November 13, 2008 Briefing Note

Funding Requested for Research on Links between Plasmacytoid Leukemia and shifts in migration timing and high mortality of sockeye salmon in the Fraser River

For over a decade, adult sockeye salmon from the Fraser River have experienced unprecedented levels of mortality (up to 95%) during up-stream migration, and Late-run stocks have exhibited early spawning migration timing. The functional genomics program has uncovered a novel conditional state afflicting the migrating salmon stocks from the Fraser River that may contribute to or cause these observations.

We conducted a multidisciplinary study that combined radio-tagging of migrating salmon to assess FW entry timing and fate, DNA stock ID to identify stock of origin of individual fish, and gene arrays to profile in biopsy samples genome-wide physiology of the fish at the time of sampling. Similar studies were performed on fish tagged in the marine environment, up to 800 km from the river, and upon entry into freshwater. This research identified in both saltwater (SW) and freshwater (FW) distinctive profiles for healthy and un-healthy sockeye salmon. The un-healthy profile fish were characterized by early river entry and high in-river mortalities (16x greater probability of dying en route to spawning grounds). Fish with un-healthy profiles were observed in each of three years, but proportions varied in different years (25% in 2005, 55% in 2006, 36% in 2007). Functional analysis revealed that un-healthy fish were responding to an intracellular pathogen, with profiles most consistent with a retroviral infection.

Although retroviruses have been observed in many fish species, only two are believed to affect salmon: the Atlantic salmon swim bladder virus, a fully characterized and sequenced virus isolated from tumours in the swim bladders of farmed salmon both in Europe and Maine, and the Salmon Leukemia Virus (SLV), postulated in the early 1990's to be the cause of Marine Salmon Anemia, also called Plasmacytoid leukemia, in BC and Washington farmed Chinook salmon. The original research on the latter virus was conducted by the Fish Health group at the Pacific Biological Station (led at the time by Mike Kent), but unfortunately they did not isolate the virus nor obtain DNA sequence from it. They did, however, show that the virus was highly infective to Chinook and sockeye salmon, and less so in coho and Atlantic salmon. We have already conducted molecular screening and **ruled out common viruses** afflicting Pacific salmon, including ISAV, IHNV, VHSV, Herpes, and IPNV. We also screened for bacterial pathogens and myxosporidian parasites, which did not yield any links to the disease profiles uncovered. The table below lists the accumulating evidence that suggests the disease afflicting our sockeye salmon is retroviral in nature and could be plasmacytoid leukemia.

Highest mortalities of sockeye were observed as fish migrated through high water temperatures. As further increases in temperature might be expected due to climate change, this disease could increase in virulence in future. Vertical transmission of the virus would also introduce the possibility of effects at other developmental stages, such as smolts.

Observation	Un-healthy Sockeye	Chinook with SLV	Retrovirus-General	Leukemia
Pale Gills	x	x		
Anemia-iron deficiency	x	x		
Look Healthy	x	x		
Temperature Sensitive	Mortality associated with high river temp	Highest mortalities in Aug-Sept	most fish retroviruses	
Salinity Sensitive	Osmoregulatory dysfunction in SW	High mortality upon transfer to SW		
Immunosuppression	In SW	?	x	x
Increased incidence of secondary infections	Fish dying in river carry multiple infections	BKD, microsporidian	x	x
Coagulation disorders	Most notably in 2003	?		x
Cancer	Strong retroviral/cancer associated profile in the brain; proliferation signals from genes specific to eye cells	eye tumours	x	x
Timing of onset	1996	1988-1994		

Given the potential devastating impacts of this disease on sockeye salmon, and possibly other Pacific salmon species, we propose research that will conclusively establish whether plasmocytoid leukemia or a similar retrovirus is, in fact, the primary cause of river entry timing shifts and higher susceptibilities of salmon to temperature stress. We propose to conduct this research through collaboration between Miller, who performed the genomic analysis implicating a viral etiology, Garver, the virologist at PBS, and Patterson, responsible for the Environmental Watch Program in the Fraser River. Tissue samples for histology and viral isolation have already been collected, but funding is required for Garver to proceed with analyses of these tissues. The proposed research would require 60K in funding for 2009/2010 and include four main objectives:

1) Molecular tools to isolate sequences of viral origin from afflicted fish (diseased fish identified originally through genomic analyses). This would include traditional PCR amplification-based approaches and application of the viral microarray used to discover SARS (the Vancouver group that did this analysis has agreed to collaborate),

2) Histological analysis of fish dying in the river in 2008,

3) Isolation and characterization of the virus from the brain, kidney and gills of afflicted fish. This research would include the development of in vitro (cell culture) and in vivo (fish) challenge models for the virus, if the virus is culturable,

4) Development of molecular markers for use in broader surveys to determine infection rates in juveniles, smolts and adults from different stocks. Genomic research already indicates that this is not a stock or late-run specific disease (although the effects to date may be largest on the late-run sockeye), and samples for this analysis have already been collected. Eventually, these markers could also be used to survey additional species.