Centre for Science Advice Pacific

FPP non-CSAS Request for Rapid Science Response

Request Information

Request Contact:	Allison Webb	Project Type: Aquaculture-Emergency response
Date of request:	June 14th, 2018	
Region of proposed impact:	British Columbia Habitat Type: Coastal	
Purpose of request:	PRV-Jaundice study Review	
Potential affected species:	Pacific salmon	
Date required:	June 21st, 2018	Request #:2018AQU03
Timeline rationale:		

Project Overview

Piscine Reovirus (PRV) and its relationship to diseases such as HSMI (Heart and Skeletal Muscle Inflammation) and Jaundice are highly topical issues given the growing body of scientific inquiry in this area; concerns being raised by the public, academics and stakeholders regarding PRV; potential risks to wild Pacific salmon; and potential implications for DFO's aquaculture management approach (including salmon transfers).

In British Columbia (BC), aquaculture licence holders must have authorization under the *Fishery (General) Regulations* prior to moving aquatic organisms. Licence holders move fish between land-based hatchery facilities, from hatchery facilities into rearing facilities in the marine environment, and between rearing facilities within the marine environment. The BC Introductions and Transfers Committee (ITC)—which includes representatives from DFO Science Branch, DFO Aquaculture Management and the Province of BC—receives applications to move aquatic organisms within BC and assesses the potential genetic, ecologic and fish health impacts of these proposed movements to fish and fish bearing habitat. For transfers of salmon between marine finfish sites, only DFO Science Branch and Aquaculture Management Division review the ITC applications as there is no provincial authorization required for these movements. DFO does not require any pre-transfer testing for specific diseases or disease agents such as PRV and/or HSMI before being transferred from hatcheries to aquaculture sites in the marine environment or before being transferred between marine aquaculture sites. However, as part of salmon transfer application review, DFO assesses the overall fish health at the source facility by examining company fish health records, any fish health and/or mortality reporting submitted as part of aquaculture licence condition requirements, and results from DFO farm audits.

Given the profile of PRV and the pace of new scholarship in this area, it is important that DFO consider new science information as it becomes available, including implications of that science for its current management approach (e.g. salmon transfers, fish health management). DFO completed a review of recent scholarship related to PRV in March 2018 but since then, there has been at least one new paper published in the primary peer reviewed literature on PRV:

Emiliano Di Cicco, Hugh W. Ferguson, Karia H. Kaukinen, Angela D. Schulze , Shaorong Li, Amy Tabata, Oliver P. Günther, Gideon Mordecai , Curtis A. Suttle and Kristina M. Miller. 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. *In press*

Aquaculture Management Division is requesting a review of this paper and other recently published studies not yet reviewed to ensure that DFO's testing and fish health management approach are informed by the latest scientific evidence and to determine whether changes to our management approach should be considered as a result of this new information.

It is requested the review consider and address the following questions:

1. How does this recently published paper, and other recently published papers not yet reviewed alter the scientific perspective on the role of PRV in the development of disease?
2. Given the recent review (DFO, 2018), how is this study relevant (or not relevant) to the testing and management of PRV in British Columbia?

1ST QUESTION

Context:

In this response we limit discussion primarily to the evidence presented in Di Cicco et al. (2018) for a link between infection with PRV to development of Jaundice Syndrome in farmed Chinook Salmon. Links between infection with PRV and Heart and Skeletal Muscle Inflammation (HSMI) in Atlantic salmon were the subject of a previous informal response and the perspective presented in that response remains valid. At this time, we are unaware of any additional science that relates to the role of PRV in the development of other diseases of relevance to the testing and management of PRV in British Columbia.

Objective/Questions:
How does this recently published paper, and other recently published papers, alter the scientific perspective on the role of PRV in the development of disease?

Importance: ☒ Essential ☐ Important ☐ Desirable

SCIENCE RESPONSE

Response:

The paper by Di Cicco et al. (2018) provides information and related inferences/interpretations on the impacts of PRV in BC farmed Chinook salmon and expands on the previously reported HSMI diagnosis. As outlined below, our detailed review of this manuscript reveals deficiencies with the data presented and the criteria used to characterize jaundice disease that render the conclusions the authors draw from these data and analyses unsupported. In addition, we note that they fail to consider previously published information that has direct relevance to the role of PRV in the development of Jaundice Syndrome. For these reasons, altering the current scientific perspective on the role of PRV in the development of disease is not recommended.

1. Use of a modified clinical definition of jaundice.

Di Cicco et al. (2018) used samples of recently dead Chinook salmon (fresh silvers) from DFO's audit program collected from 2011 to 2013 to analyze for presence of jaundice. Aquaculture Management Division's Fish Health Audit and Surveillance Program (FHASP) assigns a diagnosis of Jaundice Syndrome in a farmed Chinook population when there is "an elevated mortality rate with a substantial proportion of the carcasses presenting the characteristic yellow discoloration of the skin of the abdominal and periorbital region" (Waddington, personal communication). A summary of audit records from 2011-2013 indicated that of a total of 245 Pacific salmon examined only 3 animals (1.2% of the samples) were diagnosed by the Program veterinarians as having Jaundice Syndrome. All 3 of these diagnoses were made in 2011.

However, Di Cicco et al. (2018) utilized a significantly broader suite of signs drawn from notes made by the attending veterinarian at the time of collection to re-classify the archived samples in the audit program. Namely:

> "Chinook Salmon were classified as "jaundice/anemia" if the veterinary diagnostic comments indicated "Jaundice Syndrome" or "jaundice - no agent", and/or the gross lesions indicated "yellow fluid" or "yellow bile"

or "yellow bile like fluid" noted in the peritoneal cavity or on the pyloric caeca and/or liver. Two fish with pathological notes containing either "(inflammatory) lesions in heart or spongy layer only – CMS-like" or "acute renal tubular necrosis, suggestive of viral infection" were classified as "jaundice", as these are also features of jaundice/anemia."

As a consequence of this broader classification scheme, Di Cicco et al. (2018) characterized three times as many fish, i.e. 9 (3.7 % of the samples available to their study) of Chinook salmon as showing signs of a new disease which they refer to as "jaundice/anemia". The authors do not provide a clear description of why they broadened the definitions associated with jaundice/anemia, nor do they discuss how lesions they considered compare to those described for Jaundice Syndrome by Garver et al. (2016). Further, they do not acknowledge that several of the characteristics they included in their definition of jaundice/anemia are shared with other diseases (which leads to bias in their conclusions) and provide little consideration of the role of other pathogens (e.g. Erythrocytic Necrosis Virus – see point 3 below).

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

Di Cicco et al. (2018) propose a cause-and-effect relationship between infection with PRV and the development of jaundice/anemia yet present no direct evidence to support this. In addition, they do not consider the findings (summarized below) of previously published studies that have examined the relationship between PRV and the development of jaundice in Chinook salmon.

High loads of PRV in farmed Chinook salmon with Jaundice Syndrome have been previously reported in the literature (Garver et al. 2016). In this study, 10 Chinook salmon with Jaundice Syndrome were collected from 2 farms in Clayoquot Sound near Tofino over 2 years. Fish were classified with Jaundice Syndrome based on clinical signs (i.e. with characteristic yellow discoloration of the skin of the abdominal and periorbital region) and were found to be positive for PRV. Tissues from these fish were homogenized and injected into naive Chinook, Sockeye, and Atlantic Salmon in an attempt to recreate Jaundice Syndrome in these fish. Examination of fish after 22 weeks showed no gross or histological evidence of Jaundice Syndrome, although all of the challenged fish tested positive for high levels of PRV. More recently challenge trials carried out in Washington State examined the relationship between infection with PRV and the development of a condition referred to as Intra-erythrocytic Inclusion Bodies Syndrome (EIBS). This syndrome is characterized by anemia and the presence of inclusion bodies in red blood cells. Challenge of naïve Coho and Chinook Salmon with tissues from fish positive for PRV but not showing signs of EIBS did not cause anemia or inclusion bodies in red blood cells (see http://bcsalmonfarmers.ca/wp-content/uploads/2018/04/BCSFA-PRV-HSMI-Workshop-Cover-and-Report-Mar-29-2018.pdf). These trials indicate that even though a potential linkage between PRV presence in the occurrence of HSMI has been determined, presence of PRV does not guarantee the development of HSMI or Jaundice Syndrome in Chinook, Sockeye, Coho, or Atlantic Salmon.

3. Evaluation of Viral Disease Development is unsupported.

The authors state that individuals infected with PRV and identified as in a "viral state" by VDD biomarkers (VDD biomarkers represent a molecular categorization tool developed by Miller et al. (2017) and reviewed in a previous informal response) represent a fish that is destined to develop jaundice/anemia and die. Yet, to our knowledge the prognosis for fish which have been identified as being in a "viral disease state" based on the application of the VDD biomarkers has not been established.

Further, Di Cicco et al. (2018) note that "Samples *potentially* representing different stages of development of jaundice/anemia in Chinook Salmon" were selected to provide some insight as to how the jaundice disease progresses and lesions develop in Chinook Salmon. To accurately assess fish in varying states of disease progression, it is necessary to examine live, moribund and recently dead fish over time – yet, the Di Ciccio et al. (2018) study only examined samples that were at the same point in disease progression (i.e. recently dead) and as such, their assessment of disease progression is unsupported.

It is also important to note that mixed pathogen infections were detected in the Chinook salmon examined by Di Ciccio et al. (2018). These infections included Erythrocytic Necrosis Virus (ENV) which was present in 4 out of the 9 fish they classified as having "jaundice/anemia". Three individuals classified as jaundice/anemia contained high loads of ENV, however Di Cicco et al. (2018) concludes that ENV was unlikely to have contributed to their molecular classification (VDD signature). It is noteworthy that Di Ciccio et al. (2018) do not discuss the potential of ENV to contribute to the histopathological response they associate with jaundice/anemia.

4. Data insufficiently described/not presented.

The authors do not explicitly state what proportion of the Chinook salmon audit samples were infected with PRV and provide no data on the proportion of farmed Chinook salmon carrying PRV or the loads of PRV in apparently healthy fish. This is essential information to support their conclusions on the importance of PRV load in the development of jaundice/anemia or other diseases in Chinook salmon.

For example, the authors state in the conclusions that "The prevalence of PRV in farm audit samples of Atlantic and Chinook salmon is between 65-75% overall, with approximately 25% of salmon carrying PRV presenting with high viral loads" but none of these data are discussed or quantified, nor do the authors provide a definition of "high viral loads" in the body of the paper. Our visual assessment of Figure 2b in Di Cicco et al, (2018) indicates that approximately 21% of the farmed Chinook audit samples had PRV loads >1000 copies, but we note that many of these samples are excluded from their study without explanation.

Summary:

Di Cicco et al. (2018), demonstrated that PRV is found in association with a variety of different types of lesions in a small sample of recently dead Chinook Salmon. Di Ciccio et al. (2018) conclude that PRV is "*likely* also to be the cause of jaundice/anemia in farmed Chinook salmon". However, theirs is not a cause and effect study, but rather a retrospective analysis of archived FHASP audit samples. The authors failed to consider the findings from important published cause and effect studies, including: 1) Jaundice Syndrome challenge trials reported in Garver et al. (2016), which injected tissues from Jaundice Syndrome fish but failed to cause jaundice, although PRV was transferred, and 2) Chinook and Coho PRV challenge trials conducted in Washington State which similarly did not result in anemia or other signs of disease. Di Cicco et al. (2018) included a larger number of disease signs, many of which are shared with other diseases known to occur in Pacific Salmon, to establish a new disease which they referred to as "jaundice/anemia". By doing this the number of fish within the audit samples that are identified as having jaundice relative to the AMD's audit data is increased threefold.

2nd QUESTION

Given the recent review (DFO, 2018), how is this study relevant (or not relevant) to the testing and management of PRV in British Columbia?

SCIENCE RESPONSE

Response:

It is important to recognize that this study is not based on cause and effect, but on a set of archived samples of Chinook salmon collected as part of the FHASP. The authors developed their own set of clinical signs which they refer to as jaundice/anemia which increased the number of samples for use in their analysis to 9. However, according to the FHASP description of Jaundice Syndrome, only 3 fish out of 245 over 3 years were classified with this disease. Regardless of the clinical signs used, jaundice in farmed Chinook salmon on the west coast of British Columbia is uncommon, yet this is not discussed nor put into the context of overall factors leading to mortality of farmed Chinook Salmon. Furthermore, the authors do not demonstrate why the disease is observed in some PRV infected

fish and not others.

The statement in the paper's abstract that "Chinook salmon may be at more than a minimal risk of disease from exposure to PRV occurring on salmon farms" is not substantiated. Di Cicco et al. (2018), in summarizing the results, state that "Given that PRV-1a is the cause of HSMI in farmed Atlantic salmon, and likely also to be the cause of jaundice/anemia in farmed Chinook salmon, … illustrates that there may be very real risks associated with PRV transmission from farmed salmon (in which PRV is highly prevalent) to wild Pacific salmon". However, this statement is subsequently qualified with *"The severity and extent of those risks still remain elusive…"*. We note that the magnitude of the risk of disease was not examined in the study nor should it have been reported given the quantity and quality of the data they utilized.

References

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Responder: Mark Higgins, Science

Responder: Stewart Johnson, Science

Review Information

This response does not constitute delivery of peer – reviewed Science advice; it is intended as a rapid response to an immediate requirement for Science input.

Reviewed by: Lesley MacDougall, Coordinator, Centre for Science Advice Pacific Region

Date: June 21, 2018

Comments:

Approved by: Catherine Lamb

Date: June 27, 2018

Comments: