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SHORT COMMUNICATION

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Assessing peracetic acid for controlling post-vaccination *Saprolegnia* spp.-associated mortality in juvenile Atlantic salmon *Salmo salar* in freshwater recirculation aquaculture systems

Christopher Good[1] | John Davidson[1] | David L. Straus[2] | Susan Harper[3] | David Marancik[4] | Timothy Welch[5] | Brian Peterson[6] | Lars-Flemming Pedersen[7] | Christine Lepine[1] | Natalie Redman[1] | Thomas Meinelt[8] | Dibo Liu[8] | Steven Summerfelt[1]

[1]The Conservation Fund Freshwater Institute, Shepherdstown, WV, USA

[2]Stuttgart National Aquaculture Research Center, USDA-ARS, Stuttgart, AR, USA

[3]Beltsville Agricultural Research Center, USDA-ARS, Beltsville, MD, USA

[4]Department of Pathobiology, University Centre, St. George's University, True Blue, Grenada

[5]National Center for Cool and Cold Water Aquaculture, USDA-ARS, Kearneysville, WV, USA

[6]National Cold Water Marine Aquaculture Center, USDA-ARS, Franklin, ME, USA

[7]DTU Aqua, Section for Aquaculture, The North Sea Research Centre, Technical University of Denmark, Hirtshals, Denmark

[8]Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

Correspondence: Christopher Good, The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443, USA.
Email: cgood@conservationfund.org

Present address
Steven Summerfelt, Superior Fresh, W15506 Superior Fresh Drive, Hixton, WI, USA

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As a group, oomycete pathogens are associated with enormous economic losses worldwide in both plant and animal agriculture, including aquaculture (Wawra et al., 2012). Oomycetes of the genus *Saprolegnia* are particularly problematic for numerous species of wild and farmed fish (van den Berg, McLaggan, Dieguez-Uribeondo, & van West, 2013) and are the causal agents of the fish disease known as saprolegniasis (or, colloquially, 'fungus'). There are over 500 identified species of oomycetes (Walker & van West, 2007), with a range of *Saprolegnia* oomycete species considered ubiquitous in freshwater environments (Jiang et al., 2013) and known to be associated with disease in salmonid aquaculture (Sandoval-Sierra, Latif-Eugenin, Martin, Zaror, & Dieguez-Uribeondo, 2014). It is recognized that *Saprolegnia* spp. have been responsible for more than 10% of annual economic losses in the salmonid aquaculture industry, with previous annual production losses up to 50% in certain cases (Bly et al., 1994; van West, 2006). Methods to control saprolegniasis in farmed settings have included the use of malachite green and formalin. Many aquaculture regions around the world, however, have banned malachite green due to its toxic and carcinogenic properties (Culp & Beland, 1996). Likewise, formalin use has been reduced due to similar human safety and environmental concerns. Therefore, new control methods are currently needed to

counter the impact of saprolegniasis on farmed fish production (van den Berg et al., 2013). Over the past decade, there has been a significant research has been devoted to developing methods to control saprolegniasis in aquaculture, including the use of immunostimulants in feed (e.g. Saha, Pal, Sahu, & Saha, 2016), novel medicated feeds (e.g. fluconazole – Saha, Pal, Sahu, Saha, & Goswami, 2017), water ozonation (Hamad & Mustafa, 2018) and vaccine development (e.g. Minor et al., 2014).

At present, however, the need for effective control methods for saprolegniasis remains high. In general, fish become susceptible to infection with *Saprolegnia* spp. when injured, stressed or infected with other pathogen(s) (Duan et al., 2018; Pickering & Willoughby, 1982), while healthy, uninjured fish are normally at low risk for developing clinical saprolegniasis (van den Berg et al., 2013). In Atlantic salmon *Salmo salar* culture, specific life stages (e.g. fry stage, smoltification phase) or routine events (e.g. physical handling) are known to be associated with the development of saprolegniasis. A major risk period for saprolegniasis is during the weeks following vaccination (Greg Lambert, Cooke Aquaculture, personal communication), and a procedure carried out when juvenile salmon are typically in freshwater systems. Both the stress involved, and the tissue damage associated with intracoelomic vaccine injection, favour opportunistic *Saprolegnia* spp. to establish infection and cause clinical disease.

In the Atlantic salmon industry, there has been a significant shift in recent years away from traditional flow-through systems and towards producing smolts in land-based, closed-containment facilities utilizing water recirculation aquaculture system (RAS) technologies (Summerfelt, Mathisen, Holan, & Terjesen, 2016). One of the challenges to raising fish in RAS is that therapeutants administered to diseased populations will also circulate through the biofilter and thus have the potential to impact beneficial organisms responsible for the critical process of nitrification. Past research has focused on various therapeutants and their effects on biofilter function (Noble & Summerfelt, 1996). More recent studies have focused on applying low-dose therapeutants to RAS to develop treatment protocols that are both efficacious against pathogens and not harmful to biofilter microbial communities involved in nitrification (Pedersen & Pedersen, 2012; Pedersen, Pedersen, Nielsen, & Nielsen, 2009, 2010). Determining an effective preventive treatment for saprolegniasis during high-risk periods in the production cycle, while simultaneously maintaining RAS biofilter integrity, would be very beneficial to numerous aquaculture facilities. We therefore sought to determine whether varying (low) doses of peracetic acid (PAA), a relatively novel therapeutant, could prevent saprolegniasis during the period following vaccination while assessing potential impacts on RAS biofilter function.

Approximately 2,400 juvenile Atlantic salmon (94 ± 3 g) originating from the US Department of Agriculture (Agriculture Research Service) National Cold Water Marine Aquaculture Center were randomly allocated to 12 small (0.5 m^3) circular tanks that had been retrofitted from flow-through to experimental-scale RAS using miniature fluidized sand biofilters (American Aquarium Products), degassing columns, and submerged pumps to create and maintain water recirculation. Make-up (new) water originated from an on-site freshwater spring and was provided at approximately 4 L/min for a total system hydraulic retention time of approximately 100 min. Initial mean fish stocking density was 49.8 kg/m^3. Fish were maintained on constant photoperiod and were fed a commercial salmon diet via automated feeders at hourly intervals using a standard feeding chart. Mean water temperature over the study period was 13.9°C.

After 1 week of acclimation, all fish were anaesthetized with 75 mg/L tricaine methanesulfonate (Western Chemical, Inc.) and individually vaccinated via intracoelomic injection, following vaccine manufacturer protocols, with Forte Micro (Elanco Canada Ltd.), a salmonid vaccine containing formalin-inactivated cultures of *Aeromonas salmonicida*, *Vibrio anguillarum*, *V. ordalii and V. salmonicida*. Following vaccination, four treatment groups were randomly allocated among the 12 study tanks (n = 3) for daily pulse treatments of: (a) 0.2 mg/L PAA, (b) 0.5 mg/L PAA, (c) 1.0 mg/L PAA and (d) 5 ml deionized water (control). The PAA used was VigorOx® SP-15 (PeroxyChem), a commercial product containing 15% PAA and 10% H_2O_2. By providing vaccination stress, we attempted to induce clinical saprolegniasis by creating conditions that permit ubiquitous *Saprolegnia* spp. to cause disease. The study period lasted for 6 weeks post vaccination, with daily mortalities noted to assess post-vaccination survival. Biofilter function was assessed through total ammonia nitrogen (TAN) removal efficiency, such that mean TAN removal rates were assessed at 1, 3 and 6 weeks after treatment onset. Feeding and flow rates remained constant throughout the study. Minimum and maximum mean TAN levels entering the biofilters were 0.240 and 0.353 mg/L respectively.

At study's end, length and weight data were collected from a representative sample of fish from each tank, and health and welfare were assessed. Three fish per tank were humanely euthanized with 200 mg/L tricaine methanesulfonate, and representative samples of gill, spleen and kidney tissue were fixed in 10% formalin solution for histopathological processing and evaluation. Additionally, 20 fish per tank were euthanized and evaluated for the presence or absence of fin erosion, external haemorrhage and/or visible *Saprolegnia* spp. infection (which was supported via skin scrapes and wet-mount microscopy to observe the characteristic morphology and hyphae of *Saprolegnia* spp. oomycetes; subsequent culture and sequencing identified the agent as *Saprolegnia australis*). Survival, growth performance and biofilter TAN removal efficiencies were analysed via ANOVA followed by the Tukey procedure to determine significant (p < .05) differences among treatment groups. Survival and TAN removal efficiency percentages were first arcsine-transformed prior to ANOVA. Fin erosion, haemorrhage and external saprolegniasis data were analysed via logistic regression, reporting odds ratios. All statistical procedures were performed in STATA (StataCorp).

Overall, survival during the 6-week study period was significantly higher in all three PAA treatment groups relative to the control group (Figure 1), although elevated mortality due to saprolegniasis was not observed in the control group despite the vaccination stressor. Conversely, mean Atlantic salmon weight was significantly higher in the control fish compared to all three treatment groups (Figure 1). Whether this growth difference was a consequence of an inhibitory effect of PAA requires further investigation, as previous research with

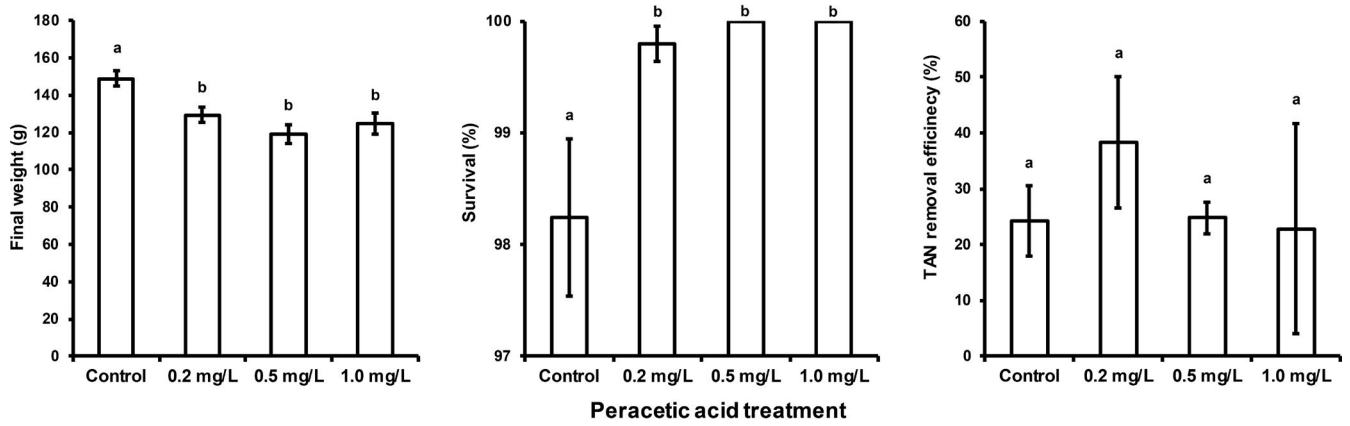

FIGURE 1 Mean salmon growth (left), survival (middle) and biofilter TAN removal efficiencies (right) observed over the 6-week study period following vaccination. Error bars represent standard errors. Different letters over chart bars indicate significant ($p < .05$) differences between treatment groups

PAA and rainbow trout *Oncorhynchus mykiss* has not demonstrated an inhibitory growth effect at low (0.2 mg/L) doses, either as pulse or continuous PAA application (Liu, Straus, Pedersen, & Meinelt, 2017). Despite the observed differences in growth performance, histopathology of the specific tissues sampled was unremarkable overall, with minimal lesions observed and no relationships between treatment groups determined. It is therefore likely that physical damage associated with PAA treatment did not impact growth performance; however, other tissue types (e.g. skin, liver, gastrointestinal tract.) should be evaluated in future PAA studies for confirmation.

Recent research (Soleng et al., 2019) has demonstrated that Atlantic salmon mount both systemic and mucosal stress responses following exposure to PAA (both at 0.6 and 2.4 mg/L), which could result in energy diversion away from somatic growth and thereby account for the growth differences observed in the present study. Fish were fed at equal rates throughout this experiment; however, due to the nature of the study tanks (i.e. no sump in which to observe wasted feed) and the 24-hr feeding period, it was not possible to compare actual feed consumption among treatment groups. Therefore, feed intake might have been lowered in response to PAA exposure, and this possibility requires examination in future studies. While fin erosion and external signs of haemorrhage were not associated with any particular treatment group(s) (Table 1), it is interesting to note that control fish were significantly more likely to exhibit external saprolegniasis compared to PAA treated fish; that is, with an odds ratio of 0.074, the odds of saprolegniasis significantly decreased with increasing PAA dosage. Finally, biofilter TAN removal efficiency was not significantly impacted by PAA treatment (Figure 1), although broad standard errors in two of the four treatment groups suggest that a higher power study is required to confirm these findings. Further research should also focus on potential effects of PAA on other biofilter types, for example moving bed, microbead and fixed bed biofilters (Malone & Pfeiffer, 2006; Pedersen, Oosterveld, & Pedersen, 2015).

TABLE 1 Observable pathologies assessed (presence or absence) at study's end following 6-week post-vaccination peracetic acid treatments

Pathology	Odds ratio (95% confidence interval)	*p*-value
Fin erosion	0.929 (0.697–1.24)	.618
Haemorrhage	0.910 (0.720–1.15)	.426
Saprolegniasis	0.074 (0.015–0.372)	.002

Note: Odds ratios reported indicate the odds of fish exhibiting the listed pathologies at increasing peracetic acid dosages relative to control fish.

In conclusion, the principal findings of this study were as follows: (a) at the dosages tested, PAA significantly reduced observable external saprolegniasis following vaccination and led to overall higher survival rates, and (b) biofiltration did not appear to be impacted by PAA exposure at the dosages, and treatment regimens tested. These results are promising with regard to the development of efficacious protocols to reduce post-vaccination saprolegniasis in Atlantic salmon RAS. Future research should expand on these findings and address the study limitations discussed.

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DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

ORCID

Christopher Good https://orcid.org/0000-0002-7383-5952
John Davidson https://orcid.org/0000-0002-6246-798X

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