Proposed 2010 DFO Funded Genomics Research relating to sockeye declines
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Anti-VIRAL-Signature Related Research Activities

1. Field HISTO-Genomic matched tissue collections: Attain tissue samples for genomic analysis (whole brain, whole blood (.3ml), spleen, gill, liver and white muscle) from the same fish sampled for HISTO
    a. Smolts leaving their natal streams (20-30 per stock)
    b. Smolts caught in the lower estuary (30-50 per survey)
        i. Some discussion of three trapping intervals, early, mid and late in the season
    c. Smolts caught early in the ocean (May) (50-100 total)
    d. Smolts caught in Beamish SOG surveys in June (100-150 fish)
    e. Smolts caught in Trudel High Seas survey in Oct/Nov (100-150 fish)

    David Patterson can supply samplers for some of these collections (already planning on sampling Chilko fence, smolt trap (one time interval), a portion of the Beamish SOG cruise, a portion of the Trudel High Seas Cruise

    If funds are available to pay a **co-op student salary (10K)**, we could use a student to help with some of the additional sampling

2. Establishing the prevalence and intensity of the viral-signature or virus itself
    a. Option 1: If we are able to isolate a sequence from the purported virus, we could develop a molecular marker to identify prevalence of the virus, and measure this quantitatively to establish intensity of infection. We have already shown in adults that fish carrying the viral-signature suffer higher en-route and pre-spawning mortalities in the river, with effects that increase with intensity of the signal (i.e. infection).
        i. Cost to run a survey of the virus alone (assuming we have the viral assay) would be $15 per fish. To date, we have over 3,000 sockeye that have been collected during adult and smolt migration from 2005-2009, and anticipate that with 2010 collections, we could have an inventory of 4,500 or more fish. Up to 2009, we have stock ID's for these fish and a large proportion are of Fraser origin, but we also have sockeye smolts from Trudel's cruises from more northern drainages and the WCVI. If we assayed all 4,500 fish, the cost would be $67,500. With this, we could establish over a 5 year period prevalence rates and intensities of infection and how these change over time and space as salmon move away from natal streams as smolts and back to the spawning grounds as adults. This could be used to establish the likelihood that this viral disease is an important factor in sockeye salmon declines. We could run a smaller portion of these samples, high-grading for certain stocks, if less funding

were available. **COST for survey with viral marker: 33-67K, depending upon number of samples**

b. Option 2: If we were not able to isolate a sequence from the purported virus, but were able to develop biomarkers to recognize the viral signature, and were able to find a way to obtain the equipment needed to cheaply and quickly assay these biomarkers, the cost to assess 4,500 samples would be 90K in disposables plus 6 months tech time=90K. We could run a smaller portion of these samples, high-grading for certain stocks, if less funding were available. **COST for survey with biomarkers: 45-90K, depending upon number of samples and availability of equipment**

3. Establishing whether or not aquaculture fish (Atlantic salmon) could also be affected by this purported viral disease, and could thus be carriers
    a. In thinking further about the idea of combining multiple individuals into a single analysis, this really is not going to work, as we are not simply looking for the presence of a molecular marker, but rather we are measuring the level of expression from individual genes. Some genes are expressed at a higher level in viral-signature fish relative to "healthy" fish, and others are lower. Hence, if you mix a viral-infected fish and a healthy fish into a single sample, you simply get some mean level of expression. Everything is relative here. Hence, we simply have to run single samples.
    b. I would suggest that to save costs, that we survey a single tissue (either the liver or the brain) of Atlantic salmon (but collect both). Incidence levels in the liver of sockeye salmon range between 20-40%, and in the brain from 30-60%, so we can easily pick up infected individuals in a sample size of 10. I would suggest that we sample 5 fish each from 5 farms (25 fish) in SW and 8-10 fish each from hatcheries (smolts would be best). With a 50-slide experiment, at a cost of $18,750, we could establish whether or not Atlantic salmon carry this signature. This could be money well spent when dealing with the press on this issue, which will get out in publication and likely as a result of the inquiry. **COST to establish using arrays whether viral-signature is present in Atlantic salmon: $18,750**
    c. It may also be possible to do this using the markers we hope to develop if we can attain a viral sequence, which would enable us to look at more fish and would be cheaper. However, we may not want to wait for this, depending upon external pressure.

In total, to establish the potential impact of the purported novel viral disease on declines in abundance of sockeye salmon from the Fraser River, without gaining significant new information on the virus itself, the cost would be 50-100K depending upon which approach and how many samples were analysed. To establish the potential that Atlantic salmon may be carriers is around 19K.