was only significantly elevated in prevalence in pink salmon in the GoA (Bass *et al.*, 2017; Teffer *et al.*, 2017). *Ca.* B. cysticola has been correlated with lower relative weight (Bass *et al*. In Prep), inflammation in coastal Chinook (Wang *et al*. In Prep), and reduced migration success in steelhead (*Oncorhynchus mykiss*) (Twardek *et al.*, 2019).

Viral encephalopathy and retinopathy virus (VER) was significantly elevated in prevalence in coho salmon. This widespread virus of marine fish and invertebrates is transmitted horizontally, vertically, and trophically (Costa and Thompson, 2016). While brain tissue was not included in this screen, in contrast to coastal salmon, the neurotropic VER can also be detected in other tissues by qPCR (Costa and Thompson, 2016). We hypothesize that the detection of VER in non-neuronal tissue could reflect a systemic viremia state of recent trophic acquisition, but we likely underestimate both the prevalence and load of this virus in GoA. The relatively high prevalence in coho salmon might reflect their higher trophic level compared to other salmon species encountered in the GoA (Somov et al., 2019).

Erythrocytic necrosis virus (ENV) often causes epizootics in Pacific herring but has recently established as a common coastal virus infecting salmon (Pagowski *et al.*, 2019). It was found in lower prevalence in the GoA, potentially due to the more coastal distribution of Pacific herring not commonly found in the open ocean limiting transmission potential.

The meso/mycetozoea protist *Sphaerothecum destruens,* transmitted in fresh water in a broad host range of fish, was found at significantly elevated prevalence in sockeye salmon (Gozlan *et al.*, 2009). Infection results in splenomegaly and nephromegaly and causes anemia (Elston, Harrell and Wilkinson, 1986). The elevated prevalence in sockeye in the GoA might be a stock of origin, as the GoA has a high proportion of Alaskan-origin fish that harbor this infection, compared to the prevalence of BC-origin fish in the coastal database.

Ichthyobodo sp. was detected at high prevalence in pink, chum, and coho salmon. This ectoparasite has been shown to be a major factor influencing chum survival at sea in the western Pacific (Urawa, 1993; Mizuno *et al.*, 2017).

Interpreting the prevalence data in the GoA compared to coastal British Columbia needs to consider the differences in life stage and season. Gulf of Alaska fish were captured in the middle of their life, whereas coastal salmon from British Columbia were primarily out-migrating post-smolts. As salmon change their diet throughout their life, e.g. increase piscivory, this might impact exposure to trophically transmitted pathogens. The heterogeneity between data sets is especially pronounced for sockeye, pink, and chum salmon that spend most of their life in the open ocean and are only rarely encountered in coastal waters. Coho salmon, on the other hand, are present in coastal waters at all life stages and offer a more robust comparison. Thus, seasonal patterns and fish size or age might influence differences in infectious agent prevalence, specifically for sockeye, pink, and chum.

Infectious agents of freshwater and coastal origin decline in prevalence in the GoA

Myxozoans, commonly observed in coastal environments, have a life cycle that alternates between fish and invertebrate hosts. Most myxozoans, specifically all *Parvicapsula spp.,* showed reduced prevalence in the GoA as invertebrate hosts such as annelids may be limiting (Yokoyama, Grabner and Shirakashi, 2012; Somov et al., 2019). As *Parvicapsula spp.* can reduce visual acuity and have been correlated with increased predation, infected individuals

might also be lost from the population (Miller *et al.*, 2014; Nylund *et al.*, 2018). *P. kabatai* and *P. minibicornis* both showed stock-specific trends in coho. *Tetracapsuloides bryosalmonae*, the causative agent of the lethal proliferative kidney disease (PKD) was absent in the GoA, with infected individuals presumably removed from the population (Sterud *et al.*, 2007).

Pacific salmon parvovirus (PSPV), a DNA virus reported in sockeye salmon with unknown pathogenicity, was the highest prevalence virus in the GoA (Miller *et al.*, 2011, 2017; Nekouei *et al.*, 2018). Several novel viruses, Salmovirus and Rhabdo virus, were detected in GoA coho correlating with hypoxia stress and VDD gene expression, as well as a novel Picornavirus in chum (Mordecai *et al.*, 2019, 2020).

The microsporidian *P. theridion (*syn. *Desmozoon lepeophtherii),* infects gill tissue but also the sea louse *Lepeophtheirus salmonis* that may act as a vector (Nylund *et al.*, 2010; Sveen *et al.*, 2012). *P. theridion* is highly prevalent in coastal salmon in spring and summer but decreases over winter and was only observed in five coho in the GoA (Tucker *et al.*, 2018; Laurin *et al.*, 2019; Bateman *et al.*, 2020).

The bacterium *Ca.* S. salmonis (Sch), which causes gill impairment, was lower in prevalence in in the GoA (Nylund *et al.*, 2015), as was *T. maritimum*, the causative agent of mouth rot, presumptively related to the poor outcome of these diseases (Avendaño-Herrera, Toranzo and Magariños, 2006).

Infectious agent profiles are associated with size and in some species stock of origin

In chum and coho salmon, infectious agent and gene expression profiles significantly correlated with size, suggesting that many infectious agents are either shed during maturation or that infected individuals are lost from the population due to mortality. Alternatively, differing prey composition (Losee *et al.*, 2014) or age-dependent mixture such as in chum where Asian-origin fish are absent from the first-year age class might explain these trends. Coho salmon showed stock-specific differences in infectious agent profile, with stocks from the contiguous United States showing distinct infectious agent profiles compared to stocks from Northern British Columbia and Alaska.

Prey availability and temperature are correlated with immunosuppression and higher pathogen prevalence in Pacific salmon in the Gulf of Alaska

Changes of the physical environment experienced by salmon at sea based on daily travel rates are negligible (0-1% on average: Sup. Table 4, Sup. Fig 11) in relation to the speed of gene expression changes that can occur in response to stress in salmonids (Ogura and Ishida, 1992; Ogura and Ishida, 1995; Houde, Akbarzadeh, *et al.*, 2019). While the abundance of prey items was more spatially variable (3-28% changes per day on average: Sup. Table 4, Sup. Fig 11), the movement of salmon at sea is not random and salmon are expected to remain in prey rich areas, once found, thus the correlation of gene expression with prey group presence might be stronger than apparent from prey distribution.

Fit-Chip analysis in all species showed large-scale changes in gene expression, specifically from biomarker panels involved in immune response (immune stimulation, inflammation, viral disease development [VDD]). In pink and to a lesser degree in coho salmon, primarily caught in warmer waters on the southern border of the survey area, reduced gene expression correlated with warmer temperatures and reduced prey availability. This could be an indicator of higher metabolic demands in malnourished individuals. In chum and sockeye, gene expression correlated positively with temperature, while low prey availability still showed a negative correlation. At lower temperatures, gene expression may simply reflect the correlation of metabolic activity with temperature in ectothermic animals. Alternatively, individuals at higher latitudes (i.e., colder waters) were experiencing extremely high abundance of the northern sea nettle *Chrysaora melanaster*, a large jellyfish *(Pakhomov et al., 2019)*. Thus, temperature might act as a proxy for the impact these large jellyfish had on zooplankton communities thereby affecting lower trophic level salmon in the north of the GoA. Indeed, chum followed by sockeye had the lowest stomach fullness indices (Somov et al., 2019).Individuals with reduced expression of most immune response genes are effectively immunosuppressed. Immunosuppression was correlated inversely with the biomass of the primary prey groups as determined by stomach content in all species except sockeye where small zooplankton had a larger effect than euphausiids, the dominant stomach content of sockeye (Somov et al., 2019). Immunosuppression was strongly correlated with Relative Infection Burden (RIB) in chum and pink salmon, and to a lesser degree in coho and sockeye. Pink salmon also showed a protective effect of high condition factor that countered immunosuppression and RIB.

Multiple ecological relationships could explain the observed link between energetics (prey availability), immunosuppression, RIB, and temperature. Low prey availability could drive salmon into energetic deficit, to which they respond by suppressing the immune system, a common response to malnutrition in many vertebrates (Latshaw, 1991; Lord *et al.*, 1998). Similar observations have been made in steelhead / rainbow trout, where fish exhibit distinct immunity and energetic programs in response to smoltification and migration (Sutherland *et al.*, 2014), as well as in Atlantic salmon where starvation negatively impacted immune response to bacterial infection (Martin *et al.*, 2010). Strikingly, immunosuppression has recently been associated with mortality in Atlantic salmon (Krasnov *et al.*, 2020). Immunosuppression would make salmon more susceptible to pathogens, explaining the elevated infectious agent loads. Immunosuppression could also explain the absence of immune response to pathogens such as *Ca.* B. cysticola and *S. destruens*, suggesting that these are opportunistic pathogens with elevated prevalence in immunosuppressed individuals. Since condition factor was inversely correlated with immunosuppression and RIB, “good performance” could have acted protectively, as such individuals are less likely to suffer from energy deficit, thus are immunocompetent and able to fend off infections.

This interpretation is corroborated by field observations, where prey groups showed heterogeneous distributions with little overlap and sockeye and chum salmon exhibited poor feeding condition (Pakhomov *et al.*, 2019; Somov et al., 2019). Specifically in chum, extremely low condition factors individuals were caught where the average water temperature were more than half a degree warmer than their preferred range (Fukuwaka, Sato and Takahashi, 2007).

Unusually warm temperatures and stratification during the weak 2018/19 El Niño event - conditions previously hypothesized to disrupt open-ocean food webs and reduce prey availability (Rand, 2002; NOAA, 2021) - could have driven the observed energy deficits of many salmon in the study area by reducing primary production or altering zooplankton communities. Accordingly, salmon in the survey area were observed to orientate towards structural elements of the water column as well as mixed layer depth, presumably to improve their energetic balance at more favorable environments (Pakhomov *et al.*, 2019; Radchenko, Somov, and Kanzeparova, 2019). Alternatively, pathogen exposure associated with certain temperature regimes could result in impaired foraging and thereby cause energetic deficits and immunosuppression.

The Fit-Chip technology was developed and validated on the premise of recognizing specific responses based on consistent patterns of coactivation of as few as 7 curated biomarkers (Miller *et al.*, 2017; Houde *et al.*, 2019; Akbarzadeh *et al.*, 2020). However, in the GoA only a subset of any given biomarker panel was co-activated in the first four principal components of gene expression. The observed trends in gene expression were primarily large-scale changes in global gene expression, such as is typical to immunosuppression, rather than responses to specific stressors. One caveat is that this study did not employ known health status controls for different stressors to classify stressor status in individual fish, as these were not available across all four species at the time. We can thus only identify relative differences, rather than classify individuals into specific stressor categories. Refinements of Fit-Chip technology including species-specific stress standards and classification systems are underway.

Cumulative effects of ocean conditions, prey availability, and infectious agents could impact overwintering salmon in the Gulf of Alaska and highlight challenges in a warming ocean

We presented the first comprehensive overview of the health and condition of Pacific salmon at the end of the winter in the open Eastern Pacific Ocean. We highlight overall trends in pathogen profiles and identify key pathogens present in the open ocean. Further, we find that all species are influenced by energetic constraints correlated with reduced prey availability that was associated with immunosuppression and increased pathogen burden. All species investigated exhibit signs of cumulative effects of stressors, with ocean conditions and prey availability being the primary associated factors. This highlights the impacts a warming ocean could have on winter survival at sea in the face of climate change, specifically in the northern part of the GoA that experienced a large sea surface temperature abnormality in 2019 (Hinch *et al.*, 1995; Miller *et al.*, 2014). Warming, with its downstream effects on salmon energetics, could be especially disruptive in the GoA, where overwintering salmon from both sides of the Pacific basin congregate due to its homogeneous environment (Rand, 2002; Beacham *et al.*, 2009; Litzow *et al.*, 2018).

With many wild Pacific salmon populations declining in abundance and productivity, interest in resolving factors that limit salmon survival at sea is strong. Most of what we understand about salmon comes from studies along the coastal margin. The present study provides the first detailed insight into the health and condition of Pacific salmon in the open ocean during the

740 winter. This work will serve as a baseline for future evaluation of the ability of the Northeast
741 Pacific to support salmon populations of North America and Asia.

742

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Tables

Table 1: Primers and probes utilized in the infectious agents and pathogen screen.

Table 2: Primers and probes utilized in the Fit-Chip biomarker gene expression survey. Biomarker panel abbreviations: ImMort: Imminent Mortality; Hypox: Hypoxia; ImmSt: Immune stimulation; VDD: Viral Disease Development; MorRel: Mortality related signature; GenStr: General Stress; TherStr: Thermal Stress; Infl: Inflammation; ClLev: Cl Levels; OsStr: Osmotic Stress; Growth: Growth hormone expression.

Table 3: Data queried for correlation with gene expression and pathogen profiles.

Figures

Figure 1: Comparison of selected infectious agents and pathogens with high prevalence in the Gulf of Alaska and Coastal BC. Asterix indicates significant differences in prevalence in the GoA with Fisher's exact test $p<0.05$. Ratio under species indicates the number of salmon in the analysis for the respective species (coastal : GoA). See Table 1 for infectious agent and pathogen abbreviations and Sup. Table 3 for all prevalences.

Figure 2: (a): Selected pathogens showing extremely high loads in samples from the Gulf of Alaska: ic_hof: *Ichthyophonus hoferi*, lo_sal: *Loma* sp., ver: Viral encephalopathy and retinopathy virus. (b): Relative infection burden of salmon in the GoA compared to coastal BC (mean value, SD, and n). (c): Shannon diversity of infectious agents and pathogens of salmon in the GoA compared to coastal BC (mean value, SD). Asterix indicates significant differences with a t-test $p < 0.05$.

Figure 3: Gene expression and pathogen profile heatmap of overwintering salmon in the Gulf of Alaska. Gene expression of salmon is depicted in the left heatmap, where the relative delta-delta cycle threshold value (RddCt) detected in the Fit-Chip analysis is shown (blue to red) and individuals (rows) are hierarchically clustered based on similarities in gene expression (dendrogram and cluster number on left). Columns correspond to genes and are sorted by Fit-Chip biomarker panel (color scheme above). Load of pathogen detections associated with the individuals are depicted on the right heatmap in relative cycle threshold value (RelCt, black to red). Annotation graphs to the far right show Relative infection Burden (RIB), temperature (TEM) at the capture site, dissolved oxygen saturation (DO), and zooplankton size class abundance (ZooS/L).

Figure 4: Gene expression analysis of salmon captured in the Gulf of Alaska during the winter 2019. (a), (b): chum, (c), (d): sockeye, (e), (f): coho, (g), (h): pink. PCA plot of gene expression is overlaid with meta-data (infectious agents, intrinsic variables, and environmental metadata). Dots depict individual salmon. Annotations (bold black) show superimposed data correlating with differential gene expression. Only data with a correlation significance of $p < 0.05$ are shown unless noted with "*". Gene expression influence summarized by biomarker panels are indicated by the colored vectors (see main text for description of specific biomarkers driving these findings). For a full figure depicting individual genes see Sup. Fig. 9. For a full list of infectious agent abbreviations and corresponding factors see Table 1.

Figure 5: Association of primary prey species biomass, Relative Infection Burden, and temperature with gene expression in the Gulf of Alaska during the winter 2019. Primary prey

1058 species such as euphausiids, hydromedusae, and pteropods are highlighted in relation to
1059 immunosuppression (Imm_Sup: inverse vector of summarized biomarker panels immune
1060 stimulation, inflammation and viral disease development).

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1062 Figure 6: NMDS of infectious agent profile overlaid with corresponding gene expression,
1063 intrinsic and environmental metadata. Dots depict individuals and infectious agent vectors are
1064 indicated by the infectious agent abbreviation (see Table 1 for abbreviations). Corresponding
1065 superimposed data with a significance of $p<0.05$ is depicted.

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Assay name	Type	Strain	Name	Reference	Primer_F	Primer_R	Probe
ae_sal	Bacterium		Aeromonas salmonicida	Miller at al. 2016	TAAAGCACTGTC TGTTACC	GCTACTTCACCC TGATTGG	ACATCAGCAGGC TTCAGAGTCACT G
c_b_cys	Bacterium		Candidatus Branchiomonas cysticola	Mitchell et al. 2013	AATACATCGGAA CGTGTCTAGTG	GCCATCAGCCGC TCATGTG	CTCGGTCCCAGG CTTTCCTCTCCCA
pch_sal	Bacterium		Piscichlamydia salmonis	Nylund et al. 2008	TCACCCCCAGGC TGCTT	GAATTCCATTTC CCCCTCTTG	CAAAACTGCTAG ACTAGAGT
pisck_sal	Bacterium		Piscirickettsia salmonis	Corbeil et at. 2003	TCTGGGAAGTGT GGCGATAGA	TCCCGACCTACT CTTGTTTCATC	TGATAGCCCCGT ACACGAAACGG CATA
re_sal	Bacterium		Renibacterium salmoninarum	Powell et al. 2005	CAACAGGGTGG TTATTCTGCTTTC	CTATAAGAGCCA CCAGCTGCAA	CTCCAGCGCCGC AGGAGGAC
rlo	Bacterium		Rickettsia-like organism (RLO)	Lloyd et al. 2011	GGCTCAACCCAA GAACTGCTT	GTGCAACAGCGT CAGTGACT	CCCAGATAACCG CCTTCGCCTCCG
sch	Bacterium		Candidatus Syngnamydia salmonis (Sch)	Duesund et al. 2010	GGGTAGCCCGA TATCTTCAAAGT	CCCATGAGCCGC TCTCTCT	TCCTTCGGGACC TTAC
te_mar	Bacterium		Tenacibaculum maritimum	Miller at al. 2016	TGCCTTCTACAG AGGGATAGCC	CTATCGTTGCCA TGGTAAGCCG	CACTTTGGAATG GCATCG
vi_ang	Bacterium		Vibrio anguillarum	Miller at al. 2016	CCGTCATGCTAT CTAGAGATGTAT TTGA	CCATACGCAGCC AAAAATCA	TCATTTCGACGA GCGTCTTGTTCA GC
vi_sal	Bacterium		Vibrio salmonicida	Miller at al. 2016	GTGTGATGACCG TTCCATATTT	GCTATTGTCATC ACTCTGTTTCTT	TCGCTTCATGTT GTGTAATTAGGA GCGA
ye_ruc_glnA	Bacterium		Yersinia ruckeri	Miller at al. 2016	TCCAGCACCAAA TACGAAGG	ACATGGCAGAAC GCAGAT	AAGGCGGTTACT TCCCGGTTCCC
ce_sha	Parasite		Ceratanova shasta	Hallett and Bartolomew 2006	CCAGCTTGAGAT TAGCTCGGTAA	CCCCGGAACCCG AAAG	CGAGCCAAGTTG GTCTCTCCGTGA AAAC
de_sal	Parasite		Dermocystidium salmonis	White et al. 2013	CAGCCAATCCTT TCGCTTCT	GACGGACGCAC ACCACAGT	AAGCGGCGTGT GCC
ic_hof	Parasite		Ichthyophonus hoferi	Miller at al. 2016	ACGAACTTATGC GAAGGCA	TGAGTATTCACT YCCGATCCAT	TCCACGACTGCA AACGATGACG
ic_mul	Parasite		Ichthyophthirius multifiliis	Miller at al. 2016	GTCTGTACTGGT ACGGCAGTTTC	TCCCGAACTCAG TAGACACTCAA	TAAGAGCACCCA CTGCCTTCGAGA AGA
IcD	Parasite		Ichthyobodo sp.	Miller at al. 2016	AAATGGGCATAC GTTTGCAAA	AACCTGCCTGAA ACACTCTAATTTT T	ACTCGGCCTTCA CTGGTTCGACTT GG
ku_thy	Parasite		Kudoa thyrsites	Funk et al. 2007	TGGCGGCCAAAT CTAGGTT	GACCGCACACAA GAAGTTAATCC	TATCGCGAGAGC CGC
lo_sal	Parasite		Loma sp.	Miller at al. 2016	GGAGTCGCAGC GAAGATAGC	CTTTTCCTCCCTT TACTCATATGCT T	TGCCTGAAATCA CGAGAGTGAGA CTACCC
my_arc	Parasite		Myxobolus arcticus	Miller at al. 2016	TGGTAGATACTG AATATCCGGGTT T	AACTGCGCGGTC AAAGTTG	CGTTGATTGTGA GGTTGG
my_ins	Parasite		Myxobolus insidiosus	Miller at al. 2016	CCAATTTGGGAG CGTCAAA	CGATCGGCAAA GTTATCTAGATT CA	CTCTCAAGGCAT TTAT
na_sal	Parasite		Nanophyetus salmincola	Miller at al. 2016	CGATCTGCATTT GGTTCTGTAACA	CCAACGCCACAA TGATAGCTATAC	TGAGGCGTGTTT TATG
ne_per	Parasite		Neoparamoeba perurans	Fringuelli et al. 2012	GTTCTTTCGGGA GCTGGGAG	GAACTATCGCCG GCACAAAAG	CAATGCCATTCT TTTCGGA
pa_kab	Parasite		Parvicapsula kabatai	Miller at al. 2016	CGACCATCTGCA CGGTACTG	ACACCACAACTC TGCCTTCCA	CTTCGGGTAGGT CCGG
pa_min	Parasite		Parvicapsula minibicornis	Hallett and Bartolomew 2009	AATAGTTGTTTG TCGTGCACTCTG T	CCGATAGGCTAT CCAGTACCTAGT AAG	TGTCCACCTAGT AAGGC
pa_pse	Parasite		Parvicapsula pseudobranchicola	Jorgensen et al. 2011	CAGCTCCAGTAG TGTATTTCA	TTGAGCACTCTG CTTTATTCAA	CGTATTGCTGTC TTTGACATGCAG T
pa_ther	Parasite		Paranucleospora theridion / Desmozoon lepeophtherii	Nylund et al. 2010	CGGACAGGGAG CATGGTATAG	GGTCCAGGTTG GGTCTTGAG	TTGGCGAAGAAT GAAA
sp_des	Parasite		Sphaerothecum destruens	Miller at al. 2016	GGGTATCCTTCC TCTCGAAATTG	CCCAAACTCGAC GCACACT	CGTGTGCGCTTA AT

te_bry	Parasite		Tetracapsuloides bryosalmonae	Bettge et al. 2009	GCGAGATTTGTTGCATTTAAAAAG	GCACATGCAGTGTCCAATCG	CAAAATTGTGGAACCGTCCGACTACGA
arena1	Virus	SPAV-1	Salmon pescarenavirus-1	Mordecai et al. 2019	CCTGCCTCTTTGCTCATTGTG	AGAAAAAGCTGTGGTACTTTAGAAAGC	ATCCGCCTAACGGTTGG
arena2	Virus	SPAV-2	Salmon pescarenavirus-2	Mordecai et al. 2019	AACATGAAGGGCGATTCGTT	CAGCCCGCGGACTGAGT	CAAGTGATGTAAGCTTG
Bafini_b	Virus		Putative bafini virus	This study	TCAATAAGGGCCAGCGACAT	CCATTGCTTATCAGGCTCTTCA	CTGTGACATGATTTTC
Circo	Virus		Putative circo virus	This study	AAGCCCTCGATGCCTACGTA	ATGGCCTCTTTCCGACTTCA	AAAAAAGAGACGAGGATCG
cov	Virus	PsNV	Pacific salmon nidovirus	Mordecai et al. 2019	GGATAATCCCAACCGAAAAGTTT	GCATGAAATGTTGTCTCGGTTTAA	CGATCCCGATTATC
ctv	Virus	CTV-2	Cutthroat Trout Virus-2	Mordecai et al. 2020	CCACTTGTCGCTACGATGAAAC	CGCCTCCTTTGCCTTTCTC	ATGCCGGGCCATC
Hantavirus	Virus		Putative hantavirus	This study	ATTGCATTCACCGCAACAAG	GTCCAGCTTTGCCGTTGTCT	CAGGACCAAGAGGTGTT
Nido2_a	Virus		Pacific salmon nidovirus sequence variant	This study	TCAACACCCCCGAAAGAAAC	AAGGAACTGGAGCTTCAGGTAGAG	TACATTTTTGTAGGAACACTACC
ortho	Virus	RbtOV	Rainbow trout orthomyxovirus	Batts et al. 2017	GGAAGCAGTGGACGCTAACC	TCGCGAAGGTCTCTCAATGTC	ATTCTTCTCATCAAAGGCA
Picorna2	Virus		Putative - picorna virus	This study	GGGAATACTAGCGCTCCTTCCT	TGGACCGACCATGAAGAAGAA	CTCTATGAGGCGGCAGG
prv	Virus	PRV-1	Piscine orthoreovirus-1	Wiik-Nielsen at al. 2013	TGCTAACACTCCAGGAGTCATTG	TGAATCCGCTGCAGATGAGTA	CGCCGGTAGCTCT
pspv	Virus		Pacific salmon parvovirus	Nekouei et al. 2018	CCCTCAGGCTCCGATTTTTAT	CGAAGACAACATGGAGGTGACA	CAATTGGAGGCAACTGTA
Qin	Virus		Putative Qin-like virus	This study	TCACCTCACGCTCAGAAAGCT	GCGAAGTCATAGCCTTCAACGT	TTCTCAAGTGTTTTGGATGTT
reov	Virus	CAV	Chinook aquareovirus	Mordecai et al. 2019	AACTTTCGGCTTTCTGCTATGC	GAGGACAAGGGTCTCCATCTGA	TTAATTGCGGTACTGCTC
Rhabdo3	Virus		Putative rhabdo virus	This study	TGAGCTAGCACTTTCACCACAGTAT	GTTGGAGCATATTGAATCTTTTAGTCA	CCTGACTGCTGATTCT
Salmovirus	Virus		SalmovirusWFRC1	NC_034441	CCGGCCCTGAACCAGTT	GTAGCCAAGTGGGAGAAAGCT	TCGAAGTGGTGGCCAG
smallUK	Virus	PRNAV	Putative RNA virus	Mordecai et al. 2020	GTACCTAATTTAACTGGAACAGTAGAC	CGTTCAGTAACACAAGTATCCAAA	TGCAACAGGCAAGTGATATGCTTGA
ven	Virus	ENV	Erythrocytic necrosis virus	J. Winton, pers. Comm.	CGTAGGGCCCCAATAGTTTCT	GGAGGAAATGCAGACAAGATTTG	TCTTGCCGTTATTTCCAGCACCCG
ver	Virus		Viral encephalopathy and retinopathy virus	Krosnes at al. 2005	TTCCAGCGATACGCTGTTGA	CACCGCCCGTGTTTGC	AAATTCAGCCAATGTGCCCC
vhsv	Virus		Viral hemorrhagic septicemia virus	Jonstrup et al. 2013	AAACTCGCAGGATGTGTGCGTCC	TCTGCGATCTCAGTCAGGATGAA	TAGAGGGCCTTGGTGATCTTCTG

Gene	Panel 1	Panel 2	Panel 3	Gene Name	Primer_F	Primer_R	Probe
AARDC	ImMort			Arrestin domain containing 2	AAGAAAGCC AAGGCGTGA GTAA	TCGGTTGCC AGGGTTAGC	TGGAGGACA AATCGGA
ATP5G3	MorRel			ATP synthase lipid-binding protein, mitochondrial precursor	GGAACGCCA CCATGAGAC A	CGCCATCCT GGGCTTTG	AGCCCCATT GCCTC
AURKB	Hypox	ImMort		Aurora kinase B-like	GAAATGTGG TCGCTTCGAT GA	CATCAGCCA ACTCCTCCAT GT	CAGCGCACT GCTAC
B2M	ImmStim			B2M	TTTACAGCG CGGTGGAGT C	TGCCAGGGT TACGGCTGT AC	AAAGAATCTC CCCCCAAGG TGCAGG
BSG	ImMort			Basigin	CGTGGCCGA GGTCATCAT	TCAGGCTTTC TCCTCTTCTC GTA	TGGTCAGCA TCATCTT
C7	MorRel			Complement component C7 precursor	GATGCTGAC CACATCAAAC TGC	ACCTCTGTC CAGCTCTGT GTC	AACTACCAG ACAGTGCTG
VAR1	VDD			VAR1	CCACCTGAG GTACTGAAG ATAAGACA	TTAAGTCCTC CTTCCTCATC TGGTA	TCTACCAGG CCTTAAAG
2-Mar	MorRel			E3 ubiquitin-protein ligase MARCH2	GCACCTGCG ATAGAAGAG CAT	GAGATGGAA TCCGCAGAA GCT	ACTTGTTTAA CCATGCTGT GCGACTCTC CT
CBEBP	GenStr	ImMort		CCAAT/enhancer binding protein (C/EBP), beta	AACTGGCCG CAGAGAATG AC	AAGTTACGC AGAGTGGCA AGCT	TTTACAAAAA CGCGTGGAG C
CD83	ImmStim			CD83	GTGGCGGCA TTGCTGATAT T	CTTGTGGATA CTTCTTACTC CTTTGCA	CACCATCAG CTATGTCATC C
CDKN1B	ImMort	Hypox		Cyclin dependent kinase inhibitor 1B	CGTCCTCAG CGAAATGGA A	CCATTCGAAT CTCCCGTTTA AT	TCGATTTTTC AAGTCAAAC
CFTR_1	GenStr			Cystic fibrosis transmembrane conductance regulator I	ACGCCTGTC CAAAGATAGT GTCTA	GCAAAGCAT TGCTCCATAT CC	AGCGAGGAT GTGGACG
CIT	Hypox			Citron Rho-interacting kinase-like	GATCTCTAG GTTTCAGCG CAAGA	TGAGCTCCA CATCCTTTTG GT	ACCTGGAGT CAGTTCT
CLASPIN	Hypox			Claspin-like	ATGCGGGCT GCCCTATC	CTCTTGAAGA ACTGGTCGA TGCT	CATGCCTGA GCCCAA
CLEC4E	ImMort			C-type lectin domain family 4, member E	CCTGAGGGC TGGATTCATG T	TCGGCCAGT CCATCTTGTC	TGAGAAATGT TACTCCTTCA GT
COX6B	Hypox			Cytochrome C oxidase	GCCCCGTGT GACTGGTAT AAG	TCGTCCCATT TCTGGATCC A	TCTACAAATC ACTGTGCCC
DEXH	VDD			ATP-dependent RNA helicase	CCATAAGGA GGGTGTCTA CAATAAGAT	CTCTCCCCC TTCAGCTTCT GT	TGGCGCGCT ACGTG
EF2_1	TherStr			Elongation factor 2	GGAATTTAGT GGATGTCTG ACCATT	TCCCATCCCT CACTCGTAC AG	CCCATTCCTT CTATTCCT
EF2_2	TherStr			Elongation factor 2	AGGTCACAG CCGCCCTTA G	ACACAGTCT CTGTCTGCA CACACA	CGACTGCGT CTCAGGT
EPD	Infl			Ependymin	ACAAGACATT CGGCCTGGA T	CGGTTCTTGT GGTTAATCGT ATACA	CCCTTCTGCT CTTCA
ERCC6L	Hypox			ERCC excision repair 6 like, spindle assembly checkpoint helicase	TTGTATGGTC TCCACAGAG ATGGT	GTCTTCCCTA AGCCCATGT CAT	TCAAGGAGG AATCCTAG
ES1	Infl			ES1 protein homolog	CGGCAACTT CCATGAAGG A	GGACCTCCC CCACTTTCTT ATT	TGGGCTGTA AACACG
FKBP10_1	TherStr			FK506-binding protein 10 precursor	ACTATGAGAA TGCCCCCAT CAC	CTCGTCCAG ACCCTCAATC AC	CCTGGGAGC CAACAA

FKBP10_2	TherStr			FK506-binding protein 10 precursor	CCTGAAGAG ATCATTGCTG ACATG	GACGATGAC CCCATCCTT GT	TCAGGAACC AGGACCG
FKBP5	GenStr	ImMort		FK506-binding protein 5	GGGCGTTCC TCTGGGTGT A	GCATGCAGC ATTCTCCTTT CT	ACAGGGCCA TGGAGA
FYNTBP	MorRel			FYN-T-binding protein	TGCAGATGA GCTTGTTGTC TACAG	GCAGTAAAG ATCTGCCGTT GAGA	CTCAACGAT GACATCCAC AGTCTCCCC
GAL3	VDD			Galectin-3-binding protein precursor	TTGTAGCGC CTGTTGTAAT CATATC	TACACTGCT GAGGCCATG GA	CTTGGCGTG GTGGC
GILT	InfI			gamma-interferon-inducible lysosomal thiol reductase (GILT)	CTGGTGCCC TATGGAAATG C	CCGTGCTGG CAGGTGAAC	ATCTTTTGAT GGGAAGAAG
GLUL	ImMort			Glutamate-ammonia ligase (glutamine synthetase)	GTTCCAGGT TGGCCCTTG T	CCTAGCTGC CCAAAGGTG ATC	AAGGCATCA GCATGGG
GPX3	Hypox	ImMort		Gutathione peroxidase 3-like	AGGCCAGTC CTTCAGTGC AT	GGCAGGACC AGGAGGTAA CA	TGGGCCTGG TAACC
H2EB1	ImMort	ClLev		Histocompatibility 2, class II antigen E beta	CAGTTGAGC CCCATGTCA GA	TCAGCATGG CAGGGTGTC T	TGAGCTCAG TGACTCC
HEP	ImmStim			Hepcidin	GAGGAGGTT GGAAGCATT GA	TGACGCTTG AACCTGAAAT G	AGTCCAGTT GGGGAACAT CAACAG
HERC6	VDD			Probable E3 ubiquitin-protein ligase HERC6	AGGGACAAC TTGGTAGAC AGAAGAA	TGACGCACA CACAGCTAC AGAGT	CAGTGGTCT CTGTGGCT
HLA2G	ImMort	ClLev		HLA class 2 gamma	CCAGGACGT TATCCTCCCA AT	GAGAAGACA CGCCAGCAC TGT	AGGGCCTCT AACAGC
HSC70	ImMort	OsStr	GenStr	Heat shock cognate 70kDa protein	GGGTCACAC AGAAGCCAA AAG	GCGCTCTAT AGCGTTGATT GGT	AGACCAAGC CTAAACTA
HSP70	TherStr			Heat shock 70 kDa protein	TCAACGATCA GGTCGTGCA A	CGTCGCTGA CCACCTTGA A	CCGACATGA AGCACTGG
HSP90al	TherStr			Heat shock proteina 90 alpha like	TTGGATGAC CCTCAGACA CACT	CGTCAATAC CCAGGCCTA GCT	CCGAATCTA CCGGATGAT
HSP90	GenStr			Heat shock protein 90	TGGGCTACA TGGCTGCCA AG	TCCAAGGTG AACCCAGAG GAC	AGCACCTGG AGATCAA
HSP90a	ImMort	GenStr	OsStr	Heat shock protein 90 alpha	ATGACCCTC AGACACACT CCAA	CCTCATCAAT ACCCAGTCC TAGCT	CGCATCTAC AGAATGA
HTATIP	MorRel			HIV-1 Tat interactive protein	CTTGTAACAG TTCGACATG GCTTATT	TGGTGAAGC ATTTCTGTAT GTCAA	TCTGTACTGA GCATCCCCG CACATTACA
ICLP2	ImMort	ClLev		Invariant chain-like protein 2	CAGCAGAAG GGTCCAACA AGAG	TCCTGCAGG TCTTTAATGT CGTT	TTCAAGATAG CTGGTTTCAC
IFI	VDD			IFN-induced protein	GCTAGTGCT CTTGAGTATC TCCACAA	TCACCAGTAA CTCTGTATCA TCCTGTCT	AGCTGAAAG CACTTGAG
IFI44	VDD			IFN-induced protein 44-1	CCACTGGAC TAACCCTCCA TGA	TGTGTCCCT CGGGTGCAT	ACTCTGGCT ATCATCAAA
IFIT5	VDD			Interferon-induced protein with tetratricopeptide repeats 5	CCGTCAATG AGTCCCTAC ACATT	CACAGGCCA ATTTGGTGAT G	CTGTCTCCAA ACTCCCA
IFNa	ImmStim			IFN-alpha	CGTCATCTG CAAAGATTG GA	GGGCGTAGC TTCTGAAATG A	TGCAGCACA GATGTACTG ATCATCCA
IGMs	ImmStim			IgM (sec.) AB044939	CTTGGCTTGT TGACGATGA G	GGCTAGTGG TGTTGAATTG G	TGGAGAGAA CGAGCAGTT CAGCA

IL_11	Infl			Interleukin11	GCAATCTCTTGCCTCCACTC	TTGTCACGTGCTCCAGTTTC	TCGCGGAGTGTGAAAGGCAGA
IL_15	ImmStim			Interleukin15	TTGGATTTTGCCCTAACTGC	CTGCGCTCCAATAAACGAAT	CGAACAACGCTGATGACAGGTTTTT
IL_17D	Infl			Interleukin17D NM 001124399	CAACAGAAGTGCGAACGATG	GATGCCACATCGCATAACAG	TGGTCGAGTATCTTTCGTGTGTTTGC
IL_1b	ImmStim			Interleukin1 beta	AGGACAAGGACCTGCTCAACT	CCGACTCCAACTCCAACACTA	TTGCTGGAGAGTGCTGTGGAAGAA
IQGAP1	ImMort			IQ motif containing GTPase activating protein 1	GAGGGTGTGGCTGTGATGAA	CAGGAAGATGAGCAGGTTGACA	CTCTTCGACAGGGCC
IRF_1	MorRel			Interferon regulatory factor 1 (IRF-1) gene	CAAACCGCAAGAGTTCCTCATT	AGTTTGGTTGTGTTTTTGCATGTAG	CTGGCGCAGCAGATA
JUN_F3	GenStr			7_4_4_6_Transcription factor AP-1	TTGTTGCTGGTGAGAAAACTCAGT	CCTGTTGCCCTATGAATTGTCTAGT	AGACTTGGGCTATTTAC
KIF15	Hypox			Kinesin family member 15	CAGGCAGGTCTTCTCCAAGCT	AGTTTGGATGATAGCCTCCTTCTG	CACAGGATCAGACTGC
KIF2C	Hypox			Kinesin-like protein KIF2C	CGGCCAAACTGGAAGTGGTA	TTCTGGCTCTTCCCTGAAAAGT	AACTCACACAATGGGAG
KRT8	MorRel			Cyclokeratin-8	CGATTGAGCGGCTGGATAA	GCATTGTTTACCTTTGACTTGAATTG	CCCCCTTCTCTACTCTCTTGCTCACCATTC
LDHba	ImMort	GenStr		L-lactate dehydrogenase B-A chain-like	GTCACTGCTCCCATTTTACACTCTAG	CCCAAACTCCCTCCCAGATAAC	CTGTTCTTAGCTTCCC
Map3k14	TherStr			Mitogen-activated protein kinase kinase kinase 14	GCTCCCTGGGTTCATGGAT	GCCTCCCTTCAGCAGAGACA	CCAGCAATAGCTTATG
MFHAS1	Hypox			Malignant fibrous histiocytoma-amplified sequence 1 homolog	CCGAGGCCTGGGTGAAC	TCAGCTGCTCCACAGAGAAGAA	TCAGTGGCTGCTAGTC
MHC_IIb	ImmStim			MHCII b chain	TGCCATGCTGATGTGCAG	GTCCCTCAGCCAGGTCACT	CGCCTATGACTTCTACCCCAAACAAAT
MMP13	Infl			Matrix metalloproteinase-13	GCCAGCGGAGCAGGAA	AGTCACCTGGAGGCCAAAGA	TCAGCGAGATGCAAAG
MMP25	Infl			Matrix metalloproteinase-25 precursor	TGCAGTCTTTTCCCCTTGGAT	TCCACATGTACCCACACCTACAC	AGGATTGGCTGGAAGGT
MX	VDD			Mx	AGATGATGCTGCACCTCAAGTC	CTGCAGCTGGGAAGCAAAC	ATTCCCATGGTGATCCGCTACCTGG
NAPEPLD2	Infl			N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D	CAGACACTCCCTGGCTATTCACT	CCTGAGTCTCACTGGAGGCTCTA	AACCTTCGCTTTAGCTTACGA
NUPR1	ImMort			Nuclear protein 1	GGAAGCCAGCGACAATACCA	GGGTTAGCCGTCCGATTTG	CACGAGCGCAAGCT
ODC1	ImMort			Ornithine decarboxylase 1	CCAGAAGGCTCCCTGTTTCA	GCAGCCATTTCCTGGAGAAG	ACAACCCAATCTCA
P_RAS	MorRel			Oncorhynchus mykiss G-protein (P-ras) mRNA, complete cds	GCAGGATGAGCAGAGGAAGAA	GGCCTGGGCAATGTAACACT	CCCCCTAAAGATGCAG
PRLR	OsStr			Prolactin receptor	GATGCCGGAGGGAAAAGAC	CCGACTGGCTCTTGGACTTG	TCCAAGATGTTGGCTGC

PSMB7	ClLev			Proteasome (prosome, macropain) subunit, beta type 7	AGGAACCCA CGTGTCGTG AT	TGGCCCCGG TACCTGAATA	CAGTAAACAT ATTACAGGA CATG
PSMB8	ImMort	ClLev		Proteasome (prosome, macropain) subunit, beta type 8	CTGGTTGTG GTAGCAGCT ATGC	CGCCTCCTC TACCGTCAT GT	TACGGAGTG ATGGACAGC
RAMP1	Hypox			receptor activity-modifying protein 1-like	CGAACCAAG TGGTGCAAG ACT	CCGGACATG CCTGGAAGA	CTTCATCCAG ATCCATTC
RGS21	GenStr			Regulation of G protein signalling 21	TCCCGACTA CAGCGCAGA T	TCCTCAGGG CTAAGTCGTT CA	TTCCCAATCC CCC
RIG1	ImmStim			Retinoic acid-inducible gene I	ACAGCTGTTA CACAGACGA CATCA	TTTAGGGTG AGGTTCTGT CCGA	TCGTGTTGG ACCCCACTC TGTTCTCTC
RPL6	MorRel			Neoplasm-related protein C140	CGCCACCAC AACCAAGGT	TCCTCAGCC TCTTCTTCTT GAAG	AGATCCCCA AGACTCTGT CAGACGCCT
RRI	Hypox			ribonucleoside-diphosphate reductase large subunit-like	GCTGGAAGC AGGGTCTGA AG	GTTGGCTGC AGGCTTGGT	CGGGCATGT ACTACCT
RRM2	Hypox			Ribonucleoside-diphosphate reductase subunit M2-like	TGCTGCTAG TGATGGCATT GT	TTTGGAAACC ATAGAAGCAT CTTG	ATTTACACAG GAAGTCCAG G
RSAD2	VDD			Radical S-adenosyl methionine domaine-containing 2	GGGAAATTA GTCCAATACT GCAAAC	GCCATTGCT GACAATACT GACACT	CGACCTCCA GCTCC
SAA	ImmStim			Serum amyloid protein a (SAA)	GGGAGATGA TTCAGGGTT CCA	TTACGTCCC CAGTGGTTA GC	TCGAGGACA CGAGGACTC AGCA
SCG2	MorRel			Secretogranin2	GGATGTGAA GAATCCAAC ACTGAT	ACACCACTTC AAACTAGCC ATACATT	CGGCTGTAT GTGCACTG
SERPIN_1	TherStr			Serpin H1 precursor (HSP47)	ACTATGACCA CTCGAAGAT CAACCT	CCCATTCGTT GATGGAGTT CA	AGGGACAAG AGGAGC
SERPIN_2	TherStr			Serpin H1 precursor (HSP47)	GAGGTCAGC GACCCAAAG AC	GCCGTAGAG GCGGTTACT GAT	CGGAACGTC ACATGGA
SFRS9	TherStr			Splicing factor, arginine/serine-rich 9	ACATTCGTGT CCACGGAGA AC	GGACCCTCT GCTTTTGTAA GGA	TGCCAGTTAT GGTCGCT
SHOP21	GenStr			Hyperosmotic protein 21 (Shop21)	GCGGTAGTG GAGTCAGTT GGA	GCTGCTGAC GTCTCACATC AC	CCTGTTGAT GCTCAAGG
TAGLN3	ImMort			Transgelin 3	TGGCTCAAG GACGGATGT G	GGATCTTCCT GATGGGCTT GT	TGTGTGAACT GATCAACAG
TGFb	Infl			Transforming growth factor β	TGAGCTCCG TCTCCTCATC A	GCGATTGGC CCATTCCTT	AGAGGCTGG AACTCTACAG
TRIM1	VDD			Fish virus induced TRIM-1	CATGATGTCT GGTGTTGAT GTATATTG	GAGACAGAG AACCAACTG AGAAAACATA	TTGTCATTCA GAACCATTG
TXN	Infl			Thioredoxin (txn)	CAAGAATGT GGTTTTCCTC AAGGT	GCATTTGATG TCACAGTGTT TGG	TGGACGAGG CAGCG
VEGFa	ImMort	GenStr		Vascular endothelial growth factor A	GGTCTGCTG TGGATATGA GTATCTTAAA	CCGTTGCAC CTCTCAGTG AA	AGCGAAATT GTGACCATA A
IGF	Growth			Growth cytokine	GACACGCTG CAGTTTGTGT GT	GTGACCGTC GTGAACTGG G	GAGAGAGGC TTTTATTTCA GTAAACCAA CGGGG

78d	Ref			S100 calcium binding protein	GTCAAGACT GGAGGCTCA GAG	GATCAAGCC CCAGAAGTG TTTG	AAGGTGATT CCCTCGCCG TCCGA
Coil	Ref			Coiled-coil domain-containing protein 84	GCTCATTTGA GGAGAAGGA GGATG	CTGGCGATG CTGTTCCTGA G	TTATCAAGCA GCAAGCC
MrpL	Ref			39S ribosomal protein L40, mitochondrial precursor	CCCAGTATG AGGCACCTG AAGG	GTTAATGCTG CCACCCTCT CAC	ACAACAACAT CACCA

Metric	Abbreviation	Source	Data generated	Comment
Mass	Mass	This study	Measured	Dissection comment
Fork length	FL	This study	Measured	Dissection comment
Fulton's body condition factor K	K	This study	Calculated	Dissection comment
Sex	Sex	This study	Observation	Dissection comment
Hatchery/wild origin	H/W	This study	Observation	Dissection comment
Presence of wounds and marks	Wound	This study	Observation	Dissection comment
Nematodes	Nematodes	This study	Observation	Dissection comment
Sea lice	Sea_lice	This study	Observation	Dissection comment
Enlarged gallbladder	Gall_bladder	This study	Observation	Dissection comment
Stock and region of origin	Stock	Provided by DFO PBS	Genetic Stock Identification	Only Coho and Sockeye
Energy density	Cal	Provided by NOAA ABL	Calorimetry	N.A.
Infectious agent load	N.A.	This study	Calculated	See table 1
Gene expression level	N.A.	This study	Measured	See table 2

Number of infectious agents detected	number_of_agents	This study	Calculated	N.A.
Relative Infection Burden	RIB	This study	Calculated	N.A.
Pteropod biomass	Ptero	Pakhomov et al., 2019	Juday Net	N.A.
Euphausiid biomass	Euphaus	Pakhomov et al., 2019	Juday Net	N.A.
Hydromedusae biomass	Medu	Pakhomov et al., 2019	Juday Net	N.A.
Caetognats biomass	Caeto	Pakhomov et al., 2019	Juday Net	N.A.
Zooplankton biomass	Zoo_S/M/L	Pakhomov et al., 2019	Juday Net	N.A.
Temperature	TEM	Pakhomov et al., 2019	CTD	Average of top 100m
Dissolved oxygen	DO_p	Pakhomov et al., 2019	CTD	Average of top 100m
Salinity	SAL	Pakhomov et al., 2019	CTD	Average of top 100m
Sea surface temperature	SST	Pakhomov et al., 2019	Temperature logger on headrope of trawl net	SBE 56 temperature sensor
Latitude	Lat	Pakhomov et al., 2019	Bridgelog	N.A.
Longitude	Long	Pakhomov et al., 2019	Bridgelog	N.A.

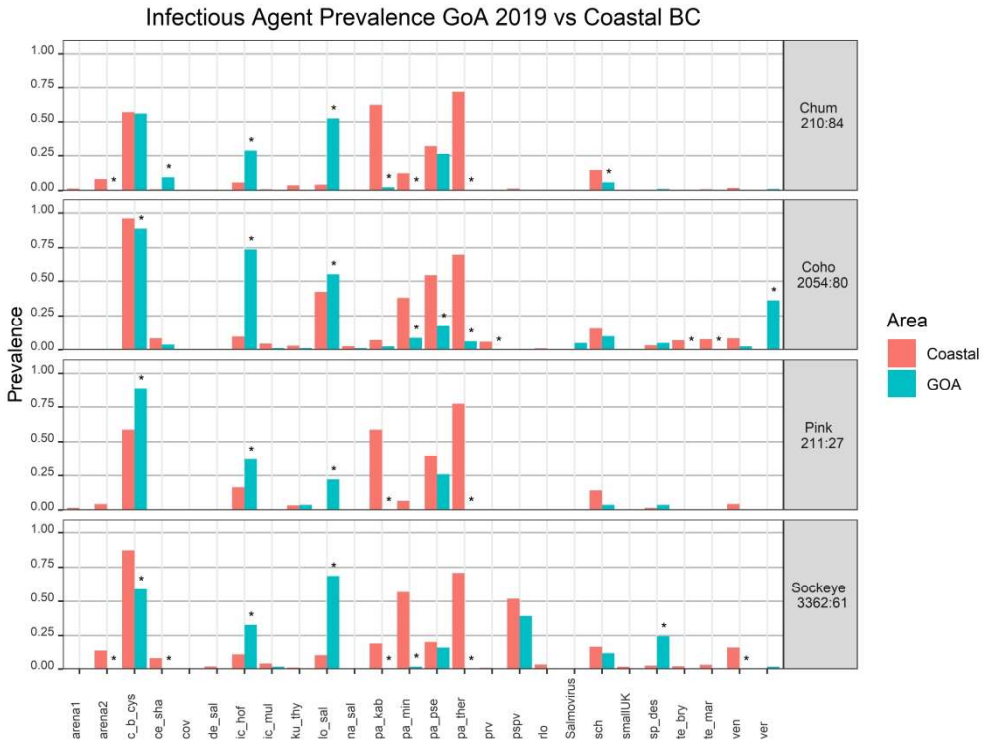

Figure 1: Comparison of selected infectious agents and pathogens with high prevalence in the Gulf of Alaska and Coastal BC. Asterix indicates significant differences in prevalence in the GoA with Fisher's exact test $p<0.05$. Ratio under species indicates the number of salmon in the analysis for the respective species (coastal : GoA). See Table 1 for infectious agent and pathogen abbreviations and Sup. Table 1 for all prevalences.

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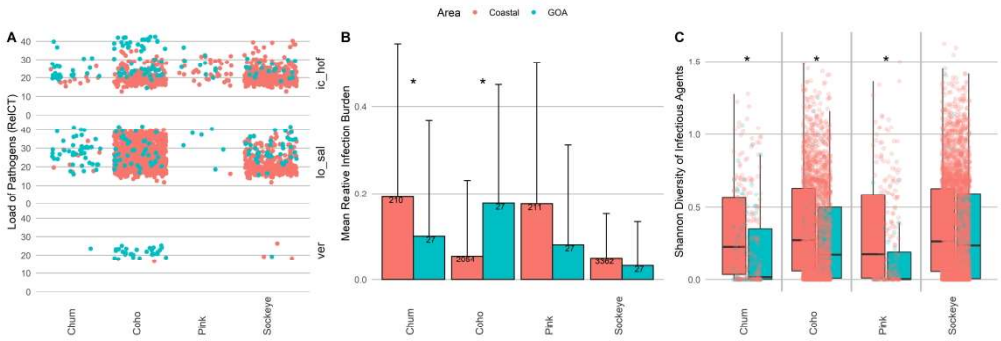

Figure 2: (a): Selected pathogens showing extremely high loads in samples from the Gulf of Alaska: ic_hof: Ichthyophonus hoferi, lo_sal: Loma sp., ver: Viral encephalopathy and retinopathy virus. (b): Relative infection burden of salmon in the GoA compared to coastal BC (mean value, SD, and n). (c): Shannon diversity of infectious agents and pathogens of salmon in the GoA compared to coastal BC (mean value, SD). Asterix indicates significant differences with a t-test $p < 0.05$.

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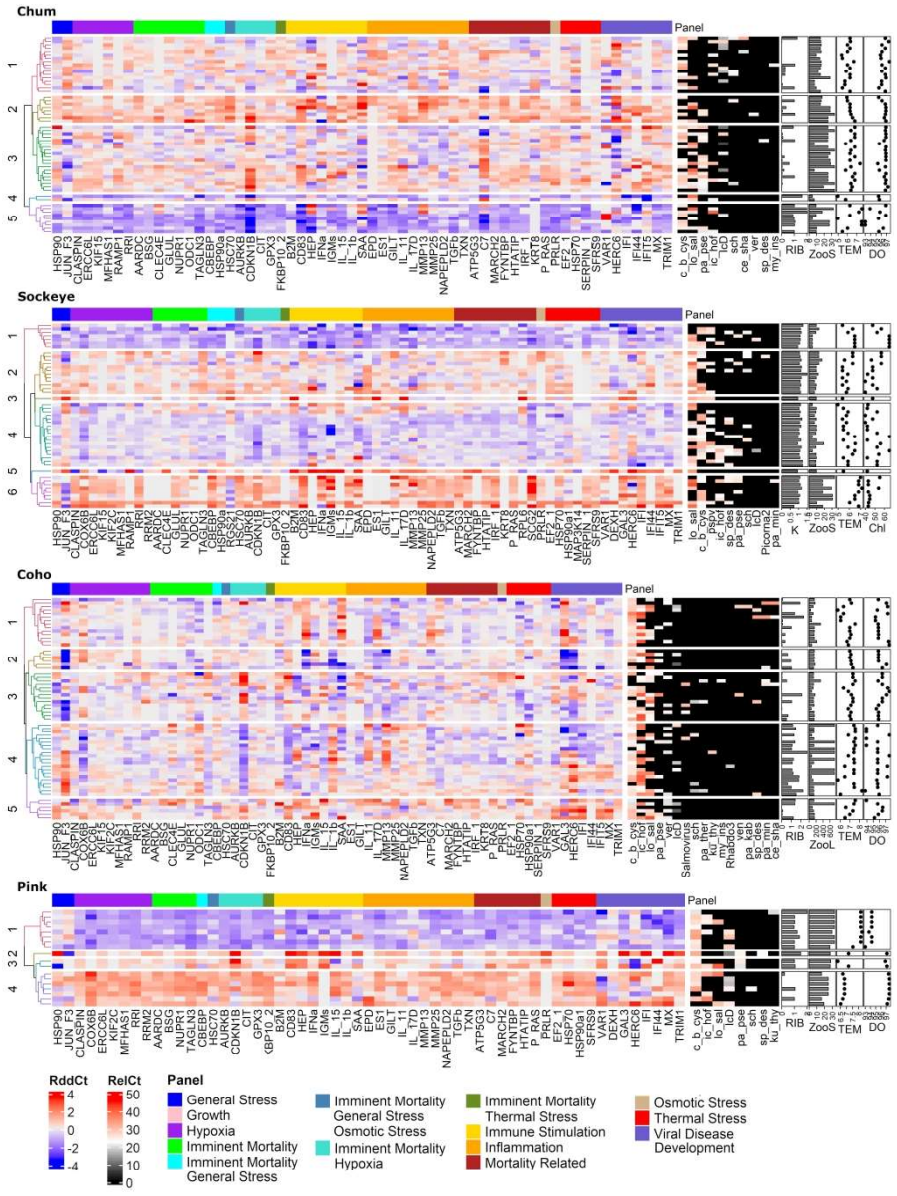

Figure 3: Gene expression and pathogen profile heatmap of overwintering salmon in the Gulf of Alaska. Gene expression of salmon is depicted in the left heatmap, where the relative delta-delta cycle threshold value (RddCt) detected in the Fit-Chip analysis is shown (blue to red) and individuals (rows) are hierarchically clustered based on similarities in gene expression (dendrogram and cluster number on left). Columns correspond to genes and are sorted by Fit-Chip biomarker panel (color scheme above). Load of pathogen detections associated with the individuals are depicted on the right heatmap in relative cycle threshold value (RelCt, black to red). Annotation graphs to the far right show Relative infection Burden (RIB), temperature (TEM) at the capture site, dissolved oxygen saturation (DO), and zooplankton size class abundance (ZooS/L).

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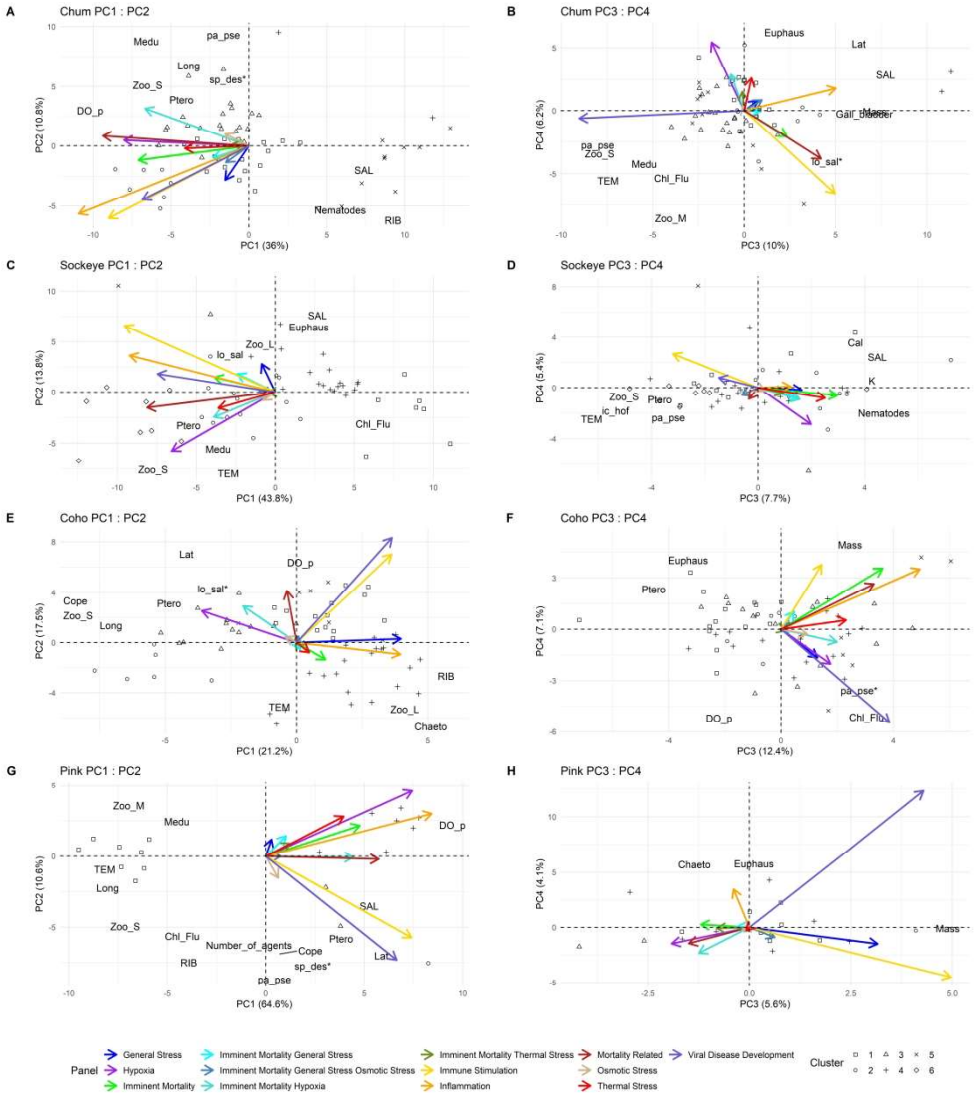

Figure 4: Gene expression analysis of salmon captured in the Gulf of Alaska during the winter 2019. (a), (b): chum, (c), (d): sockeye, (e), (f): coho, (g), (h): pink. PCA plot of gene expression is overlaid with meta-data (infectious agents, intrinsic variables, and environmental metadata). Dots depict individual salmon. Annotations (bold black) show superimposed data correlating with differential gene expression. Only data with a correlation significance of $p < 0.05$ are shown unless noted with "*". Gene expression influence summarized by biomarker panels are indicated by the colored vectors (see main text for description of specific biomarkers driving these findings). For a full figure depicting individual genes see Sup. Fig. 9. For a full list of infectious agent abbreviations and corresponding factors see Table 1.

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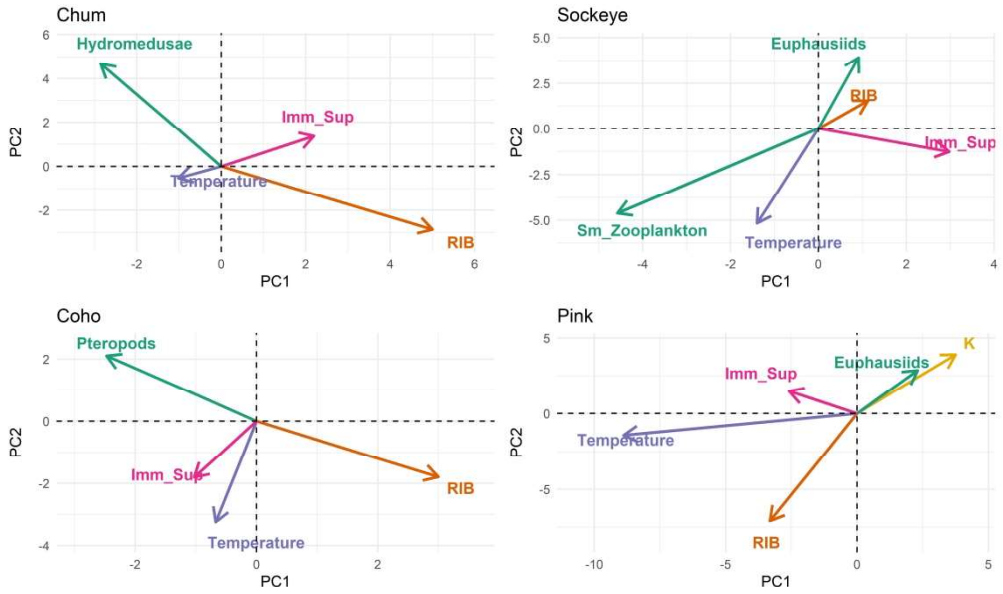

Figure 5: Association of primary prey species biomass, Relative Infection Burden, and temperature with gene expression in the Gulf of Alaska during the winter 2019. Primary prey species such as euphausiids , hydromedusae , and pteropods are highlighted in relation to immunosuppression (Imm_Sup: inverse vector of summarized biomarker panels immune stimulation, inflammation and viral disease development).

249x149mm (600 x 600 DPI)

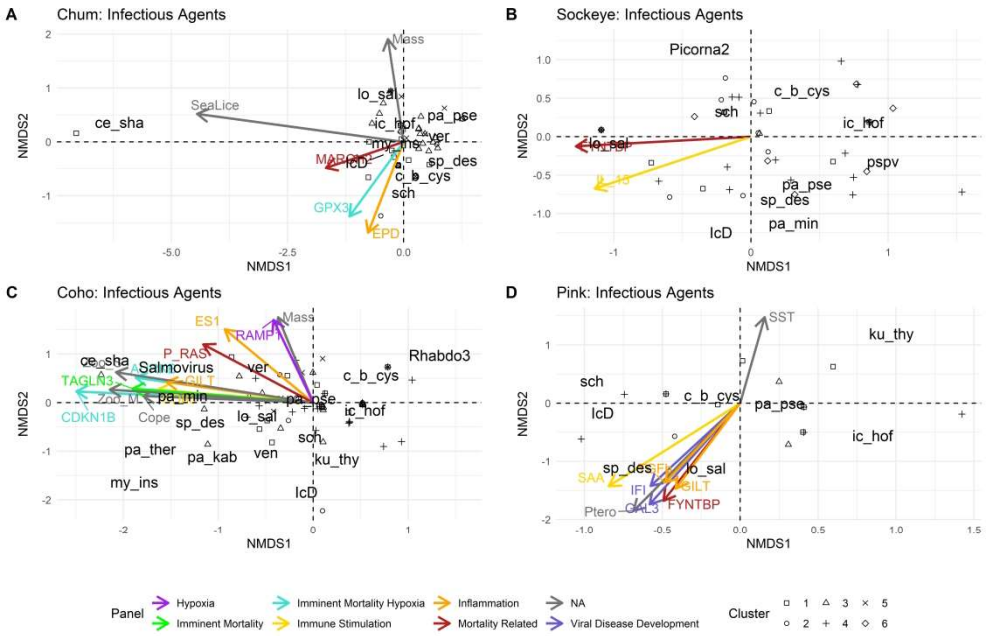

Figure 6: NMDS of infectious agent profile overlaid with corresponding gene expression, intrinsic and environmental metadata. Dots depict individuals and infectious agent vectors are indicated by the infectious agent abbreviation (see Table 1 for abbreviations). Corresponding superimposed data with a significance of $p<0.05$ is depicted.

299x199mm (600 x 600 DPI)