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# Reducing mortality associated with opportunistic infections in Atlantic salmon *Salmo salar* fry using hydrogen peroxide and peracetic acid

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## Abstract

Developing efficacious protocols for applying water disinfectants to reduce opportunistic pathogen-associated mortalities during the Atlantic salmon *Salmo salar* fry stage would be highly beneficial for producers. Atlantic salmon fry (0.47 g ± 0.02) were exposed to daily stressors over four weeks while providing daily 30-min bath treatments of 15 mg/L hydrogen peroxide ($H_2O_2$), 0.2 mg/L peracetic acid (PAA) or 0.5 mg/L PAA. Survival was tracked, and skin and gill samples were collected at 2 and 4 weeks for histopathology. Moribund fish were regularly assessed via wet-mount microscopy, with organisms resembling *Saprolegnia* spp. routinely observed on gills of affected fish. Tanks treated with $H_2O_2$ had significantly ($p < 0.05$) higher survival (83.7% ± 1.7) compared to controls (69.5% ± 5.2) while no significant differences were observed between either PAA treatments (76.6% ± 0.6 and 77.4% ± 3.0 survival in the 0.2 mg/L and 0.5 mg/L PAA groups, respectively) and controls. Interestingly, no significant differences were noted among treatments for waterborne *Saprolegnia* spp. concentrations through qPCR quantification. Lower total suspended solids (TSS) were observed in both PAA treatment groups; no other water quality differences were noted. No treatment impacts were observed through histopathology at either sampling point. These results suggest that, at the dosage and treatment regime tested, $H_2O_2$ can be a safe and efficacious water treatment for reducing Atlantic salmon fry opportunistic infection-associated mortality during periods of physical and environmental stress. Assessments of alternative PAA treatment regimens should also be considered in future research aimed at reducing early life-stage mortality in Atlantic salmon.

**KEYWORDS**
Atlantic salmon, hydrogen peroxide, peracetic acid, *Saprolegnia*

## 1 | INTRODUCTION

During the early life stages of salmonid culture, comparatively low fish immunocompetence and relatively high susceptibility to handling stress render juvenile populations vulnerable to mortality associated with opportunistic pathogens (Magnadóttir, 2006; Salonius & Iwama, 1993). Author experience during previous on-site research trials indicates that Atlantic salmon *Salmo salar* fry (up to approximately 4–5 g) in our flow-through early rearing system are predominantly affected by *Saprolegnia* spp., opportunistic pathogens that are naturally found in our spring water and fish culture systems. *Saprolegnia* spp. are ubiquitous freshwater oomycetes, with certain species being causative agents of saprolegniasis, a disease that can severely impact the health of freshwater fish and other aquatic organisms in aquaculture and natural ecosystems (Eissa et al., 2013; Fernández-Benéitez et al., 2008; Kestrup et al., 2011; van den Berg et al., 2013). Fish with physical injuries and/or reduced immunocompetence are more susceptible to saprolegniasis, while fish with no physical damage are often able to avoid infection with *Saprolegnia* spp. (van den Berg et al., 2013). A spike in saprolegniasis at an aquaculture facility can result not only in increased mortalities, but can also have deleterious economic consequences if the situation is not controlled (Kam & Leung, 2008). Previous effective therapeutants used to counter *Saprolegnia* spp. have included malachite green; however, this chemical is now banned from use in aquaculture due to its carcinogenic properties (Culp & Beland, 1996). Currently, other treatments are used or considered for controlling *Saprolegnia* spp. include formalin, benzoic acid, acetic acid, iodoacetic acid and copper sulphate, along with possible biocontrol with the use of probiotics administered in water and feed (Barnes et al., 2002; González-Palacios et al., 2018; Tedesco et al., 2018).

Developing efficacious alternative water treatment approaches to prevent or reduce mortalities associated with *Saprolegnia* spp. and other opportunistic pathogens during Atlantic salmon early rearing would be greatly beneficial for commercial producers. For this purpose, two environmentally benign (Block, 2001; Wagner et al., 2002) water treatment chemicals were investigated: peracetic acid (PAA) and hydrogen peroxide ($H_2O_2$). Peracetic acid is a clear and colourless oxidant and disinfectant that is most stable at commercially available concentrations of 10%–15% (Kitis, 2004). Previously, PAA has been used predominantly in wastewater treatment due to its antimicrobial properties, as it has been shown to prevent growth of *Escherichia coli* and faecal coliforms (Rossi et al., 2007). In aquaculture, PAA has been shown to be efficacious against the crayfish plague oomycete *Aphanomyces astaci* in freshwater European crayfish *Astacus astacus* farming (Jussila et al., 2011) and has been used to improve water quality and reduce opportunistic pathogen levels in recirculating aquaculture systems (RAS) without disturbing bacterial populations responsible for nitrification in biofilters (Pedersen et al., 2009; Suurnäkki et al., 2020). Presently, PAA has not been approved by the U.S. Food and Drug Administration (FDA) for use in aquaculture in the United States; however, PAA has been approved by the U.S. Environmental Protection Agency (EPA) for use as an aquaculture surface disinfectant (e.g. tanks and pipes) when food fish are not present (Straus et al., 2018).

In comparison, $H_2O_2$ is approved by the FDA for use in U.S. aquaculture to treat a number of diseases, including saprolegniasis, in freshwater-reared coldwater finfish; however, the comparative efficacy of $H_2O_2$ versus PAA in reducing *Saprolegnia* spp.-associated mortality in juvenile Atlantic salmon has not been assessed. Hydrogen peroxide can be effective in controlling bacterial gill disease at concentrations of 56–110 mg/L when applied as a 60-min bath, or at 56–230 mg/L for a 30-min bath (Rach et al., 2000). It has also been effective as a treatment for parasites on fin fish, such as amoebic gill disease and sea lice in net pens (Martinsen et al., 2018; Thomassen, 1993), and has been observed to improve water quality in RAS without negative impacts on fish or biofilters (Pederson & Pederson, 2012).

In the present study, we sought to evaluate $H_2O_2$ and PAA to compare the relative efficacy of these chemicals in reducing opportunistic pathogen-associated mortality in stressed Atlantic salmon fry through daily low-dose treatments of culture tank water.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental system

A total of 12 replicated tanks in a flow-through system were used for this study, with each 0.5 $m^3$ circular tank operated at approximately 0.29 $m^3$ water volume and with a flow rate of 11.4 L/min. All study tanks were independent and supplied with only new water; this water was sourced from an on-site freshwater spring and was initially treated via passage through a packed degassing column, then supplemented with liquid oxygen to obtain 100% dissolved oxygen (DO) saturation in the culture tanks. Water entered each tank from a vertical in-tank spray bar and exited through a bottom drain. The drain screens were kept at the smallest size available (0.32 cm diameter holes) throughout the study, to limit solids removal and provide an additional environmental stressor. Mean water temperature was 12.6°C ± 0.04 over the study duration. Tanks were cleaned daily following treatments (see below), using a siphon to collect any extra feed from the previous day.

### 2.2 | Atlantic salmon

Atlantic salmon (mixed-sex diploid Cascade strain, courtesy the University of Wisconsin—Stevens Point, Northern Aquaculture Demonstration Facility, Bayfield, WI, USA) were received as fertilized eyed eggs and were hatched in vertical Heath tray stacks. Following hatch, the fish were stocked into flow-through tanks until they reached the targeted initial size (approximately 0.5 g) for

the study to commence. At this mean size (68 days post-hatch), the salmon were divided evenly among 12 tanks, with 575 salmon per tank, and at a stocking density of 1 kg/$m^3$.

## 2.3 | Culture conditions

A constant 24-hour photoperiod (24 h light, 0 h dark) was provided to support 24-hour feeding. Each tank was fed every 30 min using a computer-operated feeding system (The Conservation Fund Freshwater Institute) at 120% of the typical daily feed rate (15.77 g/day/tank), calculated using the EWOS Canada Ltd. feeding chart and kept constant for the 4-week study period. Overfeeding was considered an appropriate stressor based on author experience, where wasted excess feed has been observed to accumulate on the gills of low-swimming juvenile salmon and assist in the development of *Saprolegnia* spp. growth on branchial tissue. The feed used was a commercial mash, 54% protein and 16% fat (EWOS).

## 2.4 | Treatments

Three tanks were randomly selected for each of the four treatment groups (i.e. n = 3 replication). Treatment groups consisted of (i) 0.2 mg/L PAA (VigorOx-15 [15% PAA, 10% $H_2O_2$] PeroxyChem LLC); (ii) 0.5 mg/L PAA; (iii) 15 mg/L $H_2O_2$ (Perox-Aid [35% $H_2O_2$]; Syndel); and (iv) control (deionized water). All treatments were applied as 30-min static baths, once per day for 5 days per week over the 4-week study period. During weeks 2 and 4, PAA and $H_2O_2$ concentrations were quantified at 1 min-, 10 min- and 30 min-post application during individual 30-min treatment periods, using Hach method LIT2214 and Hach drop count titration/thiosulfate method, respectively. Each tank was supplied with a diffuser to compensate for any potential reduction in DO during the static bath treatments. Daily stressors provided to each tank consisted of (i) overfeeding by 120% (described above) and (ii) physical netting of the majority of the fish population from each tank, 5 days per week. The netting stressor was applied such that captured salmon were held above water for a period of 15 seconds during each event. Netting was selected as an appropriate approach due to the well-known stress response associated with netting fish (AFS, 2014); such handling stress, in turn, is known to increase susceptibility to disease (Duan et al., 2010; Wedemeyer, 1996).

## 2.5 | Water quality analyses

Water quality profiles were assessed at the end of each of the 4 study weeks and included quantification of total ammonia nitrogen (TAN), total suspended solids (TSS), temperature, DO, pH, carbon dioxide ($CO_2$), alkalinity and total phosphorous. All water quality analyses were conducted on-site at The Freshwater Institute's Water and Environmental Chemistry Laboratory.

## 2.6 | Waterborne *Saprolegnia* spp. quantification

Three 1 L water samples were collected from each tank at three time-points (pre-treatment, 2 weeks and 4 weeks) and shipped to Bowling Green State University for quantifying waterborne *Saprolegnia* spp. concentrations via quantitative polymerase chain reaction (qPCR) and agar colony-counting assay. The timing for 2- and 4-week sample collections was approximately mid-way between treatment administrations.

### 2.6.1 | (i) qPCR

Water samples were divided into two 500 ml aliquots, with one aliquot used for qPCR and the second for plate colony-counting assays. Each water sample was first filtered through 5 µm polycarbonate track etch membranes (Sterlitech) under 5 kPA vacuum pressure. Membranes were then transferred into a 2 ml Eppendorf tube containing 800 µl of 0.1% Tween-80 and 0.1 g of 0.5 mm diameter glass beads (Benchmark Scientific Inc.). Tubes were vortexed for 1 min and pulse-centrifuged. The supernatant was then collected and heated at 95°C for 10 min, chilled on ice for a further 5 min and immediately used for qPCR reactions. The cytochrome C oxidase subunit 1 (COI) gene was used as the molecular marker (Seifert et al., 2007). The qPCR reactions were carried out in triplicates on a CFX thermal cycler (Bio-Rad) at 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 59°C for 15 s. The quantification of *Saprolegnia* spp. was determined by correlating the observed cycle threshold (Cq) values from a standard curve with the amount of *Saprolegnia* spp. genomic DNA present in the water samples. Data were then converted to waterborne concentrations (ng/L) of *Saprolegnia* spp. genomic DNA.

### 2.6.2 | (ii) Agar plate assay

The second 500 ml of each water sample was filtered as described above, and the membranes were then overlaid onto a YPS agar plates supplemented with 68 µg/ml streptomycin (Sigma) and 68 µg/ml chloramphenicol (Sigma). Plates were then incubated overnight at room temperature, in the dark. Following incubation, membranes were removed, and the number of *Saprolegnia* spp. propagules were enumerated microscopically. All assays were performed in triplicate.

## 2.7 | Fish sampling

Mortalities were removed daily and recorded to monitor survival. Moribund fish were euthanized humanely with 200 mg/L of tricaine methanesulphonate (MS-222, Syndel) and regularly assessed via wet-mount light microscopy (phase-contrast) at 400× magnification for the presence of observable pathogens on the gill tissues.

Histopathology was evaluated at 2 and 4 weeks, during which three fish from each tank were randomly selected, humanely euthanized and preserved whole in 10% buffered formalin. A cross-section was taken from each fish at the level of the opercular cavity and pharynx to histopathologically evaluate gill and skin tissue. Tissues were processed routinely, sectioned at 4 µl and stained with haematoxylin and eosin (H&E). Slides were digitized (Histowiz) and evaluated blindly by a single pathologist. All visible gill tissue was examined, and skin was evaluated on the dorsum and on each operculum. Tissues were examined for mononuclear and eosinophilic granular cell infiltrates, goblet cell density, epithelial hyperplasia and necrosis, and lamellae fusion/adhesion (gill only). Cellular and extracellular changes were semi-quantitatively scored on a 0 (no change) to 3 (mild, moderate and severe) point scale.

### 2.8 | Statistical analyses

Daily tank mortality data were summarized by week and analysed via 2-way repeated measures analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test comparing each treatment group to the control group for each of the four study weeks. This analysis was performed in GraphPad Prism v.8.2 (San Diego, CA, USA). Histopathology scoring data were analysed for each sampling event using ordered logistic regression in STATA v.16.1 (StataCorp LLC). Water quality results were averaged over the study period and compared via ANCOVA in STATA v16.1, with dummy variables created for each treatment group and with sampling date controlled as a covariate. Individual water quality parameters that did not follow a normal distribution (as assessed via the Shapiro–Wilk normality test) were compared non-parametrically using the Kruskal–Wallis equality-of-populations rank test. Waterborne *Saprolegnia* spp. data were analysed in STATA v16.1 via repeated measures mixed effects linear regression, controlling for sampling date and pre-treatment *Saprolegnia* spp. values. All tests were performed at the $p < 0.05$ level of statistical significance.

## 3 | RESULTS

### 3.1 | Water quality

Water quality analyses revealed significant differences between total suspended solids in the 0.2 and 0.5 mg/L PAA groups and the control group; all other water quality parameters assessed were not significantly different from controls (Table 1). Treatment concentrations assessed over the course of the 30-min static bath are summarized in Figure 1. All treatments did not decay significantly in concentration during bath treatments; initial and final concentrations for the targeted 0.2 and 0.5 mg/L PAA and 15 mg/L $H_2O_2$ treatment groups were as follows: 0.23 ± 0.02 and 0.21 ± <0.01 mg/L, 0.61 ± 0.04 and 0.52 ± 0.02 mg/L, and 15.0 ± 1.29 and 14.2 ± 0.83 mg/L, respectively.

### 3.2 | Survival

Survival rates over the course of the 4-week study period were 83.7% ± 1.7, 77.4% ± 3.0, 76.6% ± 0.6 and 69.5% ± 5.2 for the $H_2O_2$, 0.5 mg/L PAA, 0.2 mg/L PAA and control groups, respectively. The overall ANOVA model indicated a significant ($p < 0.05$) time-by-treatment relationship; subsequent multiple comparison testing indicated significantly less mortality in the $H_2O_2$ group versus the control group during week 2 ($p < 0.05$) and weeks 3 & 4 ($p < 0.01$) (Figure 2; Table 2). Although lower in general, mortality in 0.5 and 0.2 mg/L PAA treatment groups was not significantly different from the control group.

### 3.3 | Waterborne *Saprolegnia* spp.

Analysis of pre-treatment waterborne *Saprolegnia* spp. data indicated a significant tank effect, with mean individual tank concentrations ranging from 0.373 to 26.7 ng/L *Saprolegnia* spp. DNA and mean tank *Saprolegnia* spp. propagules ranging from 2.00 to 239

| | $H_2O_2$ | PAA 0.2 mg/L | PAA 0.5 mg/L | Control |
|---|---|---|---|---|
| Total ammonia nitrogen (mg/L) | 0.01 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 |
| Total phosphorous (mg/L) | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.01 |
| Total alkalinity (mg/L) | 276 ± 1.60 | 274 ± 1.92 | 276 ± 1.78 | 275 ± 1.58 |
| Dissolved carbon dioxide (mg/L) | 17.5 ± 0.72 | 17.6 ± 0.73 | 17.6 ± 0.76 | 17.8 ± 0.79 |
| Total suspended solids (mg/L) | 4.16 ± 1.69 | 2.93 ± 0.54* | 2.19 ± 0.63* | 5.76 ± 1.59 |
| pH | 7.58 ± 0.02 | 7.56 ± 0.01 | 7.56 ± 0.02 | 7.56 ± 0.01 |
| Dissolved oxygen (mg/L) | 10.5 ± 0.02 | 10.6 ± 0.02 | 10.6 ± 0.02 | 10.6 ± 0.02 |
| Temperature (°C) | 12.3 ± 0.04 | 12.3 ± 0.04 | 12.3 ± 0.05 | 12.3 ± 0.04 |

**TABLE 1** Water quality parameters summary (means ± standard errors) of weekly measurements throughout the study period. Asterisks represent significant ($p < 0.05$) differences between specific treatments and controls

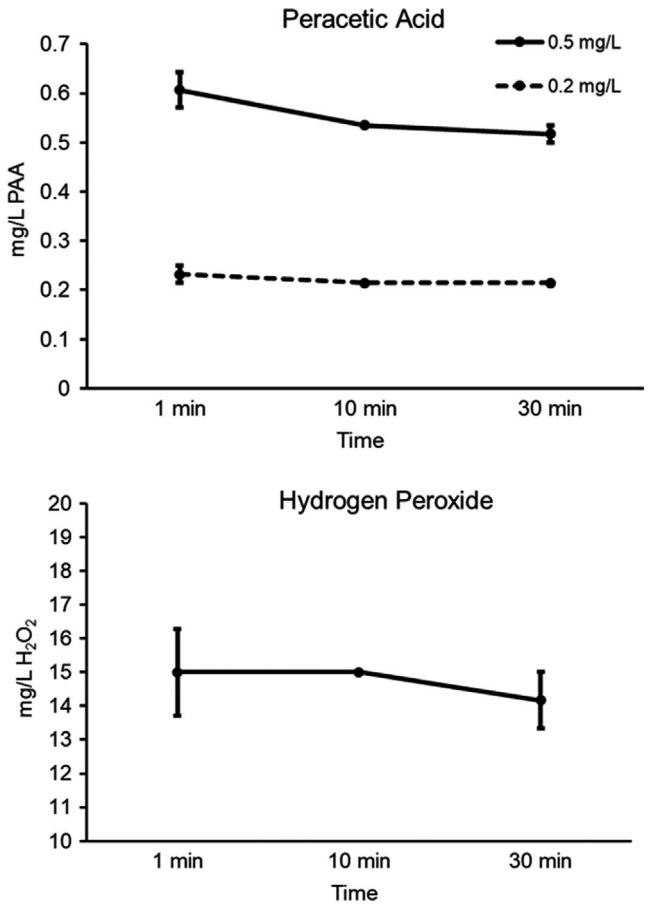


**FIGURE 1** Decay curves of peracetic acid (top) and hydrogen peroxide (bottom) concentrations during 30-min static bath applications. Error bars represent standard errors

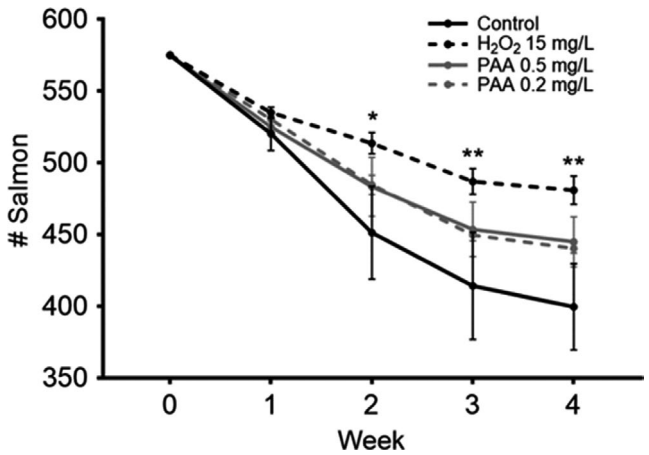


**FIGURE 2** Reduction in salmon through weekly mortality for each treatment group. Asterisks (* and **) indicate significant differences ($p < 0.05$ and $p < 0.01$, respectively) between the H2O2 treatment and control groups. Error bars represent standard errors

colonies/L. These pre-treatment quantifications demonstrated a range of 5.39 ± 1.58 to 17.8 ± 2.99 ng/L for qPCR and 52.2 ± 14.7 to 158 ± 25.3 colonies/L for agar assays among the four treatment groups (Table 3). Correlation between qPCR data and agar plate assays was observed, where water samples with relatively less *Saprolegnia* spp. gDNA content also demonstrated less colony counting and delayed growth of *Saprolegnia* spp. Nearly all tanks, controls included, demonstrated a decline in *Saprolegnia* spp. concentrations over time; however, while controlling for differences in baseline, pre-treatment tank levels, no significant differences in reduction in *Saprolegnia* spp. concentrations over time were determined among $H_2O_2$ and PAA treatment groups.

### 3.4 | Light microscopy and histopathology

Wet-mount microscopy of the gills of moribund fish invariably revealed the presence of organisms resembling *Saprolegnia* spp., that is oomycete hyphae were observed among the gill filaments and lamellae. Histopathological evaluation of gill and skin sections from randomly sampled fish demonstrated good overall tissue health, with only minor findings that were relatively consistent between experimental groups and not uncommonly observed in healthy fish. There were mild inflammatory infiltrates, consisting of lymphocytes, plasma cells and eosinophilic granular cells within the dermis and gill filaments and lamellae. Rarely, patchy branchial epithelial hyperplasia and adhesion between lamellae were observed; however, these lesions were found in only a few fish and were characterized as mild in severity. No significant statistical associations were determined at either 2 or 4 weeks post-treatment onset between treatments and observed lesions in randomly sampled fish. Fungal hyphae morphologically consistent with *Saprolegnia* spp. were observed between gill filaments of two fish, but the incidence was not high enough for statistical comparison.

## 4 | DISCUSSION

The results of our study demonstrate that daily low-dose application of $H_2O_2$ can be effective in reducing mortalities associated with opportunistic infections in stressed juvenile Atlantic salmon without negatively impacting gill and skin tissue integrity. These results contribute to the growing body of research assessing $H_2O_2$ use in aquaculture and may be particularly relevant to its application in RAS operations. Currently, $H_2O_2$'s approved use for treating saprolegniasis in coldwater salmonids consists of a regimen of three alternating-day 75 mg/L treatments (USFWS, 2020), and while this approach has been shown to be efficacious in traditional aquaculture, it is likely that the relatively high treatment concentration would be detrimental to biofilter nitrification when applied in RAS settings. For example, Pedersen and Pedersen (2012) demonstrated that a single dose of 64 mg/L $H_2O_2$ in a RAS reduces ammonia removal efficiency and nitrification, with a slow partial rejuvenation rate of 7 days, and it is therefore likely that dosing with a similarly high concentration for serial treatments without sufficient recovery time could lead to partial or complete biofilter dysfunction. Total failure of a fluidized

**TABLE 2** Results of Dunnett's multiple comparisons test following repeated measures analysis of variance, comparing weekly mortality in each treatment group relative to the control group

| Week | Comparison | Mean Difference | 95% confidence interval | *p*-value |
|---|---|---|---|---|
| 1 | Control vs. $H_2O_2$ | −14.67 | −68.79, 39.46 | 0.8470 |
| | Control vs. PAA 0.5 mg/L | −4.447 | −58.79, 49.46 | 0.9934 |
| | Control vs. PAA 0.2 mg/L | −10.00 | −64.13, 44.13 | 0.9426 |
| 2 | Control vs. $H_2O_2$ | −62.33 | −116.5, −8.207 | *0.0205* |
| | Control vs. PAA 0.5 mg/L | −32.00 | −86.13, 22.13 | 0.3481 |
| | Control vs. PAA 0.2 mg/L | −33.33 | −87.46, 20.79 | 0.3166 |
| 3 | Control vs. $H_2O_2$ | −72.67 | −126.8, −18.84 | *0.0061* |
| | Control vs. PAA 0.5 mg/L | −39.33 | −93.46, 14.79 | 0.1989 |
| | Control vs. PAA 0.2 mg/L | −35.33 | −89.46, 18.79 | 0.2730 |
| 4 | Control vs. $H_2O_2$ | −81.33 | −135.5, −27.21 | *0.0020* |
| | Control vs. PAA 0.5 mg/L | −45.33 | −99.46, 8.793 | 0.1180 |
| | Control vs. PAA 0.2 mg/L | −40.67 | −94.79, 13.46 | 0.1779 |

**TABLE 3** Waterborne *Saprolegnia* spp. quantification (means ± standard errors) via qPCR (ng/L *Saprolegnia* spp. genomic DNA) and agar assays (colonies/L) at pre-study, 2 weeks and 4 weeks. Water samples were collected approximately mid-way between treatment administrations.

| Method | $H_2O_2$ | PAA 0.2 mg/L | PAA 0.5 mg/L | Control |
|---|---|---|---|---|
| qPCR (ng/L) | | | | |
| Pre-study | 8.34 ± 0.41 | 17.8 ± 2.99 | 5.39 ± 1.58 | 8.77 ± 0.52 |
| 2 weeks | 8.03 ± 1.14 | 15.1 ± 2.23 | 7.27 ± 0.73 | 7.28 ± 0.30 |
| 4 weeks | 4.61 ± 0.31 | 6.93 ± 0.60 | 5.64 ± 0.57 | 3.51 ± 0.63 |
| Agar assay (colonies/L) | | | | |
| Pre-study | 79.6 ± 4.52 | 158 ± 25.3 | 52.2 ± 14.7 | 88.9 ± 5.01 |
| 2-weeks | 76.4 ± 8.65 | 122 ± 14.1 | 73.6 ± 5.94 | 72.7 ± 2.58 |
| 4-weeks | 54.7 ± 2.49 | 71.6 ± 2.88 | 64.2 ± 6.36 | 33.6 ± 5.22 |

sand biofilter has been demonstrated with a single dose of 100 mg/L $H_2O_2$ (Schwartz et al., 2000). Whether daily 30-min 15 mg/L $H_2O_2$ treatments are RAS biofilter friendly in addition to improving survival requires further study to confirm. Likewise, further study is warranted to assess these treatment regimes in other types of rearing units beyond circular tanks (e.g. raceways).

Similar research is also required to pinpoint appropriate concentrations of PAA dosing in RAS. In the present study, we were limited to conducting research in a flow-through system; however, previous on-site research has demonstrated no impact on fluidized sand biofiltration when RAS were exposed to low-dose (up to 0.3 mg/L) PAA on a semi-continuous basis (Davidson et al., 2019). A further study demonstrated that TAN removal efficiency was not impacted when fluidized sand biofilters were exposed to a daily pulse dose of 0.2 mg/L, 0.5 mg/L or 1.0 mg/L PAA, and that these treatments decreased saprolegniasis following intracoelomic injection vaccination (Good et al., 2020). In comparison, concentrations of 2–3 mg/L PAA can disrupt biofilter nitrification, leading to increases in nitrite levels in RAS (Pedersen et al., 2009). When considering other potential water quality improvements associated with PAA usage, our results demonstrated a significant reduction in TSS concentrations in both PAA treatment groups compared to control and $H_2O_2$ treatments. These results differ from previous on-site research that showed no reduction in TSS with low-dose PAA application (Davidson et al., 2019); however, the PAA concentrations used in that study were generally lower (0.05, 0.10 and 0.3 mg/L) and, more significantly, were applied on a semi-continuous basis in RAS, an approach which the authors theorized might have contributed to biofilm growth of PAA-adapted bacteria and, in turn, increases in TSS through bacterial proliferation. The drop in TSS observed in the present study might therefore be related to both PAA dose and application method, and warrants further research given the potential benefits that reduced TSS might confer (e.g. assisting a facility to stay within its TSS discharge limits).

At higher doses, PAA is known as an effective water disinfectant, with temporary improvements in water quality by means of reduced ammonium levels and/or removal of biofilm on the inner surface of the tank (Liu et al., 2017; Suurnäkki et al., 2020). The potential for PAA to improve water quality in aquaculture, both in flow-through systems and RAS, is likely considerable, and optimizations in concentration and application technique remain a necessary area of investigation. While the use of PAA in aquaculture with food fish present is currently approved in the European Union, additional results obtained from domestic research are required for U.S. approval of PAA beyond its current use as a disinfectant for aquaculture equipment and systems when no fish are present.

Although generally higher, PAA-treated fish did not demonstrate significantly increased survival relative to controls. Further research is therefore required to investigate alternative treatment regimens for PAA in order to determine optimal approaches for applying this chemical. A safe no-observed-effect concentration (NOEC) for PAA use is well above the concentrations tested in this study. Published PAA NOEC values for aquatic species range from 1.9 to 5.8 mg/L and are species-dependent (Straus et al., 2018); however, no PAA toxicity studies have been published to date focusing on Atlantic salmon. It has been shown that twice weekly PAA administration (1 mg/L for 3 h) will confer a range of benefits, including an up to 90% decrease in waterborne bacterial density, improved gill morphology and lower circulating plasma cortisol (Liu et al., 2018); however, the study by Liu et al. (2018) focused on mirror carp *Cyprinus carpio*, a relatively robust species compared to Atlantic salmon. In flow-through systems, rainbow trout *Oncorhynchus mykiss* have been observed to tolerate 8.9 mg/L PAA, with no apparent gill, kidney or liver damage observed 60 days post-treatment and with treated fish simultaneously demonstrating higher immunocompetent blood cell populations (Hushangi & Shekarabi, 2018). Although baseline research has recently been conducted to characterize the physical and physiological responses of Atlantic salmon following exposure to PAA (Lazado, Haddeland, et al., 2020; Lazado, Sveen, et al., 2020; Lazado et al., 2021; Soleng et al., 2019), further research is necessary to fully characterize PAA's toxicity (e.g. $LC_{50}$ studies) to Atlantic salmon which, in turn, will be useful in assisting in the development of efficacious water treatment protocols in Atlantic salmon aquaculture.

The decrease in *Saprolegnia* spp. concentrations over the course of the study, irrespective of treatment conditions, does not align with the survival differences noted between treatments. It is therefore unknown at present why relatively higher *Saprolegnia* spp. counts were not observed in tanks with (presumably) higher *Saprolegnia* spp.-associated mortality. As with other saprotrophic opportunistic pathogens, it is difficult to determine a *Saprolegnia* spp. infection threshold, given that the quantity of agent does not directly correlate with infection intensity (Merikanto et al., 2012). Furthermore, infection by *Saprolegnia* spp. does not necessarily result in disease or death of the hosts. In general, *Saprolegnia* spp. infections are considered to emerge following some form of injury or deleterious condition, including infection by other micro-organisms (Pickering & Willoughby, 1977). Additionally, *Saprolegnia* spp. are known to form biofilms with other micro-organisms, which can contribute to this pathogen group's overall virulence (Ali et al., 2013). Given the relatively crude assessment (light microscopy) of moribund fish in the present study, it is possible that additional opportunistic pathogen(s) were involved in the disease and mortality observed, and therefore, subsequent research should consider a microbiome assessment approach to identifying changes in waterborne opportunist populations in relation to treatment conditions and survival. If other opportunistic pathogens were involved in the mortality observed in the present study, these would likely have been relatively susceptible to $H_2O_2$ (and to a lesser extent, PAA) at the concentrations applied, in order to explain differences in survival relative to controls. Alternative hypotheses to consider when assessing the *Saprolegnia* spp. concentration results are as follows: (i) $H_2O_2$ acted to decrease the virulence of *Saprolegnia* spp., leading to the higher survival rate in the $H_2O_2$ treatment group in the absence of any significant differences in *Saprolegnia* spp. concentrations among treatment groups, and (ii) exposure to $H_2O_2$ conferred some form of beneficial immune and/or micro-anatomical response (similar to findings by Liu et al., 2018), rendering the salmon more resilient to *Saprolegnia* spp. infection pressure. Follow-up research is required to confirm or refute these or additional hypotheses.

In conclusion, our study demonstrates the potential for $H_2O_2$ to reduce mortality in stressed Atlantic salmon fry, and while *Saprolegnia* spp. pathogens likely contributed to this mortality (based on light microscopy observations) additional research is needed to fully characterize the opportunistic pathogen involvement. Further research is also required to refine and optimize application approaches for PAA and $H_2O_2$ in Atlantic salmon aquaculture, in terms of concentrations and exposure durations, and this is particularly important in RAS given its increasing usage in Atlantic salmon culture and the potential impacts of water treatments on RAS biofiltration. Future studies should also focus on different life stages of Atlantic salmon to determine whether the observed benefits of $H_2O_2$ are similar for parr, smolt and post-smolts.

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## DATA AVAILABILITY STATEMENT

Data are available upon request to the corresponding author.

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