Timeline of Genomic Research relating to the Mortality-related Genomic Signature Hypothesized to be associated with a potentially Novel Virus

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A. **July 2008**: **Discovery of mortality-related signature (MRS), suggestion of viral activity**. The first time I suggested publicly the potential role of a virus was at the *Biology of Fish Meeting in Portland Oregon* July 30, 2008. At this point, we had just completed an initial analysis of the first two tagging studies on adults migrating back to the Fraser River in 2006. These fish were tagged and biopsy sampled in the SW and FW environments, and we discovered that the same genomic signature was associated with premature mortality en route to spawning grounds, which we termed the "mortality-related signature (MRS)" or sometimes the "unhealthy" signature, regardless of whether they were sampled in the marine or freshwater environment. These "discovery" data were the basis of the paper published Jan 14, 2011 in Science. At this point in time, we did not know about the existence of this signature in other tissues, years, species or life-history stages.
B. **November 2008**: **Discovery that MRS is in more than one tissue, potential retroviral-type activity, directing some focus on Plasmacytoid Leukemia**. At the *launch of our Genome BC project* in November 2008, I gave a talk to our executive about this signature and the potential for viral involvement. I had determined that the dominant genomic signature observed in adult brain from fish migrating in 2003 and in 2005 was highly correlated with our MRS from gill tissue. Importantly, this signature in brain was also correlated with migration route around Vancouver Island, and contained indications of advanced maturation in MRS fish. I had also done more work annotating the individual genes involved in the signature and had begun to notice that many of the most significant genes were associated with retroviral-type infections in other studies. When I looked for information about retroviruses in salmon, there were only two that had been implicated, one affecting the swim bladders of Atlantic salmon, not on the Pacific coast, that was characterized and sequenced, and a second purported virus, the salmon leukemia virus, that was suspected to be the causative agent of Plasmacytoid Leukemia affecting Pacific salmon in the Northwest. While there was not a sequence or culture available for this purported virus, challenge studies showed that sockeye salmon were highly susceptible to this disease/syndrome observed off our coast since the late 1980's. Naturally, given the geographic and temporal consistency with our observations, this became a disease of specific focus. At this point, I had begun discussing these data with Kyle Garver, our virologist at DFO, and we wrote a Southern Endowment Proposal for funding (never received due to the funds being pulled in the economic decline).
C. **January-February 2009**: **Discovery of highly correlated MRS in brain, liver and gill of 2005 adult returns, but variance in tissues affected among**

individual fish. I gave a seminar in January 2009 at the *UBC Fisheries Centre* that also contained some discussion about this signature and potential role of an intracellular pathogen, but no more detail than already presented above. In a re-analysis of a study based on 2005 return migrating Fraser River sockeye salmon, I discovered that the MRS was also present in adult liver tissue. Moreover, this study contained the same suite of individuals with analysis over three tissues, gill, liver and brain, and we found that while a signature highly correlated with the MRS from the 2006 gill studies was present in each of these tissues in 2005, individuals tended to carry this signature in only one or two of the tissues, rarely in all three. This very finding, verified later to also exist in smolts, made it highly unlikely that such a signature could result from an environmental stressor, which, depending upon the stressor, should elicit a highly similar response in the same subset of affected tissues (e.g. a toxicant would elicit a strong response in the liver of all affected fish, and perhaps more subtle responses in other tissues, but one should not see heterogeneity in affected tissues within individuals), and added further weight to the hypothesis that a pathogen was responsible for eliciting this signature. While pathogens generally target a specific subset of tissues, individuals will often vary in which tissues are affected. We soon also discovered that this signature could be easily resolved via Principle Component Analysis, generally as the first eigenvector, meaning that it explained the largest source of individual variance in the data—i.e. it affected the activity of more genes than any other signature. Moreover, given that the signature contained some signs of "damage"—DNA damage, apoptosis, shifts in cellular proliferation, inflammation, immune response—we hypothesized that this signature can be associated with disease.

D. **March 2009**: **Collaborated with Kyle Garver (DFO virologist) and Patrick Tsang (BC Centre for Disease Control) in novel study using viral arrays, which showed greater binding of DNA/RNA from MRS than "healthy" tissue**. In a *report I provided to Genome BC* (Quarter 2 of our project) also at the end of February 2009, I again noted potential important linkages of our fate signature and viral involvement. I had also obtained 5K from the SAFE Division in DFO to work with Kyle Garver (DFO virologist) and Patrick Tang at BC Centre of Disease Control to try running their Viral Arrays (that had been applied to identify the SARS virus) on DNA and RNA from tissues containing the mortality-related genomic signature (that we often also termed "unhealthy"). These arrays contain probes for most animal viruses that have been fully sequenced and have been effectively applied to identify the family of origin of unknown viruses. Most of the work using these arrays, however, had been applied in mammals and from viruses obtained through cell culture. We were attempting to use them for the first time on fish (fish viruses were not well represented on these arrays) and using tissues from affected individuals as opposed to cell cultures (hence lower viral titres and with contamination from fish tissue). This study took place at the BC Centre for Disease Control over the next several months.

    a. While we could not definitively identify a virus to family in the same way that they do in mammals (where they have worked out the expected

patterns of binding for each family), we did find that tissues (brain, liver and gill) carrying the MRS contained 6-times greater signal:noise on the array than tissues with a "healthy" signature, suggesting greater binding to viral probes on the arrays. There was a slight over-representation of retroviral family binding, but we had to be cognisant that retroviruses also contain endogenous sequences (retroviral sequences that exist within the salmon genome and function as oncogenes), which we knew were stimulated in our MRS, so one could not use this as a definitive proof of an exogenous (i.e. not in the salmon genome) retrovirus.

E. **June 2009**: **New statistical approach further substantiates the linkage of MRS with viral activity and leukemia**. I had run a new analysis program that enabled us to statistically test hypotheses about associations of our signature with signatures from the literature relating to various biological phenomena (e.g. viral activity, bacterial activity, leukemia, hypoxia, temperature, etc… processes that through manual curation appeared to be linked to many of the significantly affected genes in various signatures). When applied to our MRS signatures for gill, liver and brain, we repeatedly found statistically significant associations with various terms associated with viral activity and with leukemia. The preliminary results of these analyses were presented at a *UBC meeting reviewing the NSERC and FishManomics programs on June 8*.
F. **July 2009**: Attended a *Pacific Salmon Foundation* meeting that brought together a multidisciplinary group of researchers to begin formulating a proposal to better understand factors in the Strait of Georgia that may be important in declines in stocks abundances of coho and Chinook salmon. I gave a talk that included some information on our MRS and viral linkage, and presented a schematic I developed from data in the literature about predicted climate change impacts on smolts. The following were proposed hypotheses that came out of this meeting, with some focus on what genomics could add to hypothesis testing. This large collaborative project, led by Brian Riddell of the PSF, has not yet received any funding.
G. **August 2009**: **Discovery of brain lesions**. Given our hypothesis that there may be a connection between our MRS genomic signature and Plasmacytoid leukemia, we began to look at other aspects of that disease to see what additional evidence of a connection we might be able to resolve, as we had not had any luck trying to obtain samples of fish afflicted with this disease/syndrome. One of the observations by Mike Kent had been the occurrence of optic tumours in some of the affected individuals. Given that we had observed a highly correlated MRS in the brains of returning adults, we began to look anatomically to see if there was any evidence of optic tumours in 100's of brains of fish we had not already used for microarray experiments. There were no photos available of these tumours, so we dissected out the optic lobe of these brains to look for any abnormalities. We found that a portion of the adult brains contained enhanced vascularization and what appeared to be highly vascularized dark pink "growths" on that varied in size, sometime filling the entire lobe, and other times only appearing a few cell layers deep. We showed these to Stewart Johnson, head of fish health, to find out if he had observed anything like this before and if he could tell whether these could be tumours, and he suggested that we run histology on these brains (which

were run Oct/Nov with additional funding from the SAFE Division). I put two technicians onto these brain dissections and they developed a ranking system to assess the prevalence and intensity of these abnormal growths (hereafter referred to as lesions) that we thought could be tumours and dissected hundreds of brains from adult salmon collected during their return migration in 2006, 2008 and 2009 and smolts entering the ocean in 2008 and 2009.

H. **September 2009**: **Found prevalence of brain lesions declined over migration for smolts and adults; briefing note to Minister started.** Given the very striking results that were coming from our brain dissections, which showed a much higher incidence and intensity of lesions in returning fish 2009 than in 2006 or 2008, and a decline in proportion of affected fish from the ocean to spawning grounds (in 2008 where we had enough fish to do this analysis), I wrote an email to the executive of the FishManOmics project outlining our findings to date (Sept 8, 2009) and suggesting that we get together to talk about next steps. I also *discussed these results early in September with Mark Saunders, our fish health experts Stewart Johnson and Kyle Garver, and Laura Richards*. In these conversations, I asked about obtaining departmental funding to continue pursuing research on these findings. Laura suggested that we should start by writing a briefing note for the Minister on our findings to date; this was the first briefing note pertaining to any of the research coming out of our genomic program. The briefing note took two months to draft (did not go out until November), with many edits back and forth between myself, the fish health group, Laura R., Mark, and I believe Paul Sprout's office.

I. **September 30, 2009**:**MRS, potential linkages with Plasmacytoid leukemia, and brain lesion data presented at DFO internal science workshop.** The first time I spoke of these new results publicly was at the *DFO internal science workshop* aimed at the development of working hypotheses pertaining to the declines in abundance of Fraser River sockeye salmon and abnormally low returns in 2009. We were asked to present the state of our sockeye salmon research programs and hypothesize on factors that may impact salmon performance, and ultimately salmon declines. Moreover, we were also asked what additional research would be required to decipher the relative impacts on declines of the factors being hypothesized. Given that we were actively pursuing these brain lesions at the time, these were a significant focus in my talk, recognizing that we still had not established a direct link between the genomic signature and these brain anomalies, whether they were, indeed, similar to lesions observed with Plasmacytoid leukemia, or whether they were, in fact, tumours (In hindsight, I should have called them lesions throughout this talk). These caveats were outlined by me verbally, but may not be clear when simply viewing the slides. My feelings at the time were that there were enough indirect linkages between the genomics and brain lesion data and Plasmacytoid leukemia to warrant a closer look at this disease. Moreover, the weight of accumulating evidence suggested that the MRS and the brain lesions likely associated with a negative impact on performance throughout the salmon life-cycle, and were present at prevalence rates that could have a major impact on population. Of import, there were no researchers in BC still studying Plasmacytoid leukemia (often

called a syndrome as there is no identified infective agent, although some believe that Nucleospora salmonis is the agent responsible), and while it had been studied in the 1980's-early 1990's in association with mortalities in Chinook salmon net pens, with the exception of one survey in the early 1990's it had not been looked at extensively in wild fish. Interest in this disease/syndrome waned once Chinook farming was taken over by the farming of Atlantic salmon, that did not appear to have an issue with this disease/syndrome, and researchers working on it moved out of the province. The content of this talk has already been made quite public through the inquiry and various websites.

J. **October 2009**: **Tried to redirect Genome BC funding to concentrate more on the MRS-viral research.** I gave a talk to the *Scientific Advisory Committee for the Genome BC FishManOmics project* year end Oct 22, 2009 that contained information on these and other findings. I had asked the board to consider changing some of our objectives so that we could focus more time and money on this one issue. They allowed us to replace one of our microarray studies to establish whether there was a linkage between the brain lesions and the MRS, which we followed up on in November/December. However, while they were highly interested in these results, they wanted to keep our program more general and focussing on the discovery of a broad array of genomic signatures that may compromise the performance of fish rather than focusing on this one signature.

K. **November 2009**: **Outlined ideas for new viral-focussed research proposal to Genome BC; Briefing note went to Minister's office**. I gave a talk at the *Genome BC staff meeting* that focussed primarily on our findings relating to the MRS and brain lesions. I outlined ideas for a new salmon genomics project focussing on viral disease characterization using novel genomic tools. I was asked to give a similar talk at the Genome BC Winter Symposium in January.

L. **October/November 2009**: **Brain histology reveals that lesions are likely aneurisms (haemorrhaging) not tumours.** Bill Bennet, the histologist for DFO, prepared histology slides from brains we had classified for lesions. For most brains, he took a single section through the middle of the brain, but for one or two, he did serial sections. I was concerned at the time that these single sections could easily miss region where we observed masses in the optic lobe. He sent 12 slides each to Dr. Gary Marty, from the provincial lab, and Mike Kent, one of the scientists who had worked on Plasmacytoid Leukemia in the past when he was a scientist at DFO, but who is now a professor at Oregon State. I was interested in his consultation on these brains to determine if he though there were consistencies with the optic tumours he had observed previously. What I learned was that these optic tumours were actually discovered at the back of the eye, not in the optic lobe (they had never actually looked in the optic lobe or at the brain itself); hence no way to match the two observations. We never collected eyes, as brains were generally removed from the carcass in the field. The histology report from Dr. Marty found mild to severe hemorrhages in the brains (with severe consistent with our scores of 4-5), and identified the presence of myxosporean spores in 11/12 brains (not a differential with our observations). The report suggested that the haemorrhaging was probably a result of the fish being killed with a blow to the head. Importantly, however, these fish had not been killed with a blow to the

head, and care had been taken in the handling of these fish to minimize damage to the brain. I was never very satisfied with this diagnosis, as a handling artefact would not explain the consistent reductions in incidence levels (of severe haemorrhaging) in both migrating adults and smolts, especially given that we used the same group of collectors to sample brains of smolts and adults over their migration between river and ocean over multiple years. Interestingly, if these hemorrhages were due to head trauma unrelated to capture and sampling, as has been observed in salmon from the Columbia system, the incidence rates were lowest at spawning grounds, where one might expect that they would be the highest, unless they were associated with mortality.

M. **December 2009 (B): Discovered that the MRS was also present in smolt brains well before salmon left natal rearing areas; moreover, while the signature did not disappear in the ocean, prevalence rates declined from summer to fall.** This information came from a relatively large Fraser River sockeye salmon smolt genomic study (about 250 fish) that tracked the genomic changes in the brain from pre-smolts in the river to the first 6-9 months in the ocean. The same study was later performed on liver, white muscle and a modified version on gill tissue. The discovery of a declining prevalence of this signature in the ocean could point to a potential impact of this signature, and whatever mechanism causes it, on early marine mortality, but at this point, we could not completely discount the possibility that some individuals "recovered" from the signature.

N. **January 2010 (A)**: **Discovered that there was NO correlation between MRS and hemorrhagic lesions in the optic lobes of the brain**. We completed a microarray brain study that contained both smolts and adult sockeye salmon (with a small number of Chinook and coho also analysed) that had been scored for the lesions. Analysis of this study revealed that there was no correlation of the lesions scores with the MRS, but revealed for the first time that the MRS was also present in Chinook and coho salmon, and was present in the brains of smolts. We did identify a small number of genes associated with the lesions, but have not followed up on this extensively, given the lack of correlation with the genomic signature of interest (largely of interest because we had already demonstrated that it was associated with premature mortality). I am still interested in pursuing more research into the brain hemorrhages, which if not a sampling artefact could still associate with mortality and have been observed in the Columbia system as well (http://www.pnl.gov/main/publications/external/technical_reports/PNNL-14748.pdf).

O. **January 2010 (B)**: **Discovered the MRS in smolt livers, again declining in prevalence between summer and fall in the ocean. Moreover, discovered that 2007 smolts carried a very high prevalence of MRS (potentially >90% affected), and may have been additionally compromised by hypoxia (heterosigma induced?), reduced feeding and a high metabolic rate in the ocean**. These data came from a smolt study similar to the brain study mentioned above, but that also incorporated 10 2007 Fraser River smolts sampled in Hecate Strait at the end of June. Analysis of this dataset revealed that the MRS was also

present in smolt livers, with highest prevalence levels before they left their natal rearing areas (as had also already been shown for smolt brains). Moreover, we found that virtually all of the 2007 fish carried the MRS in June in the ocean, compared with <40% of 2008 smolts, with the intensity of the signature also greater in 2007. Moreover, an analysis that contrasted only the MRS fish from both years revealed the stimulation of genes associated with hypoxia (oxygen deprivation), and some potential evidence of reduced feeding and a higher metabolic rate of the 2007 fish. Together, these analyses would suggest that 2007 smolts may have been compromised by a variety of potentially interactive factors. To determine whether the hypoxia signals could have resulted from exposure to harmful algal blooms (which other researchers had shown were strong in 2007)—we tried to amplified Heterosigma off the gills of some of these same fish, using the gills of net penned Atlantic salmon exposed to Heterosigma as controls. We showed for the first time that wild fish can be exposed to harmful algal blooms and may not simply avoid them by remaining deeper in the water column, which had been suggested by other researchers. However, at this point, we have still not definitively shown that the hypoxia signals in these fish were the result of heterosigma, this will require laboratory study. The signals suggesting reduced feeding (but not outright starvation) were consistent with the observations of other researchers that found lower growth rates and more empty stomachs in 2007 smolts compared to other years.

P. **February 2010 (B): Presented research results at annual PSC meeting**. I attended the *Pacific Salmon Commission meeting* in Portland Oregon and presented the results of our genomic studies to the Fraser Panel and to the Chinook Technical Committee. The presentations were largely geared to begin a dialog about the types of information that can be provided using genomic approaches, what we are discovering that may be of importance to management, how we intend to incorporate this information into new management models, and how we could work together with managers to ensure that the information provided is useful and timely.

Q. **May 2010 (A)**: **ADM Briefing.** DFO held a small internal meeting to *brief the ADM and RDG* on research and hypotheses pertaining to sockeye salmon declines that took place May 6, 2010. My talk outlined the discover of the MRS, present in multiple tissues and life-history stages, association with premature mortality in adults and declines in prevalence potentially associated with mortality of smolts in the ocean, and additional indicators of condition of 2007 smolts in the ocean.

R. **May 2010 (B)**: **Analysis of temperature holding study on gills of Chilko and Lower Adams sockeye salmon reveals that the MRS is highly associated with mortality, and prevalence increases after 1 week of holding**. A laboratory study was performed by UBC student Ken Jeffries that held adult Fraser-river caught Chilko and Adams sockeye salmon at different temperatures for a period of a week, with gill biopsy samples take before the start of the experiment, when fish became moribund, and of survivors at the end of one week of holding. We discovered that while approximately 40% of fish carried the MRS before the start of the signature, all of the fish that became moribund contained the signature, and

few that survived to the end. Combining moribund and surviving fish, prevalence after holding was closer to 70%, consistent with, but not definitive proof of, infectivity.

S. **June-October 2010: DFO funded microarray study to contrast genomics in multiple tissues of 2007 and 2008 out-migrating smolts.** Given the potentially informative information on the condition of 2007 out migrating smolts we obtained from contrasting 2007 and 2008 livers in the same microarray study, I obtained additional funding from the SAFE Division to conduct a series of such experiments in multiple tissues (liver, brain, gill, and white muscle) so that a broader picture of the physiological condition of 2007 migrants could be resolved. These experiments were conducted on a new microarray platform that we had not used previously (essentially a new slide with a different content of genes printed from smaller oligonucleotide fragments of each gene). In these studies, we contrasted 10 each of 2007 Fraser River smolts sampled in the river (April) and ocean (end of June) with 2007 West Coast Vancouver Island smolts sampled in the ocean and 2008 smolts from all of the same locations/time points. We were missing freshwater livers from 2007, as these were too degraded to run on arrays.

T. **June 2010: Pacific Salmon Commission-DFO workshop identifies disease as a potential major contributor to sockeye declines.** At this workshop on Fraser River sockeye salmon declines, I was asked to present a talk outlining the evidence surrounding the hypothesis "Genomic studies suggest that some disease has infected sockeye and has become an important contributor to the Fraser River sockeye situation". Each speaker was asked to address four key issues within our talks: Explanatory power, whether there was direct or indirect evidence of impact, specific research required, and potential management actions. Talks were followed by breakout groups, and a panel of experts prepared a report that outlined which hypotheses were most likely to be key contributors to declines. The potential for pathogenic diseases to carry an impact of the magnitude observed in the declines was considered by the panel to be high. It was recognized that while the purported novel pathogen discovered using genomic data was one potential piece of this puzzle, it was not the only potential disease that could be a factor in these decline, but few data existed pathogens affecting wild sockeye salmon fish.

U. **June/July 2010**. I was invited to present talks at the Society for Experimental Biology Animal Symposium on Intra-specific diversity in aquatic animals, Sète, France, June 25-27, 2010 and the AFS 9th International Congress on the Biology of Fish, Barcelona Spain, July 5-9, 2010. I gave similar talks at both of these meetings on our genomics program, largely, but not solely, focussed on the MRS.

V. **September-October 2010. Attempts to isolate a virus from MRS tissue**. We worked with Kyle Garver to apply sucrose gradients in an attempt to isolate viral particles from MRS-positive tissue. We did this on liver, brain and gill, but concentrated most of our efforts on liver. We obtained a band in MRS-positive tissues that was not apparent in "healthy" tissues that did not contain this signature. We repeated this in smolts and adults.

W. **November-December 2010. Sent DNA and RNA concentrated on sucrose gradients to Genome Centre for 454 sequencing.** We had them sequence >500,000 reads (combined) from one each positive smolt and adult liver.

X. **November 2010-March 2011. Analysis of 2007-2008 smolt contrast studies reveal that physiological divergence between the years starts in the freshwater environment and *potential* for MRS-associated mortality early in the ocean.** Studies based on liver, gill and brain tissue revealed a very high prevalence of the MRS in 2007, estimated at >90% in each tissue as smolts left the river. Importantly, in both the gill and brain, none of the 10 Fraser River smolts sampled in the ocean at the end of June, approximately 2 months after leaving the river, were positive for the MRS. Alternately, in 2008, whereas we observed 20-30% decline in prevalence of this signature in brain and liver from summer to fall in the ocean, no decrease in prevalence was observed between the freshwater and summer in the ocean. These data could potentially imply that in a year when ocean conditions were poor, the MRS is less tolerated—i.e. fish with this signature in multiple tissues perish quickly; however, given the limited sample size of fish available for 2007, we must treat this hypothesis with some degree of caution. Prevalence of the MRS was not the only factor differentiating 2007 and 2008 Fraser River fish. When sampled in the ocean, in addition to signs of reduced feeding and hypoxia, 2007 Fraser smolts also showed signs of immunosuppression and stress, and potentially a slower rate of growth relative both to 2008 Fraser smolts and 2007 smolts from West Coast of Vancouver Island stocks. Importantly, while these differences existed in the ocean, even if we exclude the MRS, 2007 fish were also highly divergent before leaving the river, suggesting that ocean conditions alone do not explain all of the physiological variation among years, and there may be additional factors in the river that should be considered.

Y. **January 2011. Science paper comes out.** This paper outlines the initial discovery of a genomic signature linked to premature mortality of salmon in the river, and the hypothesis that this mortality-related signature is associated with a virus. The most important message in this paper, in my view, was that salmon were already physiologically compromised before they entered the river, hence environmental conditions in the river may not explain all of the premature mortality occurring therein. I believe this to also be the case for smolts entering the ocean, that premature mortality may be both affected by the condition of the fish entering the ocean and the environmental conditions they encounter early in ocean life.

Z. **January-February 2011. Bioinformatic analysis of 454 sequencing data uncovers novel parvovirus**. This analysis was largely done in my lab, where we took the sequence contigs and used them to search sequencing databases for identities. Given that our starting material was salmon tissue, most of the reads were of salmon origin, and these had to be discovered and filtered out. Among the DNA contigs, we discovered a 2,214 base sequence that was observed in 76 sequencing reads that had a very high probability hit to a Parvovirus. This was the only high level viral hit among the sequences.

AA. **March-April 2011 (A). Amplification of Parvovirus from affected tissues.** We developed molecular markers for the salmon Parvovirus and showed that we could pick it up in a portion of smolt and adult livers, and in brain and gill tissue, but not in DNA from operculum punches (that we use for stock ID). These data were consistent with the sequence emanating from an exogenous virus. Moreover, we found that the presence of the virus was correlated with the presence of the MRS in liver tissue of smolts and adults. This is promising, but until we have validated that the virus is associated with the MRS in multiple tissues, we cannot conclude definitively that the two are always associated. We also amplified this virus in heart, kidney and spleen tissues, and are getting histology done on parvovirus-positive tissues from smolts, and we are working on the development of a quantitative assay for the virus and the attainment of a full viral sequence (we have about half of the sequence). This work is ongoing.

BB. **DFO follow-up internal workshop on Fraser River sockeye salmon declines—Presentation of new data contrasting 2007 and 2008 smolts.** See above.

CC. **May 2011. Parvoviral Disease Challenges**. We have identified a stock of sockeye salmon fry in Okanogan Lake that appears to be negative for the salmon Parvovirus that we will use for disease challenge research. This is being done by DFO virologist Kyle Garver. This work will establish whether this virus is infective, and whether it causes disease in fry. This should be repeated at a later date in smolts to better understand the role of shifting salinity environments on disease progression.

DD. **Moving forward**: While funding for continued work on the Parvovirus and MRS is uncertain at this point, we are conducting research to definitively establish whether the novel salmon Parvovirus is causative of the MRS, and will continue exploring the potential role that this novel disease may have on salmon declines in multiple species. We will follow, in general, the research plans put together in 2009, pending availability of funds.