Review of Fishery Data Series No. 07-64

Assessment of *Ichthyophonus* in Chinook Salmon within the Yukon River Drainage, 2004

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General comments:

This report covers the first year (2004) of a three-year study (2004 - 2006) conducted by ADF&G to evaluate the impact of *Ichthyophonus* on Yukon River Chinook salmon. The study follows a previous 5-year study (1999 – 2003) that revealed a significant *Ichthyophonus* related pre-spawn mortality in Chinook salmon at three terminal spawning tributaries in the Yukon and Tanana Rivers (Kocan et al. 2004).

The present study consists 6 stated objectives, four of which were not addressed at all (*), and six conclusions, none of which are supported by the data presented.

Each comment in this review is accompanied with a citation to the page number in the ADF&G report. To follow the comments in this review it is recommended that the reader have the original ADF&G report and refer to the page numbers cited in this review in order to clearly understand the comments.
Can be downloaded at: http://rapidsresearch.com/ADF_G_ICH__Study_2004.pdf

Objectives (pg 2, para 2)

1) * The Yukon River was <u>not</u> the object of this study, which focused on the Tanana, Chena and Salcha rivers with a baseline sample taken at Emmonak. No middle or upper Yukon River data were taken (above Tanana River confluence).
2) * Only one “non-lethal” test (not tests) was used. This PCR test utilized muscle tissue, which is known from previous studies to be highly variable as the fish move upriver, thus making the results unrepeatable.
3) Spawning success was evaluated (but incorrectly interpreted), but pre-spawn mortality was not addressed.
4) * Radio telemetry study was a failure – no reliable baseline of infection was established and too few fish were sampled (9) after tagging.
5) * Juvenile fish were examined from Canadian sites with no connection to the Tanana and lower river study sites.
6) * Temperatures for 2004 are presented but there is no attempt to “correlate” these with infection prevalence or pre-spawn mortality.

Conclusions (pg 22)
There are six "conclusions" presented in this report. For clarity they are numbered and summarized below and immediately followed by an evaluation of each.

1. "This study demonstrated that cardiac muscle is the most reliable tissue type for *Ichthyophonus* monitoring"
2. "PCR is an adequate alternative to histology and culture techniques"
3. "Gear type is likely the main bias inadvertently introduced to *Ichthyophonus* studies… (and) may lead to a presumably higher prevalence of *Ichthyophonus* in 'weaker' fish or large fish (i.e. females) with shore preference"
4. "…clinical pathology associated with *Ichthyophonus* is ambiguous and therefore the correct identification of the parasite using this non-specific immune response is unreliable"
5. "Results of this study show no difference in spawning success…between infected and uninfected females"
6. "*Ichthyophonus* prevalence is potentially correlated to age and sex…."

A comparison of these "conclusions" with the data presented in the report reveals that none of the conclusions is supported by the data presented, and some are actually refuted. A discussion of these discrepancies follows:

<u>Conclusions (pg 22)</u>
Conclusion #1. This study only examined two tissues; cardiac muscle (requiring lethal sampling) and skeletal muscle (non lethal sampling). Numerous published studies on *Ichthyophonus* have shown that these are the most and least severely affected tissues respectively. The same literature also shows that stomach, spleen, liver, kidney, gonad and brain are also target organs for *Ichthyophonus*, and thus may be better tissues for evaluating infection prevalence. Since none of these organs was evaluated during this study the "conclusion" should state: "Cardiac muscle is more suitable for monitoring *Ichthyophonus* prevalence than skeletal muscle, the tissue least likely to be affected".

Conclusion #2. PCR is **not** an adequate alternative to histology or in vitro culture. Even though PCR is a highly sensitive technique for the detection of nucleic acids (DNA & RNA) it does not distinguish between living and dead organisms, or even the presence of an intact organism. Therefore, PCR can only reveal if a fish has been exposed to *Ichthyophonus* DNA. The Yukon River drainage has at least 4 species of fish known to be infected with *Ichthyophonus*. These infected fish release the organism into the water during the course of their infection as well as after they die and decompose. Therefore, the entire drainage should be considered contaminated with DNA from *Ichthyophonus*, thus making contamination of samples with *Ichthyophonus* DNA highly probable. Visualization of *Ichthyophonus* in the host is the only <u>conclusive</u> method for determining its presence. Histology allows the observer to see the preserved parasite and is the only way to evaluate tissue damage and immune response. Verification of the identity of the parasite can also be accomplished with special stains, such as PAS. Likewise, in vitro culture verifies the presence of the living organism and is irrefutable because the living parasite can be directly observed.

When detection sensitivity between PCR and histology or culture is compared (Table 1) there is no significant difference between PCR and histology or in vitro culture. Therefore, not only is PCR inappropriate for detecting infection prevalence, it offers no advantage in sensitivity over histology or in vitro culture.

Conclusion #3. Nowhere in this study is a scientific evaluation of gear-type effectiveness described. To do this, similar numbers of fish should be sampled using both gear types at the same location at the same time, and then compared with the same test for infection prevalence. Since this was not done, this conclusion should be considered an "opinion".

However, if fish wheels do bias the sample (as suggested by the authors) toward higher infection prevalence, it would be expected that the Tanana (fish wheel) sample would have significantly higher infection prevalence than the Emmonak (gill net) sample. Values from Appendix B6 of this report show that the *Ichthyophonus* infection prevalence in females sampled by gill net at Emmonak was 30.5% while at Tanana, where fish were sampled by fish wheel, the prevalence was 26.1% (4.4% lower than Emmonak). A statistical evaluation of these two samples reveals that there was no difference between the two sample groups, ($X^2 = 0.016$; $n = 82$; $P = 0.69$), thus proving this hypothesis incorrect.

Although samples from fish wheels are singled out as "biased" in this report, there is no discussion of potential gill net bias. If large mesh gill nets were used, as they have been in the past, then the gill net samples should be biased toward larger fish while the fish wheels sample all fish without regard to their size and age.

Conclusion #4. The accurate identification of *Ichthyophonus* infection by gross lesions (aka "white spots") is highly correlated with the training, experience and skill of the observer. Having said this, no reputable pathologist would offer a diagnosis of infection or disease based solely on visible gross lesions. Sound biomedical and scientific practice dictates that any visual evaluation be confirmed with a laboratory test(s) for the presence of the pathogen. If visual evaluation is eliminated from the field diagnoses as suggested, then clinical disease cannot be determined, thus eliminating a crucial epidemiologic parameter needed to evaluate disease within a population.

Conclusion #5. Not only are these conclusions not supported by the data, they contradict it. Among Chena River females 9% fewer infected fish successfully spawned compared with uninfected females, while in the Salcha River 13% fewer females successfully spawned out. Although these spawning successes are not statistically different they are biologically significant. In fact, for all three years of this study, infected Chena River females underperformed uninfected females by 9%, 5% and 19% respectively (*ADF&G Ichthyophonus Results 2004-2006; presented to the Joint Technical Committee 2006*). Likewise, for two of the three years, infected Salcha River females underperformed uninfected fish by 8% and 6%. This repeated poor spawning performance by infected fish in these two rivers over a 3-year period is strong evidence that *Ichthyophonus* is depressing spawning success.

Reduced spawning success is also evident in the decrease in infected fish observed on the spawning grounds compared with the Yukon-Tanana mainstem. Although infected Chena River spawn-outs exhibited the highest infection prevalence anywhere in the river (this study only), infected Salcha River females were significantly under represented. This same reduction in infected females also occurred from 2001-2003 (published data) and again during 2005 and 2006 (ADF&G data; JTC report 2006) (figure 1).

Conclusion #6. The authors' statement on pg 9 (last para) "…*Ichthyophonus prevalence was not significantly different between females and males*…" contradicts the conclusion (pg 22) that "…*Ichthyophonus prevalence is potentially correlated to age and sex*…". However, the data on pg 9 show that a correlation does exist. Data in Appendix B6 shows an infection prevalence in females nearly 3 times that of males (30.5% vs 11.6%) at Emmonak, with a X^2 value of 6.2 and a P = 0.01). This difference is highly significant! How was this huge difference missed?

Although this study claims a correlation between infection prevalence and age, a previous 5-year study showed no correlation between age (size) and infection prevalence. More than a single year of data is needed to disregard a 5-year data set.

Specific Comments

Results: (pg 8 – 15)

Pg. 9; Prevalence within drainage.
The authors claim no difference in infection prevalence between males and females at Emmonak - this is incorrect. Females were more than twice as likely to be infected (23.5%) than males (10.8%). Statistically (X^2) this is significant at p = 0.01.

Pg. 10, Figure 4.
Standard Deviations in this graph are inappropriate because they are generally used to compare arithmetic means while confidence limits are used to compare proportions.

Pg 11; Spawning success
Although there was no statistical difference in spawning success between infected and uninfected females in both the Chena and Salcha Rivers, the difference is biologically significant. In both cases fewer infected females successfully spawned-out and more infected females did not spawn at all. Partial spawn-outs should be classified as "spawning failure" because it is difficult to envision a female retaining half of her eggs and still defending the redd – especially if found dead!

The most significant issue associated with *Ichthyophonus* infection is pre-spawn mortality. However, there is no discussion of the loss of infected fish between the

Yukon-Tanana mainstem and post-spawn fish. Figure 1 shows data from a previous study compared with this study, including data from 2005-2006. Clearly there is evidence of a significant decrease in infected fish among the post-spawn females relative to the Yukon-Tanana mainstem. Since *Ichthyophonus* is effectively a "biomarker", then significant loss of spawners occurs before infected fish reach the terminal spawning sites.

The last two paragraphs of this section (pg 13) are a repeat of the 2nd and 3rd paragraphs on the previous page.

Pg 14, Table 3.
Conclusion #2 states that "*Gear type is likely the main bias…. for fish wheels tend to catch a larger number of small males*…" This conclusion cannot be reached based on data from this study because no age/size data was collected from the fish wheel samples.

The authors suggest that "weaker" infected females would swim closer to shore to take advantage of the slower current, thus being more likely to be caught by the fish wheel. This would assume that "sick" fish were smarter than healthy fish because they know the easiest route to the spawning grounds.

These two arguments (small healthy males and large infected females) are opposite arguments for fish wheel bias. One argues that the bias is toward fewer sick fish while the other argues that the bias is for more sick fish; the logic here is elusive.

The authors suggest (pg 18) that the decrease in infected fish at terminal spawning sites represents some "…*Yukon Chinook salmon stocks more susceptible than others to Ichthyophonus infection,*…". If some stocks are at greater risk, then each stock should be managed separately for the greatest protection to the most susceptible stock.

Pg. 13; Radio telemetry
This study is seriously flawed because no solid baseline (cardiac tissue) data were collected and not enough radio-tagged fish were recovered from this project to be of any scientific significance. Skeletal muscle was used as the infection "baseline" but should be considered unreliable because it changes over time and is unpredictably variable.

However, the authors did claim that 78% (7 of 9) of the PCR-positive fish tagged at Tanana "…*were located in known spawning tributaries*…". In the Discussion (pg. 22) it is suggested that the "…*majority of infected fish are not dying during migration*". A different interpretation of this small data set says that 22% of the infected fish entering the Tanana River do not make it to the spawning grounds – probably due to mortality.

The radio telemetry data also reported that 5% of the tagged fish were swept down-river after release and 11% could not be accounted for. This is an overall loss of 16% of the radiotagged fish. The authors offer several possible scenarios to explain the missing fish, but do not include the most plausible explanation – pre-spawn mortality!

Pg 15; Environmental data

Water temperatures for Emmonak, Tanana Chena and Salcha rivers was recorded and presented in Figure 8 (pg 17). However, there is no attempt to correlate these data with infection prevalence and/or pre-spawn mortality as proposed in "Objective 6" (pg. 2).

General observation:

From 2001 - 2003, and again from 2005 – 2006, there was a significant drop in the number of infected post-spawn females observed in the Chena and Salcha Rivers relative to the Yukon–Tanana mainstem. Because Chinook enter the Yukon River already infected, the parasite serves as a "biomarker" that can be used as a biological tag to monitor the fish as they migrate upstream. When the proportion of infected fish drops, this indicates that the infected fish are no longer present in the population. Consequently, when a large proportion of infected pre-spawn fish do not appear on the spawning grounds (Chena & Salcha Rivers), their absence must be explained. The most plausible (parsimonius) explanation is that they died before reaching the spawning grounds and have been swept down stream. An alternate explanation, offered by the authors, that infected fish are diverting to unsampled streams, relies on a highly improbable assumption that populations from specific streams are isolated from the remainder of the Yukon River Chinook population when they are exposed to *Ichthyophonus*. This would also imply that some streams (unsampled, of course) would have populations of spawning fish with infection prevalences significantly higher than those observed in the Yukon-Tanana mainstem.

One of the most difficult components of this study to understand is the huge increase in infection prevalence reported for the Chena River spawners in 2004. Appendix B6 shows infection prevalence for males was 11.6% at Emmonak, 8.7% at Tanana and 34.2% in post-spawn Chena fish (>300% increase!). For females this was 30.5%, 26.1% and 37.5% respectively (Figure 2). For a real increase in infection prevalence to occur in the Chena River fish, they would have to become infected during their fresh water migration, or a large number of healthy (uninfected) fish would have to die en route, leaving a large proportion of infected fish to spawn. Neither of these arguments is plausible, making the 2004 Chena River data suspect.

Conversely, the 2004 Salcha River female infection prevalence significantly decreased relative to Emmonak and Tanana fish (X^2; n = 184; p= 0.01), identical to what was reported in 2001 thru 2003 and again from 2005-2006, opposite of the 2004 Chena River data (Figure 3).

Discrepancies of this magnitude, especially when they are contrary to data that repeats for multiple years, must be addressed. If data from all three years of this study had been included in this report a more complete picture of the host-parasite relationship would emerge and the 2004 "outlier" might be less problematic. However, with the limited data presented the 2004 Chena River data must be viewed with caution.

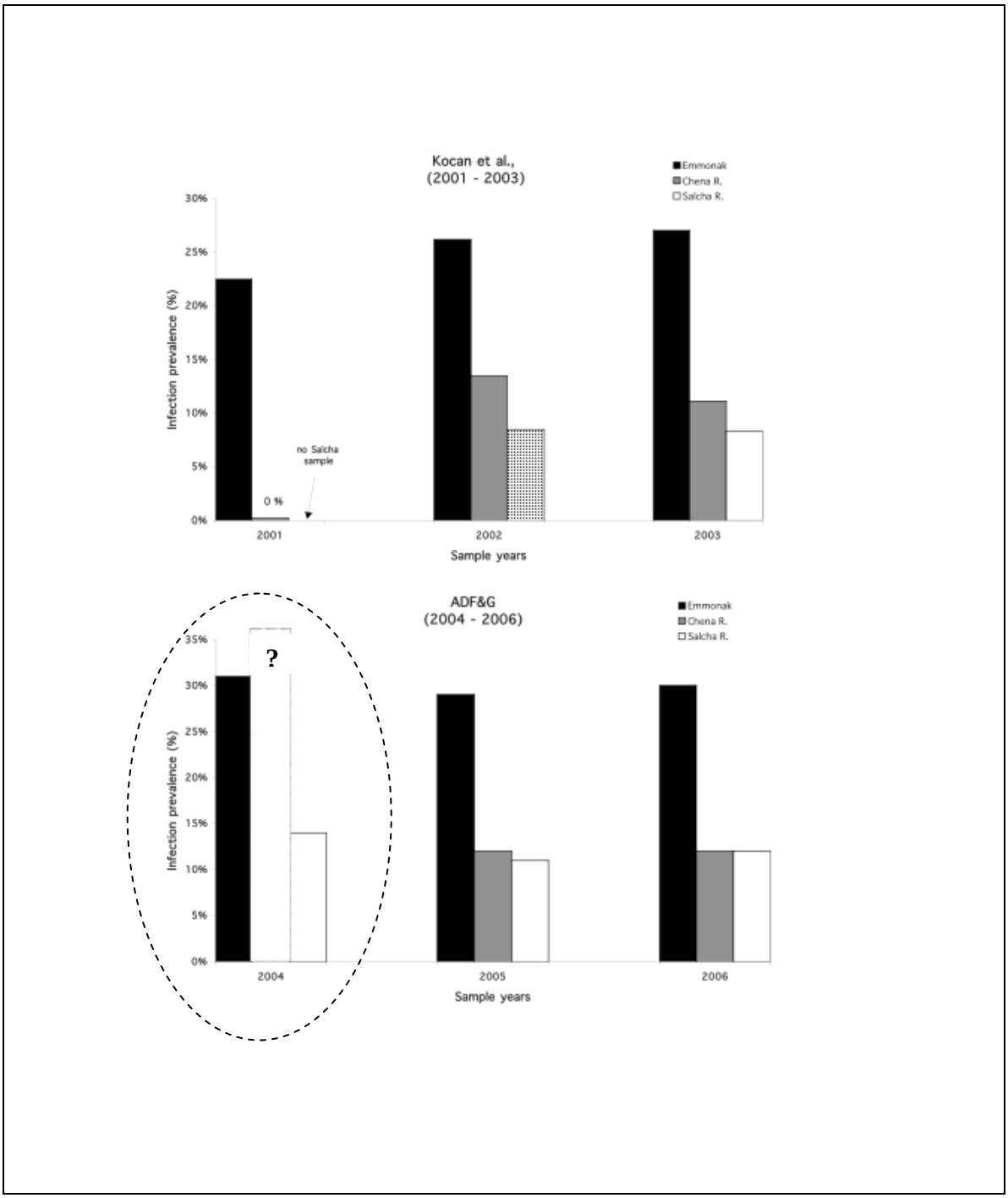

Figure 1. Loss of infected females between Emmonak and the terminal spawning grounds. (2001 – 2003; published data) and (2004-2006; ADF&G data). With the exception of the "unusual" high infection prevalence in the Chena River in 2004 (circled), all other years showed a significant decrease in the number of infected females reaching the spawning grounds in both the Chena and Salcha Rivers.

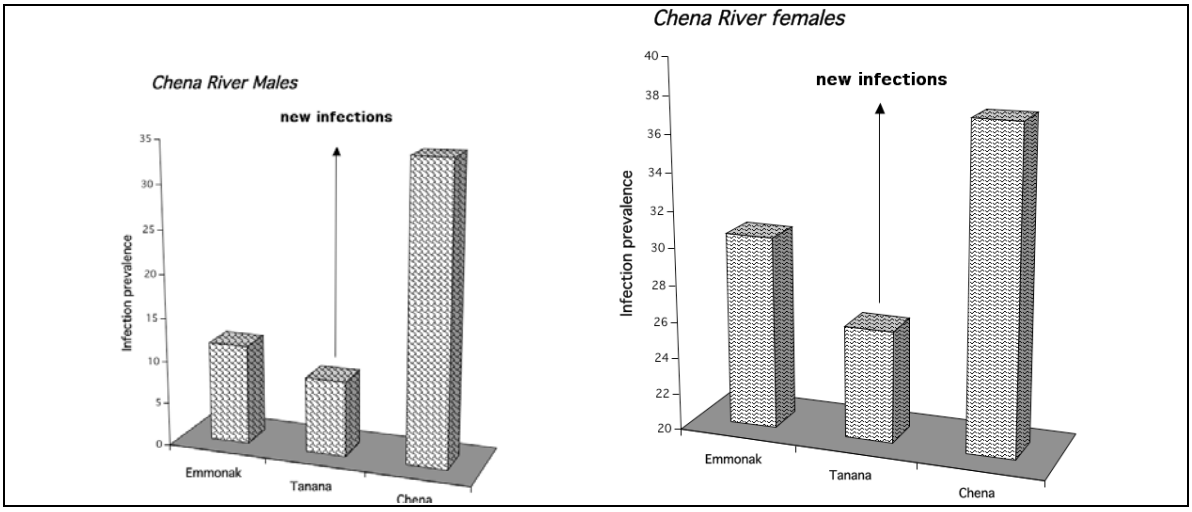

Figure 2. ADF&G data showing a highly improbable increase in *Ichthyophonus* infection prevalence in post-spawn Chinook salmon from the Chena River in 2004 relative to the Yukon and Tanana mainstem. This increase did not occur in Salcha River fish.

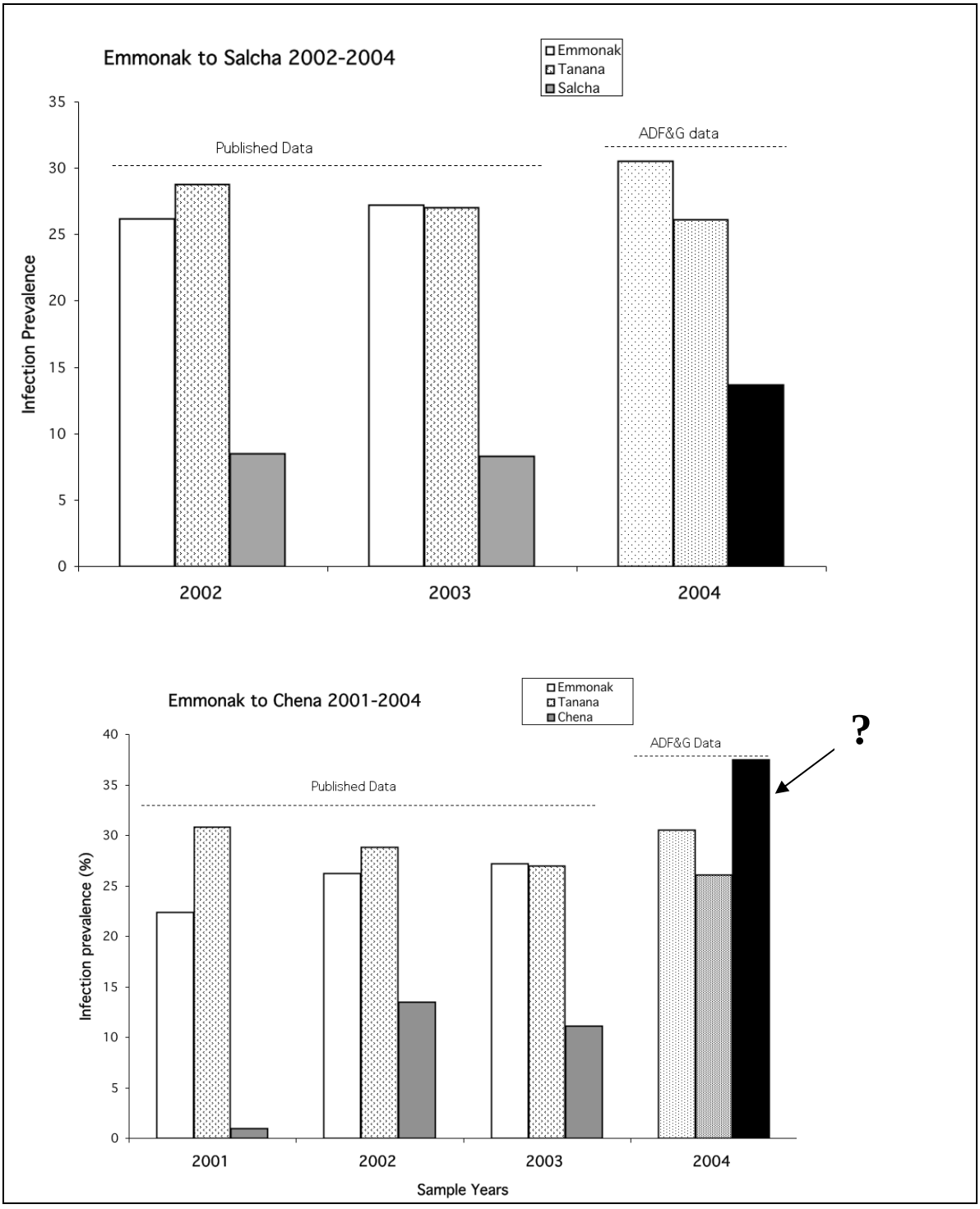

Figure 3. Comparison of *Ichthyophonus* infection prevalence in post-spawn females from 2002 – 2004 (Salcha River) and 2001 – 2004 (Chena River) relative to the Yukon-Tanana mainstem, showing a significant loss of infected females in every year with the exception of Chena River females in 2004.