Pacific Salmon Foundation, 300 – 1682 West 7th Avenue, Vancouver, BC V6J 4S6

Dr. Carmel Lowe,
Region Director of Science, Pacific Region
Department of Fisheries and Oceans,
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RE: Centre for Science Advice Pacific, report on PRV – jaundice study review, June 2018

July 30, 2018

Dear Dr. Lowe:

As a partner in the Strategic Salmon Health Initiative undertaken by Genome BC, DFO Science and the Pacific Salmon Foundation, I support having the best possible scientific review of our SSHI findings. However, I must address concerns over a recent internal report that presents DFO Science Advice in response to a request submitted by DFO Aquaculture Management Division. I am referring to an evaluation of the relevance of the Di Cicco et al. 2018[1] publication to the testing and management of PRV in British Columbia; signed by yourself on June 27, 2018. My concern for this process/report is not the request for regulatory advice, but for the review process and exclusion of the primary authors or any independent reviewers.

During my career within DFO, I don't recall such a rapid response process, but the process is now clearly presented on the CSAS/SCCS website ((http://www.dfo-mpo.gc.ca/csas-sccs/process-processus/srp-prs-eng.htm). Unfortunately, the minimum standards described in these guidelines have not been adhered to in this example; and are at odds with government commitment to open, objective and transparent science. Further, in non-government circles, this will again be criticized as contrary to a precautionary approach.

Most concerning to me, was that no advice on the relevance of the study findings to PRV testing and management in BC, was requested from the DFO-affiliated researchers that authored the Di Cicco et al. 2018 study. The study authors were not informed of this review and they were not provided the opportunity to respond to criticisms of their study methods. This is more troubling since the CSA report is internal and does not receive peer review like the publication in question did. Since I received the CSA report for persons outside of DFO Science, I must assume the criticisms and recommendations in the report are now in the public. Certainly, the reports has now unfairly led to targeted comments in the media about the Di Cicco et al. paper and researchers involved.

[1] Di Cicco, E., et al. 2018. The same strain of Piscine orthoreovirus (PRV) is involved with the development of different, but related, diseases in Atlantic and Pacific Salmon in British Columbia. FACETS 3: 599-641.

Consequently, I have asked the authors to comment on the review content and I provide a summary of their comments for your consideration (attached). Following the SSHI report on PRV/HSMI in Atlantic salmon in 2017, the logical next question for people was the risk of PRV to Pacific salmon. The Di Cicco et al. (2018) study of cultured Chinook salmon using audits samples already assessed by DFO, demonstrates the susceptibility of Chinook salmon to PRV and its expression as Jaundice/anemia. Given these observations and the potential risks to Pacific salmon, and the comments attached, I submit that the recommendations in the CSA review should not be accepted and that a more thorough scientific review is necessary and appropriate.

Yours Truly,

Brian E Riddell, PhD.

CEO/President, Pacific Salmon Foundation, and

Project Co-Lead, Strategic Salmon Health Initiative

Cc. R. Reid, RDG Pacific DFO

S. Greenwood, Genome BC

J. Hudson, Chairman, PSF

Attachment: Scientific Reply to comments in Science Advice Pacific, report on PRV – jaundice study review, June 2018

Scientific Reply to comments in Science Advice Pacific, report on PRV – jaundice study review (submitted June 2018)

Comments are aligned by the four categories described in the CSA reply, July 29, 2018

1) **Use of Modified clinical definition of jaundice.**

We are aware of the definition provided by Garver et al. (2016) but these authors only utilize "yellow discolouration of the abdominal and periorbital region" and "pale livers". As jaundice/anemia has received little study to date, there is no standard case definition of this disease, and hence the Di Cicco et al (2018) study utilized the clinical manifestations described in similar diseases (HSMI-like and EIBS) caused by PRV (cause and effect now established for both of these diseases) in Pacific salmon worldwide (a table of publications and manifestations is available if desired). Given the lack of sufficient study of this disease in BC, this broader clinical definition of jaundice/anemia enabled the analysis of samples at a potentially earlier stage of disease development. Moreover, in most cases, multiple fish within a farm were classified with combinations of these clinical signs. In fact, the study showed that the pathological lesions in fish classified as jaundice through the DFO Audit program were highly overlapping with those carrying our broader range of clinical signs, with the same pattern of PRV localization within the regions of damage.

In the paper, we lay out both the clinical signs and the pathological findings that define jaundice/anemia and their linkages with findings in studies worldwide. We show that 6 of 34 farms carry multiple Chinook salmon affected by PRV-related disease (17.6%), with three farms where PRV-related disease was the only known contributor to mortality (hence 8.3% of farm audits). To put this into context, a farm-wide diagnosis (at least two of the sampled fish diagnosed with a specific disease) of Furunculosis (*Aeromonas salmonicida*) occurred in 1 of 34 farms, *Loma salmonae* – branchitis in 5 farms, *P. salmonis* (piscirickettsiosis) in 1 farm, BKD/Rs infection in 10 farms (many of these with only 2 fish impacted). Most remaining farms were not diagnosed specifically or diagnosed with unknown diseases. Hence, the farm-level occurrence of jaundice/anemia as the primary source of mortality on three farms is well within the range of occurrence of other diseases known to be important sources of mortality in farm audits (Piscirickettsiosis, Furunculosis, Loma, and BKD), hence not inconsequential, as Johnson/Higgins conclude.

2) Previous studies examining PRV as a causative agent of Jaundice Syndrome in Pacific salmon have not been considered.

To the contrary, the authors are fully aware of the Garver et al. (2016) paper, the only citation to previous studies provided in Higgins and Johnson (2018). As outlined below, this study is an outlier to the worldview on the role of PRV in disease development and suffers several flaws that render its conclusions uncertain. Higgins and Johnson (2018) also state that Di Cicco et al. (2018) did not use "standard well accepted approaches to study disease." In fact, histopathology, which is considered the "gold standard" in disease diagnostics, played a strong role in the Di Cicco study and was led by two histopathologists, one of which, Dr. Ferguson, is a world renowned pathologist who was among the first

to describe HSMI in Europe. We note that histopathology had a significantly weaker role in the Garver et al. (2016) study (see below). The Di Cicco study also employed in situ hybridization, a technique that is on the cutting edge merging molecular and histopathological techniques to visualize pathogens, and has often been applied in medical research. Finally, the molecular VDD panel to recognize early stages of viral disease was, in fact, first developed and validated for diagnosis of human influenza, but is completely novel to studies in fishes. The combinations of these tools was paramount in providing the first mechanistic hypothesis to explain how the same virus could cause an inflammatory disease in one species and a necrotic disease in another.

The Garver et al (2016) paper has been used by industry and DFO as the key piece of evidence that PRV does not cause disease in BC salmon (Atlantic or Pacific) as the study claimed that they did not produce HSMI or jaundice syndrome from PRV infected tissue. However, this result now runs counter to the majority of evidence around the world that PRV does cause disease, including evidence reported under the Strategic Salmon Health Initiative (SSHI) being conducted in partnership with other DFO Science staff. The authors of Di Cicco et al. (2018) chose to focus on their research findings and place them into context by the evidence worldwide, but given the criticism by Higgins and Johnson, it seems necessary to point out issues in the Garver et al. (2016) study that render its conclusions of no disease inconclusive, and unjustified as the basis of advice to regulators.

a. The definition of "disease" used in the study required death as an endpoint, or evident clinical signs, before they performed any assessments of pathology. But in other research trials linking PRV with HSMI in Norway and elsewhere around the world (where pathological lesions have been recapitulated), clinical signs have rarely been observed and death has never been shown in laboratory studies, including the Wessell et al. (2017) study recognized around the world as definitively establishing a cause and effect relationship between PRV and HSMI in Atlantic salmon. Hence it is unclear why BC researchers would expect that laboratory challenges in BC salmon should include mortality as the endpoint rather than the pathological lesions that are the standard elsewhere around the world.
b. Because the study required death as the endpoint to study, they did not perform pathological investigation until the end of the study, 22 weeks post challenge. All other challenges performed on this virus have shown disease manifests between weeks 7-12, and recovery thereafter. Hence this study likely would have missed the peak disease window, something that the Norwegian experts have already pointed out; most recently at the PRV-HSMI workshop put on by the BCSFA (November 2017).
c. Despite the above concerns, the analyses reported in Garver et al. did in fact, identify lesions consistent with jaundice/anemia (and related diseases EIBS and HSMI-like around the world) in their description of histopathology, especially for Chinook salmon (hepatocellular cytoplasmic iron-rich pigment granules (87% affected), renal erythrophagocytosis (87% affected), hepatocellular cytoplasmic vacuoles (33%), leucocytic hepatitis (33%), renal tubular cytoplasmic protein droplets (20%), renal glomerular protein deposits (20%), myocardial karyomegaly (20%) and lymphohistiocytic endocarditis (60%), all exclusively observed in challenged fish.). However, they discounted all of these findings, stating "because the study included only four control fish,

> confidence intervals around control fish prevalence values are large." Hence, the study was acknowledged as having very limited power to evaluate whether pathological evidence of disease developed in the challenge fish relative to controls.

These lesions and the design limitations (only mentioned in the results section), do not support the conclusive nature assigned to this one study. Further, since this publication, we are also aware of subsequent research between Dr. Garver and Norwegian scientists that have demonstrate infection and disease expression in Atlantic salmon using BC variant PRV. Perhaps more importantly, the weight of evidence world-wide is that PRV does cause disease in Atlantic and Pacific salmon, which certainly supported the authors (Di Cicco et al. 2018) assessment of risk in British Columbia. This broader view of the evidence was not considered in the review by Higgins and Johnson (2018).

Higgins and Johnson also state that Di Cicco et al. (2018) failed to cite an unpublished Chinook and Coho PRV challenge trial in Washington State. Being unpublished and therefore not peer reviewed, there is no merit to their statement.

3) Evaluation of viral disease development is unsupported.

It should be noted that there are strong alternative opinions to that expressed by Higgins and Johnson. An editorial included in the journal in which the VDD paper was published stated:

> "The implications of this new method *(referring to the VDD paper)* are profound. Researchers can now diagnose viral infections in salmon using a tiny, non-invasive biopsy that leaves fish unharmed. This method also allows for early detection of viral infections, which means timely treatment will be possible in farmed populations. Wild populations will benefit as well. With a better understanding of the viruses that impact wild fish, scientists can start tracing the origins of these diseases with the hopes of eradicating them in the future. Indeed, this new methodology couldn't have come at a more critical time, since viruses are predicted to have a greater impact on fish populations as water temperatures increase in the future." [2]

The VDD tool is novel but has been validated in Miller et al. (2017). Moreover, the use of the VDD panel on farmed and migratory salmon, coupled with DNA sequencing, has led to the discovery of eight new viruses in BC salmon, some of which are fairly prevalent and others fairly rare. In this study, the VDD panel was applied to simply differentiate fish that were mere carriers of PRV versus those showing molecular signs of disease. This application is similarly, but much more specific in its association, to studies that utilize Mx expression to denote when an individual becomes responsive to a virus (used in the Garver et al. 2016 study in fact).

As there has considerable debate on why PRV can be carried at high loads in Atlantic salmon with no evidence of "disease", the Di Cicco study utilized this tool on PRV infected fish as an initial measure to differentiate fish that were truly not responding to a viral infection (i.e. no disease) from those

[2] T.D. Laubenstein. 2018. Conservation Physiology 5: doi: 10.1093/conphys/cox051
https://academic.oup.com/conphys/article-abstract/5/1/cox051/4101674

that were at various stages of disease development, including those with clinical signs/diagnosis of jaundice. By application of this approach, the team could begin to unravel a mechanistic understanding of the two diseases, and were able to show that for both Atlantic and Chinook salmon, when the VDD was not stimulated, the virus was not observed outside of red blood cells (RBCs) but when it was stimulated, there was evidence of the virus infecting other tissues. In addition, in Chinook salmon, for fish classifying as VDD, there was also evidence of RBC lysis, which the authors hypothesize is the key factor that leads to the development of necrotic lesions in this species. Hence, the novel approach was able to describe, for the first time, how the same virus could potentially cause two divergent but related diseases, with results consistent with the world-view of PRV-related diseases.

4) Data insufficiently described/not presented

The study provided explicit information on all samples with moderate to high copy numbers of PRV, and showed a figure (1) to support this representation, by copy number. In Figure 1, we also show which fish were designated "Jaundice positive". However, as the paper focusses on disease rather than on the mere presence of the virus, and the proportions of PRV infected audit fish were previously reported (Miller et al. 2017), the Di Cicco study did not go into details again on high background levels of PRV, but certainly this is presented in the discussion.

For the in situ analysis, which is quite expensive, the study attempted to balance the numbers of fish assessed in each category. As the goal of the study was to document the progression of the disease(s) and whether there was evidence of PRV involvement/localization during this progression (i.e. does the virus track the location of the lesions), we did not need to examine every fish available. Instead, we attempted to simplify the dataset by removing some of the fish diagnosed with other diseases (e.g. one farm with a high incidence of BKD), but of course as these were all dead-sampled fish, this made it difficult to find truly clean fish as a comparator. However, we presented all of the lesions present in fish utilized in the in situ analysis for our study, and highlighted those that were localized consistently with the virus. These turn out to be the lesions that have been classically associated with PRV-related diseases in Pacific salmon worldwide. Furthermore, by contrasting our results in the less studied Pacific salmon with those from the more highly characterized disease HSMI, we were able to show that the same pattern of co-localization of virus occurred in both diseases.

It is important to note that nowhere in their review did Higgins and Johnson even mention the in situ analysis, which is the workhorse and key innovation that brings the Di Cicco (2018) study together, and shows very clearly the intimate relationship between PRV localization and lesion development in BOTH HSMI and Jaundice/anemia in all of the fish that were studied (regardless of whether the AMD veterinarian recognized jaundice syndrome as a disease). A rigorous review of the science would have included an alternate explanation for the in situ findings in both species if they wish to continue to contend that PRV is a bystander virus of no impact.