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Title: Viral survival and infectivity in effluent from a fish processing plant

Report by:

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Overview: Fish processing plant effluent has the potential to be a point source of organics and infectious agents. While organic material loads are relatively easily measured, assessing the infectivity of aquatic pathogens in discharged effluent can be challenging. Consequently, the risk of processing effluent as a point source in the spread of endemic aquatic pathogens is not easily quantified. In particular, viral agents known to be common to cultured salmon in British Columbia such as piscine orthoreovirus (PRV), has raised concern regarding the potential release of live virus through fish processing effluent. In this study, we examine the efficacy of a dissolved air floatation (DAF) treatment system installed at Brown's Bay Processing Plant in reducing the load of PRV in effluent generated from the processing of farmed Atlantic salmon infected with PRV.

Experimental Design:

Immersion exposure

Approximately eighty to one hundred liters of DAF treated effluent was collected every hour from 9 am to 2 pm on April 11, 2022 from Browns Bay Processing Plant while farmed Atlantic salmon were being processed at the plant. The collected effluent was transported in insulated totes to the Pacific Biological Station where it was acclimated to 10-12^{o}C overnight. The following morning, 200 liters of the DAF treated effluent was placed into a circular tank. In another tank, 100 liters of DAF treated effluent was mixed with 100 liters of saltwater (32 ppt, 10-12 ^{o}C). In each tank, 10 Atlantic salmon were immersed in the static effluent baths for 2 hours after which saltwater (32 ppt, 10-12 ^{o}C) was added to each tank at a rate of 7 liters per minute. Fish were monitored daily for 6 weeks at which time, all fish were euthanized and blood was collected and analyzed for the presence of PRV.

Injection exposure

Twenty milliliters of DAF treated effluent and twenty milliliters of untreated blood water, collected during the April 11, 2022 processing of Atlantic salmon, were each centrifuged at 400 G for 5 minutes. Post centrifugation, the aqueous phase was removed and passed through a 0.45 micron filter whereby the resulting filtrate were used as inoculums in the injection exposure groups. In one tank, ten Atlantic salmon received an intraperitoneal injection of 500 microliters of the DAF treated effluent while in a second tank ten Atlantic salmon received an intraperitoneal injection of 500 microliters of the blood water. The injected fish were placed in saltwater (32 ppt, 10-12 ^{o}C) and monitored daily for four weeks. At the end of four weeks, fish were euthanized and blood was collected and analyzed for the presence of PRV.

Screening for PRV

Blood was obtained from a caudle puncture using a 1 milliliter syringe and 22 gauge needle and transferred to a 1.5 milliliter microtube on ice. Total RNA was extracted from 100 microliters blood, untreated blood water and DAF treated

effluent in Trizol reagent (Life Technologies) as per manufacturer's instructions that implemented a 5 millimeter steel bead and TissueLyser II (Qiagen) operating for 2 minutes at 25 hertz. A portion of eluted RNA (1.0 μg) was denatured for 5 minutes at 95°C, immediately cooled to 4°C, and reverse-transcribed using a High-Capacity cDNA Reverse Transcription kit (Life Technologies) following the manufacturer's instructions. Resulting cDNA was used directly as template for qPCR analysis in a StepOne-Plus real-time detection system (Applied Biosystems) using previously described primers and TaqMan probe (Zhao et al. 2021). Samples were assayed in duplicate and were considered positive if both technical replicates reported a Ct value < 40 cycles, inconclusive if only one technical replicate reported a Ct value < 40 cycles, or negative if both technical replicates failed to fluoresce beyond the preset threshold (Δ Rn 0.01) in 40 cycles.

Results:

No mortality or external signs of disease were noted in any of the treatment groups over the entire duration of the experiment. Blood sampled from ten Atlantic salmon that remained unexposed to effluent proved negative for PRV demonstrating the stock of fish used in the effluent exposure experiment were free of PRV prior to exposure. Four to six weeks post effluent exposure, Atlantic salmon tested positive for PRV in the blood. A summary of PRV RT-qPCR screening results across all treatment groups is shown in Table 1.

Table 1. Number of PRV positive, inconclusive and negative RT-qPCR results in blood samples collected from Atlantic salmon either immersed or intraperitoneally injected with DAF treated processing plant effluent or blood water.

Route of Effluent Exposure	Treatment group	PRV RT-qPCR result (n=10/treatment)
Immersion	DAF treated effluent	5 positive 3 inconclusive 2 negative
	1:1 Diluted DAF treated effluent (1 Seawater: 1 DAF treated effluent)	4 positive 6 negative
Injection	Blood water	10 positive
	DAF treated effluent	4 positive 6 negative

Conclusion:

DAF treated processing plant effluent showed a 100-fold reduction in the load of PRV in comparison to untreated blood water. Nevertheless, the treated effluent did contain infectious PRV, as Atlantic salmon exposed to effluent either through a 2 hour static immersion or via an intraperitoneal injection, acquired PRV blood infections. Consequently, the DAF treatment process installed at Browns Bay Processing plant will require additional disinfection processes to eliminate infectious PRV in blood water generated during the processing of PRV infected Atlantic salmon.

References:

1. Zhao J, Vendramin N, Cuenca A, Polinski M, Hawley LM, Garver KA. Pan-Piscine Orthoreovirus (PRV) Detection Using Reverse Transcription Quantitative PCR. Pathogens. 2021 Nov 27;10(12):1548. doi: 10.3390/pathogens10121548. PMID: 34959503; PMCID: PMC8707331.