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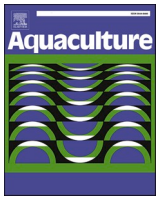

Acute dose-response exposure of a peracetic acid-based disinfectant to Atlantic salmon parr reared in recirculating aquaculture systems

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ABSTRACT

There is a high regard for peracetic acid (PAA)-based disinfectants in recirculating aquaculture systems (RAS) because of the low risk of bioaccumulation, fast degradation with neutral residuals and minimal impact on biofilter performance. However, the no-observed-effect concentration in Atlantic salmon parr is unknown. The present study evaluated the effect of an acute PAA exposure on Atlantic salmon parr health and welfare by evaluating survival, swimming behaviour, appetite and histopathological alterations in the gills and skin. Nine experimental RAS units were employed, where each unit was dedicated for one PAA concentration (0.0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L). Fish were exposed to the target PAA concentration in a static system for 1 h and the exposure protocol was repeated after a 52 h recovery. The fish survival was 100%, 80% and 0%, respectively $\leq$1.6, 3.2 and 6.4 mg/L. Fish swimming behaviour was normal in PAA $\leq$ 1.6 mg/L, whereas it become erratic with air gasping for PAA $\geq$ 3.2 mg/L. The fish appetite did not change among the different PAA treatment groups. Skin and gill histopathological alterations were pronounced in PAA $\geq$ 3.2 mg/L, characterized by a poorer skin condition and necrotic gill lamella. The skin acidic mucous cells density was 55% lower in the 6.4 mg/L group than the 0 mg/L group. The sub-lethal water pH values observed in the 6.4 mg/L group after PAA administration may have played a confounding and compounding factor to the PAA toxicity response in this group. In conclusion, the current study identified the no-observed-effect concentration for PAA to be below 1.6 mg/L for Atlantic salmon parr and provided insights into its use as a water prophylactic strategy in RAS. Toxicity of PAA-based disinfectants is influenced by its acidified nature, which can interfere with the water pH of low alkalinity aquaculture systems. Further studies should evaluate the health and welfare consequences of a long-term PAA exposure in Atlantic salmon parr.

1. Introduction

Atlantic salmon (*Salmo salar*) global production had an extraordinary growth (900%) in the last three decades and seems to have now stabilized in the last years with around the 2.4 million tonne per year figure (FAO, 2020). The lack of suitable areas available to expand sea-cage production and the biological constrains with pathogens are shifting thousands of tonnes of salmon production to alternative aquaculture production systems.

Recirculating aquaculture systems (RAS) are among the most environmentally sustainable systems to culture fish due to their reduced water usage, low released nutrients to the environment and virtually no fish escapees (Martins et al., 2010). Despite the high biosecurity features in RAS, pathogen outbreaks in parr and smolt have accounted for several mass mortality events (Murray et al., 2014). *Yersinia ruckeri* and Ca. *Branchiomonas cysticola* are some of the major pathogens that pose a significant challenge to Atlantic salmon production in freshwater RAS (Murray et al., 2014; Wiik-Nielsen et al., 2017). Moreover, the re-use water which characterizes RAS, leads to the accumulation of substances (e.g. dissolved solids, ammonia and hormones) in the water (Mota et al., 2014) and, this imposes restrictions to the efficiency of disinfection strategies. The disinfection strategy needs also to consider that the biological filtration unit depends on specific bacterial species to convert a toxic fish metabolite (ammonia) to a less toxic form (nitrate), and these beneficial bacteria should not be severely impacted by the disinfection protocol (Eding et al., 2006; Pedersen et al., 2009). To

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control pathogens in the water-phase without negatively affecting the fish or the biofilter bacteria is challenging, and strategies applied in flow-through systems are usually not applicable in RAS.

Disinfection solutions developed for RAS can be categorized as continuous and periodic disinfection. Continuous water disinfections include the use of ozone (O_3) and UV irradiance in the full flow of RAS. Ozone is a powerful oxidant agent that is used to improve water quality and reduce pathogens in aquaculture (Davidson et al., 2021; Powell and Scolding, 2018). However, as shown by Stiller et al. (2020), its application needs a complex understanding of water chemistry and an effective way to monitor and control the O_3 system, otherwise it that can result in poor fish health and welfare, and even in fish mortality. Periodic disinfection typically comprises of chemical disinfectants such as formalin, copper sulphate, chloramine-T, hydrogen peroxide (H_2O_2) and peracetic acid (PAA, CH_3CO_3H) (Pedersen and Pedersen, 2012). These chemicals can be effective against a wide spectrum of pathogens (Good et al., 2020; Meinelt et al., 2015), but when not managed properly, can result in compromised fish health and biofilter performance (Pedersen et al., 2013).

Peracetic acid is a strong disinfectant with a wide spectrum of antimicrobial activity and a higher oxidation potential when compared to other chemical disinfectants used in aquaculture (Pedersen et al., 2009; Yuan et al., 1997). The oxidizing agents PAA and hydrogen peroxide are the main constituents of PAA-based disinfectants, and these are stabilized by acidification with for example acetic acid. A main advantage of PAA is that it easily degrades into harmless residues (acetate, water and carbon dioxide) (Pedersen and Lazado, 2020). PAA has been used to eradicate fish ectoparasites including such as causative agent of white spot disease (*Ichthyophthirius multifiliis*) from freshwater systems with Nile tilapia (*Oreochromus niloticus*) (Abu-Elala et al., 2021). Pathogenic bacteria, such as *Vibrio anguillarum*, *V. harveyi*, *Vibrio alginolyticus*, and *Photobacterium damselae* were also effectively eradicated with the use of PAA in Gilthead seabream (*Sparus aurata*) production systems (Acosta et al., 2021). Furthermore, PAA has been used in commercial open recirculated rainbow trout (*Oncorhynchus mykiss*) farms to control fish parasites (Pedersen et al., 2013) and a continuous application of 0.2 mg/L PAA caused no stress to fish (Liu et al., 2017a). Another study in rainbow trout demonstrated that growth, survival, and feed conversion ratio were not affected at doses ranging from 0.05–0.30 mg/L PAA (Davidson et al., 2019). Moreover, the no-observed-effect concentration (NOEC) was suggested to be in the range of 0.5–2.7 mg/L for bluegill (*Lepomis macrochirus*), < 1 mg/L for zebrafish (*Brachydanio rerio*) and 1.3–2.2 for channel catfish fry (*Ictalurus punctatus*) (reviewed in (Straus et al., 2012). However, an excessive exposure to PAA can affect fish as shown by initiating a stress response in rainbow trout (Liu et al., 2017a) and common carp (*Cyprinus carpio*) (Liu et al., 2017b). The studies cited above indicate that there is species-specific PAA toxicity, which illustrates the need to test the PAA toxicity for each target species before its application.

Atlantic salmon studies on PAA toxicity are scarce. One study in Atlantic salmon post-smolts exposed to 0.6 and 2.5 mg/L, found that fish responded to the exposure by altering several systemic (i.e., plasma cortisol, glucose, lactate, total antioxidant capacity) and mucosal (i.e., expression of antioxidant coding genes in the skin and gills) defence parameters (Soleng et al., 2019). Another study also showed that the skin mucosa of Atlantic salmon is affected by PAA exposure, though the changes were marginal and the authors suggested that the PAA does not present a significant challenge to the mucosal health of salmon in the tested concentration range (< 2.5 mg/L). (Lazado et al., 2020b). Moreover, PAA exposure between 0.2 and 1 mg/L seems to not impact Atlantic salmon smolts survival (Good et al., 2020). However, a NOEC for Atlantic salmon remains unknown. Moreover, it is likely that a NOEC to be life-stage dependent, especially in a fish species like Atlantic salmon with marked life phase differences in freshwater (parr) and seawater (post-smolt). Therefore, the objective of the present study was to evaluate the effect of a peracetic acid acute exposure on Atlantic salmon parr welfare and health. To this end, fish were exposed to nine PAA concentrations (0.0–6.4 mg/L) and their survival, swimming behaviour, appetite and histopathological changes in the gill and skin tissues were recorded.

2. Material and methods

2.1. Experimental design

Nine PAA concentrations (0.0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L) were tested in this study. Atlantic salmon parr were exposed to 1 h of PAA twice with a 52 h recovery interval between the 1st and 2nd exposure. During the PAA exposure the water flow to the fish tanks was interrupted, aeration was provided to facilitate the mixing and the PAA solution was added to the water. After 1 h, the water flow was resumed. The PAA-based product (Aqua Des ™) was obtained from Aquatic Chemistry AS (Lillehamer, Norway). The disinfectant is a stabilized PAA solution (5% *v*/v PAA, 23% Hydrogen peroxide and 10% Acetic acid), and the actual amount of PAA and Hydrogen peroxide in the solution was experimentally verified by the DTU Aqua laboratory (Pedersen and Lazado, 2020) to be at approximately 6.6% and 23.4%, respectively.

2.2. Experimental fish and RAS

All procedures involving fish were approved by the Norwegian Food Safety under FOTS ID 24128.

Atlantic salmon eyed eggs (AquaGen Atlantic QTL-innOva PRIME, AquaGen AS, Trondheim, Norway) were hatched and fish were raised in a flow-through system (Tromsø Aquaculture Research Station, Kårvik, Norway) at ±7.5 °C under continuous light (LD24:00) photoperiod until approximately 11 g of body weight.

Atlantic salmon parr ($N = 180$; 11.3 ± 1.8 g) were captured from the flow-through system holding tank and randomly distributed over nine experimental tanks ($N = 20$/tank). Each experimental tank was part of a RAS unit. Each individual RAS unit consisted of: a cylindro-conical fish tank (V = 0.5 m^3); drum filter (micro-screen mesh size = 40 μm); a moving bed bioreactor (V = 0.2 m^3, 750 m^2/m^3 bio-media), a gas exchange unit (CO_2-degasser cylinder), a foam fractionator (protein skimmer), low pressure oxygen cone (0.6 Bar) and a temperature control unit. The total RAS water volume was 0.8 m^3, water flow to the fish tank was 1500 L/h, fish tank hydraulic retention time (HRT) was 20 min., and photoperiod was L24:D00.

Fish were acclimatized (3 days) to the environmental parameters maintained during the whole experimental period including dissolved oxygen >85% saturation (> 9.2 mg/L), pH 7–7.5, temperature = 11.5–12.5 °C, salinity = 0 ppt. The make-up water alkalinity = 20 mg/L as $CaCO_3$. Fish were fed continuously (approx. 23 h day) with a commercial diet (1 mm pellet size, Nutra Olympic, Skretting, Norway) delivered through an automatic belt feeder.

2.3. Water sampling and analysis

Water samples for PAA analyses were taken immediately before exposure and after 1 h of exposure. The method to determine PAA was a modified DPD (N,N-diethyl-*p*-phenylenediamine sulfate salt) photometric method (Pedersen and Lazado, 2020). Briefly, DPD reacts with PAA at pH 6.5 to give a red color complex (DPD^+). The reaction is catalyzed with potassium iodide (KI) and has a maximum absorption value at 550 nm (Pedersen et al., 2009). To measure the PAA concentration, water samples (2.5 mL) were pipetted to a cuvette (4 ml) and reagent 1 (EDTA•$2H_2O$, H_2SO_4 96% and DPD salt) was added (250 μL). Thereafter, reagent 2 (Na_2HPO_4•7 H_2O, KH_2PO_4 and KI; 250 μl) was added. The solution was mixed and allowed to equilibrate for 30 s before the absorbance at λ = 550 nm was measured in a spectrophotometer (PharmaSpec UV-1700, Shimadzu®, Japan).

Dissolved oxygen, pH and temperature were measured daily using a portable meter (FDO 925 and Sentix 940 sensors, Multi 3630 IDS, WTW, Germany). Ammonium (NH_4-N), nitrite (NO_2-N) and nitrate (NO_3-N) were measured daily from water samples using a spectrophotometer (Test Kit 1.14558.001, 1.14776.0001and 1.14942.0001, Spectroquant ®, Merck, Germany). Ammonia (NH_3−N) concentrations were calculated from the ammonium concentration as a function of pH, temperature, and salinity (Johansson and Wedborg, 1980). Turbidity was measured daily from water samples using a portable meter (ORION AQ4500, Thermo Scientific®, Thermo Fisher Scientific, USA). All measurements and water samples were obtained from each fish tank.

2.4. Fish sampling and analyses

Appetite assessment was carried by manually feed (08.30–09.30 h) each individual tank until apparent fish satiation every morning during the three experimental days. After each meal the uneaten pellets were weighed and recorded. During this three-day estimation window, the automatic feeding amount was reduced to 50% and the feeder period was reduced from 24 h/day to 12 h/day (18:00–06:00).

Fish swimming behaviour during the 1 h PAA exposure was recorded using a camera on the top of each fish tanks (Hero 7 and Hero 8, GOPRO, USA). Swimming behaviour was evaluated every 5 min. Using a 1 to 3-point system, where 0 indicates no reaction to PAA and 3 indicates intense swimming activity or abnormal behaviour such as gasping for air. This behavioural scoring was specifically developed for this trial, which was based on previous PAA exposure trials in Atlantic salmon (Soleng et al., 2019).

Fish individual body weight and length was measured from all fish (N = 20/tank) immediately after the end of the second PAA exposure, except for 3.2 and 6.4 mg/L groups that were measured immediately after the end of the first PAA exposure. Fish were humanely euthanized with a bath overdose of benzocaine (Benzoak vet, 200 mg/ml, Euro-Pharma, Leknes, Norway). Skin tissue (N = 5 fish/tank) was sampled below the dorsal fin and gill tissue (N = 5 fish/tank) was collected from the second arch from the right operculum. The tissue samples were placed in 10% neutral formalin containers (BiopSafe®, Mermaid Medical, Denmark) and stored at 4 °C until analysis. Tissue samples for histology were embedded in paraffin, sliced tangentially into 3-μm-thick section, stained with Periodic Acid Schiff-Alcian Blue (AB-PAS), and digitised using a slide scanner (Aperiod CS2, USA). Gill sections were evaluated by randomly selecting six filaments per fish, and in each analyse 40 lamellae, totalizing 240 lamellae evaluated per fish. Histopathological alterations were identified based on 7 key common cases: unaffected, lamellar clubbing, lamellar fusion, hypertrophy, hyperplasia, epithelial lifting and necrosis (Stiller et al., 2020). Skin sections were evaluated by focusing on the epidermal surface morphology using a semi-quantitative method: general appearance of the epidermis and surface quality of the epidermis. A 3-point scale scoring system was used, where 0 indicates excellent skin condition and 3 indicates poor skin condition (Stiller et al., 2020).

2.5. Statistics

Statistical analyses were performed with IBM® SPSS® Statistics V27 (IBM, Corp., USA). Homogeneity of variance was assessed using Levene's test and normality using the Shapiro-Wilk test. One-way ANOVA followed by post-hoc Tukey HSD test (equal variances assumed) or Games-Howell test (unequal variances) were used to compare differences among the PAA treatment groups. A significant level (α) of 0.05 was used for all analyses. All data are presented as mean ± standard deviation (S.D.).

3. Results

3.1. Fish survival and behaviour

The survival of the fish exposed to PAA in the range of 0.0 to 1.6 mg/L was 100%. In contrast, fish exposed to PAA 3.2 and 6.4 mg/L had a survival of 80% and 0% within the first 60 min-exposure (Fig. 1). Both groups were terminated at the end of the first PAA exposure.

The fish swimming behaviour in the groups 3.2 and 6.4 mg/L PAA demonstrated as intense erratic activity with occasional gasping for air, scoring 2.8–2.9 (Fig. 2A). This behaviour was significantly different (*P-value* < 0.001) from the control group (0 mg/L) that scored 1.2. The remaining groups, i.e., 0.05 to 1.6 mg/L did not differ from the control group and the score ranged between 1.0 and 1.6.

Fish appetite did not significantly differ among the different treatment groups (*P-value* = 0.419) (Fig. 2B).

3.2. Skin and gill histology

Histopathology revealed that the skin surface quality of the groups exposed to 0.8, 3.2 and 6.4 mg/L PAA was significantly different (*P-value* < 0.001) from the control group (0 mg/L) (Fig. 3A). In particular, the surface quality score for the 3.2 and 6.4 mg/L PAA groups averaged 1.7–2.1, representing a fair - poor skin condition. Likewise, general appearance of the skin showed that 3.2 and 6.4 mg/L PAA groups score was significantly higher (*P-value* < 0.001) compared to control group (0 mg/L) (Fig. 3B). This is illustrated in Fig. 4, where the compromised skin barrier structure was characterized by rough patchy surface and a significant part of the epidermis was missing in these two high PAA groups. The acid mucous cell density in the skin was significantly lower (↓ 55%) in the 6.4 mg/L PAA group, 28.5 ± 14.8 cells/μm^2, when compared to the control group, 63.1 ± 17.3 cells/μm^2. (Fig. 3D). No significant differences were observed in the neutral mucous cell density; treatment groups average was 6.8 ± 5.6 cells/μm^2 (Fig. 3C).

Quantitative histopathology revealed that gill structures were compromised at >1.6 mg/L PAA, as shown in Fig. 6. The prevalence of healthy lamellar was significantly higher (*P-value* < 0.001) in the treatment groups ≤1.6 mg/L PAA compared to 3.2 and 6.4 mg/L groups. The first 7 groups exhibited healthy lamellae over 85% of the total evaluations (Fig. 5B), with minor prevalence of lamellae lifting (Fig. 5C) and hyperplasia (Fig. 5E), whereas the groups 3.2 and 6.4 mg/L showed 100% necrosis of lamella tissue (Fig. 5D).

3.3. Water quality

The water quality measured in the fish tanks throughout the 52-h trial did not significantly (*P-value* = 0.419) differ among the groups and was on average: dissolved oxygen = 100.3 ± 2.4% saturation (10.8 ± 0.3 mg/L), temperature = 11.8 ± 0.2 °C, salinity = 0 ppt, turbidity = 0.9 ± 0.1 NTU, NH_4-N = 0.7 ± 0.2 mg/L, NH_3-N = 1.4 ± 0.8 μg/L, NO_2-

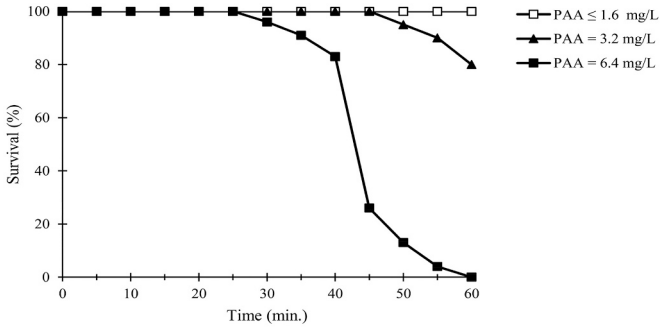

Fig. 1. Percentage survival (%) of Atlantic salmon parr exposed to different PAA concentrations. (N_{start} = 20 fish per tank).

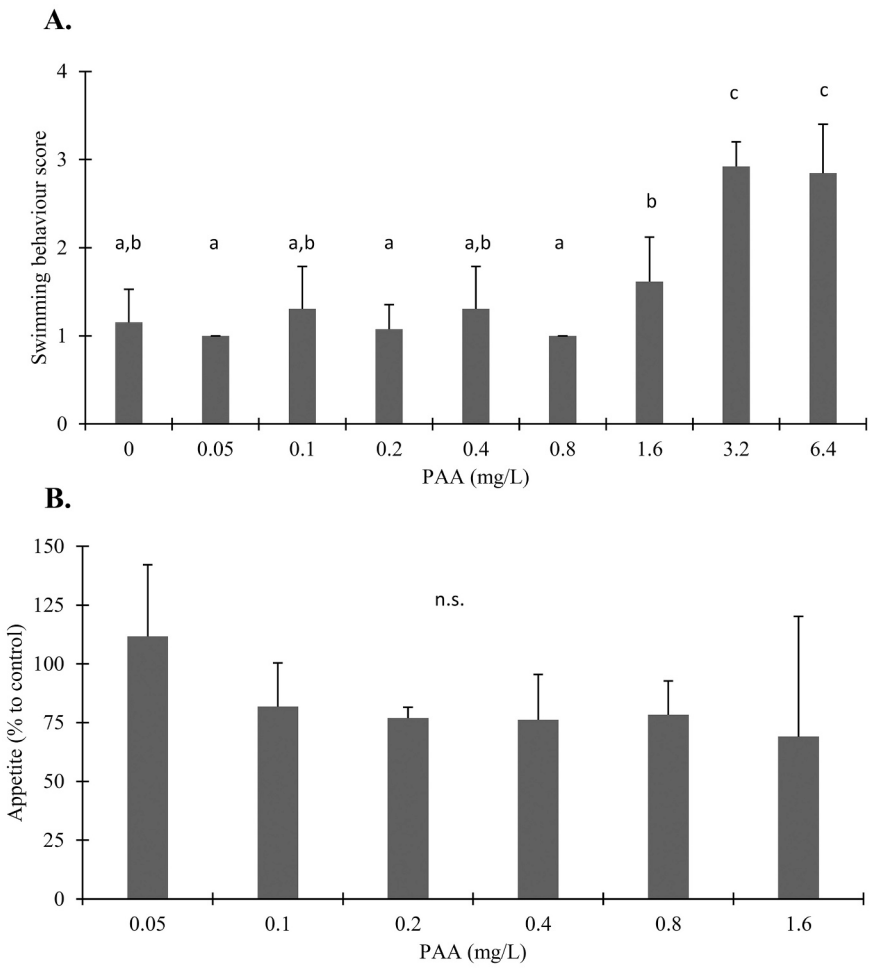

Fig. 2. Fish behaviour characterized by swimming activity and appetite. A) Swimming behaviour during 1 h PAA exposure was assessed every 5 min using a 1 to 3-point system, where 0 indicates no reaction to PAA and 3 indicates intense swimming activity ($N = 13$). B) Appetite (%) during three days in relation to the control group (0 mg/L). PAA 3.2 and 6.4 mg/L are not shown due to the early termination of these two groups ($N = 3$). Different superscript letters represent statistically significant differences among treatment groups (*P-value* < 0.05).

$N = 0.003 \pm 0.005$ mg/L and pH $= 6.9 \pm 0.4$. The latter, pH, despite not statistically significant, had a drop due to the PAA administration as shown in Fig. 6. PAA groups $\leq$1.6 mg/L had a pH after 60 min. exposure $\geq$6.5, whereas for the 3.2 mg/L group pH was reduced from 7.2 (T0min.) to 5.8 (T60 min.) and for the 6.4 mg/L group pH was reduced from 7.1 (T0min.) to 4.8 (T60 min.).

4. Discussion

Fish toxicity to PAA has been described in several species. Straus et al. (2018) revealed PAA toxicity in fingerlings of twelve aquaculture fish species through a 24 h exposure, revealing that the median lethal concentration (LC50) values ranged from 2.8 to 9.3 mg/L. It was further indicated that the estimated the 24-h no-observed-effect concentration (NOEC) ranged was from 1.9 to 5.8 mg/L PAA. Similarly, a recent study with Gilthead seabream (*S. aurata*) found >40% mortality within 24 h in fish exposed to 10 mg/L PAA (Acosta et al., 2021). Pathological changes associated with the acute toxicity of PAA included severe gill damage and moderate degeneration of the renal tubule epithelium within the posterior kidney (Straus et al., 2012). PAA is a chemical stressor that could also trigger a strong stress responses, both at the systemic (i.e., plasma cortisol, glucose, lactate, total antioxidant capacity) and mucosal (i.e., expression of antioxidant coding genes in the skin and gills) levels (Soleng et al., 2019). The induction of stress response indicated the adaptive physiological changes the fish mounted as a countermeasure for PAA. In salmonids, minor or negligible effects were found in the range of 0.2–1.0 mg/L for rainbow trout (Davidson et al., 2019; Liu et al., 2017a) and of 0–2.5 mg/L for Atlantic salmon smolts and post-smolts (Good et al., 2020; Lazado et al., 2020b; Soleng et al., 2019). In the current study, 1 h PAA exposure lethal concentrations for Atlantic salmon parr ranged between 3.2 and 6.4 mg/L, whereas no mortality was observed in concentrations equal or below 1.6 mg/L. This result indicates that the upper threshold for PAA in Atlantic salmon parr (±11 g) without resulting in mortality was between 1.6 and 3.2 mg/L PAA, which is somehow within the range (1–2 mg/L) suggested for prophylactic use (Liu et al., 2021).

Swimming behaviour of fish is impaired by exposure to different waterborne chemicals and it can be used as an indicator of sublethal toxicity in fish. Changes in fish swimming behaviour have been observed during exposures to various contaminants at concentrations below 5% of their LC50 values and at concentrations that subsequently reduced growth (Little and Finger, 1990). Several toxicants have been shown to affect the swimming performance of salmonids, including ammonia (Wicks et al., 2002) and copper (De Boeck et al., 2006). In the present study, changes in fish swimming behaviour were identified at PAA exposure equal or higher than 1.6 mg/L. Fish exposed to these concentrations exhibited an intensive and erratic activity, either by standing too close to water outlet structure or by swimming close to the surface and gasping for air. These behavioural manifestations suggest that fish identified the danger in the environment and the escape strategy is often a classic response to evade dangerous stimuli, including toxicants. As a strong oxidant, PAA is likewise an irritant, which can interfere with respiratory functions, as shown by hyperventilation and intense air gasping. The alterations in the swimming activity showed to be an early warning sign of the potential distress, suggesting a NOEC between 0.8 and 1.6 mg/L PAA for Atlantic salmon parr.

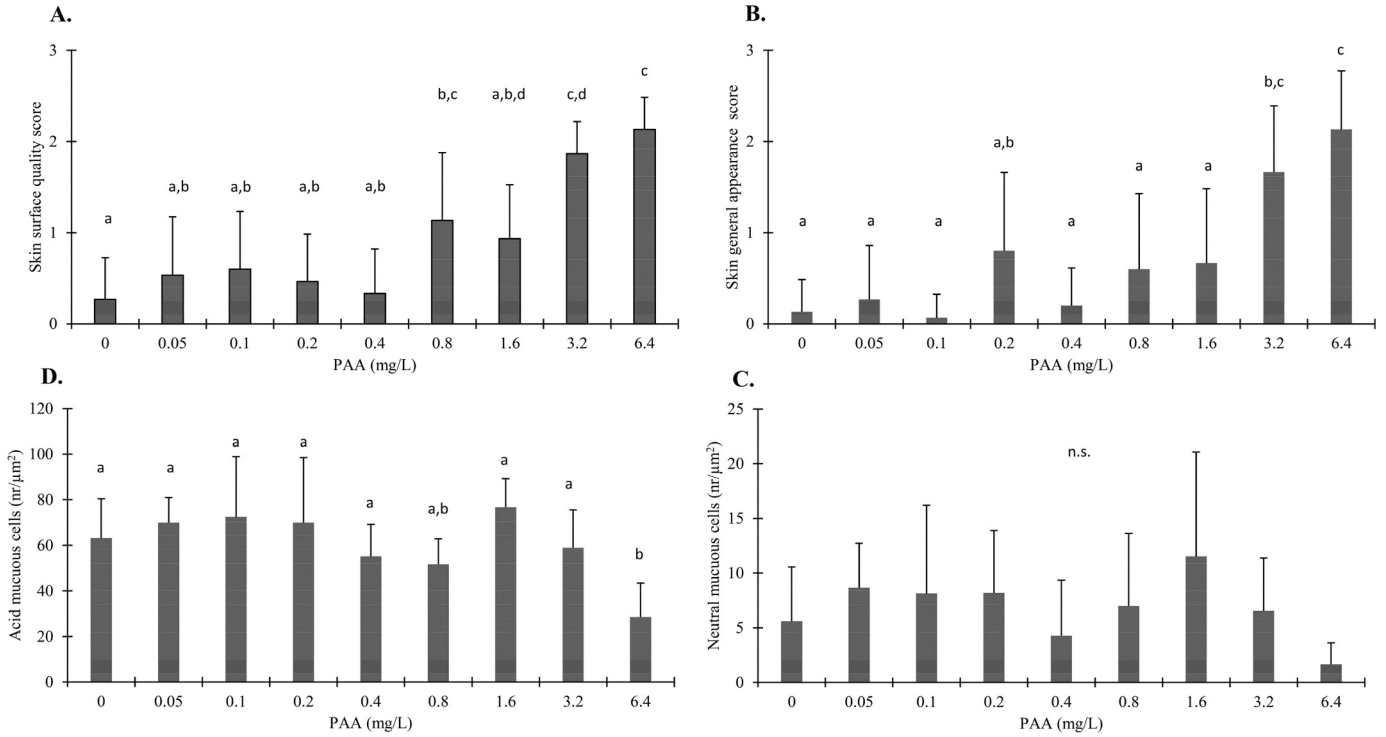

Fig. 3. Histological assessment of skin health. Skin health was evaluated using 2 key parameters: A) general appearance and B) surface quality, using a score system of 0 (good) to 3 (poor) condition. Mucous cells density (nr cells/μm^2) C) acid cells and D) neutral cells. Different superscript letters represent statistically significant differences among treatment groups (*P-value* < 0.05). $N = 5$ fish per tank.

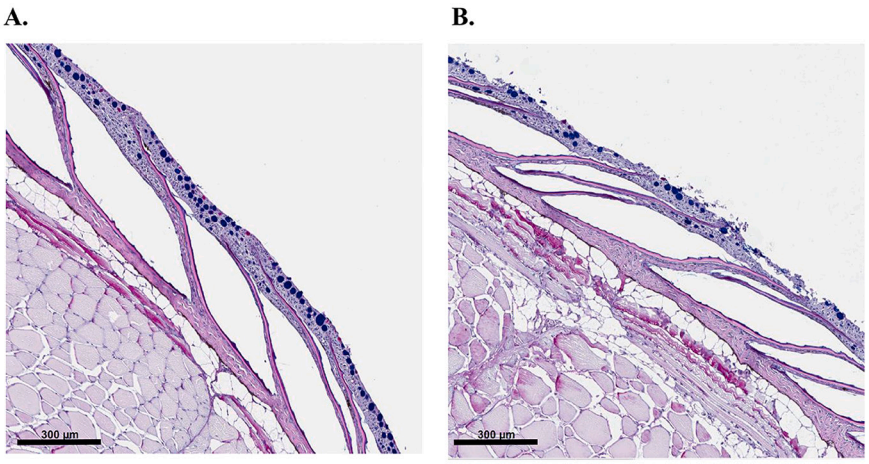

Fig. 4. Representative photographs of A) healthy skin, with defined structures, smooth surface and intact epithelium and B) a compromised skin with rough surface and missing epidermal layer. Note mucous acid cells (in blue) and neutral cells (in purple) in these two photographs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Regulation of fish appetite is a complex mechanism that relies on endogenous and external signals. Stress is known to impact the mechanisms regulating appetite in fish (Conde-Sieira et al., 2018). In the current study, no differences were found on a 3-day appetite assessment in fish exposed to PAA between 0.05 and 1.6 mg/L. The fish appetite estimation showed a non-significant but lower appetite when compared to control fish. Future studies should focus on the effect of sub-lethal PAA doses on fish appetite and feed intake.

Mucosal surfaces are the utmost fish barrier to the surrounding environment variations and besides their role on defence, they have crucial physiological functions such as skin in osmotic balance and sensory reception, and gills on ion transport, osmoregulation and acid-balance (Cabillon and Lazado, 2019). The skin tissue changes were previously observed in Atlantic salmon exposed to different toxicant gradients including carbon dioxide (Mota et al., 2019), ozone (Stiller et al., 2020) and PAA (Soleng et al., 2019). Atlantic salmon post-smolts exposed to 2.4 mg/L PAA showed a moderately compromised epithelium with a rough surface (Lazado et al., 2020b). In the current study, PAA exposure resulted in compromised skin with rough surface and parts of the epidermis missing in the two high groups. PAA directly contacts the skin, and the observed missing epidermis can be a form of epidermal desquamation resulting from toxicity of the oxidant. Mucous cells are sentinels of the mucosa and they produce the mucus layer that provides the a bio-physical barrier. Neutral mucous cells stain purple

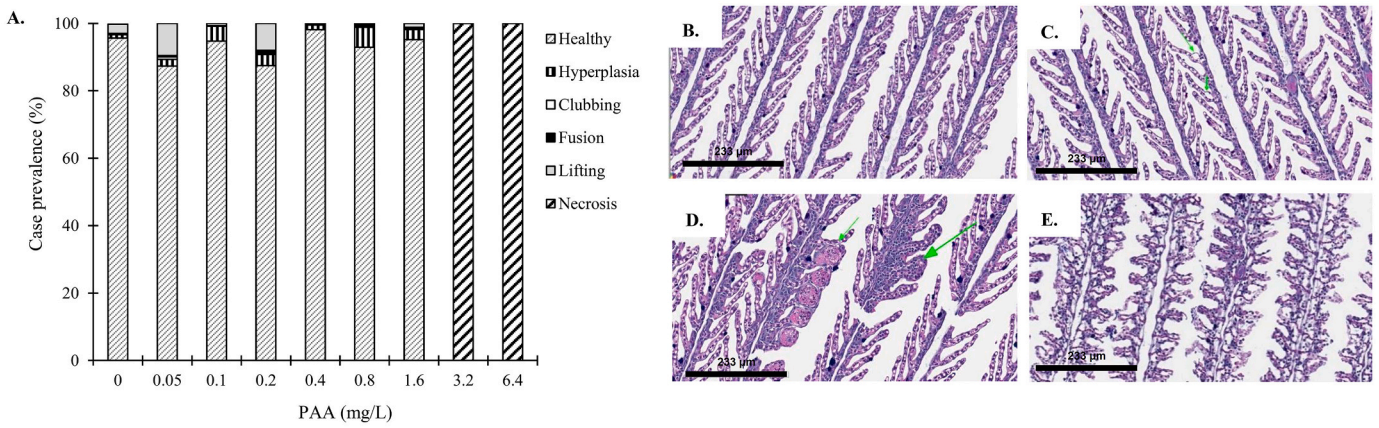

Fig. 5. Histological assessment of gill health. A) Gill health was assessed by counting the prevalence of pathologies often observed following oxidant treatments in Atlantic salmon, including PAA exposure. Representative photographs of B) healthy gills with well-defined structures, C) epithelial lifting in the secondary lamella (arrows), D) necrotic gills with dead tissue (arrows) and E) aneurysm and hyperplasia of epithelial cells. (N = 5 fish per tank).

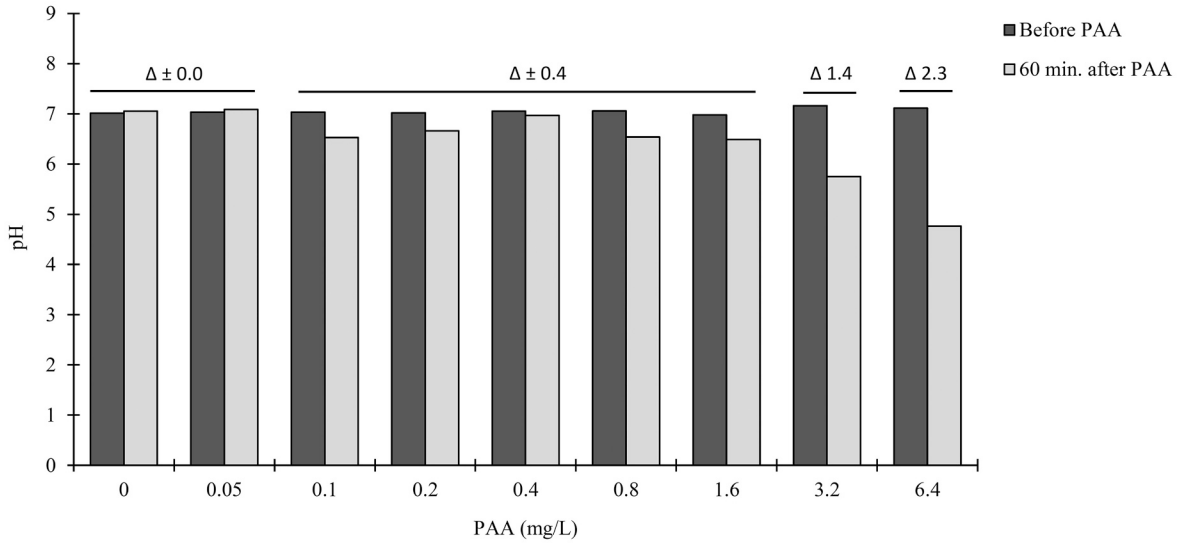

Fig. 6. Water pH of each fish tank before and 60 min. After PAA administration. Δ (delta) -value between pH at 0 min. and pH at 60 min.

and have a higher pH, whereas more acid mucous cells blue with AB/PAS. The density of acidic mucous cells decreased in the current study by 55%, whereas no changes were observed in the neutral mucous cell density. A similar reduction on mucous cells density was observed on rainbow trout exposed to PAA-based disinfectant (Liu et al., 2020). However, the response of mucous cells was unclear in Atlantic salmon post-smolts repeatedly exposed to PAA (Lazado et al., 2020b). Changes on the density of the mucous cells may be PAA exposure duration sensitive or maybe dependent on the commercial PAA product. Nonetheless, the finding that acidic and neutral mucous cells behaved differently to PAA indicates that the chemical may influence mucous cell biochemistry, which is interesting for future studies.

Teleost fish gills comprise of 4 pair of gill arches, each supporting numerous filaments and each filament consists of numerous lamellae folded in a monolayer of mixed cells. This complex gill structure is ideal for gas and ions exchange with the environment, but it makes the gills vulnerable to external toxicants. PAA exposure effect on fish gill health varied depending on PAA concentration and frequency of exposure. For example, rainbow trout periodically (1 mg/L/24 h twice a week) exposed to PAA-based disinfectant developed gill lamellae hyperplasia, whereas such pathological alteration was not evident in continuous exposure (0.2 mg/L) (Liu et al., 2020). Similarly, in the current study, fish exhibit a healthy gill lamella when exposed to PAA concentration equal or below 1.6 mg/L, which may indicate that these PAA concentrations did not pose a risk to acute gill health. In contrast, high (3.2.and 6.4 mg/L) PAA concentrations led to severe necrosis of gill lamellae which likely compromised gill functions. This partly provides support as to why mortality was very high in these two groups. Other studies that exposed Atlantic salmon to adverse external environment, such as ozone, found a similar result with severe morphological damages of the gill resulting in fish mortality (Stiller et al., 2020).

Peracetic acid (PAA)-based products are stabilized using an acidified mixture of acetic acid and hydrogen peroxide, resulting in a low pH solution. For example, the PAA-based product used in the current study has a pH value <2, according to the manufacturer (Aqua Des ™, Aquatic Chemistry AS, Lillehamer, Norway). Previous work found that adding PAA to the water cause a pH reduction, and that the pH reduction was lower depending on PAA concentration and water alkalinity levels (Liu et al., 2021). Moreover, the same study found that the decrease of water pH played a determining role in PAA toxicity in low alkalinity water, where the toxicity as LC50 of zebrafish embryos to PAA was lower at high alkalinity (630 mg/L as $CaCO_3$) compared to a medium (120 mg/L as $CaCO_3$) and low (12 mg/L as $CaCO_3$) alkalinity water. Similarly, another study found that the toxicity of PAA to zebrafish embryos to be

negatively correlated with water alkalinity (Marchand et al., 2013). These authors exposed zebrafish embryos to PAA ranging from 0 to 9 mg/L in low (25 mg/L as $CaCO_3$), medium (250 mg/L as $CaCO_3$) and high (2500 mg/L as $CaCO_3$) alkalinity water. The lowest LC50 value was 2.24 mg/L PAA in the low alkalinity water, and the highest LC50 value was 7.14 mg/L PAA in the high alkalinity water. In current study, the water quality during the experiment was, except for pH, within recommended levels for Atlantic salmon (Gutiérrez et al., 2019; Thorarensen and Farrell, 2011). The minimum pH value without impact on Atlantic salmon parr is indicated to be 5.6 (Kroglund et al., 2008). This limit suggests that the results from the 6.4 mg/L group, particularly the mortality and gill histopathological changes, can be from the interaction of the toxic effect of the PAA and sub-lethal pH values. Moreover, the low water alkalinity (± 20 mg/L as $CaCO_3$)) at that this toxicity study was conducted may explain the higher susceptibility found on Atlantic salmon parr to PAA when compared to other studies in full-seawater strength with high alkalinity values (Lazado et al., 2020b; Soleng et al., 2019).

In conclusion, the present study identifies that the no-observed-effect concentration of PAA for Atlantic salmon parr to be below 1.6 mg/L thus providing support for its eventual use as a routine water disinfectant. Fish mortality and the health status of skin and gill was severe compromised at PAA concentrations equal or higher than 3.2 mg/L, whereas changes in swimming behaviour were pronounced at concentrations equal or higher than 1.6 mg/L. Fish swimming activity could provide an early warming to the distress and eventuality to mortality, that are associated with PAA exposure. The strong acidification effect of PAA-based products should be considered on the product toxicity and on the pH reduction of low water alkalinity aquaculture systems. The data from this study is relevant for potential application of PAA as a routine RAS loop water disinfectant.

CRediT authorship contribution statement

Vasco C. Mota: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Maia L. Eggen:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Carlo C. Lazado:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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