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Assessing bactericidal activity of peracetic acid to selected fish pathogens in RAS water

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By Christopher Good, DVM , Natalie Redman , Megan Murray , David L. Straus, Ph.D. and Timothy J. Welch, Ph.D.

Peracetic acid concentrations and exposure duration data for three key pathogens

Peracetic acid (PAA) has been identified as an efficacious alternative to common disinfectants used in aquaculture settings to reduce fish pathogen loads. Compared to other aquaculture disinfectants like formalin, PAA is considered relatively environmentally friendly, given that it degrades to acetic acid, oxygen and water when applied in an aqueous environment, and does not leave toxic residues. Additionally, PAA has been shown to be a useful therapeutic in recirculation aquaculture systems (RAS) due to its antimicrobial properties at low doses that, in turn, do not impair biofilter nitrification.

The effectiveness of PAA, however, is impacted to an extent by certain water quality characteristics. Given the unique culture environment of RAS, wherein numerous substances such as nitrogen species, waterborne metabolites, nutrients, suspended solids, fine particles, heterotrophic bacterial counts and dissolved metals accumulate over time before reaching a steady state, RAS producers need to consider their specific water chemistry characteristics when developing effective PAA treatment regimens.



This research studied the bactericidal activity of peracetic acid (PAA) to selected fish pathogens in recirculating aquaculture system (RAS) water and determined PAA concentrations and exposure duration data for the reduction of three important bacterial fish pathogens (*Yersinia ruckeri*, *Weissella ceti* and *Flavobacterium columnare*). This is valuable and practical information for RAS producers using PAA to reduce populations of bacterial pathogens – like this Columnaris disease caused by *Flavobacterium columnare* in the gills of a chinook salmon. Photo by U.S. Fish and Wildlife Service (public domain).

Previous studies have demonstrated the toxicity of peracetic acid to a range of fish pathogens, but little research has been devoted to evaluating the bactericidal effects of PAA on fish pathogens in RAS water. Considering the increasing adoption of RAS technologies in the global aquaculture industry, we sought to characterize *in vitro* PAA efficacy against three important bacterial fish pathogens in water with representative chemistry profiles of salmonid RAS production. The three selected bacterial pathogens were *Yersinia ruckeri*, *Weissella ceti* and *Flavobacterium columnare*; these pathogens are the causative agents of the diseases Enteric Redmouth disease, Weissellosis and Columnaris respectively. Columnaris and Enteric Redmouth Disease have long been identified as major diseases of fish, while Weissellosis is currently recognized as an important emerging disease in salmonid aquaculture, with outbreaks reported in China, Brazil, the United States, Mexico, Japan and Peru.

This article – adapted and summarized from the **original publication** (<https://doi.org/10.1111/are.16031>) (Good, C. et al. 2022. Bactericidal activity of peracetic acid to selected fish pathogens in recirculation aquaculture system water. *Aquaculture Research* 2022; 00:1–6) – reports on a study to determine the PAA concentration and exposure duration associated with complete elimination of specific pathogens, as determined by colony count reduction in water from a salmonid RAS.



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Study setup

To carry out PAA experiments within RAS water, initial water samples were collected in sterile 1-liter glass bottles from the culture tank side drain of an on-site experimental-scale freshwater RAS, as described in previously published studies. At the time of sampling, the RAS was operated at low exchange, was stocked with post-smolt Atlantic salmon (*Salmo salar*) reared at normal commercial densities and provided with a standard commercial feed at hourly intervals via automated feeders with feeding rates adjusted periodically based on observations related to satiation. Feed loading was approximately 800 liters of makeup water per kg of feed.

This RAS provided suitable water for successful experiments involving *Y. ruckeri* and *W. cети*; however, this same water hindered the assessment of *F. columnare*. Therefore, a second on-site RAS was used as a water source for this bacterium; it was identical in scale to the initial RAS but was operated at a higher flushing rate.

Based on preliminary range-finding experiments to determine the relative sensitivity of each bacterial pathogen to PAA, the following concentrations and exposure times were selected: 0, 2, 5 or 10 mg/L PAA for 0, 2, 5 or 10 minutes of exposure time for *Y. ruckeri* and *W. cети*, and 0, 1, 2, 3 or 5 mg/L PAA for 0, 2, 5 or 10 minutes of exposure time for *F. columnare*. Each selected concentration of PAA was applied to separate flasks of the prepared pathogen-RAS water suspension in triplicate. Samples were collected and analyzed. All microbiological work was carried out in a sterile biological safety cabinet. Plates were incubated at 30 degrees-C for 24 hours, after which colonies were enumerated and CFU/20 µl were calculated and graphed for each PAA concentration and exposure duration, for each pathogen.

For detailed information on the experimental design and analytical procedures, and statistical analyses, refer to the original article.

Results and conclusion

Complete reduction to 0 CFU/20 µl was achieved for all three bacterial pathogens examined. In the first experiment using low-exchange RAS water, *Y. ruckeri* was completely eliminated following 5 minutes of exposure to 5 mg per liter PAA, while *W. cети* was completely eliminated following 10 minutes of exposure to 10 mg per liter PAA (Fig. 1). In the second experiment utilizing water from the relatively high-exchange RAS, *F. columnare* was completely eliminated with 3 mg per liter PAA following 5 min exposure time (Fig. 2).

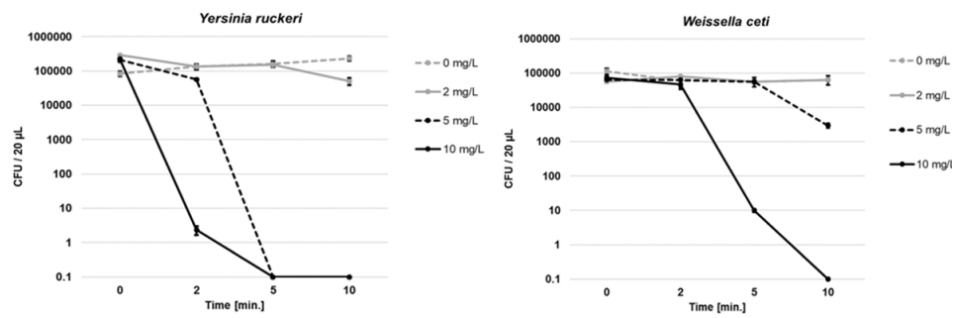


Fig. 1: Colony-forming units (CFU) of *Yersinia ruckeri* (top) and *Weissella ceti* (bottom) following increasing exposure time to various nominal peracetic acid concentrations in RAS water. Data points represent means \pm standard errors, with $n = 3$ replication.

Our results provide RAS producers with useful information regarding the effectiveness of PAA to reduce populations of specific bacterial pathogens residing in “typical” RAS water and contribute to the growing scientific knowledge base that will inform regulators when evaluating PAA’s potential use in aquaculture settings. Our findings need to be considered in combination with other studies focusing on (i) PAA toxicity to specific aquaculture species, (ii) the safety of PAA for use in RAS and its potential effects on biofilter nitrification, (iii) the impact of differing water quality profiles on PAA efficacy and kinetics, and (iv) the toxicity of PAA to other important fish pathogens in order to develop comprehensive strategies to effectively combat waterborne pathogens in RAS.

Similar to hydrogen peroxide (H_2O_2), which has been shown to be useful and biofilter-safe in commercial RAS, PAA has strong potential for application as a water disinfectant in operational aquaculture systems; however, PAA is a much more potent disinfectant in comparison to (H_2O_2).

It is unknown what water quality characteristic(s) inhibited *F. columnare* from fully entering into suspension in the RAS water used during Experiment 1. Previous research has demonstrated a significant water quality effect on the pathogenicity *F. columnare*, with these authors considering divalent cation concentration (i.e., water hardness) being a major reason for reduction in virulence. In our study, hardness likely did not contribute to the observed *F. columnare* clumping. Although hardness was not measured, alkalinity and hardness tend to correlate and, as such, the hardness in the second trial (in which *F. columnare* easily entered into suspension) was likely higher than in the first trial, based on alkalinity data and our historical alkalinity/hardness measurements.

Among other water quality parameters measured, nitrate-nitrogen, nitrite-nitrogen and total ammonia nitrogen differed significantly between trials; however, little research has been carried out examining the effects of these parameters on *F. columnare* viability and pathogenicity. Other authors have determined that elevated total ammonia limited *F. columnare* infection in channel catfish but, as with hardness, total ammonia nitrogen was actually higher in our second, successful experiment.

Beyond the water quality profile differences observed between experiments, other speculative chemical and/or biological causes for the apparent inactivation of *F. columnare* in low-exchange RAS water include the effects of accumulated humic substances or *F. columnare*-specific bacteriophages [viruses that infect and replicate inside bacteria]. Significant additional research is required to understand the observed effects of low-exchange RAS water on *F. columnare* viability.

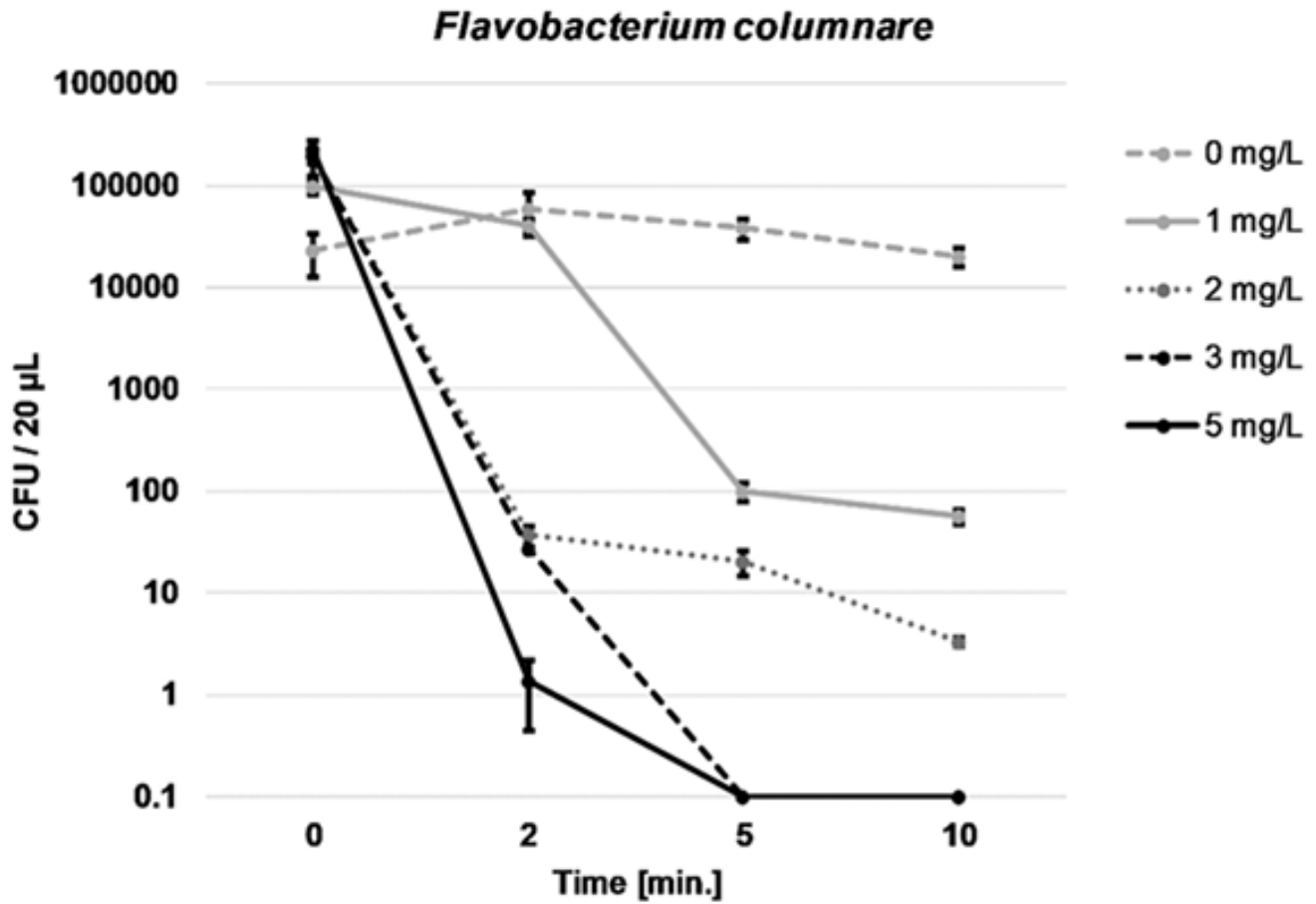


Fig. 2: Colony-forming units (CFU) of *Flavobacterium columnare* following increasing exposure time to various nominal peracetic acid concentrations in RAS water. Data points represent means \pm standard errors, with n = 3 replication.

Parasite treatment reduces *F. columnare* infection in tilapia

The authors conducted a study to evaluate whether treatment of Trichodina-parasitized tilapia with formalin would improve fish survival and reduce *F. columnare* infection. Tilapia not treated with formalin showed significantly higher mortality than treated fish.



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The higher resistance of *W. ceti* to PAA, relative to *Y. ruckeri* and *F. columnare*, may be related to *W. ceti* being a Gram-positive (vs. Gram-negative) bacteria. Gram-positive bacteria tend to have thicker cell walls, ranging from 20–80 nm in thickness (vs. 1.5–10 nm thick in Gram-negative bacteria), and these thick cell walls have been shown to assist Gram-positive bacteria in being resistant to disinfection, although this is not always the case across different approaches to disinfection (e.g. photoinactivation).

The majority of important bacterial fish pathogens are Gram-negative, and as such are likely more susceptible to disinfectants such as PAA; however, further research is required to determine differences in susceptibility between additional important Gram-negative (e.g. *Aeromonas salmonicida*, *Edwardsiella tarda* and others) and Gram-positive (e.g. *Streptococcus iniae*, *Renibacterium salmoninarum* and others) pathogens of farmed fish.

Perspectives

Based on our results, we provide PAA concentration and exposure duration data for 6-log reduction in three important bacterial fish pathogens while in RAS water. Further research should examine, among other things, additional fish pathogens and/or varying profiles of RAS water quality.

Authors



CHRISTOPHER GOOD, DVM

Corresponding author

The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, West Virginia USA

cgood@conservationfund.org (<mailto:cgood@conservationfund.org>).



NATALIE REDMAN

The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, West Virginia USA



MEGAN MURRAY

The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, West Virginia USA



DAVID L. STRAUS, PH.D.

Stuttgart National Aquaculture Research Center, USDA-ARS, Stuttgart, Arkansas, USA



TIMOTHY J. WELCH, PH.D.

National Center for Cool and Cold Water Aquaculture, USDA-ARS, Kearneysville, West Virginia, USA

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