**2005 YUKON RIVER CHINOOK SALMON *ICHTHYOPHONUS* UPDATE**
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My comments will address the four (preliminary) conclusions.

1. Non-lethal sampling is unreliable.

   Non-lethal sampling was an objective of the 2004 sampling season and appears to have been based on (biopsied) muscle. The muscle was examined by PCR (Tanana) or by culture (Lower Chena and Lower Salcha Rivers). Heart was also cultured from Tanana, Chena River and Salcha River specimens. The percent of positive samples (prevalence) was consistently higher for cultured heart compared with muscle examination at all sites. I have the following concerns: were these paired samples (were heart and muscle samples obtained from the same fish)? If not, were the heart and muscle samples obtained from different fish at the same time? The Lower Chena and Lower Salcha sites appear to be different from the Chena River and Salcha River sites. If samples are based on different fish collected at different times it is likely that some of the variation between methods will be due to actual differences in prevalence and not to the reliability of the detection method.

   Based on the information provided, there does not appear to be good evidence to support this conclusion.

2. Clinical signs increase as fish migrate upriver

   Clinical (visible) signs of *Ichthyophonus* as a percentage of all *Ichthyophonus* infections appeared to vary among sample sites in 2004 and 2005. In 2004, base-line data obtained by PCR at Emmonak showed ~43.8%. This increased to 93.7% at Tanana then declined in spawning ground samples to 60.9% (Chena R.) and 50% (Salcha R.). The overall lower prevalence at Salcha R. may have been due to the application of different carcass selection criteria. In 2005, the Emmonak baseline was 55.7% and this increased to 93.3% and 71% at Chena and Salcha, respectively using criteria 1 and to 100% and 47.3% at Chena and Salcha, respectively using criteria 2. In 2005 the application of criteria 2 appears to be associated with overall lower prevalence, consistent with 2004. The ability to culture from the spawning ground samples will depend on the quality of the sample (the condition of the carcass) which reflects how long it's been dead, temperature, desiccation, etc. Reduced cultivability would tend to skew upwards the percent clinical cases. Even with criteria 1, if these fish have been dead for a sufficiently long time, there will be a tendency for the percent clinical cases to be increased due to a loss of cultivability. The criteria could be validated by sampling repeatedly from fresh fish collected at Emmonak or Tanana and left in a secure spot on the bank. Over time the condition of the eyes, heart, gills, etc would be noted and cultivability compared. This would also provide an estimate of false-negatives on the spawning ground due to loss of cultivability.

   The data in 2004 appear to show an increase in percent clinical cases between Emmonak and Tanana. The percent clinical cases at the spawning ground may or

may not be accurate depending on carcass quality and it's not clear that either criteria has been validated with respect to cultivability.

3. Upriver infection rates are similar between gender
   The data indicate similarities in prevalence for males and females at Chena (2004) and at Chena and Salcha (2005). In contrast prevalence appears to be much lower in males compared to females both at Emmonak (both years) and at Tanana (2004). It is not clear whether some of these differences were due to unequal numbers of males and females at each of the sample sites.
4. Infected fish spawn successfully
   The data for 2005 suggest that a similar proportion of infected and uninfected females were spawned out at Chena and at Salcha. In contrast, while the proportions of infected to uninfected spawned out males was similar for the Chena it appears that fewer infected males spawned on the Salcha.

Other comments: Until a complete statistical analysis is completed on these data, it is unclear what is significant and what is not. Infected chinook evidently survive to the spawning grounds and some of these fish spawn successfully. The variabilities associated with sampling and analytical methods and with sampling locations may be influenced to various degrees by other factors that require some control. For example, the carcass selection criteria will benefit by a validation process, as suggested above. Similarly, the non-lethal detection methods should be validated by comparing samples from the same fish. This could be built into the carcass criteria validation exercise. Additional interventions (e.g. holding marked salmon at a mid-stream location and following disease progression, sexual maturation, etc. over time) may also be necessary to assess the outcome of infection and to help interpret the in-river observations and to predict impacts of infection. The low rate of returns of the radio-tagged fish suggests this may not be a useful approach to assess the impacts of *Ichthyophonus*.