Responses to Pathogen Infections in River-deployed Juvenile Fall-run Chinook Salmon (*Oncorhynchus tshawytscha*)

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Introduction

- Infectious disease is one of many factors contributing to the decline of wild salmonid populations[1]
- Salmonids are under constant exposure to multiple pathogens in the aquatic environment and have subsequently developed immunological coping mechanisms[2]
- Major knowledge gaps remain in our understanding of the occurrence of co-infections and their impact on wild salmon populations
- We hypothesized that deployed salmon would commonly be infected with multiple pathogens, resulting in complex host-pathogen dynamics and potentially affecting overall health

Objectives

A. Determine the prevalence and abundance of eight pathogens in gill and kidney tissue using quantitative PCR (qPCR)

B. Determine the consequences of pathogen exposure by examining expression profiles of 11 genes related to immune response, stress, and development using qPCR

Materials & Methods

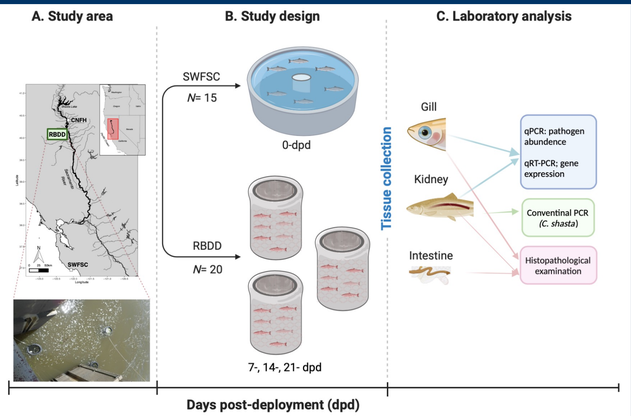

Fig. 1:

A) Map of the study area and fish facilities - Coleman National Fish Hatchery (CNFH), Red Bluff Diversion Dam (RBDD), and the Southwest Fisheries Science Center (SWFSC)

B) Fish (N=15) were sampled at SWFSC as reference samples on day 0, and (N=20) at 7-, 14-, and 21-days post deployment (dpd) at the RBDD

C) Pathogen presence and host response were assessed by a combination of qPCR and conventional PCR with supporting histopathological evaluation

A. Pathogen Screening:

- Three parasites of concern (***Ceratonova shasta, Parvicapsula minibicornis* & *Ichthyophthirius multifiliis***) and two bacteria (***Flavobacterium columnare* & *Rickettsia*-like Organism)** were detected in deployed fish at RBDD in the Sacramento River **(Fig 2)**
- *C. shasta* detection in kidney was relatively consistent with the detection in intestine, the parasite's primary infection site **(Table 1)**

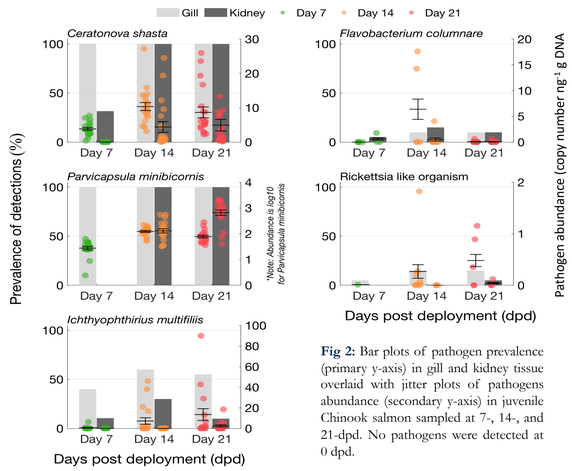

Fig 2: Bar plots of pathogen prevalence (primary y-axis) in gill and kidney tissue overlaid with jitter plots of pathogens abundance (secondary y-axis) in juvenile Chinook salmon sampled at 7-, 14-, and 21-dpd. No pathogens were detected at 0 dpd.

Table 1: *C. shasta* in intestinal tissue. Amplicons were visualized by gel electrophoresis, PCR result was given as negative ("-" no band), weak positive ("w+" band weaker than standard ladder), positive ("+" band about same intensity as ladder) and strong positive ("++" band much brighter than ladder).

C. shasta	Negative	Weak	Intense
0 dpd	15/15	0	0
7 dpd	9/20	11/20	0
14 dpd	0	0	19/19
21 dpd	0	0	20/20

B. Host Gene Expression (Fig 3):

- Gene expression followed a different pattern in gill compared to kidney tissue
  - **Gill:** ↑ TNFα, IL-1β , SAA , and IL-8
  - **Kidney:** ↑ TNFα, and IL-6
  - **Both:** ↑ IL-10
- IgT and the systemic immunoglobulin IgM were shown to be upregulated in response to pathogens infections
- BDNF showed no significant difference in gills, however, kidney showed a significant upregulation …..Remodeling?

Principal component analysis (PCA; Fig 4):

Gill: PC1 separated fish sampled at 0-dpd from deployed fish sampled at 7-, 14-, and 21-dpd

Kidney: PC 1 separated fish sampled at 0-, and 7-dpd from those at 14- and 21-dpd

Results

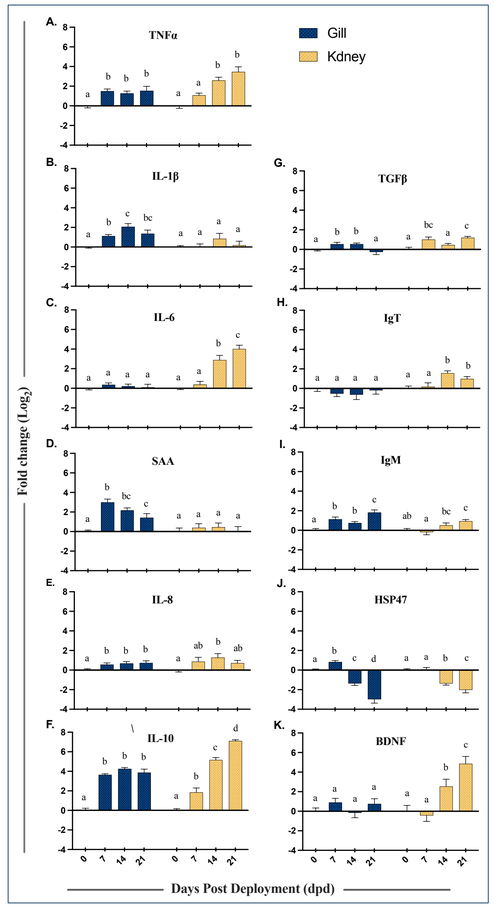

Fig 3: Gene expression at 0-, 7-, 14-, and 21-dpd in **gill** and kidney tissue presented in log_2 fold changes of selected genes: Pro-inflammatory genes: **A)** tumor necrosis factor α, **B)** interleukin-1β, **C)** interleukin-6, **D)** acute phase protein serum amyloid A, **E)** interleukin-8, Anti-inflammatory genes: **F)** interleukin-10, **G)** transforming growth factor β, Adaptive immune genes: **H)** immunoglobulin T, **I)** immunoglobulin M, General stress gene **J)** heat shock protein 47, and Development; **K)** brain derived neurotrophic factor. Lowercase letters denote significantly different ($p < 0.05$) means (±SEM). Gene expression was normalized against the housekeeping genes ACTβ1, GAPDH, and RPL7 and calibrated against fish sampled at 0-dpd.

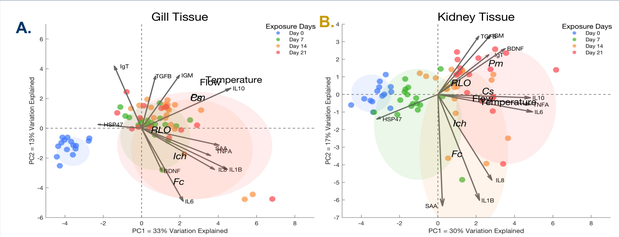

Fig 4: Principal component analysis (PCA) plot for gene expression profiles in the **A) gill** and **B) kidney** tissue for juvenile Chinook salmon sampled at 0-, 7-, 14-, and 21-dpd using log_2 fold-changes and pathogens; *C. shasta (Cs), P. minibicornis (Pm), I. multifiliis* (Ich), *F. columnare (Fc),* and *Rickettsia*-like organism (RLO) as copy number ng^{-1} gDNA.

C. Histopathological Examination:

- By 21-dpd, infection of the intestine by *C. shasta* and the kidney by *P. mimbicornis* was evident in all deployed fish, and was mostly accompanied with minimum to no histological signs of inflammation

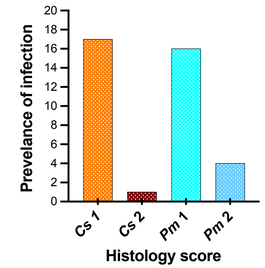

Fig 5: Prevalence of infection (number positive individuals) for *C. shasta* (*Cs*) in intestine, and *P. minibicornis* (*Pm*) in kidney specimens (1= infection with little disease, 2= disease state) at 21-dpd

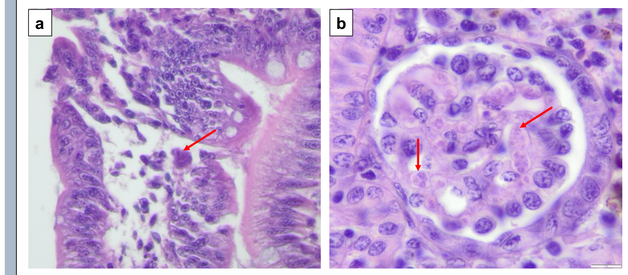

Fig 6: Hematoxylin and eosin-stained histological sections of intestine and kidney. Arrows: **a)** *C. shasta* trophozoite within lamina propria (Space is an artifact of processing), **b)** *P. minibicornis* trophozoites within glomerulus, the parasite's primary infection site, with a moderate degree of glomerulonephritis.

Conclusions

- Prevalence and infection load of the selected pathogens varied depending on the pathogen type, tissue examined and time post deployment, ranging from absent or rare (0–8%) to ubiquitous (100%)
- Altered expression of immune genes could be predictive of negative outcomes that are irreversible in the wild, posing a threat to juvenile Chinook salmon survival
- This study establishes the foundation for further investigation into the most prevalent infectious agents of out-migrating Chinook salmon in the Sacramento River and their potential pathogenicity
- Future research will also investigate roles of innate and adaptive immune genes during the early stages of infection

Literature cited

1. Kent (2011). Infectious diseases and potential impacts on survival of Fraser River sockeye salmon.
2. Lehman et al. (2020). Disease in Central Valley Salmon: Status and Lessons from Other Systems.

Acknowledgements

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