Are Smolts Healthier in Years of Good Ocean Productivity?

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Pacific salmon entering the Strait of Georgia in British Columbia (BC) experience profound annual variance in ocean survival. Ocean productivity in the early marine environment is hypothesized to be a key driver of this variation, with other biological stressors such as harmful algal blooms (HABs) and pathogens potentially acting as secondary factors. The question is what do we really know about the cumulative nature of these stressors on the health of salmon? We have developed a program that applies genomic technologies to assess the health and condition of out-migrating smolts and the biological agents to which they are exposed. Additional metrics associated with growth, feeding, energy, hormones, and ion regulation are also collected. These studies not only reveal the variance in salmon condition within and between years, environments, stocks, and species, but can be used to discover the key stressors that may impact salmon performance.

Extensive sampling programs were initiated in 2008 to support this research program, starting with sampling at natal rearing areas in the Fraser River and involving extensive ocean sampling over the first 6-9 months of ocean residence for sockeye, Chinook, and coho salmon. Our collections have included sampling of smolts over an area up to 2,000 km of migration. Individual genetic stock identification has been applied to each fish so that genomic assessments can track the physiological progression of individual stocks over time and space. Contrasts in the physiological variation among stocks, species, hatchery versus wild fish, life-history types, environments, seasons, and years were of interest. Variations associated with growth (IGF-I was used as a measure of instantaneous growth), size, hematocrit values, and other variables were measured (Fig. 1). Importantly, we were also interested in the elucidation of physiological variation among fish using approaches such as principle components analysis to identify the major physiological trajectories in the data (that may or may not correlate with measured variables) to contrast levels of variation between environments and years of good and poor ocean productivity.
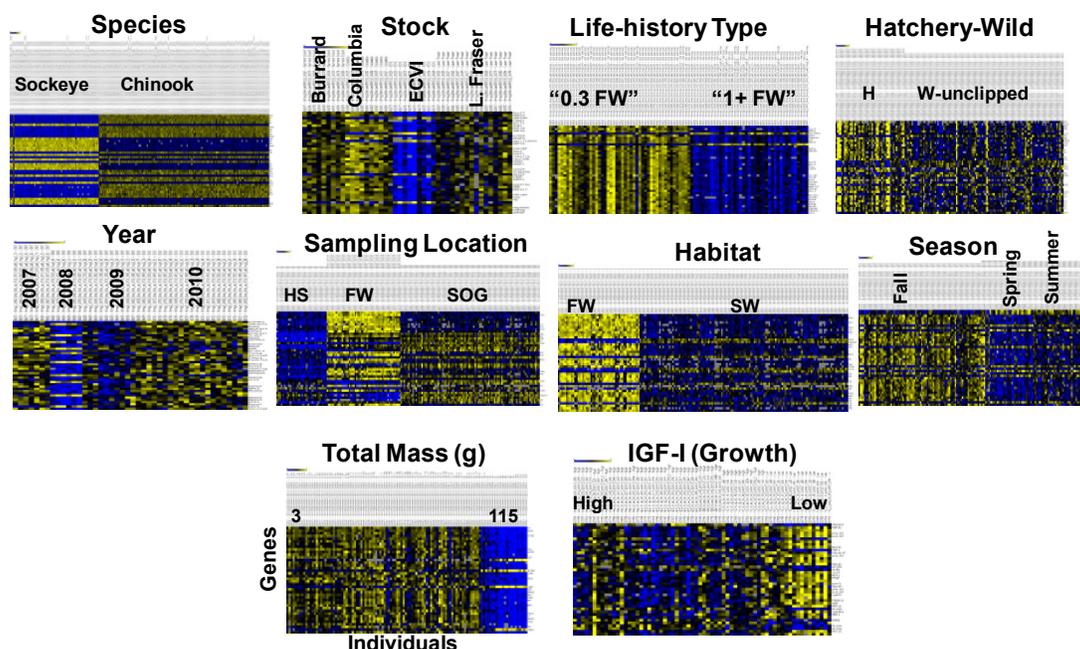

Fig. 1. Heatmaps showing gene expression signatures associated with specific measured variables of interest. Genes are differentiated on the y-axis, individuals on the x-axis, with up-regulated genes in yellow and down-regulated genes in blue. In each heatmap, the top 100 differentially regulated genes are shown.

In our functional genomics studies, microarrays, which are slides that have printed on their surface tens of thousands of salmon gene probes, were applied to assess the activity of the genes in each of four key tissues: brain, liver, gill and white muscle. As the expression (transcription) of genes to make proteins depends upon the physiological function of a given tissue, by surveying multiple tissues with distinct functions we are able to gain a snapshot of the genome-wide physiology of the fish.

Activity of genes expressed in the brain is important for controlling a wide range of behavioural and physiological processes. The most profound shift in the brain transcriptome (expressed genes) over migration occurred in the fall in all three species assessed (sockeye, coho, and Chinook salmon). The signature observed in Chinook and coho salmon showed strong loading of the same genes, suggesting a high functional correlation in these two species. Moreover, this shift was even stronger, and perhaps earlier in hatchery coho than wild coho salmon. Among the most affected physiological processes was the histamine H2 receptor mediated signaling pathway, which was highly up-regulated in the fall. In mammals, this pathway controls feeding and motor activity. Given that we know many Chinook and coho salmon stocks maintain residence in the Strait of Georgia during the summer and begin migrating northward in the fall, we hypothesize that this signature could relate to a signal for continued migration. If this were the case, our data may suggest that hatchery fish may undergo this behavioural migratory shift earlier than wild fish, a hypothesis we plan to test in future.

There is a growing body of literature that suggests that survival of hatchery fish in the early marine environment is lower than that of wild fish, especially when environmental conditions are stressful (e.g. poor ocean productivity; Beamish et al. 2012). If this were the case, we would expect that hatchery fish may retain a degree of physiological distinction from wild fish in the common ocean rearing environment, or at least may respond to environmental stressors differently. Our studies show that while hatchery fish do not remain phenotypically distinct from wild fish, brain and liver profiles show strong differentiation in prevalence of common signatures. Given the functions of these tissues, these data suggest that behavior, metabolism, and feeding may be the most differentially affected processes between hatchery and wild fish.

One of the most important questions we were interested in addressing was the variance in physiology associated with years of good and poor ocean productivity. We were fortunate to sample at least a small number of sockeye smolts leaving the Fraser River in 2007, a year-class that was associated with the lowest returns in over 75 years and resulted in the formation of the Cohen Commission of Inquiry. More extensive sampling was conducted in 2008, an out-migrant year-class that brought back record numbers of sockeye salmon to the Fraser River. We contrasted annual variation in gene expression in all four tissues from 2007 through 2010 (and 2011 for brain) and found that the two out-migrant years with the lowest (2007) and highest (2008) returns were the most physiologically distinct across multiple tissues collected from fish in the ocean. Importantly, this distinction was not limited to the marine environment. Gill tissue collected from migrating smolts in freshwater in 2007 showed the highest variance of all, with a powerful signature associated with differential signaling of immune cells of the gill that affected all sampled Chilko smolts, but the signature was absent six weeks later when the smolts were in the ocean (Fig. 2). This same signature was observed only sporadically in other years, and in sockeye and coho salmon sampled both in the freshwater and marine environments. Contrasts between 2007 and 2008 fish in freshwater and saltwater across multiple tissues revealed that annual variances in gene expression in fish collected from freshwater were often times as great as those observed in fish in the marine environment. Similar patterns of freshwater and saltwater variance across years were observed in coho salmon. These data suggest that the potential for conditional difference among fish entering the ocean should not be discounted as a factor that could undermine the ability of salmon smolts to adapt and respond effectively to stressors in the marine environment.
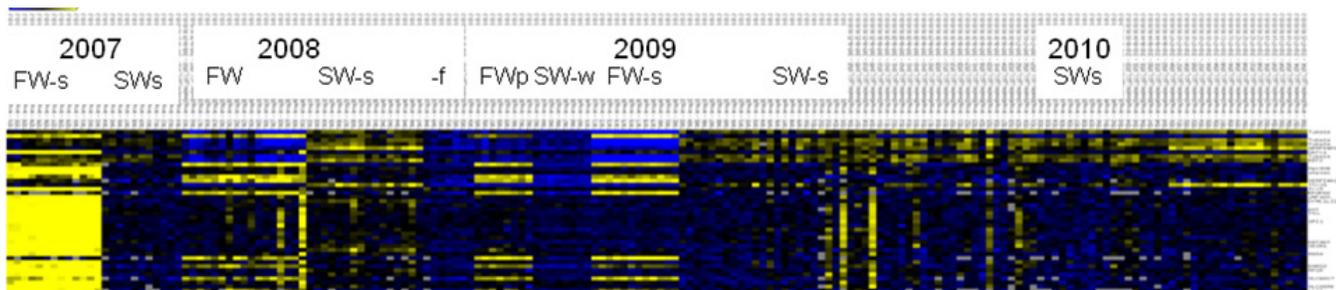

Fig. 2. Heatmap showing the top 100 genes significantly associated with annual variance in gill tissue of sockeye salmon. Note that 2007 fish in freshwater (FW) were highly distinctive from all other years and freshwater samples, as well as the 2007 samples taken in saltwater (SW) six weeks later.

As a whole, the genomic data indicated a poor conditional state of the 2007 out-migrant year-class from the Fraser River, with signs of enhanced anti-viral activity, immunosuppression, hypoxia (potential for harmful algal bloom exposure in the ocean), unusual osmoregulatory shifts in freshwater (potentially prepared for freshwater too early), and signatures in liver and muscle consistent with poor feeding and growth, but not outright starvation.

Chinook salmon populations that migrate to the ocean as yearlings are generally experiencing lower productivity than sub-yearling stocks. Genomic profiling showed that unlike hatchery and wild fish, yearling and sub-yearling smolts are highly distinctive well into ocean residence and across brain, liver and muscle tissues. Brain profiles were consistent with potential differences in feeding behavior, stress response, motility-activity, emotional reactivity, regulation of pain sensation, and learning. In fact, one of the most differentially-affected signaling pathways was that of endogenous cannabinoid signaling, which was highly stimulated in yearling stocks in the ocean. In mammals, this pathway is the same as that stimulated by exogenous cannabis intake, with profound effects on feeding, stress, and pain thresholds. Finally, and perhaps most importantly, annual variance within seasons was almost exclusively observed in yearling populations, consistent with the hypothesis that the yearling life history type is more susceptible to or possibly more exposed to environmental stressors.

Differential immune stimulation was commonly observed among the principal components explaining the highest sources of variation between co-migrating salmon in contrasts between years or stocks with low and high productivity and in adults dying prematurely in the river. We hypothesize that some of these signals may be associated with infectious diseases. To test this hypothesis, we began undertaking research aimed to identify which profiles may be associated with infectious agents. We first conducted a small suite of quantitative PCR analyses using published assays to known infectious agents to elucidate infectious agents associated with key profiles. Using this approach, we found that one signature profoundly affecting fish in the marine environment was associated with the presence and load of a gill microsporidian parasite, *Paranucleospora theridion*. This parasite, associated with proliferative gill disease in Europe, is known to be transmitted through sea lice, but was only recently identified in BC (Nylund et al. 2011; Jones et al. 2012). By combining acoustic tracking and genomic profiling, we also identified an association between infection with IHNv, a virus endemic to BC, and down-stream migration mortality. A strong interferon-type response was also correlated with load of this virus and with fate of fish in the river. Finally, we identified the piscine reovirus (PRV) in both Chinook and sockeye salmon, with virus load increasing in the fall and winter months. Unfortunately, this virus was not prevalent enough in individuals from our microarray studies to identify a unique genomic signature using existing arrays.

We also undertook next generation sequencing to determine if there were any novel viruses associated with our genomic signatures. We obtained a full genome sequence of a novel salmon parvovirus—the first parvovirus to be identified in a fish species. This virus is present in a high load in sockeye salmon smolts migrating to the ocean, with load and prevalence generally declining from summer to fall in the ocean. Prevalence of this virus varies greatly among stocks and years, but we do not yet have data to determine whether it is associated with disease. A full genome sequence of the PRV in BC salmon was also uncovered through next generation sequencing. The PRV was not previously known to be present in BC, and it is suspected to be causative of heart and skeletal muscle inflammatory (HSMI) syndrome in Europe (Palacios et al. 2010). Associations with disease in BC are unknown.

Recently, we have embarked on a new project that aims to develop and validate a high-throughput microfluidics platform to simultaneously conduct quantitative analysis of 45 microbes across 96 samples. Once developed, this platform will be applied across thousands of fish to determine what microbes associated with salmon diseases worldwide are carried by BC salmon. We anticipate retrospective mining of microarray data will facilitate the identification of genomic signatures correlated with the loads of specific microbes, information that could be used to assess their potential to associate with tissue damage and disease. With this approach, we will continue to determine which signatures that we have already identified as showing strong differential immune stimulation may be associated with infectious diseases. Ultimately, by merging data from gene expression profiling, physiological biomarker monitoring (for stress hormones, growth, osmoregulation, and energy), and quantitative microbe assays, we can elucidate patterns of microbe exposure and response across environments, seasons, stocks, species, and life-history types, and the interactions between microbe exposure and signatures potentially associated with differences in feeding, growth, and behaviour. This will allow us to begin to assess cumulative impacts of infectious disease and other stressors on performance of wild salmon in the ocean.

We show that in some years, smolt condition can be compromised before they enter the ocean, potentially exacerbating their ability to survive the additional stressors encountered when ocean feeding conditions are poor. In the marine environment, the condition of salmon is highly divergent between extreme years of good and poor ocean productivity. We show that wild and hatchery salmon are exposed to and potentially impacted by HABs in some years. Finally, we find that differential immune stimulation is a key driver of many of the most powerful conditional signatures both within and between years and hypothesize that at least some of these are in response to infectious agents. Preliminary data from a new microbe surveillance program is revealing a broad range of infectious agents carried by smolts in the ocean, some of which are present in high load and associated with strong immune stimulation in the early marine environment, and some decrease precipitously over time. We hypothesize that biological stressors may be less tolerated and associated with higher levels of mortality in years of poor ocean productivity.

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