Benchmarking healthy gills in Atlantic salmon (*Salmo salar)* in seawater recirculating aquaculture system after repeated peracetic acid exposure

Sindre Haddeland

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Sindre Haddeland

Supervisor: Karin Pittman

Department of Biology

Faculty of Mathematics and Natural Sciences

University of Bergen

Bergen, Norway.

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Abstract

Peracetic acid (PAA) is considered as an alternative to hydrogen peroxide in disease treatment and as prophylaxis. PAA is applicable in both RAS-systems and sea-cages. Since it is applied in the water, the effect of PAA on fish health can be answered by the innate immune system and the mucous cells in the barrier tissues of gills and skin and the degree of alteration on the gills. Gills are a good indicator of fish health and responds quickly to a stimulus. We studied the effect of repeated exposure to a range of low doses of PAA on the gills and some skin of Atlantic salmon (*Salmo salar)* from 100-300g in Hirtshals at DTU Aqua. Twelve tanks in a seawater RAS system stocking 30 fish each were repeatedly exposed to 6 doses ranging from 0 to 2.4 ppm PAA. The first exposure lasted 5 minutes and 15 days later the second exposure lasted for 30 minutes. Sampling was done 2 hours, 2 days and 2 weeks after each exposure making a total of 60 gill samples and 10 skin samples treatment. The volumetric density, mean area of the mucous cells and the barrier status was measured using Veribarr mucosal mapping technology in the gill filament and lamella and histopathological alteration index was constructed for the gill tissues groups exposed to 0.6, 1.2 and 2.4 ppm PAA and the control. However, for skin, only the groups exposed to 0.6 ppm, 2.4 ppm PAA and the control was analysed, and the maximum lamellar diffusion distance was measured on the control and the group exposed to 2.4 ppm PAA.

The repeated exposure to PAA had some effect on the mucous cell density on the skin where it for some fish decreased to under 10% of the volumetric density in the second exposure, which is associated with harsh treatment. However, the repeated exposure had no effect on the mucous cell density on the lamella which consistently was unchanged from the control group with a mean mucous cell density of 1.48%. The lamellar mucous cell area in the groups exposed to 0.6 ppm PAA and 1.2 ppm PAA was significantly larger than that in the group exposed to 2.4 ppm 2 hours after first exposure. However, the 0.6-, and 1.2 ppm PAA remained stable during the repeated exposure. The mucous area of the control and the group exposed to 2.4 ppm PAA trended to increase after second exposure and was equal to all groups with a mean mucous area of 54μm^2 and a barrier status of 0.34. There were no significant differences in mucous cell area or density in the gill filament mucosa between the treatment groups. The barrier status on the lamella and filament showed a trended response with decreased barrier status after the first

exposure. However, the second exposure had little effect on the mucosal barrier status. The Histopathological Alteration Index showed that 94.2% of the gills investigated were healthy. The maximum diffusion distance of the lamella increased 2 hours after the first exposure and was stable for the rest of the trial around 6 µm. This allows us to benchmark healthy gills in seawater RAS with the parameters summoned in Table 1.

Table 1: Benchmark of healthy salmon gills in seawater RAS. N=240 Atlantic salmon (Salmo salar) exposed twice to peracetic acid. Sampling was done 2 hours, 2 days and 2 weeks after each exposure for groups exposed to 0.6-, 1.2-, 2.4 ppm PAA and the control, making a total of 60 gill samples per treatment groups

Tissue	Mucous cell Area (µm^2)	Mucous cell Density (%)	Barrier status	Maximum Diffusion distance (µm)	HAI-Score
Lamella	49.98	1.48	0.29	6.11	4.4
Filament	85.27	9.48	1.11		

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1. Introduction

Production of Atlantic Salmon (*Salmo salar)* has in the last 50 years increased to 1.3 million tons (2017) and the Norwegian government has set their goal to increase this production 5 times (Hovland et al, 2014; SSB, 2018; Regjeringen.no, 2014) The industrialization of fish production has favoured higher density of fish stocks compared to the wild counterparts, and the epigenetic changes in life history have led to an increase in multiple health and welfare challenges including diseases (Miller et al, 2014), water quality (Qun et al., 2016) and mechanical stress such as crowding, handling, sorting and transport (Basrur, 2010; Barton & Iwama, 1991). These factors stimulate stress that requires the individual to spend energy towards maintaining homeostasis, and not on growth, reproduction or normal behaviour (Damsgård et al., 2006).

1.1 Fish Welfare

The importance of animal welfare is commonly acknowledged, but there is not yet a consensus about the criteria for fish welfare (Noble et al, 2018). Brambell (1965) developed the five freedoms of animal welfare that were formalized by the Farm Animal Welfare Council (1979). The five freedoms are listed as:

1. Freedom from thirst, hunger or malnutrition.
2. Appropriate comfort and shelter.
3. Prevention or rapid diagnosis and treatment, or injury and disease.
4. Freedom to display most normal Pattern of behaviour.
5. Freedom of fear.

Animal welfare can further be approached from the animal's biological function, the animal's natural life, and the animals subjective experience of its situation (Damsgård et al, 2006).

Fish welfare can be approached by biological function and the health (Damsgård et al, 2006). Good fish health can be defined as the ability to maintain homeostasis and normal biological function, reflected in high production rates and the absence of disease (Segner et al, 2012). The result of deviation from homeostasis is stress. The acute stress response is an adaptive response that mobilizes energy for the fight or flight response. Repeated stressors can lead to chronic stress that may affect welfare, health and mortality (Damsgård et al, 2006). Atlantic salmon stressed once daily had 34% lower growth rate than the control, reduction in growth was

replicated in Atlantic salmon stressed once every week (McCormick et al, 1998; Basrur et al, 2010).

Fish health can be measured by the robustness of the fish (Pettersen et al., 2014). A robust fish was defined by Castro et al., (2011) as a fish that has strong disease resistance, an ability to cope with environmental challenges and the capability of combining fast growth with normal organ development. The disease resistance is dependent on the innate and the adaptive immune system (Secombes & Wang, 2012). Long term stressors have been shown to impact the immune system in sea bass (Vazzana et al., 2002) and sea bream (Sunyer & Tort, 1995). Stressors that impacts the fish health can vary in aquaculture, where the stressors can be environmental and physical (Tort, 2011). Clearly microorganisms, suboptimal water quality, inadequate nutrition and "routine" husbandry or transport can all contribute to weakening of the immune system and the protective barriers tissues of gill, skin and gut.

1.2 Recirculating aquaculture system

Fish health can be challenged uniquely in different systems, and welfare indicators are important to understand if the fish are thriving in its environment. Culturing of fish in recirculation aquaculture system (RAS) has increased in the recent years due to technical advances and increased demand for fresh water resources. RAS-systems provides more stable and controlled environmental conditions than flow through-systems (Summerfelt et al, 2009), but also a narrower microbial environment which is impacted by every change in feed, fish and the water (Kitano & Oda, 2006)**.** In flow through systems, uncontrolled change in water quality can produce a stressor that disrupts the homeostasis of the fish which can lead to reduced growth and performance (Kolarevic et al., 2014). Thus, it could seem plausible that a RAS-system provides better conditions for fish health compared to traditional production. A RAS-system is dependent on efficient water treatment, to keep environmental parameters below hazardous values. Environmental parameters such as ammonia, carbon dioxide and oxygen have been challenging to manage (Badiola et al., 2012).

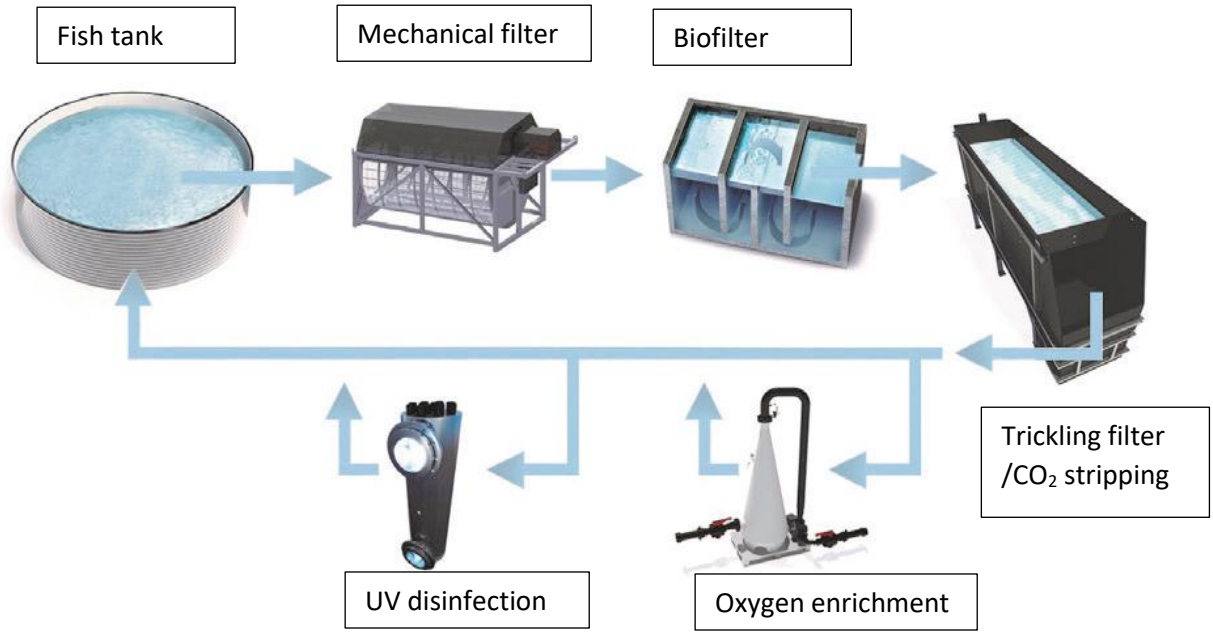

Figure 1: Principle of a RAS system and the path for cleaning and degassing of water. The mechanical filter removes the large organic waste particles, biofilter denitrifies remaining particles, trickling filter removes CO2, oxygen cone adds oxygen and UV disinfects the water. Figure retrieved from Bregnballe, 2015

Recirculation of water relies on the removal of particles and conversion of NH_3 to $NH_3^-$by autotrophic bacteria such as *Nitrosomas spp.* and *Nitrospira spp* (Dittmann et al., 2017; Wold et al., 2014). Autotrophic bacteria can be challenged by heterotrophic bacteria in RAS-systems and compete for space and oxygen. Heterotrophic bacteria are important due to their metabolic by-products and their risk of causing disease (Michaud et al., 2006). Accumulation of particles in a RAS-system can enhance proliferation of heterotrophic bacteria, disrupt the biofilters and impact the fish health (Wold et al., 2014). Surplus in organic materials disrupt the carbon/nitrogen (C/N) ratio and favours opportunistic bacteria. Low organic carbon and a low C/N ratio favours autotrophic bacteria (Michaud et al., 2006). The favouring of K-strategists in RAS-communities allows for more mature and stable bacterial community (Attramedal et al., 2014). In RAS-systems there is a constant struggle to keep the water clean, to reduce the possibility of opportunistic bacteria and to reduce the number of solids in the water (Rurangwa & Verdegem, 2014).

1.3 Barrier tissue

1.3.1 Gill Structure and function

Mucus epithelium represents the biological interface between the fish and the external environment. Mucosal surfaces cover all fish surfaces in contact with the environment (gill, skin and gut), and constitute a barrier as a first line defence against pathogens and other harmful compounds. In addition to a first line of defence in the gills, the mucosal surfaces are responsible for vital physiological processes such as osmoregulation, waste excretion and respiration. (Koppang et al., 2011). The mucus layer is dynamic in both quantity and quality in substances present. It is continuously secreted from mucous cells to prevent colonization of bacteria (Esteban, 2012). Mucous cells which are the producers of mucus have measurable and repeatable patterns of size and volumetric density in response to stress, diet, handling and pathogens which are of clinical importance (Pittman et al., 2011; Pittman et al., 2013; Torrecillas et al., 2015; Dang et al., 2019; Rantty, 2015; Hallberg, 2017; Thorsen, 2016; Cabillon & Lazado, 2019).

The mucosal surface protects the epithelium against infection, dehydration, chemical- and physical injury (Pittman et al, 2011; Alverez-Pellitero, 2008; Peatman & Beck 2011). The mucosal surface is part of the innate immune system and consists of both bactericidal and bacteriostatic compounds (Pittman et al, 2011). Mucin is produced by mucous secretion cells and contains bioactive immune components, such as immunoglobulin, lectins, lysosome, proteolytic enzymes, esterase, antimicrobial peptides and haemolysin (Alverez-Pellitero, 2008). Mucous cell stimulation increases the protection around the epithelium, and thus provides better protection against environmental agents (Strzyżewska-Worotyńska et al, 2017).

The gills are one of the main portals of entry for pathogens due to its vast surface area and the short distance at approximately 0.006 mm between water and blood. The protective properties of mucous cells in gills have not been as fully investigated compared to skin, but a high similarity between the function in gills and skin have been indicated (Koppang et al, 2011). The function of the mucosal barrier, including composition, structure and thickness of the mucosal layer can vary (Torrecillas et al., 2015). The function of mucosal epithelium depends on their somatic location within an organ (Pittman et al., 2013). The mucosal barrier can be affected by mechanical, physiological and immunological effects (Castro & Tafalla, 2015).

Gills are constantly exposed to the external environment and are one of the main entry points for pathogens, environmental toxicants, and are easily affected by compounds like suspended

solids in the water (Lee & Neff, 2011; Badiola et al., 2012). Gill tissue are also the first to react to unfavourable environmental conditions (Poleksic & Mitrovic-Tutundzic, 1994). Lesions on the gill tissue can be visible ahead of behavioural changes (Strzyżewska-Worotyńska et al., 2017). The quick reaction makes gills effective to monitor the health of the fish (Palaniappan et al., 2010).

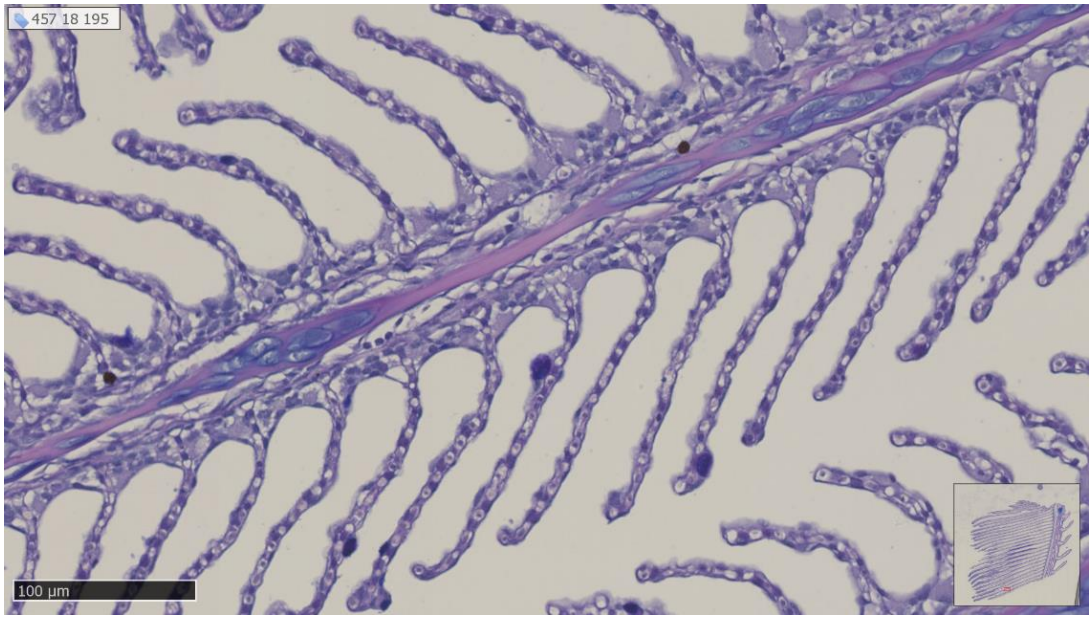

Figure 2: Healthy gills from sampling pool of 240 Atlantic salmon exposed to four different doses of peracetic acid. The doses ranged from control to 2.4 ppm PAA. Atlantic salmon was exposed twice with the first exposure lasting 5 minutes and second exposure lasting 30 minutes. Sampling was done 2 hours, 2 days and 2 weeks after each exposure.

Gill tissue has functions in osmoregulation, respiration and acid-base balance (Evans et al., 2005**).** Gills have a large respiratory surface that constitutes approximately 50% of the total area of the fish (Braunbeck et al., 1998). Structurally, the gills are divided between the filament and the lamella. The filament has a simple design with a cartilaginous rod as support and covering two blood vessels. Lamella ascend from the filament as a secondary circulatory system and provides gills a vast respiratory surface. Lamella are internally supported by pillar cells, that also isolates blood plasma from the environment. A double layer of epithelial cells covers the both the lamella and the filament. The epithelial layer contains mucous- and chloride cells among other specialized cells. The epithelial layer enhances the surface area by the presence of microridges which also enhances the adherence of mucous (Koppang et al., 2015; Kryvi & Poppe, 2016). Mucous secreting cells is uniquely positioned to cover intracellular junctions in the epithelium with mucus (Peterson, 2011).

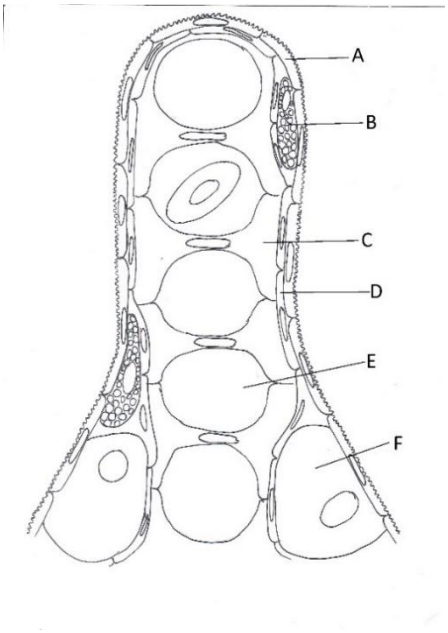

Figure 3: Histological anatomy of the lamella, A=Epithelium, B=Mucous cell, C= Pillar cell, D=Inner epithelium, E= Blood vessel, F= Chloride cell. Figure by Kaja Moe.

Accumulation of particles in RAS systems can directly impact the gill tissue (Randall, 1984). Gill cover deformities can further enhance the exposure of the gills. Shorten opercula can lead to shortening and thickening of gill filaments and make handling of fish more challenging (Pettersen et al., 2014). All these factors affect the gills and the performance of the fish. The first line of response to antigens is the innate immune system, including physical barriers, humoral and cellular responses. Pathogens are usually blocked by the physical barriers such as mucous, epithelium and cellular responses (Castro & Tafalla, 2015).

Environmental stimuli can impact the gill tissue. Alterations on gill tissue have been shown to be caused by pathogens (Munday et al., 2002), pollution (Evans, 1987) and particles (Randall, 1984) among other stimuli. Gills exposed to suspended materials have shown increased mucous production, hypertrophy and hyperplasia (Reynolds et al., 1989) Histopathological alterations in the gill can affect the robustness of the fish due to altered epithelium and thus reduced function (Henriksen, 2013; Tort et al., 2011). Alterations can vary from minor damages like clubbing and lifting of the epithelium to necrosis of gill epithelium. To quantify the degree of alteration, indexes like Histopathological alteration index have been structured (Poleksic & Mitrovic-Tutundzic, 1994). Factors affecting the large and sensitive gill tissue will also trigger the immune response to protect the fish.

Morphological alterations in the gills can be divided into lesions that results from direct damage of environmental stimuli, and lesions that results from defence mechanisms (Strzyżewska-Worotyńska et al., 2017). Gill lesions as a defence mechanism such as lifting, hypertrophy and hyperplasia increases the distance between the blood and the environment, resulting in reduced absorption in the gills. (Velasco-Santamaria & Cruz-Casallas, 2008; Strzyżewska-Worotyńska et al., 2017). The diffusion distance between blood and water, is one of the most important dimensions of gills for fish respiration (Flajšhans & Piačková, 2006). The water is separated from the gills by pavement cells, mucous cells and chloride cells. Proliferation of any of these cells can cause an increase in the water to blood distance, although proliferation of mucous cells enhances the protection of the epithelium with an increased layer with mucous (Strzyżewska-Worotyńska et al., 2017).

1.3.2 Skin Structure and function

The body surface of the fish is a physical barrier against potential pathogens and prevents against leakage of water and nutrients. Skin have multiple purposes including maintaining body shape and protecting the body against physical damages. The skin barrier is a semipermeable barrier with chemical and biological properties (Raj et al., 2011). Fish skin secretes mucous that exhibits immune functions (Minniti et al., 2017). The presence of mucous cells in fish skin are highly numerous and are an evolutionary adaptation due to the high density of pathogens in sea water (Xu et al., 2013; Magnadottir, 2010).

The skin is morphologically, divided in to the inner and the outer layer, called epidermis and dermis. The epidermis is stratified and can be subdivided into the surface, intermediate and basal layer. It consists mainly of squamous cells and mucous cells. The surface layer contains a single layer or squamous cells and keratin that creates micro ridges on surface that helps contain the mucous (Kryvi & Poppe, 2016; Esteban & Cerezuela, 2015; Speare & Ferguson, 2006). The intermediate layer contains a variety of cells, including mucous cells, sensory cells, pigments cells, ionocytes and immune cells. The basal layer is a single cell layer that links the epidermis with the dermis. The dermis contains dense connective tissue and scales, and the hypodermis contains loosely organised collagen fibres and blood vessels (Esteban & Cerezuela, 2015).

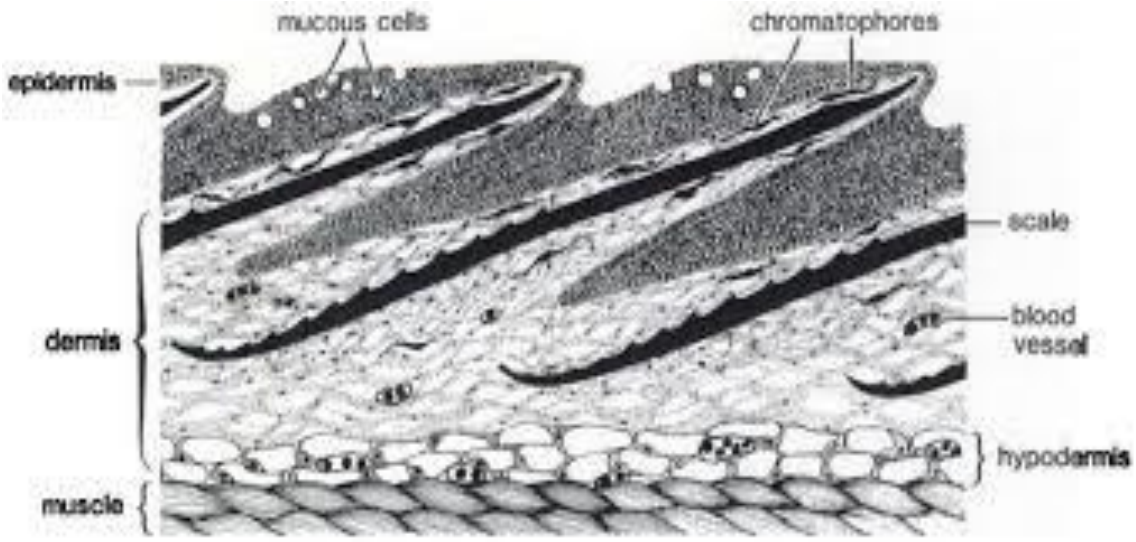

Figure 4: Longitudal histological section of skin in Atlantic salmon. Retrieved from Stead & Laird (2002).

1.4 Chemotherapeutic treatment

The outer barriers are continuously challenged by the environment (Segner et al., 2012), and oxidising agents are being used in commercial aquaculture in disease treatment and as prophylaxis to prevent potential diseases. Hydrogen peroxide (H_2O_2) have been used in aquaculture as diseases treatment and prophylaxis (Overton et al., 2018). The welfare aspects of H_2O_2 have been investigated, and high accumulated mortality have been recorded in commercially used H_2O_2 treatments (Overton et al., 2018).

The toxicity of H_2O_2 increases with temperature and exposure time (Johnson et al., 1993), and flight response has been observed during treatment (Henriksen et al, 2013). Acute toxicity of H_2O_2 in Atlantic salmon has also been shown to induce intensive epithelial lifting and necrosis in gill tissue (Johnson et al, 1993). The tolerance of H_2O_2 correlates with the size of rainbow trout and it has been hypothesized that hydrogen peroxide toxicity increases with increased gill surface area (Rach et al, 1997). The effect of H_2O_2 on gills are summoned up by Henriksen et al (2014) and includes increased epithelial cells, edemas, fusion of lamella, hyperplasia, granularity, swelling and lifting of epithelium.

Peracetic acid (PAA) is considered an option to hydrogen peroxide. It is an organic acid and is considered a strong disinfectant with an oxidative potential that is larger than chlorine, hypochlorite, hydrogen peroxide, and have a high treatment efficiency. (Pedersen et al, 2009). PAA is a mixture of hydrogen peroxide, acetic acid and water (Yuan et al., 1997). PAA in water undergoes hydrolysis through this reaction:

$CH_3COOH + H_2O_2 \rightleftharpoons CH3COOOH + H_2O$

This reaction is reversible and commercial. PAA usually has stabilizers such as phosphonates to maintain its chemical state (Wagner et al, 2002; Yuan et al., 1997).

PAA is effective against microorganisms due to its high oxidation potential and is not affected by catalase or peroxidase (Pubchem, 2018). PAA have high antimicrobial activity and parasitical effect in various temperatures (Pedersen et al, 2009). The bactericidal effect of Peracetic acid is summoned in table 1. The effect of PAA on *Flavobacterium columnare* was dependant on the H_2O_2: PAA ratio, where higher amounts of H_2O_2 increased the bactericidal effect on *Flavobacterium columnare* (Marchand et al., 2012).

Table 2: Bacterio-, parasit-, and fungicidal effect of peracetic acid on various fish diseases in vitro.

Disease	Dosage of PAA (ppm)	Effect	Source	Study
Ichthyophthirius multifiliis	2	Toxic	Meinelt et al, 2009	In vitro
Flavobacterium columnare	1.0	Reduced growth	Marchand et al, 2012	In vitro
Saprolegnia parasitica	4.0	Growth Inhibition	Marchand et al, 2012	In vitro
Yersinia ruckeri	1.0	Reduced growth	Meinelt et al, 2015	In Vitro
Aeromonas salmonicida	1.0	Reduced growth	Meinelt et al, 2015	In Vitro

The welfare aspects of PAA have been investigated on several fish species. Liu et al (2017a) found that 2 mg/L PAA induces an immediate stress response in carp (*Cyprinus carpio*) with elevated levels of cortisol, while repeated exposures decreased the cortisol levels. Liu et al. (2017b) found that the stress induced by pulse application of PAA (1 mg/L) is manageable for rainbow trout (*Oncorhynchus mykiss)*, this was indicated by downregulation of cortisol and unaffected growth compared to the control group. These findings were confirmed by Gesto et al. (2018) that tested the application of PAA for 6 weeks and found that rainbow trout are truly able to habituate to pulse treatment (1 mg/L) of PAA. This can be explained by the ability of cells in tissues to learn and to tolerate. The behaviour of a system or cells can be influenced by

earlier experiences. Repeated stressors can decrease the probability of a response (Tang & Marshall, 2018). Tolerance can also be induced by local and systemic unresponsiveness, induced by multiple cellular and molecular processes. Tolerance to a stimulus are mainly studied in mammals, where multiple processes are needed to ensure tolerance to innocent stimuli (Rombout, et al., 2014). PAA can also cause high acute toxicity. Channel catfish fry (*Ictalurus punctatus*) had a high acute toxicity towards PAA dosages over 1.3 ppm. severe degeneration of gill epithelium was observed in the group given 2.2 ppm PAA. The degeneration of gill epithelium resulted in accumulation of cell debris between lamellae. (Straus et al, 2012).

We investigated the effect of repeated exposure of PAA in doses ranging from control to 2.4 ppm on fish health. To test the effect of PAA on fish health, we conducted a study in Seawater-RAS where 12 tanks was exposed twice to 6 different dosages. Sampling was done 2 hours, 2 days and 2 weeks after each exposure. We investigated the effect of PAA on salmon performance and health by measuring the effect of these doses PAA on mucous cell size, volumetric density and barrier status of the mucous cells in gill lamellae, filament and the dorsal skin, and the effect on gill diffusion distance for oxygen uptake.

1.5 Objectivity of mucosal mapping

The size and density of mucous cells in the epithelium in skin gill and gut can be measured using tangential histological sections (Pittman et al., 2011; Pittman et al., 2013). The analysis is a result of tailor-made design-based stereological analysis and now trademarked as Veribarr. The tangential sectioning represents 2 cm^2 of mucous cells that can potentially surface and interact with the microbiome on the fish and in the environment. The analysis gives a reproducible detection of mean area and volumetric mucous cell density in the mucosal epithelium of any organism (Pittman et al, 2013). The data that are generated through mucosal mapping are objective, statistically robust and can be compared across species, tissue, time and treatment (Noble et al, 2018).

1.6 Peragill

This project is part of an FHF-funded project named Peragill that propose peracetic acid as a potential treatment against amoebic gill diseases in Atlantic salmon. My thesis investigates the effect of peracetic acid on salmon health my measuring and investigating the morphological and physiological parameters of the gills. Gene expression, cortisol measurements and histopathology among others was done in addition to mucosal analysis to determine whether peracetic acid had chronic or immediate effects on the welfare of post-smolt salmon.

2. Materials and Methods.

2.1 Trial Design and husbandry

The experiment was conducted in a land-based sea water-RAS facility in Hirtshals (Denmark) at the Danish Technology University (DTU). The experiment was conducted in April-Mai 2018, where post-smolt Atlantic salmon were kept in the RAS-facility for 7 weeks. The RAS system had a 40 µm drum filter, submerged fixed bed biofilter and a trickling filter with a water exchange of approximately 0.4 m^3/h corresponding to a water retention time of 1.5 days. The photoperiod in the experimental hall was kept to the natural photoperiod of April-Mai (16L:8dD). The fish were fed 1-1.5% of the biomass with Biomar, EFICO, 4.5mm through a belt feeder. All the environmental parameters were kept within safe limits (Table 2).

Table 3: environmental parameters measured daily in the recovery tanks in the RAS-facility. The tanks had an approximately volume of 600L and was inhabited by 60 post-smolt salmon. The RAS-facility included drum filter and fixed bed biofilter.

Parameter	Value	Unit
TAN	≤ 0.2	mg N/l
NO_2^-N	≤ 0.2	mg N/l
NO_3^-N	≤ 5	mg N/l
pH	7,5-7,7	
Temp	15 ± 1	°C
Oxygen	80-90	%saturation
Oxygen	7,8-9,1	mg O_2/l
Salinity	33-35	Ppt
Water exchange	~ 10	m^3/d
Daily feed	0,6-1,1	kg/d
Water holdup time	~ 1,5	D

Post-smolt salmon weighing 149 ± 7g were sorted and 360 fish were transferred to twelve individual 1 m^2 tanks of approximately 600 L, making a total of 60 fish per treatment group. The twelve tanks were divided into 6 treatment groups with replicates and exposes twice to different dosages of PAA: 0 (control) 0.15 ppm, 0.3 ppm, 0.6 ppm, 1.2 ppm and 2.4 ppm PAA with two replicate tanks. The Peracetic acid (Divosan Forte, PAA) was supplied by Lilleborg AS in Oslo, Norway. Divasan Forte is a stabilized Peracetic acid solution with the concentration of 15% PAA. Feeding was ceased 24 hours prior to PAA exposure. The fish were transferred to an equivalent exposure tank as shown in Figure 3 for PAA exposure. The fish was given 10

minutes rest after transfer before the concentration was added. The first exposure lasted for 5 minutes and 15 days later the second exposure lasted for 30 minutes. Fish were immediately returned to recovery tank after the exposure, and feeding was resumed 2 days after each exposure.

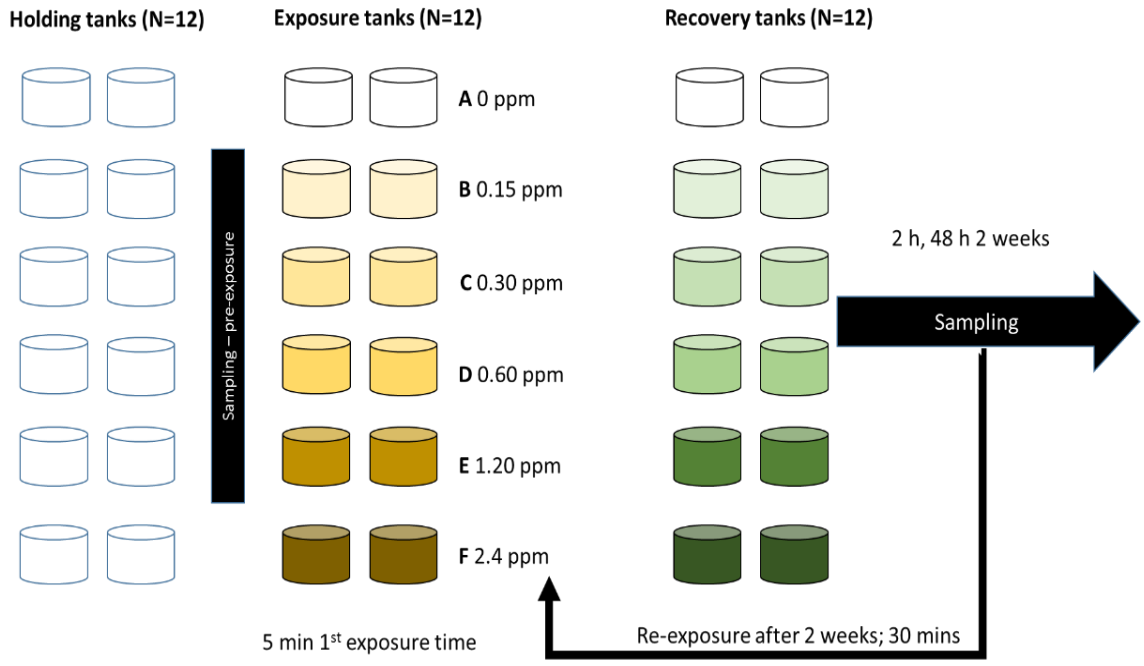

Figure 5: Trial design; Salmon was divided into 6 different groups dependant on the PAA dosage. Each dosage had 2 replicates. Fish was transferred from holding tanks to exposure tanks, and to recovery tanks after exposure. From Lazado et al (unpublished data).

Sampling was done 2 hours, 2 days and 2 weeks after each exposure as shown in Figure 5. Samples was taken from 5 fish from each tank at each sampling time, giving the total amount of 60 samples per dosage. The fish was euthanised with an overdose of benzocaine solution. The length and weight were measured, and an external check was done before sampling. Operculum deformities were recorded but not further analysed. Tissue sampling was done after Quantidoc`s standard sampling protocol. The second gill arch from the right gill cover was sampled from each fish, providing 60 gill samples per sampling day. Skin was sampled under the dorsal fin with a 1-2 cm times 1-2 cm sample. The skin was sampled inconsistently due to few histocassettes. Total amount of skin samples was 28 divided equally into control, 0.6 ppm and 2.4 ppm PAA. All samples were preserved in 10% buffered formalin (SigmaAldrich).

2.2 Histological preparation and mucosal analysis

Gill samples from the control and the groups exposed 0.6 ppm, 1.2 ppm and 2.4 ppm PAA, and skin from the control and groups exposed to 0.6 and 2.4 ppm PAA was processed histologically according to Quantidoc`s standard mucosal mapping staining protocol (2017). Gill and skin tissue were embedded in paraffin, sliced tangentially at 3µm thick sections and stained with PAS – Alcain Blue. All samples were scanned by Hamamatsu slide scanner to high resolution digital images (NDPI format).

Mucosal analysis was done after Pittman et al (2011, 2013). Veribarr™ and Mucomaster were used to analyse and estimate the volumetric density and the mean area of mucous cells on filament, lamella and skin samples. The mean area and volumetric density were used to calculate the barrier status of the mucosal epithelium. The equation for the barrier status is:

$$\frac{1}{Area/Density} * 1000$$

2.3 Diffusion Distance

The diffusion distance (the distance from blood to water) of the lamella was measured on the control group and the group exposed to 2.4 ppm PAA. Measurements were done using VIS image analysis software (Visiopharm, 2018). Approximately 20 random sites on the gill were measured for lamella thickness. The thickness was divided by 2 for maximum diffusion distance in each fish. This does not consider the volume of the capillary channel and the pillar cells as these were irregularly visible.

2.4 Histopathological Alteration Index

Histopathological alteration index (HAI) was done to assess histological alterations in relation to Peracetic acid. HAI was done after Poleksic & Mitrovic-Tutundzic (1994), where gill lesions are given a score according to the severity of the alteration (Table 3).

The score is divided into three stages:

First stage are changes from which the gills can under improved conditions. However, without improvement the damage evolves to second stage that are more severe lesions. These damages can be repaired by water quality amelioration, but if large quantities of the gill tissue are affected, and if the situation doesn’t improve they evolve to third stage lesions. Third stage

lesions are damages that aren`t reversible, and these lesions will impair vital gill functions and mortality (Poleksic & Mitrovic-Tutundzic, 1994).

Table 4: Gill lesion scores adapted from Poleksic & Mitrovic-Tutundzic (1994). The score is given in 3 stages from 1-3 depending on the severity of the damage.

Gill lesion	Stage
a) Hypertrophy and hyperplasia of gill epithelia	
Hypertrophy of respiratory epithelium	1
Lifting of respiratory epithelial cells	1
Leukocyte infiltration of gill epithelium	1
Thinning of respiratory epithelium	1
Rupture and peeling of the lamellar epithelium	2
Focal hyperplasia of epithelial cells	1
Hyperplasia from the base of approximately half of the secondary lamella	1
Irregular (Chaotic) hyperplasia of epithelial cells	1
Fusion of tips of secondary lamella	1
Uncontrolled thickening of proliferated tissue	2
Hyperplasia of sponge like eosinophilic cells	1
Fusion of several secondary lamellae	1
Shortening of secondary lamellae	1
Complete fusion of all the secondary lamellae	2
b) Changes in mucous and/or chloride cells	
Hypertrophy and hyperplasia of mucous cells	1
Empty mucous cells or their disappearance	1
Hypertrophy and hyperplasia of chloride cells	1
Chloride cells present in secondary lamellae	1
c) Blood vessel changes	
Lamellar telangiectasis	1
Filament blood vessel enlargement	1
Haemorrhages with rupture of epithelium	2
Stasis	2
d) Gill Parasites	
e) Terminal stages	
Scar tissue – fibrosis	3
Necrosis	3

The alteration index is calculated using the sum of the amount of lesion types in the formula:

$$I = \sum_{i=1}^{19} a_i + 10 \sum_{i=1}^{5} b_i + 10^2 \sum_{i=1}^{2} c_i$$

Where I = the degree of changes in a single fish gill

a = first stage alterations

b = second stage alterations

c = third stage alterations

The degree of changes in the gill is given in a scale where:

0-10 = Functionally normal gills

11-20 = Slightly moderately damaged gills

21-50 = Moderate to heavily damaged gills

>100 = Irreparably damaged gills.

2.5 Statistics

R studio (R studio, 2017) was used to test statistical variances in the dataset. $P \leq 0.005$ was considered to be statistically significant. A Linear mixed effect model (LME) was used to compare the difference in mean area, barrier status and diffusion distance between treatments at each day. A quasi generalized linear model (GLM quasi) was used to compare the difference in density between treatments. A Pearson correlation test was done for correlation between mucous cell area and diffusion distance.

3. Results

3.1 Growth and Performance

There was no mortality during the trial. All the environmental parameters were within safe limits. There was no significant difference in weight between the treatment groups (Figure 6). The group treated with 2.4 ppm PAA had a significance difference in weight from 2 hours after first exposure to 2 weeks after second exposure (p=0.0325) with a significant growth rate (SGR) at 0.33 during the trail (Table 4). The other treatment groups trended to growth during the trial, where the control had an SGR of 0.19 (p=0.0714), the 0.6 ppm PAA group had an SGR of 0.23 (p=0.07) and the 1.2 ppm PAA had an SGR of 0.20 (p=0.099). There was no significance difference between the initial k-factor and the final K-factor for the groups (Table 4) (Figure 6).

Table 5: Difference in weight, significant growth rate (SGR) and K-factor between the groups exposed to different dosages of PAA. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

Parameters	Control	0.6 ppm PAA	1.2 ppm PAA	2.4 ppm PAA
Initial mean weight	156.57±22.2g	153.72±28.14g	169.11±36.93g	139.29±26.74g
Final mean weight	199.95±31.77g	199.54±27.65g	220.04±43.09g	211.94±33.02g
Mean SGR	0.19	0.23	0.20	0.33
Initial K-factor	1.05±0.21	1.08±0.32	1.10±0.25	1.07±0.07
Final K-factor	1.03±0.19	1.05±0.14	1.03±0.09	1.07±0.19

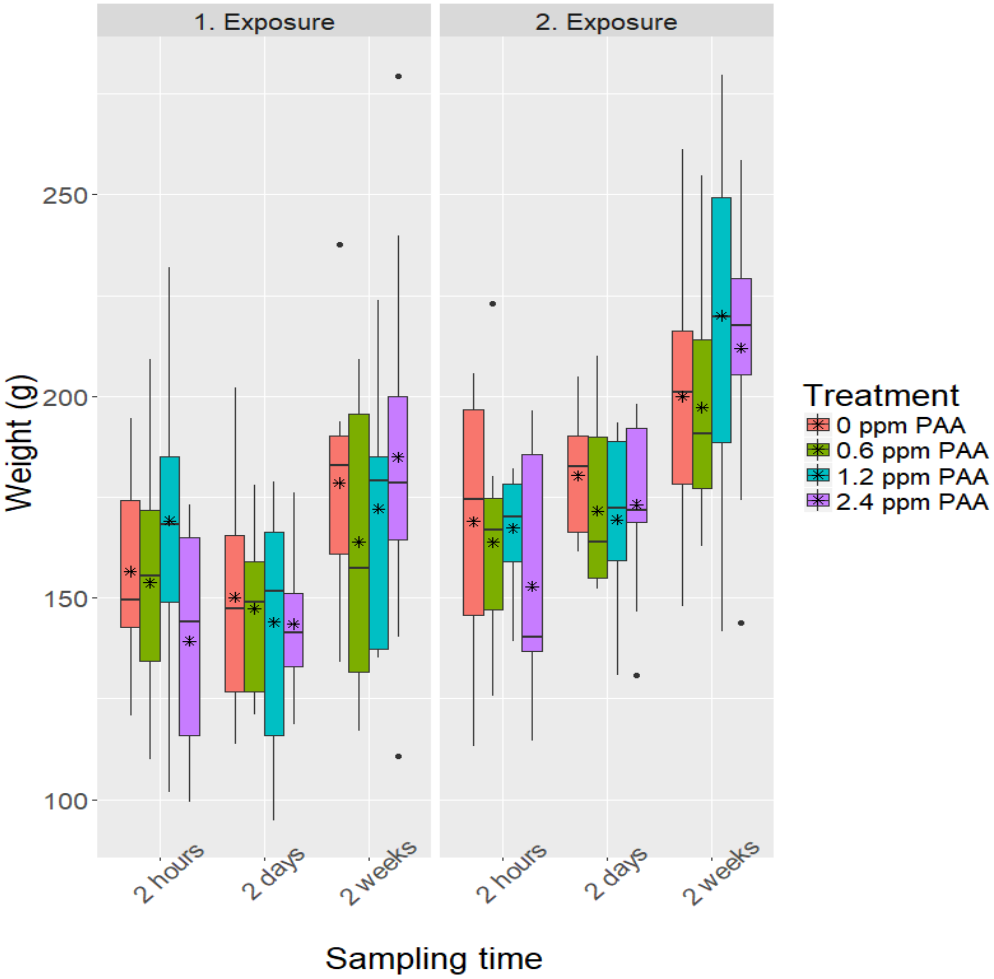

Figure 6: The weight in grams for each treatment group at every sampling time. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment was conducted in sea water RAS and lasted from 29. April to 29. May with 6 sampling times.

3.2 Lamella

3.2.1 Mean mucous area on Lamella

There was no significant difference in mean mucous area between the control (mean 36.025μm^2) and 2.4 ppm PAA group (mean 39.238μm^2) 2 hours after first exposure (Figure 6). The group exposed to 1.2 ppm PAA (mean 54.284μm^2) trended towards larger mucous cells (p=0.0646) than the control. However, the group exposed to 0.6 ppm PAA (mean 60.136μm^2) group had significantly larger mucous cells than 2.4 ppm group (p=0.0246) and the control group (p=0.027) (Figure 7).

The mucous cell area of the group exposed to 2.4 ppm trended to increase (p=0.0789) to a mean of 49.053μm^2 2 days after first exposure. The control increased likewise insignificantly (p=0.0939) to a mean of 51.542μm^2. The mean Mucous size of the groups treated with 1.2 ppm and 0.6 ppm PAA had no significance difference from 2 hours to 2 days after first exposure. Within 2 days after first exposure, there was low difference between the groups with a mean varying from 49.053μm^2 to 52.191μm^2 (Figure 7).

The mucous cell area trended towards decrease for the group exposed to 2.4 ppm PAA (p=0.0511, mean=37.790μm^2) 2 weeks after first exposure. The mucous cell area of the control (mean41.725μm^2), the 0.6 ppm (mean 55.091μm^2) and the 1.2 ppm PAA (mean 60.008μm^2) had no significant difference between 2 days and 2 weeks after first exposure. Within 2 weeks after first exposure there was no significant difference in mucous cell area between the control and the groups exposed to PAA. The 2.4 ppm group had significantly lower mucous cell area than the 1.2 ppm group(p=0.0301) and the 0.6 ppm group(p=0.0129).

The second exposure gave no immediate response in the mucous cell area with no significant difference between the end of first exposure and 2 hours after second exposure. Within 2 hours after second exposure, there was no significant difference between the control (mean 38.974μm^2) and the 2.4 ppm PAA (mean 41.730μm^2). However, the groups exposed to 1.2 ppm (mean 51.059μm^2, p=0.0192) and the 0.6 ppm PAA group (mean 59.095μm^2, p=0.0427) had significantly larger mucous cell area than the control.

There was no significant change from 2 hours to 2 days after second exposure. The group exposed to 0.6 ppm PAA continued to have significantly larger mucous cells than the control

(p=0.0305) and the 2.4 ppm (p=0.0206). There was no significant change for the treatment groups from 2 days to 2 weeks after second exposure, and there was no significant difference between the treatment groups within 2 weeks after second exposure (Figure 7)

There was no significant difference in the mean mucous cell area 2 hours after first exposure and 2 weeks after first exposure for the treatment groups. The mean mucous cell area in the control increased significantly from 2 hours after second exposure to 2 weeks after second exposure (p=0.04), however none of the other treatment groups had a significant change. The mucous area had no significant change for the treatment groups from 2 hours after first exposure to 2 weeks after second exposure for the treatment groups.

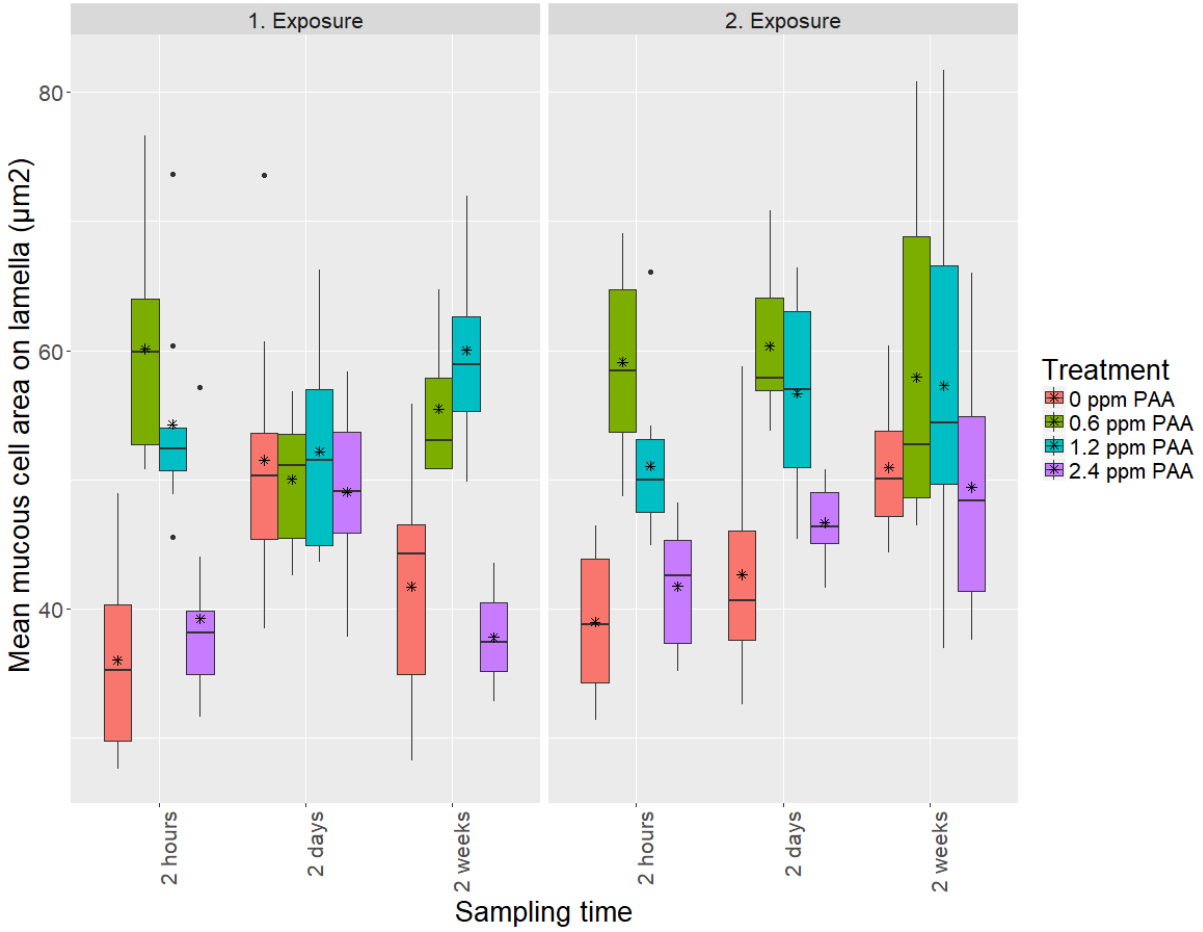

Figure 7: The mean mucous area of the treatment groups at the different sampling times at 1. And 2. Exposure. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.2.2 Mucous cell density on lamella

The first exposure had no significant difference in the mucous cell density on lamella between the treatment groups. 0.6 ppm had the highest variance with densities ranging from 0.8% to 3.5% and a mean of 2.1% of the mucosal epithelium. The mucous density of all the treatment groups trended towards decrease from 2 hours to 2 days after first exposure. However, there was no significant difference between the treatment groups 2 days after first exposure. The means of the mucous density ranged from 0.967% to 1.22% in the mucosal epithelium (Figure 8). There was no change in mucous cell density between 2 days and 2 weeks after first exposure for the treatment groups. The control (1.25%), 0.6 ppm (1.2%) and 1.2 ppm PAA (1.6%) had a trended increase in mucous cell density, and the group exposed to 2.4 ppm PAA had a trended decrease (1.05%) There was no significant difference between the groups within 2 weeks after first exposure (Figure 8).

The second exposure didn't give any immediate effect on the mucous cell density. The group exposed to 0.6 ppm (1.69%), 2.4 ppm PAA (1.18%) and the control (1.36%) had a trended increase in mucous cell density. The mucous cell density had no significant change 2 days after second exposure. The mucous cell density of the control (1.57%), 0.6 ppm (1.87%) and the group exposed to 2.4 ppm PAA (1.52) continued with the trended increase. However, the group exposed to 1.2 ppm PAA (1.51%) had low change in mucous cell density. There was no significant change from 2 days to 2 weeks after second exposure. The control (1.73%) and the groups exposed to 0.6 ppm (2.09%) and 1.2 ppm (2.27%) had a trended increase (Figure 8)

The mucous cell density on lamella had no significant difference from 2 hours after first exposure to 2 weeks after second exposure. The mucous density trended to increase during the trial for the control and the group exposed to 1.2 ppm PAA, and there was low difference between the groups exposed to 0.6 ppm PAA and 2.4 ppm PAA.

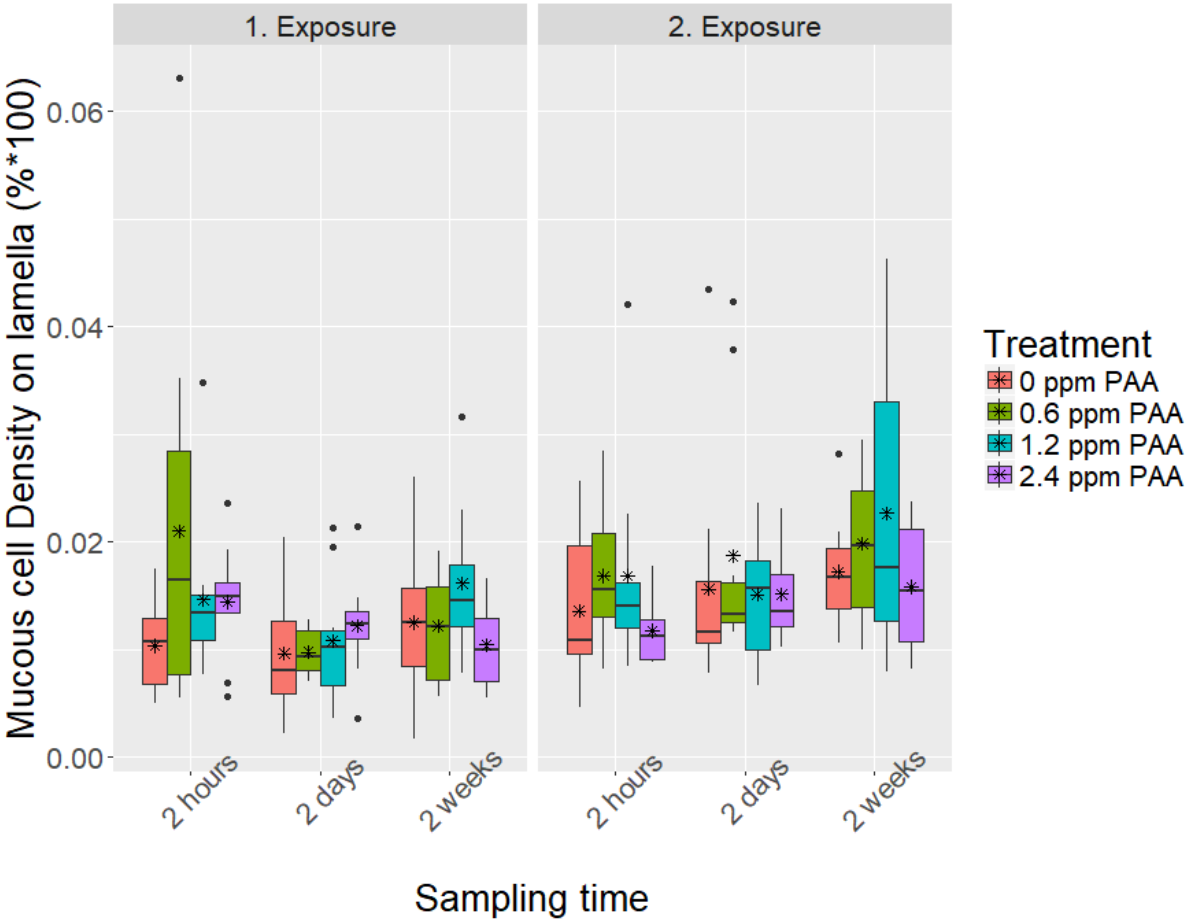

Figure 8: Volumetric mucous cell density on the lamella. The density is given in %*100 of the mucosal epithelium. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.2.3 Barrier status in Lamella

The barrier status on the lamella had no significant difference the treatment groups 2 hours after first exposure. The group treated with 2.4 ppm PAA (mean 0.3780) trended to have larger barrier status than the control (mean 0.2833) and the group exposed to 1.2 ppm PAA (mean 0.2627). There was no significant difference in the barrier status of the treatment groups between 2 hours and two days after first exposure. However, all the treatment groups trended towards decrease in barrier status. Within 2 days after first exposure had no significant difference and low variation between the treatment groups (Figure 9). The barrier status trended to increase for all treatment groups two weeks after first exposure, however there was no significant difference between the treatment groups (Figure 9).

The second exposure had no immediate effect on the barrier status, with no significant change two hours after second exposure. From 2 hours to 2 days had no significant change in barrier status for the treatment groups, with a trended increase for the group exposed to 2.4 ppm PAA (mean= 0.3227), and a trended decrease for the groups exposed to 0.6 (mean=0.2812) and 1.2 ppm PAA (mean=0.2615). However, there was no significant difference between the treatment groups within 2 days after second exposure. There was no significant change from 2 days to 2 weeks after second exposure. The groups exposed to 0.6 ppm (mean=0.3542) and 1.2 ppm PAA (mean=0.3714). The control (mean 0.3402) and the group exposed to 2.4 ppm PAA (mean 0.3208) had low change.

None of the treatment groups changed significantly from 2 hours after second exposure to 2 weeks after second exposure, and there was no significant change from 2 hours after first exposure to 2 weeks after second exposure.

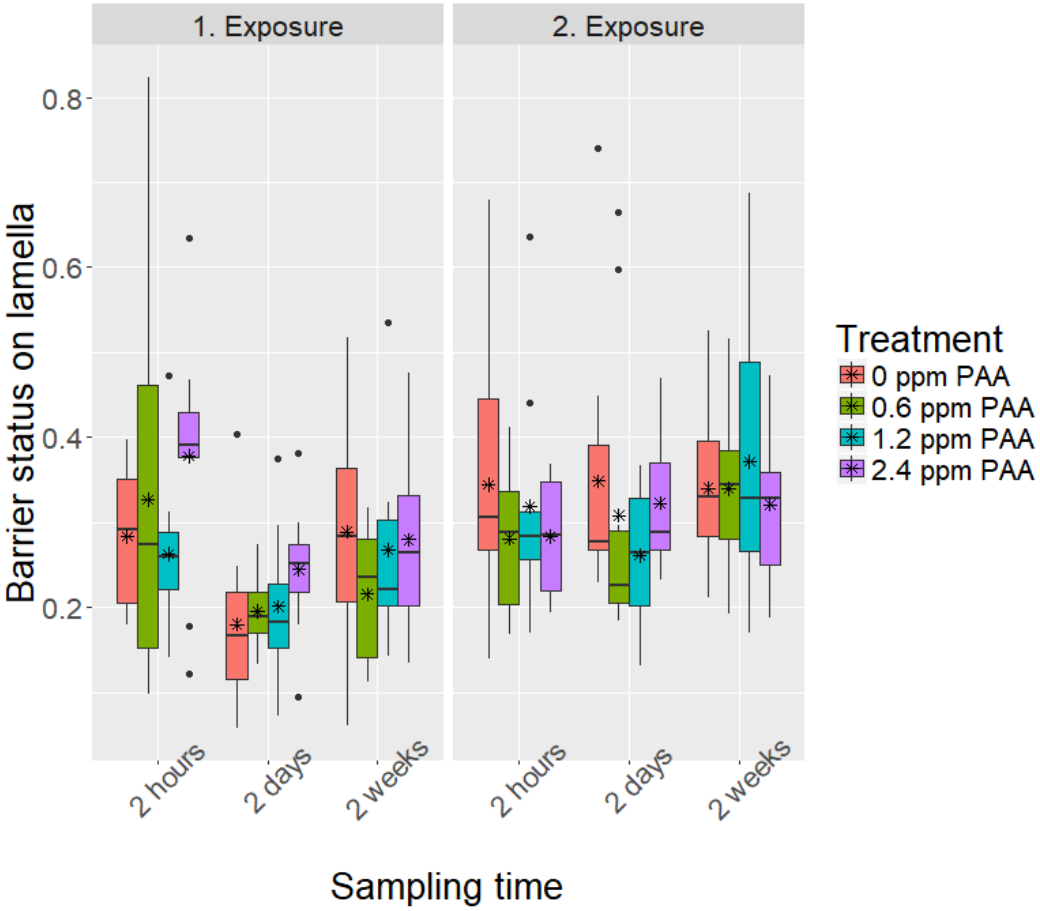

Figure: 9 showing the barrier status of the lamella at every sampling time during the trial. The barrier status is a function of the mucous density and the mean mucous area. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.2.4 Diffusion Distance of the lamella

The diffusion distance in the sampling pool varied from 3.94μm to 9.84μm with a mean of 6.11 μm.

The diffusion distance had no significant difference between the group exposed to 2.4 ppm PAA (mean 5.023μm) and the control (mean 4.864μm) 2 hours after first exposure. The diffusion length for the control increased significantly ($p=0.0202$) to a mean of 6.457μm from 2 hours to 2 days after first exposure. The 2.4 ppm PAA group trended towards increase ($p=0.0998$) in diffusion distance to a mean diffusion length of 6.473μm, but there was no significance difference between them within 2 days after second exposure. The control group trended towards decrease in diffusion distance to a mean of 5.895μm from 2 days to 2 weeks after first exposure. However, the 2.4 ppm PAA group (6.503μm) was stable (Figure 10).

The 2.4 ppm PAA group trended towards a decrease in diffusion distance 2 hours after second exposure to a mean of 5.762μm, and the control trended towards an increase to a mean of 6.413μm. There was no significant difference between 2 hours and 2 days after second exposure. The 2.4 ppm group trended towards increase in diffusion distance (mean 6.392μm) to 2 days after second exposure, and the diffusion distance of the control (6.10μm) trended towards a decrease in diffusion distance. The group exposed to 2.4 ppm PAA continued with a trended increase to a mean of 6.935μm 2 weeks after second exposure. The control trended towards increase in diffusion distance to a mean of 6.601μm (Figure 10).

There was a significant correlation between the diffusion length and the density and barrier st atus of the lamella. However, there was a significant correlation between the mucous area on t he lamella and the diffusion distance ($cor=0.47$, $p=8.2*10^{-8}$) (Figure 11).

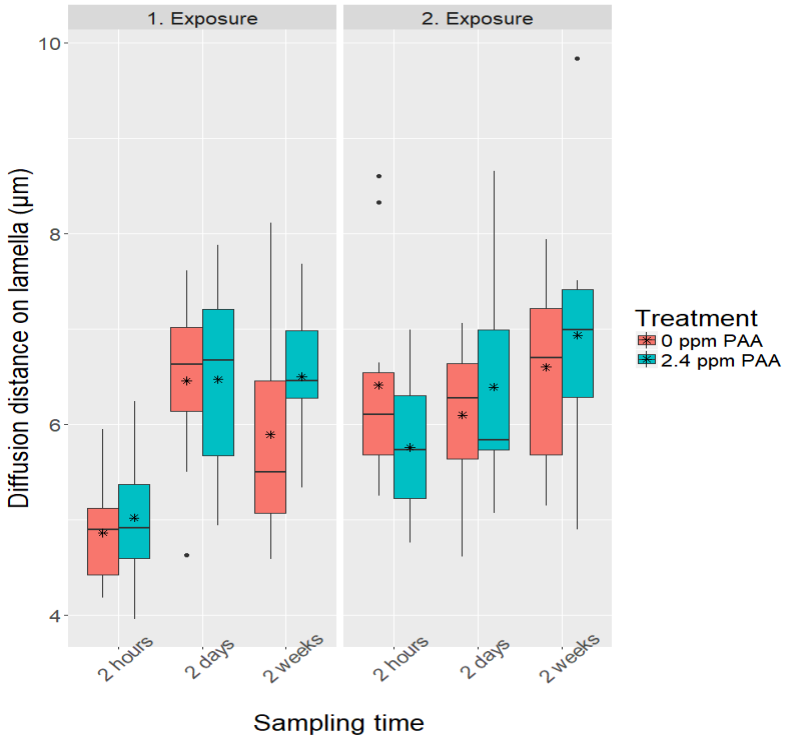

Figure 10: Diffusion length on the lamella given in µm. The red colour represents the control, the blue represents 2.4 ppm PAA, the graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 120 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

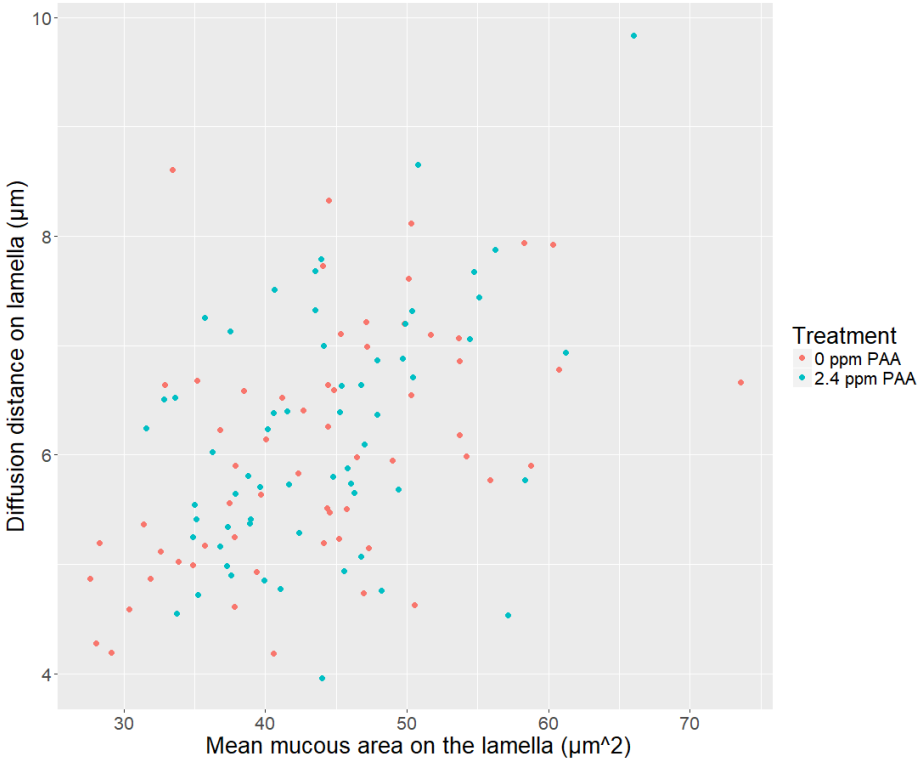

Figure 11: Correlation between Diffusion distance and mucous area on the lamella. N=120 from two exposures of Peracetic acid. The correlation was significant cor=0.47, $p=8.2*10^{-8}$. The fish was reared in Sea water RAS with sampling 2 hours, 2 days and 2 weeks after each exposure of PAA. The first exposure lasted for 5 minutes and the last exposure lasted for 30 minutes.

3.3 Filament

3.3.1 Mean mucous area on filament

The first exposure with PAA gave no significant difference in the mucous area between the control and the treatment groups. The group exposed with 1.2 ppm (mean 90.902µm^2) and 0.6 ppm PAA (mean 88.805µm^2) trended to have larger mucous cell area than the control (mean 75.057µm^2) and the group exposed to 2.4 ppm PAA (mean 74.661µm^2). There was no significant change in mucous cell area for the treatment groups between 2 hours and 2 days after first exposure. Within 2 days after first exposure, there was no significant difference between the mucous cell area in the treatment groups. There was low variation in means, ranging from 81.022µm^2 and 86.821µm^2. There was no significant change in the mucous cell area in the treatment groups between 2 days and 2 weeks after first exposure. The control (76.248µm^2), the groups exposed to 1.2 ppm PAA (mean 101.849µm^2) and the group exposed to 2.4 ppm PAA (77.279µm^2) trended towards decrease in mucous cell area. However, the group exposed to 0.6 ppm PAA had low change in mean area (81.409µm^2). Within 2 weeks after first exposure had no significant difference in mucous area between the groups (Figure 12).

The second exposure didn't give any immediate reactions. The group exposed to 1.2 ppm PAA (97.232µm^2) continued to have a trend of larger mucous cells than the control (72.612µm^2) and the 2.4 ppm group (73.6810µm^2). The group exposed to 0.6 ppm PAA trended to increase to a mean of 90.186µm^2. There was no significant difference in mucous cell area between 2 hours and 2 days after second exposure for the control, the group exposed to 0.6 ppm PAA and the group exposed to 1.2 ppm PAA. The mucous cell area of the group exposed to 2.4 ppm PAA increased significantly ($p=0.0424$) from 2 hours to 2 days after second exposure to a mean of 85.162µm^2. Within 2 days after second exposure, there was no significant difference between the treatment groups. There was no significant change between two days and two weeks after second exposure, with low change in mucous cell area for the treatment groups. Within 2 weeks after second exposure showed no significant difference (Figure 12).

There was no significant difference between the treatment groups between 2 hours after first exposure and 2 weeks after second exposure, and there was no significant difference between 2 hours after second exposure and 2 weeks after second exposure, however the group exposed to 2.4 ppm PAA trended towards an increase ($p=0.09$).

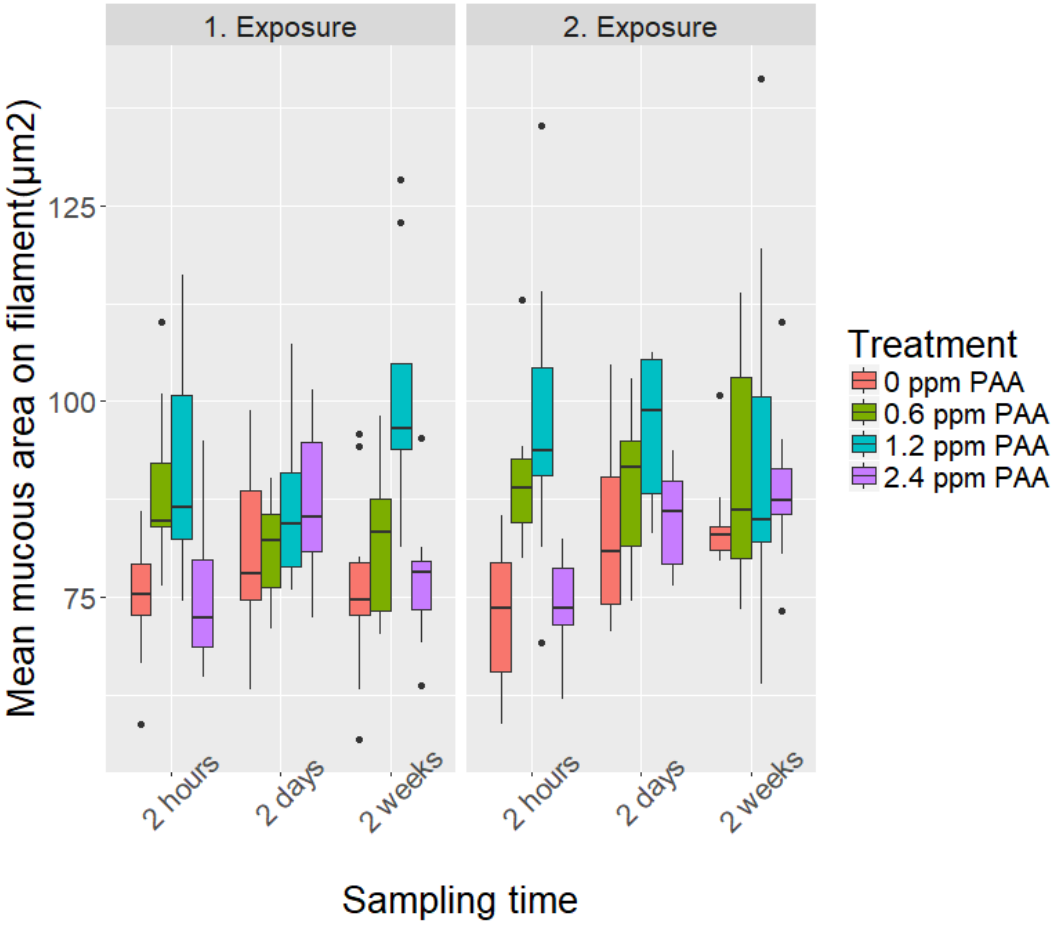

Figure 12: showing the mean mucous area on the filament for all the treatment groups. The mean Area is given in μm^2. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.3.2 Mucous cell density on filament

Two hours after first exposure had no significant difference between the treatment groups. The mean density varied from 8.73% (control) to 10.57% (1.2 ppm PAA) of the mucosal epithelium (Figure 12). Between 2 hours and 2 days after first exposure, the mucous cell density trended to decrease in all treatment groups, but there was no significant difference. Within 2 days after first exposure, the group exposed to 1.2 ppm PAA (mean 8.78%) trended to have ($p=0.0604$) higher mucous cell density than the control (6.34%). However, there was no significant difference in the mucous density of the treatment groups. The group exposed to 2.4 ppm PAA decreased to a mucous cell density of 7.36% and the group exposed to 0.6 ppm PAA decreased to a mucous cell density of 7.44% two days after first exposure. Between 2 days and 2 weeks after first exposure, the control (8.51%), 1.2 ppm PAA (10.17%) and the group exposed to 2.4 ppm PAA (9.35%) trended to increase. The group exposed to 0.6 ppm had low change (mean 6.967%). Within 2 weeks after first exposure had no significant difference in mucous cell density between the treatment groups (Figure 13).

The second exposure didn't give an immediate response in the mucous cell density. Within 2 hours after second exposure, there was no significant difference in mucous density between the groups. Between 2 hours and 2 days after second exposure the control (from 8.42% to 10.83%) and the 2.4 ppm PAA (9.11% to 11.3%) trended to have an increase in mucous density, and the groups exposed to 0.6 and 1.2 ppm PAA remained stable. Within 2 days after second exposure the mucous density had low variation ranging from 10.58% to 11.39% for all treatment groups. The mucous density of the treatment groups had no significant change from 2 days to 2 weeks after second exposure. and there was no significant difference between the groups 2 weeks after second exposure. The Density of the mucous cells didn't change significantly between 2 hours after first exposure and 2 weeks after second exposure.

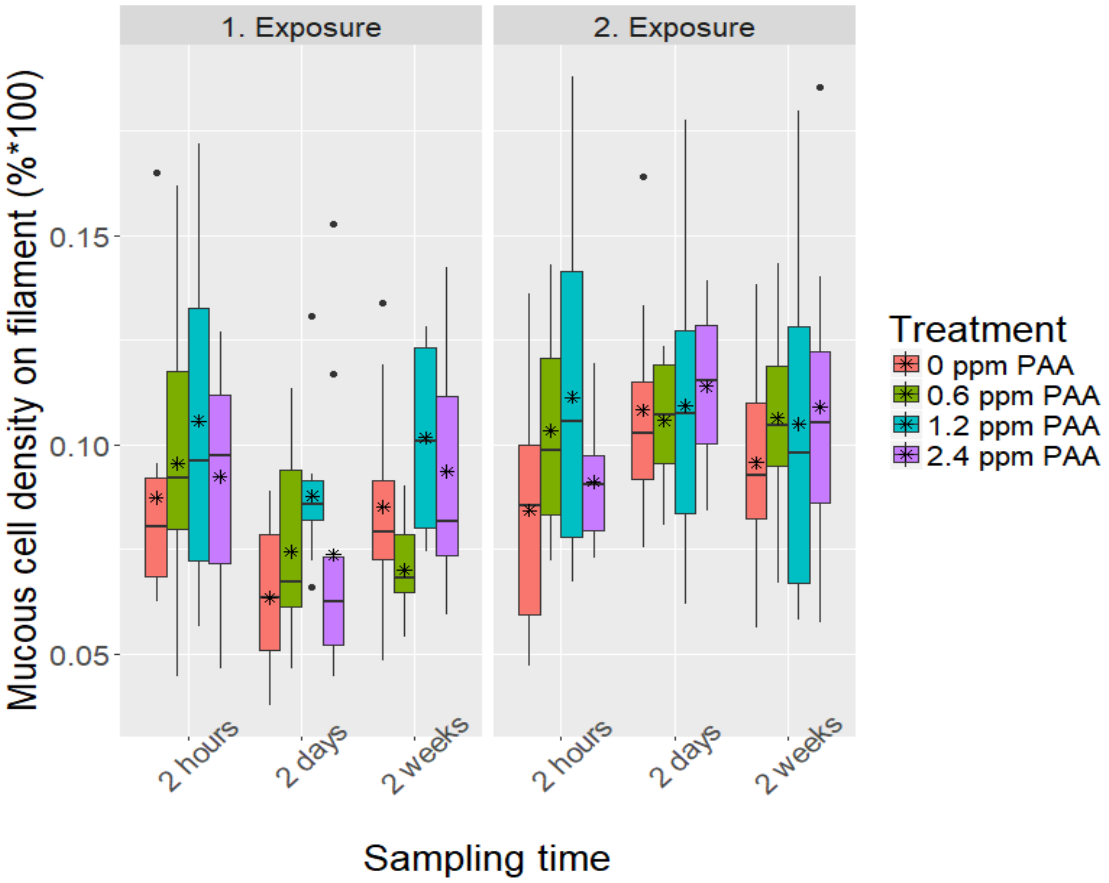

Figure 13: The volumetric Mucous cell Density on Filament (%*100). The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.3.3 Barrier status on Filament

There was no significant difference in barrier status on the filament two hours after first exposure. Between 2 hours and 2 days after first showed no significant difference, but the group exposed 2.4 ppm PAA (from 1.230 to 0.843) and the control (from 1.1587 to 0.7803) trended have a decrease in barrier status. The barrier status of the fish exposed to 0.6 ppm PAA (from 1.063 to 0.911) and 1.2 ppm PAA (1.154 to 1.006) had a minor decrease (Table 11). Within 2 days after first exposure had no significant difference in barrier status between the treatment groups. Between 2 days and 2 weeks the barrier status increased significantly for the control ($p=0.0214$) to a mean of 1.127. The group exposed to 2.4 ppm PAA (mean 1.205) trended to have an increase in barrier status. The groups exposed to 1.2 ppm PAA and 0.6 ppm PAA had no significant difference. Within two weeks after first exposure there was no significant difference between the barrier status of the treatment groups. The barrier status didn't change significantly between two hours after first exposure and 2 weeks after first exposure (Figure 14).

The second exposure didn't give any immediate significant effect on the barrier status of the treatment groups. The control (mean 1.147) and 2.4 ppm PAA (mean1.236) had little change in barrier status, while the barrier status of the groups exposed to 0.6 ppm PAA (mean 1.155) and 1.2 ppm PAA (mean 1.125) trended to have an increase in barrier status. Between 2 hours and two days after second exposure showed no significant difference in barrier status between the treatment groups (Figure 13). Within two days after second exposure had no significant difference between the treatment groups. The group exposed to 2.4 ppm (mean 1.336) and the control (mean 1.290) trended to have higher barrier status than the group expose to 0.6 ppm PAA (mean 1.182) and 1.2 ppm PAA (mean 1.118). There was no significant difference between 2 days and 2 weeks after second exposure for the barrier status of the treatment groups, but all treatment groups trended to have a decrease in barrier status (Figure 13). Within 2 weeks after second exposure showed no significant difference. There was no significant difference between the treatment groups 2 days after first exposure and 2 weeks after second exposure (Figure 14).

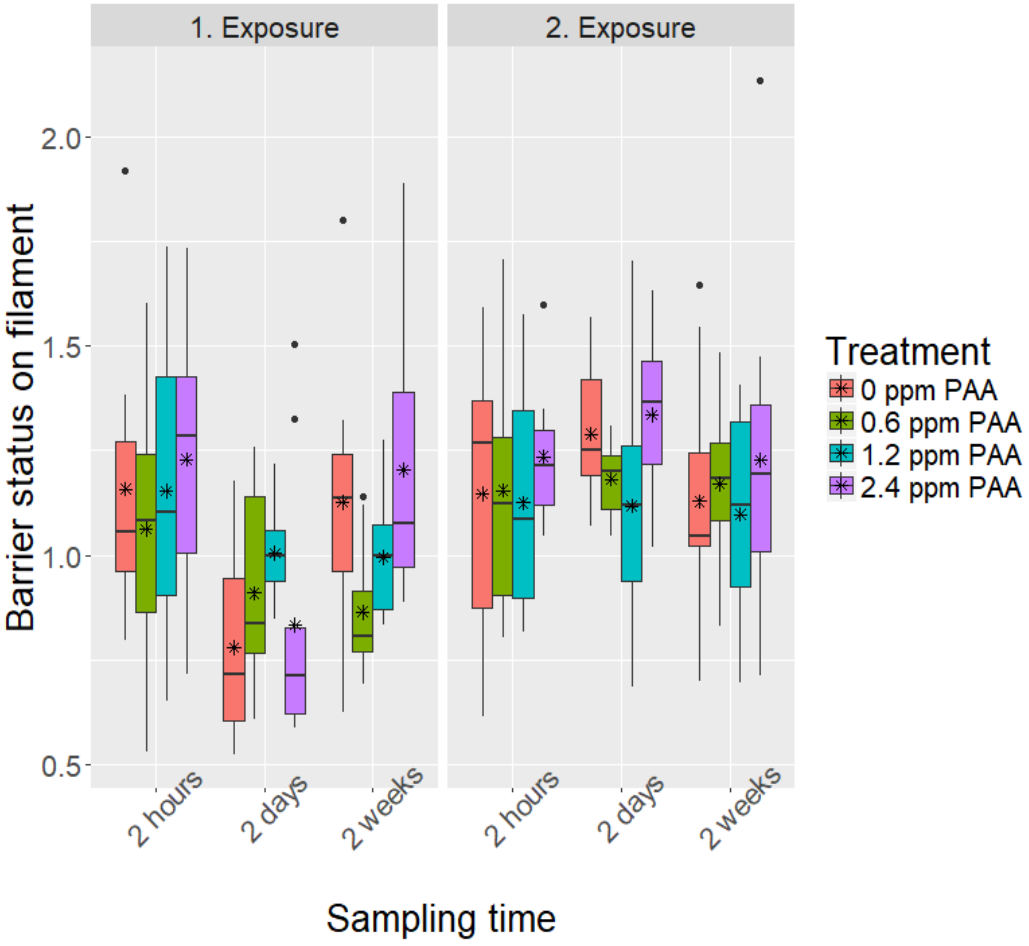

Figure 14: showing the barrier status on the filament for all treatment groups through the trial. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.4 Differences in mucous between filament and lamella

3.4.1 Mean Area

The mucous cell area on the lamella varied from 27.62 μm^2 to 81.7 μm^2, with a mean mucous cell area of 49.98 μm^2, and the mucous cell area on the filament varied from 56.9 $\mu m^2$to 141 μm^2 with a mean mucous cell area of 85.3μm^2 (Figure 15).

The Mucous cell area on the filament was significantly larger than the mucous area on the lamella in all treatment groups and the control 2 hours, 2 days and 2 weeks after first exposure. The mucous area on filament was significantly larger 2 hours and 2 days after second exposure. The group treated with 2.4 ppm PAA and the control had significantly larger mucous cell on the filament than the lamella 2 weeks after second exposure. The group treated with 0.6 ppm (p=0.1288) and 1.2 ppm PAA (p=0.1464) had insignificantly larger mucous cell mucous cell on the filament than the lamella (Figure 15).

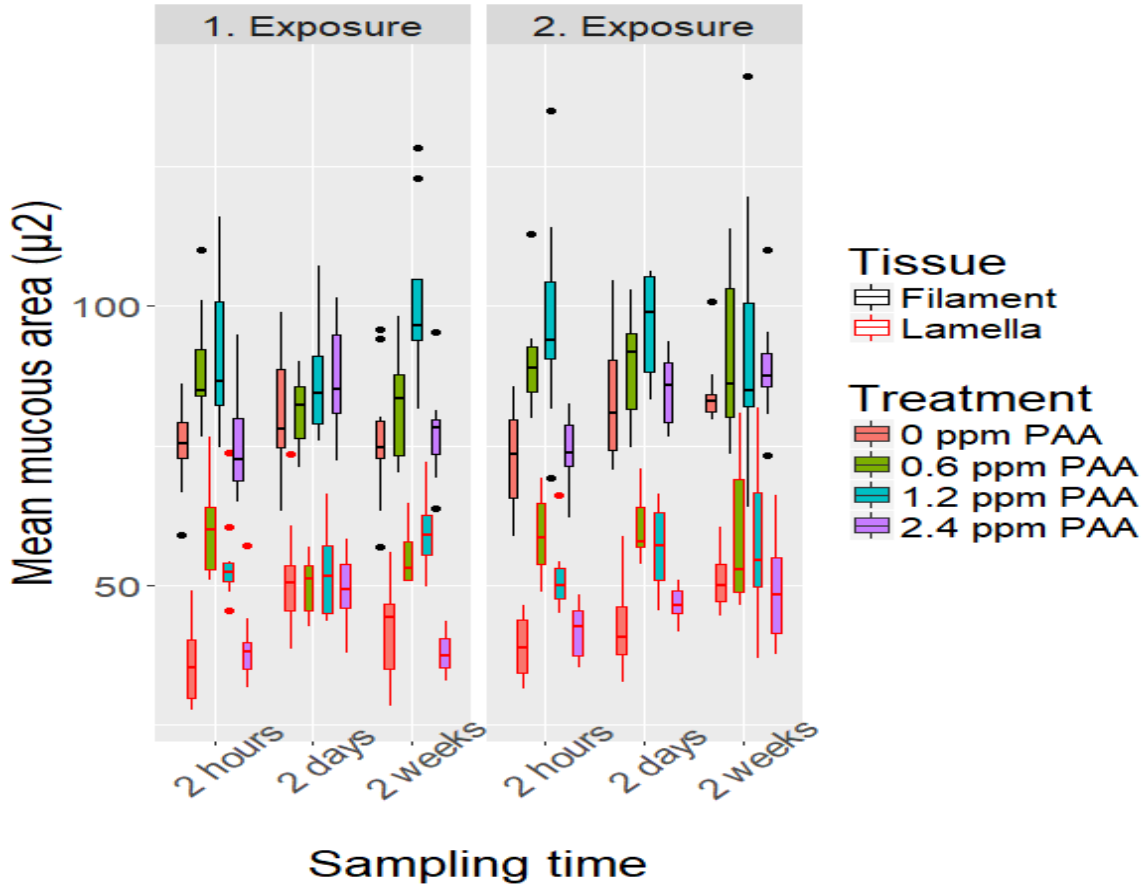

Figure 15: mean mucous area of the lamella and the filament. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.4.2 Density

The mucous cell density on the filament ranged from 3.7% to 18.8% with a mean density of 9.49% of the mucosal epithelium. The mucous cell density on the lamella varied 0.17% to 6.3% with a mean of 1.48% of the mucosal epithelium. The mucous cell density on filament was significantly larger than the lamella for all the treatment groups (Figure 16).

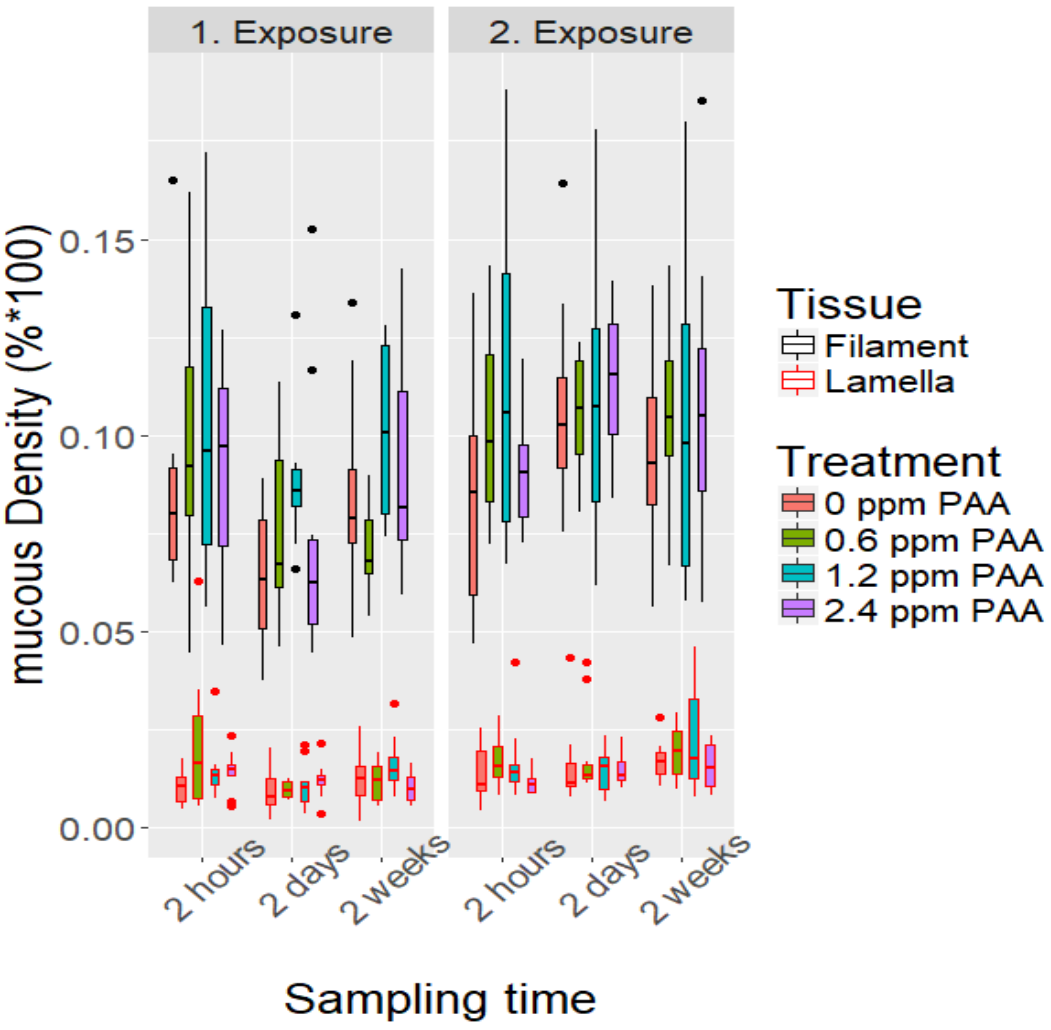

Figure 16 Mucous density on the filament and the lamella. The red colour represents the control, the green represents 0.6 ppm PAA, blue represents 1.2 ppm PAA and the purple represent 2.4 ppm PAA. The graph is divided in 2 exposures. Second exposure was 15 days after first exposure. The sampling pool consisted of 240 post-smolt Atlantic salmon, with 60 salmon per treatment group. The experiment lasted from 29. April to 29. May with 6 sampling times.

3.5 Histopathological alterations

3.5.1 Histopathological Alteration Index

The majority of the gills analysed (n=225, 94.1%) was healthy and had a HAI-score between 0-10 that was categorized in the histopathological alteration index as functionally normal gills. The rest of the gills was in the category slightly moderately damaged gills (n=14, 5.4%) and in the category Moderate to heavily damaged gills (n=1, 0.04%) (Figure 17).

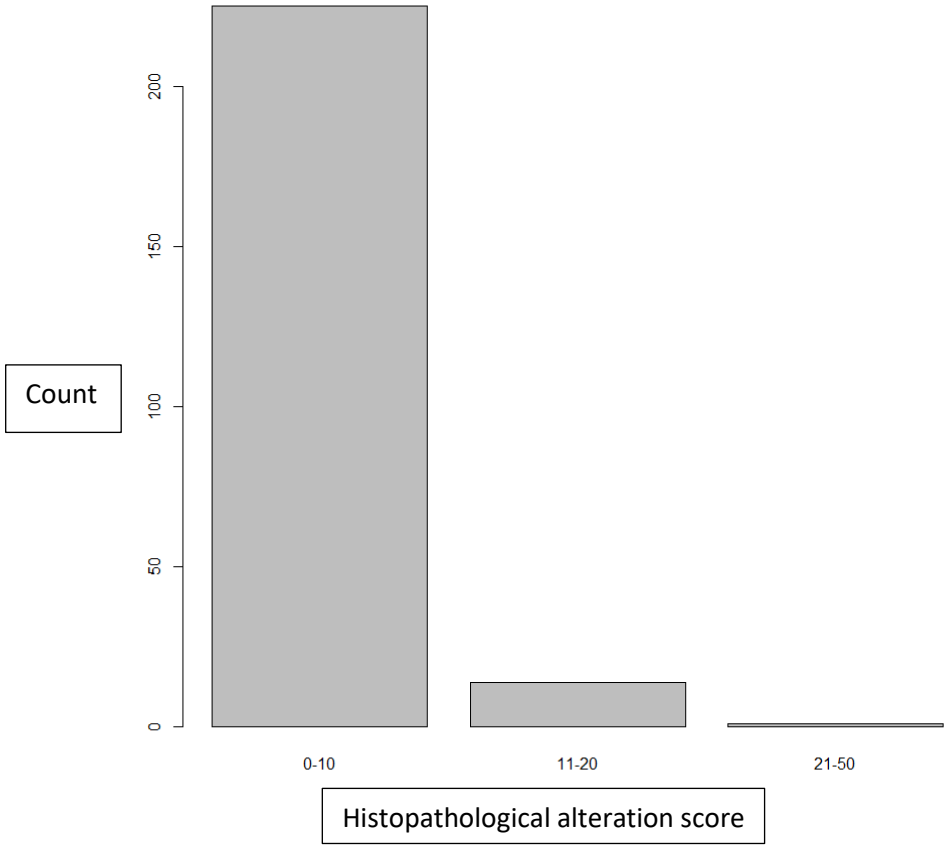

Figure 17: Histopathological alteration index (HAI) after Poleksic & Mitrovic-Tutundzic (1994) for control, 0.6-, 1.2- and 2.4 ppm PAA. 240 gills were analysed making 60 gills per treatment groups. Atlantic salmon was exposed to PAA twice with 2 weeks of rest between. The first exposure lasted for 5 minutes and the second lasted for 30 minutes.

The histopathological alteration index (HAI) was in the category functionally normal gills all the treatment groups 2 hours and 2 days after first exposure. Two weeks after first exposure showed an increase in HAI for the group exposed to 2.4 ppm PAA. However, only 3/10 samples had HAI score in the category slightly moderately damaged gills. All the treatment groups were within the category of functionally normal gills after the second exposure (Figure 18).

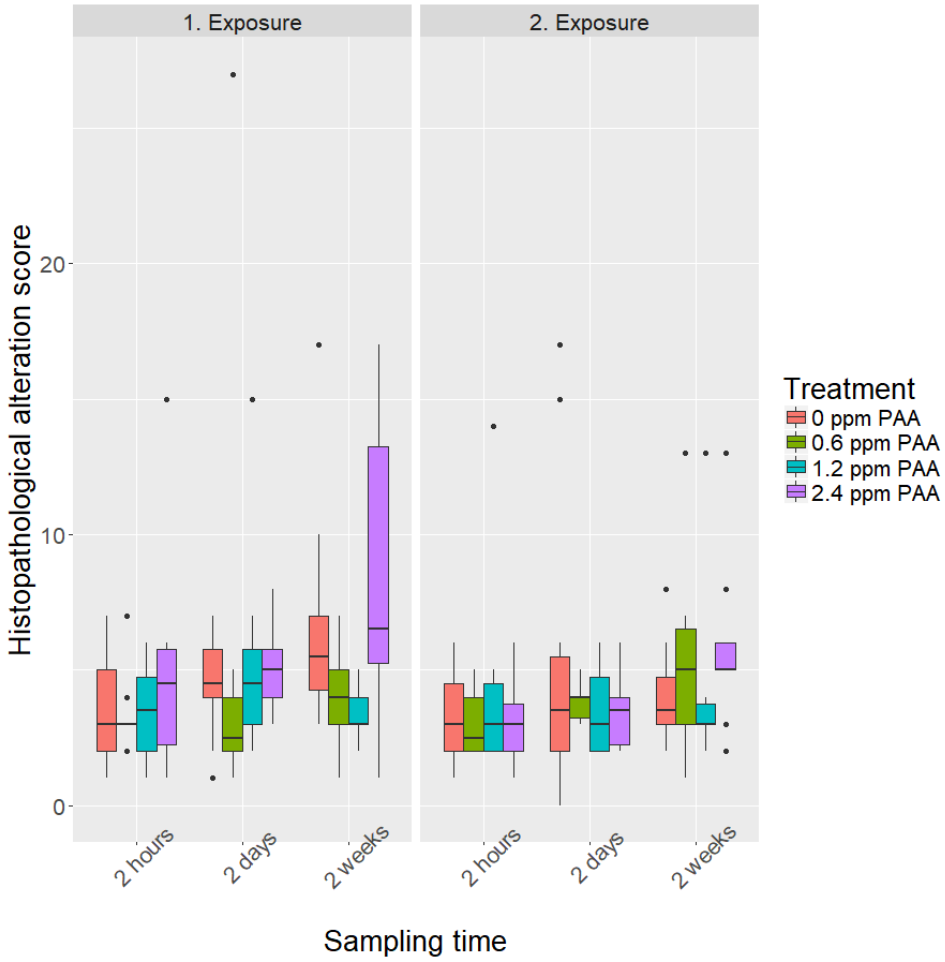

Figure 18: Histopathological alteration index for the treatment groups at every sampling time. 240 gills were analysed, making 60 gills per treatment group. The fish was exposed to Peracetic acid twice where the first exposure lasted for 5 minutes and the second lasted for 30 minutes.

3.5.2 Histopathological findings

The majority of the gill tissue investigated was healthy as shown in figure 17. Histopathological alterations were found side by side with healthy gill tissue. Histopathological damages included clubbing, hypertropia, hyperplasia, fusion, lifting**,** telangiectasias and aneurisms (Figure 19). A lot of the histopathology was found at the edge of the gills.

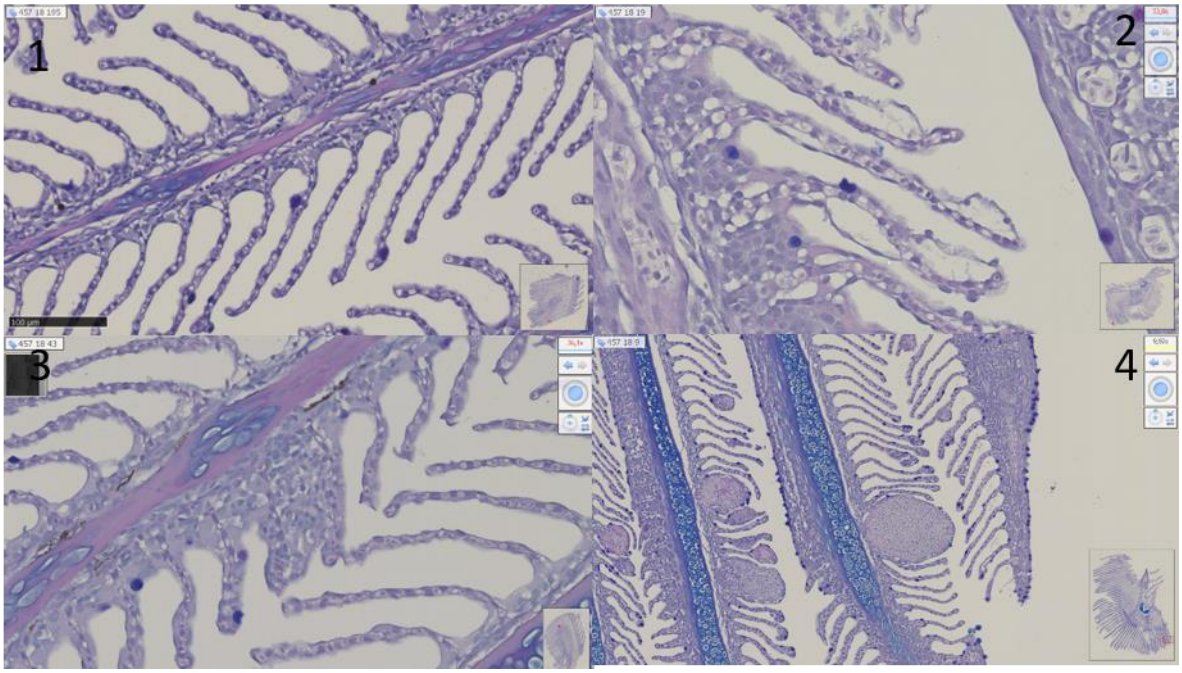

Figure 19: Extremes of histopathological alterations in the gills of the fish in the trial. 1= healthy gills, 2=lifting, 3= hypertrophia, 4= Aneurism,

3.6 Skin

3.6.1 Mean mucous cell area on skin

The mean mucous area ranged between 105.9 µm^2 to 268µm^2 with a mean of 176.35µm^2. The sampling pool showed low variation in size with the majority of the sampling pool ranging from 150 to 200 µm^2 (Figure 20).

The control had a trended increase in mucous cell area from the first exposure (mean 163.8 µm^2) to the second exposure (mean 182.6 µm^2). However, there was no trended difference between the treatment groups. The group exposed to 0.6 ppm PAA had low change in mucous cell area from first exposure (mean 173.7 µm^2) to the second exposure (mean 170.23µm^2). However, the group exposed to 2.4 ppm PAA had a trended decrease from the first exposure (200.9 µm^2) to the second exposure (165.1 µm^2)

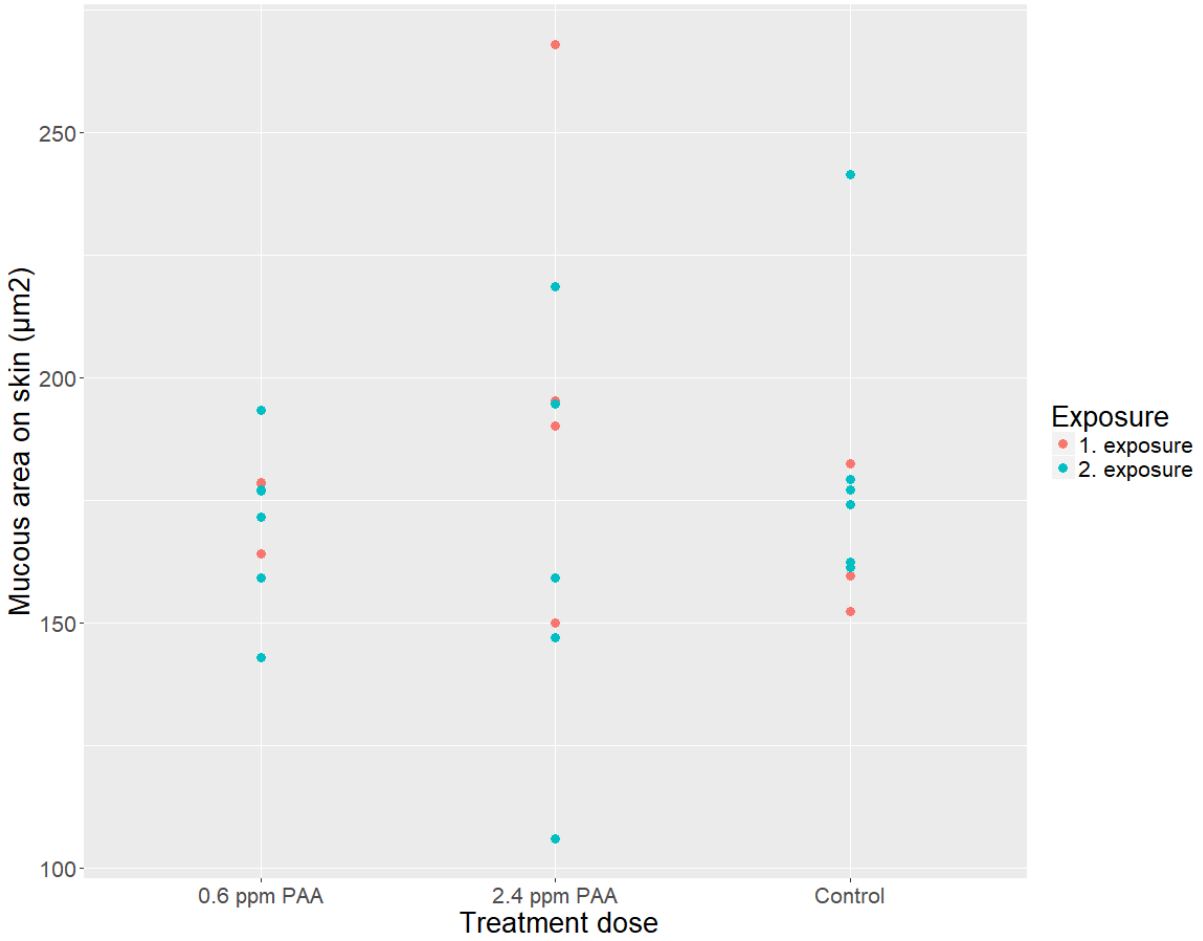

Figure 20: Mean mucous cell area on the skin (µm^2). Twenty-eight skin samples were analysed from fish exposed to 0.6 ppm, 2.4 ppm PAA and the control. Atlantic salmon was exposed to peracetic acid twice with sampling 2 hours 2 days and 2 weeks after each exposure. The first exposure lasted for 5 minutes and 15 days later the second exposure lasted for 30 minutes.

3.6.2 Mucous cell density on skin

The mucous density ranged between 5% of the epithelium to 30% with a mean of 13.7% of the mucosal epithelium (Figure 21). The majority of the skin samples had a mucous cell density between 10 and 20%. However, fish with mucous cell density under 10% was only present after the second exposure in all treatment groups (Figure 21).

The mean mucous cell density of the control had no trended change between the first (mean 13.7%) and the second exposure (mean 14.6%), and there was no trended difference between the control and the groups exposed to 0.6 ppm and 2.4 ppm PAA. The group exposed to 0.6 ppm PAA had low change in mucous density between first (mean 12.34%) and second exposure (mean 11.48%). However, the group exposed to 2.4 ppm PAA had a trended decrease in mucous density from first exposure (mean 19.4%) to second exposure (11.8%).

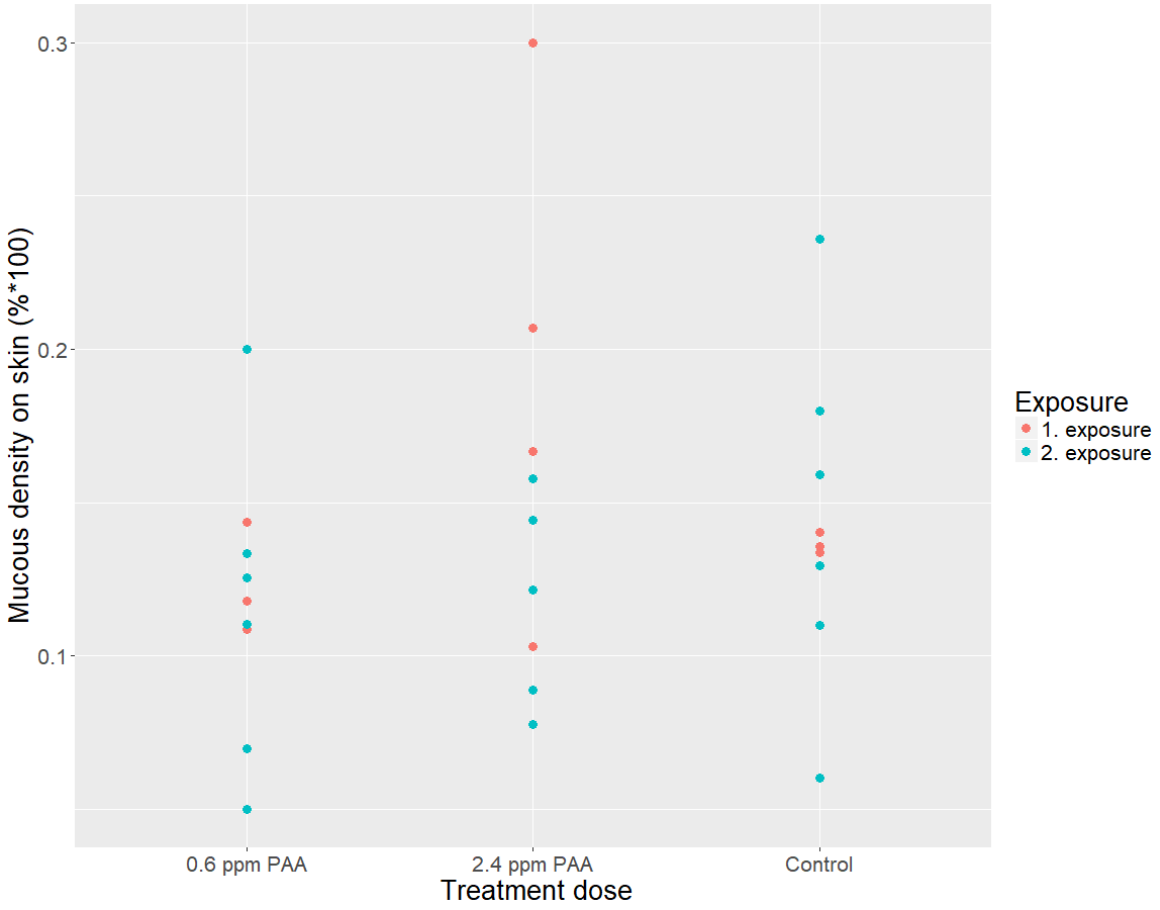

Figure 21: Volumetric Mucous cell Density on the skin (%*100). Twenty-nine skin samples were analysed. The fish was exposed to peracetic acid twice over a period of 4 weeks, with 15 days between each treatment. The first exposure lasted for 5 minutes and the second exposure lasted for 15 minutes.

3.6.3 Barrier status on skin

The barrier status on the skin ranged from 0.35 to 1.12 with a mean of 0.76 (Figure 22). The barrier status of the control had low change from first exposure (mean 0.83) to the second exposure (mean 0.78). The barrier status of the group exposed to 0.6 ppm PAA (mean 0.71 to 0.66) and 2.4 ppm PAA (mean 0.94 to 0.71) had a trended decrease in barrier status from the first exposure to the second exposure.

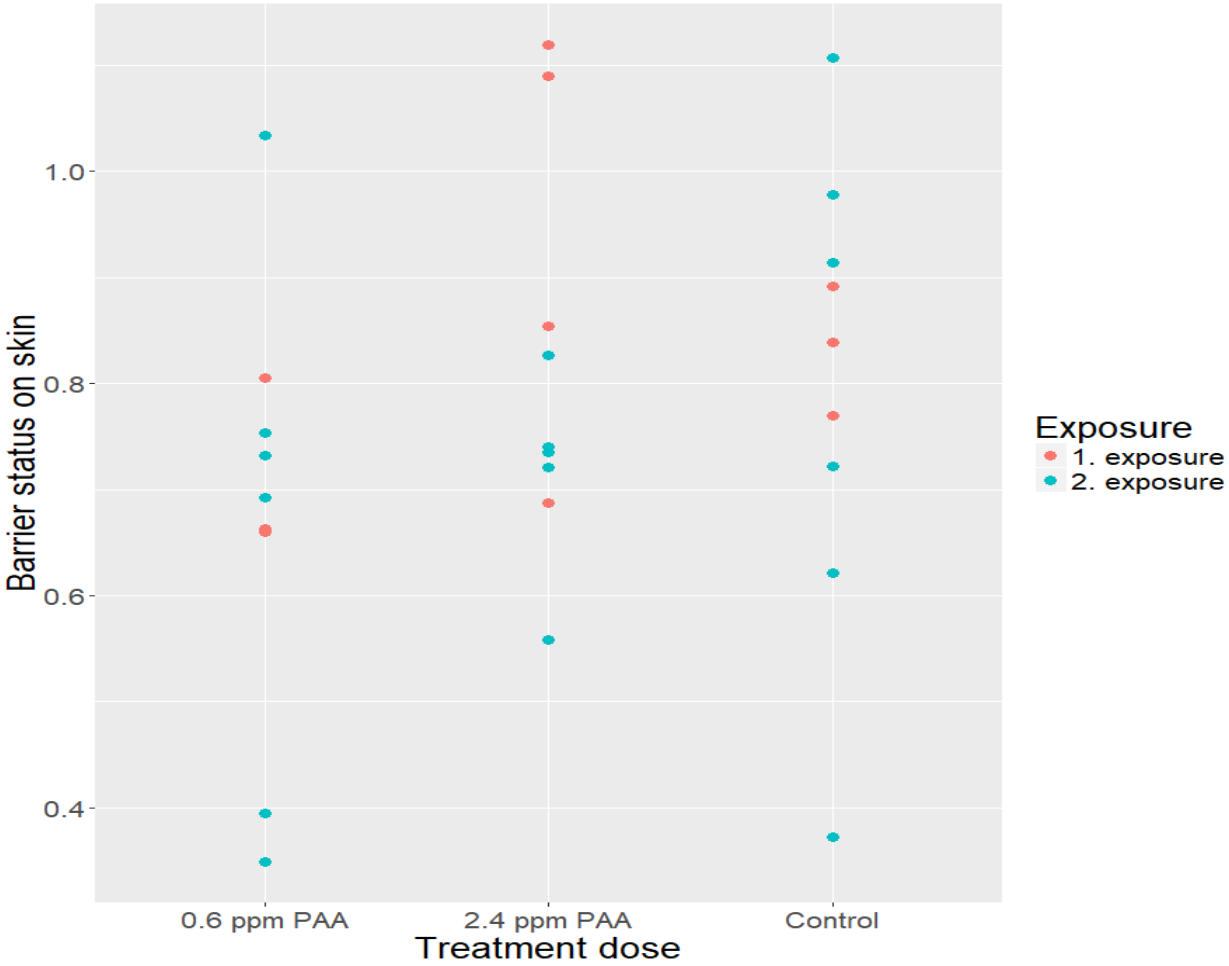

Figure 22 Barrier status on skin. Twenty-eight skin samples were analysed from fish exposed to 0.6 ppm, 2.4 ppm PAA and the control. Atlantic salmon was exposed to peracetic acid twice with sampling 2 hours 2 days and 2 weeks after each exposure. The first exposure lasted for 5 minutes and 15 days later the second exposure lasted for 30 minutes.

4. Discussion

4.1 Summary of results.

The group exposed to 0.6 ppm PAA had significantly larger mucous cell area than the control and the group exposed to 2.4 ppm PAA on the lamella 2 hours after first exposure. The mucous cells of the control and the 2.4 ppm increased to about the same size as the 0.6 and 1.2 ppm groups 2 days after first exposure. The following 2 weeks the control and the 2.4 ppm group decreased to the same values as 2 hours after first exposure, however the 0.6 and 1.2 ppm was stable. The second exposure had no effect on the groups exposed to 0.6 ppm and 1.2 ppm and the mucous cell area of the control and 2.4 ppm PAA had a trend to increase. The density on the lamella remained stable throughout the trial in all groups.

There was no significant difference in mucous area on the filament 2 after the first exposure, but the groups exposed to 1.2 ppm PAA trended to have larger mucous cell area. The second exposure had no immediate significant effect on the treatment groups. The group exposed to 2.4 ppm PAA increased significantly from 2 hours to 2 days after first second exposure. However, between 2 days and 2 weeks after second exposure, the mucous cell areas were constant across treatments. The majority of the gills analysed (94.1%) was considered healthy and the remaining 5.9% had some histopathological alterations. The diffusion distance increased from 2 hours to 2 days after first exposure, with significant increase for the control. However, there was no significant difference the rest of the trial. The range of the means is summarized in Table 5.

The mucous cell density and the barrier status on the skin had low difference between the first and the second exposure for the control and the group exposed to 0.6 ppm PAA. The mean mucous area trended to decrease in the control between the first and the second exposure. However, the group exposed to 2.4 ppm PAA trended to have a decrease in mean mucous area, mucous cell density and barrier status for the first exposure to the second exposure.

Table 5: Range of means for mucosal values and diffusion distance for all treatment groups. The mucosal measurements were done on 4 treatment groups with a total of 240 fish, and the diffusion length was measured on 120 gills at random points. Atlantic salmon was exposed twice to peracetic acid with 2 weeks of rest between.

	Diffusion length	Mucous cell Density	Mean mucous cell area	Barrier status
Range lamella	4.864±0.54µm to 6.94±1.35µm	0.97±0.6% to 2.27±1.44%	36.03±7.19µm^2 to 60.14±8.24µm^2	0.180±0.09 to 0.371±0.17
Range filament		6.34±1.89 % to 11.39±1.91%	72.61±9.29 µm^2 to 101.85±15.1µm^2	0.780±0.23 to 1.336±0.19
Range Skin		11.48±5.2% to 19.4±8.2%	164.78±15.7µm^2 to 200.9±49.2µm^2	0.66±0.25 to 0.94±0.20

4.2 Effect of dose on response variables

In this project, Atlantic salmon was exposed to PAA dosages ranging from control and up to 2.4 ppm. As seen in Table 1, treatment dosages between 0.225 ppm and 4 ppm affect multiple salmonid diseases in vitro (Meinelt et al., 2009; Marchand et al., 2012; Meinelt et al., 2015)**.** Hydrogen peroxide have a potent amoebicidal effect on AGD-infected salmon at 1000 ppm for more than 10 minutes (Adams et al., 2012). In vitro, 100 ppm of H_2O_2 kills amoeba (Powell et al., 2003). Peracetic acid has higher oxidative potential than H_2O_2 and higher potency against pathogens (Pedersen et al., 2009**).** The question this is: were the dosages used in this experiment was too gentle for the post-smolt salmon compared to potential treatment dosages to be used in vivo? This can be answered by evaluation by the response variables of the innate immune system, and the mucous cells in the gill and skin.

The mucous cell area on the filament had no significant difference between doses and varied from 56.9µm^2 to 141.1µm^2 with a mean of 85.3µm^2. This was smaller mucous cells than what was found on filament of commercially produced salmon (97.14µm^2) exposed to 1500 ppm of H_2O_2 (Rantty, 2015). The mean mucous area on the filament of shorthorn sculpins at a gradient of exposure to heavy metals (Pb and Zn) (*Myoxocephalus scorpius*) was 87.63µm^2 (Dang et al., 2019). The similarity in mucous cell size between different exposures and species analyzed with the same method (Pittman et al., 2011; Pittman et al., 2013) seems to suggest a pattern of similar mucous cell area across species on the filament. However the filament cell size was correlated with liver lead levels, supperting the hypothesis that filament mucous cells are associated with excretion of metabolics and toxins (Dang et al., 2019).

The mucous cell area on the lamellae varied from 27.62 µm^2 to 81.7 µm^2, with a mean mucous cell area of 49.98 µm^2. The mean mucous cell area on salmon exposed to PAA in sea water-RAS was smaller than what was found by Rantty (2015) (70.26 µm^2) on salmon exposed to H_2O_2 and Campo et al (unpublished) found on farmed Atlantic salmon (141.21µm^2). The small mucous size on Salmon in seawater RAS may derive from the effect of environmental stimuli, or the size of the fish. The fish investigated by Rantty (2015) and Campo (unpub. Data) was from a commercial sea cage, and the fish in this research was in controlled sea-water RAS**.** The size difference can also be a sign of the effect of treatment dosages on the mucous cell. 1500 ppm H_2O_2 is more likely to cause hypertrophy or size increase in mucous cells than dosages

from control to 2.4 ppm PAA (Henriksen et al., 2014). Healthy lamella has small and few mucous cells, suggesting healthy lamella in this project.

The mucous cell areas on the lamella of the groups exposed to 1.2- and 0.6 ppm were significantly larger for than the Control and those exposed to 2.4 ppm PAA 2 hours after first exposure. The histopathological alteration index (HAI) showed no difference in histopathology in these groups. Cortisol measurements for the group exposed to 0.6 ppm PAA (11.45 ng/ml) in the first exposure showed that the group didn't recover to the level of pre-exposure (6.38 ng/ml) 2 weeks after the first exposure, in comparison to the control (5.43 ng/ml) and 2.4 ppm PAA (6.35 ng/ml) (Soleng, 2019). Mucous cell densities in the skin have been negatively correlated with plasma cortisol in ballan wrasse (*Labrus berggylta*) (Jonassen et al., 2019). However, the correlation was not seen in the gills, which seem to have a more rapid response potential. This seems to suggest that there is tissue specific immune responses including the correlations with somatic measures of cortisol and other physiological parameters. The mucosal tissue of the lamella and filament give significantly different mucosal measurements (Rantty, 2015; Dang et al., 2019; Quantidoc unpub. Data) and can suggest different reaction to stimuli and some differences in basic functions of these two gill segments.

The mean mucous cell area on the lamellae of Atlantic salmon in this project (49.98μm^2), close to the mean mucous area on lamellae of wild chinook salmon (*Oncorhyncus tshawytscha*) (46.02$\mu m^{2)}$ (Pittman et al., unpublished data). The mucous cell density of Chinook was 2.6% in the lamella while Atlantic salmon in this project had a mean of 1.48% of the mucosal epithelium in the lamella. The data show a trend towards low mucous cell density on the lamella compared to other mucosal tissue (Rantty, 2105; Pittman et al., 2013; Pittman et al., 2011).

The mucous cells on the filament tended to be larger than the mucous cell on lamellae, where the mean cell size was 85.3μm^2 vs 49.98 μm^2. This trend has previously been indicated by Rantty (2015) and can further be supported by this experiment**.** This can indicate that the filament and the lamella have two different mucous populations. Mucous cells in the gills of Shorthorn sculpins (*Myoxocephalus scorpius*) had significantly larger mucous cells in the filament than the lamellae and their size was positively correlated with liver lead loads (Dang et al., 2019). The data that is generated through mucosal mapping can be compared across species, tissue, time and treatment (Noble et al., 2018). Thus, different mucous populations on filament and lamella can be further supported.

The mucous cell density on the filament ranged from 3.7% to 18.8% of the mucosal epithelium with a mean density of 9.49%. Rantty (2015) found that the mean mucous cell density on the filament of salmon exposed to 1500 ppm of H_2O_2 was only 4% and Shorthorn sculpin had a mean density of 3.01% (Dang et al., 2019). The higher density in the filament of the fish reared in sea water RAS may be because of fewer stress related situations due to a more gentle husbandry. Stress from handling acetic exposures have been known to stimulate secretion of mucous (Speare & Ferguson, 2006; Segner et al., 1988), and short-term stress and acetic exposure has been shown to cause reduction of mucous cells in the skin and reduction in mucosal barrier (Segner et al., 2013). The density of mucous cells in the mucosal epithelium is a good measurement for the status of the mucosal barrier compared to number of mucous cells. These results can confirm that exposure up to 2.4 ppm PAA was a gentle treatment compared to commercial H_2O_2 treatment.

The mucous cell density on the filament was significantly denser than the lamella. The densities of mucous cells on the filament and lamellae are usually different, and usually the smallest, fewest mucous cells are on the lamellae which is the respiratory surface (Quantidoc, unpublished data). However this relationship can reverse when the gills are exposed to harsh treatment, as when salmon was deloused with 1500 ppm H_2O_2 (Rantty, 2015).

4.3 Effect of time on response variables

In this experiment, salmon were exposed twice with 2 weeks between each exposure and had returned to original values prior to the second exposure. Rantty (2015) recommended that commercial treatment with hydrogen peroxide should at least have 2-3 weeks between them for the mucosal barrier to heal. Trout exposed to 1000-1500 ppm showed significant decline in lesions caused by H_2O_2 3 weeks after exposure (Speare et al., 1999). The severity of 1000-1500 ppm H_2O_2 on the gill lamellae is not comparable with exposure up to 2.4 ppm PAA, although the recovery process is comparable and measurable.

The mucous cell area on the filament for salmon exposed to 2.4 ppm PAA showed the same dynamics in the first exposure as Rantty (2015) found for salmon exposed to hydrogen peroxide. Both acids showed a trend to have increased mucous area a couple of days after exposure, and then a decrease in mean area the following two weeks. This may indicate the adaptive mechanism of the mucous cells (Strzyżewska-Worotyńska et al 2017). Or a process in

the mucosal tolerance. Mucous production increases with stimuli from pollutant and parasites, and size, number and distribution of mucous cells increases in response to pathogens, seen as a stereotypic response (Speare & Ferguson, 2006). The same response was in this case also seen in the first PAA exposure and treatment with H_2O_2. Suggesting equal immune response to oxidising irritants.

The mucous cell area on the lamellae used 2 weeks to return to the same size as 2 hours after first exposure. The secondary exposure showed a trended to increase for the group exposed to 2.4 ppm PAA. This may be the result of acclimation to the stressor. Allostasis, or acclimation to a stressor lets the animal change or adjust physical systems to handle predictable and unpredictable situations (Segner et al, 2012). The expression of mucins in the mucosal barrier is known to adapt to mucosal changes to keep good protection in higher animals (Corfield, 2018). In fish, it has been shown that the mucosal immune system is able to respond and adapt to environmental changes (Cabillon & Lazado, 2018). Thus, these results seem to suggest that the mucous cells of the groups exposed to 2.4 ppm PAA adapted to a new set point to maintain efficient protection, and it would be interesting to see how the mucosal barrier managed to overcome a third exposure, with stronger dosages.

The group exposed to 2.4 ppm was the only group that the mucous cell density on the filament responded to the second exposure. Damage from H_2O_2 exposure is mainly on the gills where larger fish are more susceptible (Rach et al., 1997). Peracetic acid is a combination of H_2O_2 and acetic acid, and it is likely that any damage is first seen on the gills with PAA exposure. High cumulative mortality of channel catfish fry (*Ictalurus punctatus*) has been caused by the effect of PAA on gills (Straus et al., 2012)**.** In this study, the low effect on the mucous density over two exposures suggests these PAA doses have a gentle effect on post-smolt salmon.

The barrier status on the filament of the group exposed to 2.4 ppm PAA was significantly lower 2 days after first exposure than two days after second exposure. This dynamic suggest that the mucous cells reacted different at each exposure. The first exposure decreased the mucous barrier as explained at the skin by Segner et al (2012). It remains unknown if the reduced barrier status is a response of exhausted mucous cells, or if it is the oxidation effect of the acid. However, the second exposure showed a trend to increase in barrier status to the level of 2 hours after first exposure, further indicating that the fish were able to acclimate to the stressor and the mucosal epithelium were able to learn.

4.4 Other results

Operculum deformities were relatively common in the sampling pool with a prevalence of 18%, but the severity was not analysed (Figure 29, Appendix). The gill cover deformities were possibly a result of the rearing before treatment with Peracetic acid. Operculum deformities was also present in all treatment groups, suggesting that it wasn't an effect of peracetic acid. Operculum deformities often makes the fish less resistant to stress and handling and can cause shortening, thickening and deformation of the gill filament, and affect the mucosal measurements (Branson, 2008; Quantidoc, unpublished data). Gill cover deformities exposes gill tissue to external disturbance that can cause abnormalities in the exposed gill tissue (Pettersen et al., 2014). It's still not clear why these damages occur (Noble et al, 2018), but it is certain that deformities leave the gill tissue more exposed to substances in the water and pathogens and are a welfare challenge that may affect the fish health (Noble et al, 2018).

The second exposure showed an 84% increase in the plasma cortisol of the group exposed to 2.4 ppm PAA compared to the control (Lazado et al., unpublished). Similar responses were not observed in the mucosal barrier. There was no difference between the barrier status of the group exposed to 2.4 ppm and the control, which suggest that these 2 systems react independent of each other. However, a correlation between plasma cortisol and mucous density on the skin have been shown in Ballan wrasse (*Labrus bergylta*). Mucous density and size are different between tissue (Pittman et al., 2013; Rantty, 2015; Dang et al., 2019).

The skin had a mucous density ranging from 4.99 to 30% with a mean of 13.7% of the mucosal epithelium. Healthy wild Chinook salmon had a mucous density of 31% (Nowak et al., unpublished data), and salmon exposed to H_2O_2 had a mean density of 12.7% (Rantty, 2015), The similar density to H_2O_2 and some fish with density equal to salmon treated twice suggest that the Peracetic acid had an effect on the mucous cells on the skin. However, the sampling pool was low with 1-2 samples per group per sampling day for the skin. This makes it hard to conclude if 13.7% is a representable mucous density for fish exposed to doses up to 2.4 ppm PAA. However, the density is similar to Salmon treated commercially with H_2O_2.

All the skin samples with mucous cell density under 10% was after the second exposure. Mucous cell densities under 10% have previously been seen after handling and delousing of Salmon (Quantidoc, unpublished data). This suggests that the repeated exposure and handling can have an effect on the skin mucous. Netting and short handling have shown to affect the skin mucous density (Segner et al., 2012), which may also have an effect in this trial. However, these

results are only indications, due to few samples. Further investigation of the effect of PAA on the skin mucous is recommended.

The mean mucous cell area on the skin ranged from 105.9 μm^2 to 268 μm^2 with a mean of 176.35 μm^2. Mucous cell area on the dorsal skin was measured to be 161.5 μm^2 in rainbow trout (Pittman et al., 2011) and 155.39 μm^2 in farmed Atlantic salmon (Pittman et al., 2013). However, Atlantic salmon exposed to 1500 ppm hydrogen peroxide had a mean mucous size of 158.1 μm^2 (Rantty,2015). Suggesting that the mucous cell size is not deviating from other measurements. However, the variation can be a result of handling before sampling. Scale loss in the skin was observed in various degree, suggesting variation in handling. Atlantic salmon smolt are known to have loose scales which can easily detach under handling and can affect the mucosal barrier.

4.5 Correlation between response variables

There was no difference in mucous cell density between the control and the treatment groups on the lamella. This suggest that the peracetic acid had low effect on the mucous cell density on the gills. Rainbow trout (*Onchorhyncuss mykiss*) exposed to 5 ppm of Chloramine-T twice a week for a month had equally no significant difference in mucous cell numbers between the control and the exposed fish (Powell et al., 1994). In Olive flounder, 100 ppm of hydrogen peroxide had no effect of the number of mucous cells number in lamella (Hwang et al., 2014). This can indicate that the mucous cell density (or number) is able to withstand various acetic treatment. For the rainbow trout, 10 mg/L was sufficient to get significant reduction in lamellar mucous number (Powell et al., 1994), and 300 mg/L provided a significant change in mucous cells on Olive flounder (Hwang et al., 2014). Peracetic acid is considered a stronger oxidising agent than H_2O_2 and Chloramine-T.

The Barrier status in filament and lamella decreased from 2 hours after first exposure to 2 days after first exposure for all sampling groups. This reaction can be put in relation with gill transcriptome, where the cell reticulum and immune responses responded to PAA (Lazado et al., unpublished data; Appendix table 1). This then allows the understanding of cellular and architectural changes to mucosal tissues are reflected in their underlying genetic changes.

The maximum diffusion distance was defined as the distance between blood and water in the lamella and have a significant correlation with mucous cell size. Mucous cell stimulation is an

adaptive mechanism which increases the amount of mucous produced and hence increases the epithelial protection (Strzyżewska-Worotyńska et al., 2017). Ultsch & Gros (1978) hypothesized that an increase in mucous around the gill will decrease the diffusion efficiency. The positive correlation between mucous cell size and diffusion length indicates that an immune response can lift the epithelial cover and increase the tissue thickness leading to the internal blood supply as shown in by Figure 2. Fish exposed to higher doses or diseased fish can increase the correlation between mean mucous area and diffusion distance by increasing the mucous cell area.

The diffusion distance was not significantly different between the control and 2.4 ppm PAA from 2 days after first exposure to 2 weeks after second exposure. The mean diffusion length varied between mean of 5.76µm to 6.93µm. McDonald et al (1991) found that the diffusion distance on Rainbow trout varied from 4.3 to 6.0µm depending on the pH of the water. Marine pelagic fish have shorter diffusion distance than less active fish (Kisia & Hughes, 1991). Suggesting that swimming itself has a beneficial effect on gill structure and respiration. Whether this effect can be transferred to salmon remains to be seen. Hughes (1972) found that the diffusion distance of Rainbow trout was 6 µm. The diffusion distance of the gills of Atlantic salmon had thinner barrier between water and blood than what Hughes (1972) found on Rainbow trout 2 hours after first exposure. From 2 days after first exposure to the end of the trial the diffusion distance was both larger and smaller than rainbow trout. The diffusion length was not significantly different between the group exposed to 2.4 ppm and the control. This could in indicate that it was not the peracetic acid that caused the increase in diffusion length from 2 hours to two days after first exposure.

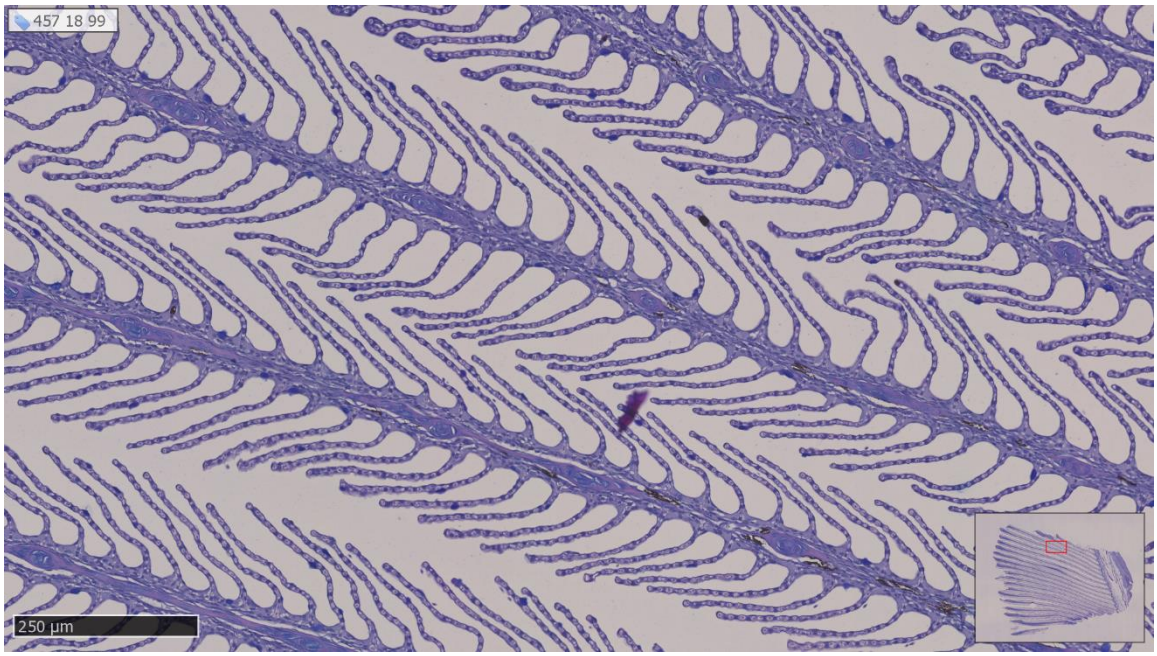

Figure 23: Healthy gills from Atlantic salmon exposed to 1.2 ppm PAA. The sampling pool of 240 Atlantic salmon exposed to four different doses of peracetic acid. The doses ranged from control to 2.4 ppm PAA. Atlantic salmon was exposed twice with the first exposure lasting 5 minutes and second exposure lasting 30 minutes. Sampling was done 2 hours, 2 days and 2 weeks after each exposure.

Histopathological analysis is a subjective analysis of the tissue. Healthy and pathological tissue can be present side by side, and the histological health status may be biased. However, Histopathological alteration index (HAI) allows objective histopathological analysis by its focus on presence of histopathological alterations, not the degree of the alteration (Poleksic & Mitrovic-Tutundzic, 1994). Histopathological analysis of 120 lamella per fish on the second gill arch on the left gill-cover showed that at least 95% of the lamellae was healthy 2 weeks after each exposure (Lazado et al, unpublished data) (Figure 24). This correlates with the histopathological index on the right gill cover (in this project), where 94.1 % of the gills were characterized as healthy (Figure 23). The HAI was done for all the gills sampled during the experiment, and thus it is likely that the HAI analysis identified more histopathology. However, lamellar spacing in the gills (50-60 µm) was not affected by exposure with peracetic acid 2 weeks after each exposure (Figure 24). This can be linked to the low difference in diffusion distance between 2 weeks after first exposure and 2 weeks after second exposure.

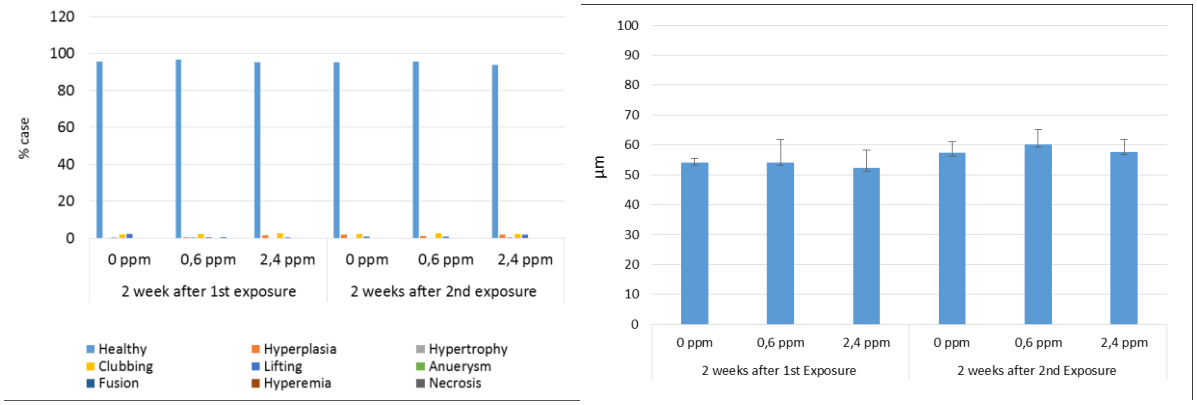

Figure 24: adapted from Lazado et al (unpublished data). The left graph showing Histopathological findings on the second gill arch on the left gill cover. 120 lamellae were analysed per fish for control 0.6 ppm and 2.4 ppm PAA 2 weeks after each exposure. The right graph shows lamellar spacing for the same groups at the same time.

The overall external welfare score to be below 1 for all the groups 2 weeks after each exposure (Figure 25). Skin damage and operculum deformity was prevalent during the trial (Lazado et al., unpublished). Skin damaged were mostly in the case of scale loss, which can be common with handling of post-smolt due to their loose scales and can be a result of netting during transport and before killing (Stefansson, 2016). These results together with HAI and histological analysis (Lazado et al., unpublished) confirms that these fish can be characterized as healthy salmon.

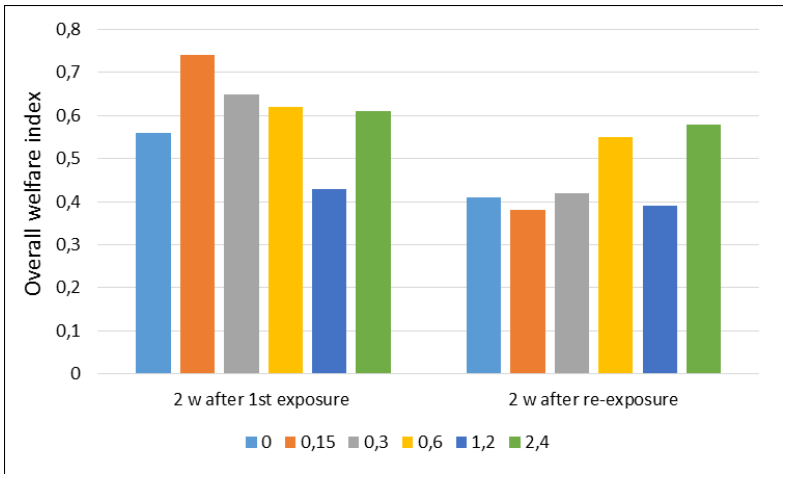

Figure 25: Overall welfare index from 2 weeks after first and second exposure. From Lazado et al (unpublished data). The welfare index is adapted from Nobel et al (2018) and shows welfare score ranging from 0-3, where 0 indicated unaffected and 3 means severely affected. The overall welfare index is the average composition of all indicators.

4.6 Benchmarking healthy gills

Post-smolt Atlantic salmon used in this trial were reared in a controlled environment where unidentified stressors such as toxins or sudden change in water quality were eliminated. This

allows us to study only the effect of peracetic acid on the gills. The gills of Atlantic salmon used in this experiment were predominantly healthy with low amounts of histopathological signs. “Gills responds to insult in the same manner, irrespective of the cause by increasing mucus production and by cell proliferation” (Dykes, 2014). Monitoring of the mucosal epithelium by the mucous size and density can detect gill responds to a stressor or a stimulus very early with a detection limit of $7\mu^2$ (Pittman et al., 2013).

Tools to analyse gill health have been previously developed (Mitchell et al., 2012; Poleksic & Mitrovic-Tutundzic, 1994), focusing on the presence or absence of histopathology to index gill health. Gills are quick to respond to change in the environment and unfavourable husbandry conditions (Poleksic & Mitrovic-Tutundzic, 1994). The status or the strength of the mucosal barrier is an indication of good fish and gill health (Segner et al., 2012).

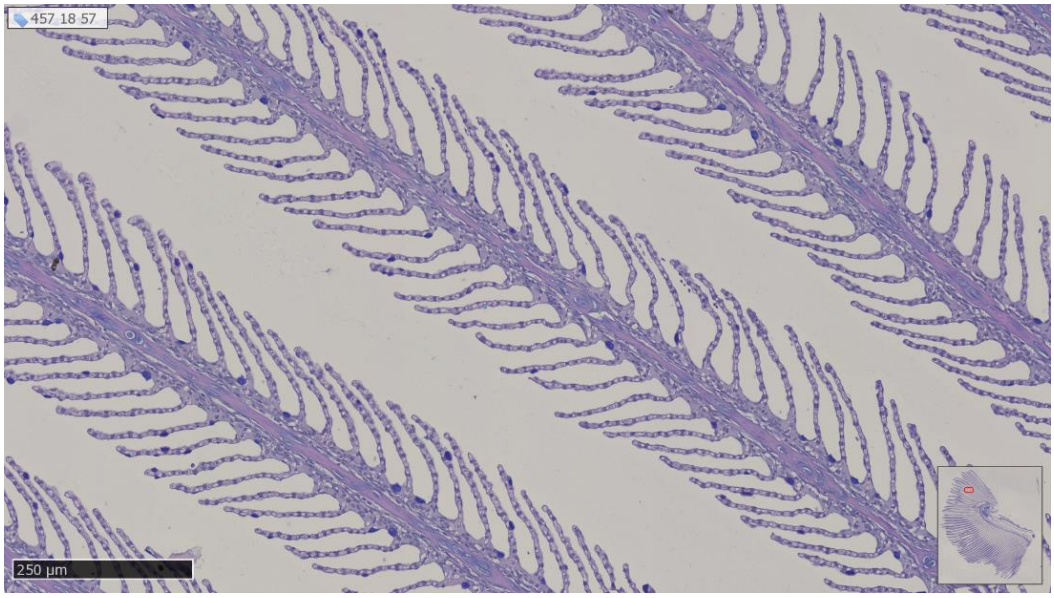

Figure 26: Healthy gills from Atlantic salmon exposed to 2.4 ppm PAA. The sampling pool of 240 Atlantic salmon exposed to four different doses of peracetic acid. The doses ranged from control to 2.4 ppm PAA. Atlantic salmon was exposed twice with the first exposure lasting 5 minutes and second exposure lasting 30 minutes. Sampling was done 2 hours, 2 days and 2 weeks after each exposure.

The objective analysis of 240 gills by mucosal mapping and HAI and measurement of 120 gills for diffusion distance. In addition, histological analysis of gills and measurement of lamellar spacing (Lazado et al., unpublished data) gives a benchmark of gill health to which others can compare the health of post smolt salmon in other rearing systems (Figure 26). The ability to heal from the first exposure and resist the secondary treatment is a sign of maintaining homeostasis and good fish health. Fish welfare is a function of good health (Figure 27) In this study, the barrier status was unaffected by the longer secondary exposure and suggests healthy

gills and good fish health. We propose that a first benchmark of gill health accords with the measures shown in Table 6.

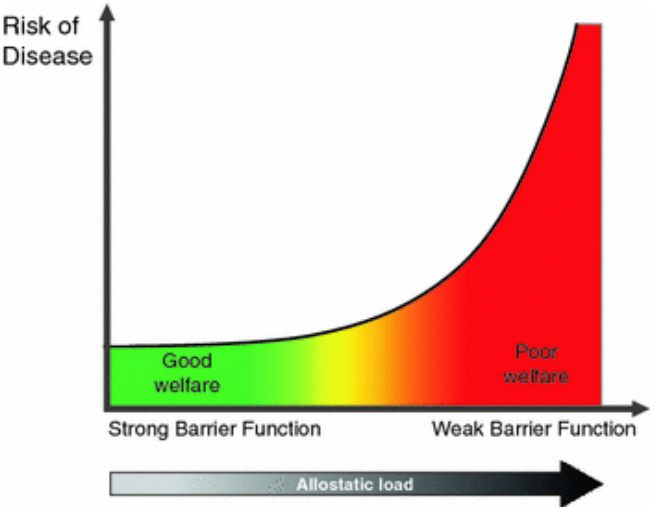

Figure 27: Showing the effect of stress on the welfare. Low stress given a strong barrier function and thereby good welfare. Figure retrieved from Segner et al (2012).

Table 6 Benchmark of healthy salmon gills in seawater RAS. N=240 Atlantic salmon (Salmo salar) exposed twice to peracetic acid. Sampling was done 2 hours, 2 days and 2 weeks after each exposure for groups exposed to 0.6-, 1.2-, 2.4 ppm PAA and the control, making a total of 60 gill samples per treatment groups.

Tissue	Mean mucous cell Area (µm^2)	Mucous cell Density (%)	Barrier status	Maximum Diffusion distance (µm)	HAI-score
Lamella	49.98	1.48	0.29	6.11	4.4
Filament	85.27	9.48	1.11		

5. Conclusion

We can conclude that exposure of PAA up to 2.4 ppm PAA for 30 minutes is habitable for post smolt Atlantic salmon in sea water RAS, and that the mucous cells of the mucosal barrier are able to learn from previous experience.

Benchmarked healthy gills have mean mucous area of 49.98µm^2 and a mucous cell density of 1.48% with a barrier status of 0.29 on the lamella and a mean mucous area of 85.27µm^2 and a mucous cell density of 9.48% with a barrier status of 1.11 on the filament with low HAI-score.

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7. Appendix

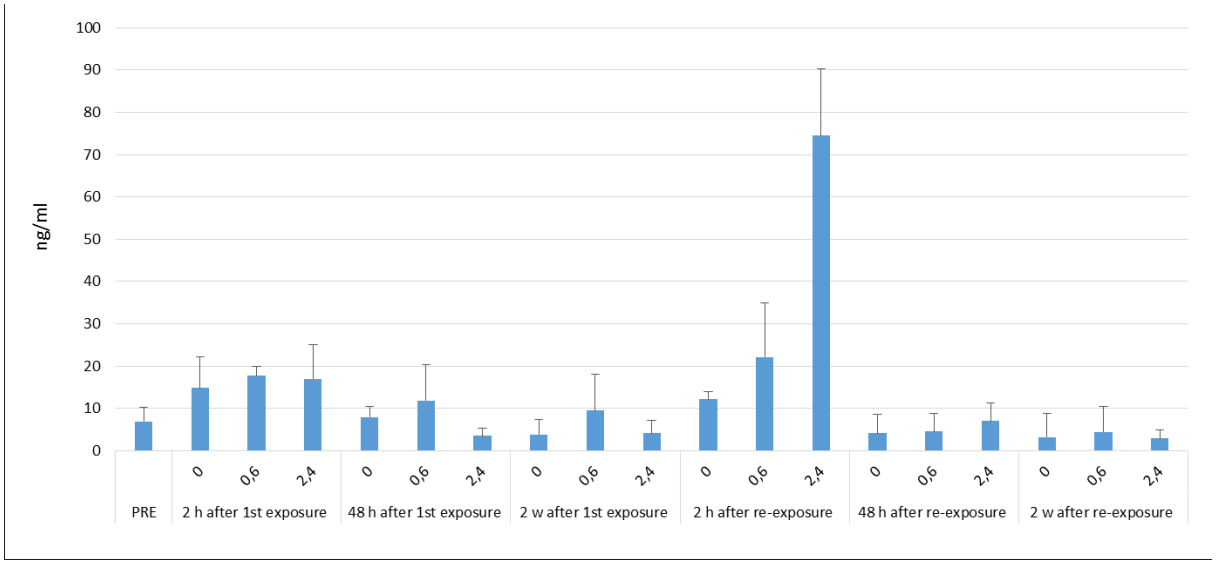

Figure 28: Plasma cortisol levels in the control, and the groups exposed to 0.6 and 2.4 ppm PAA. Retrieved from Lazado et al, (unpub. Data)

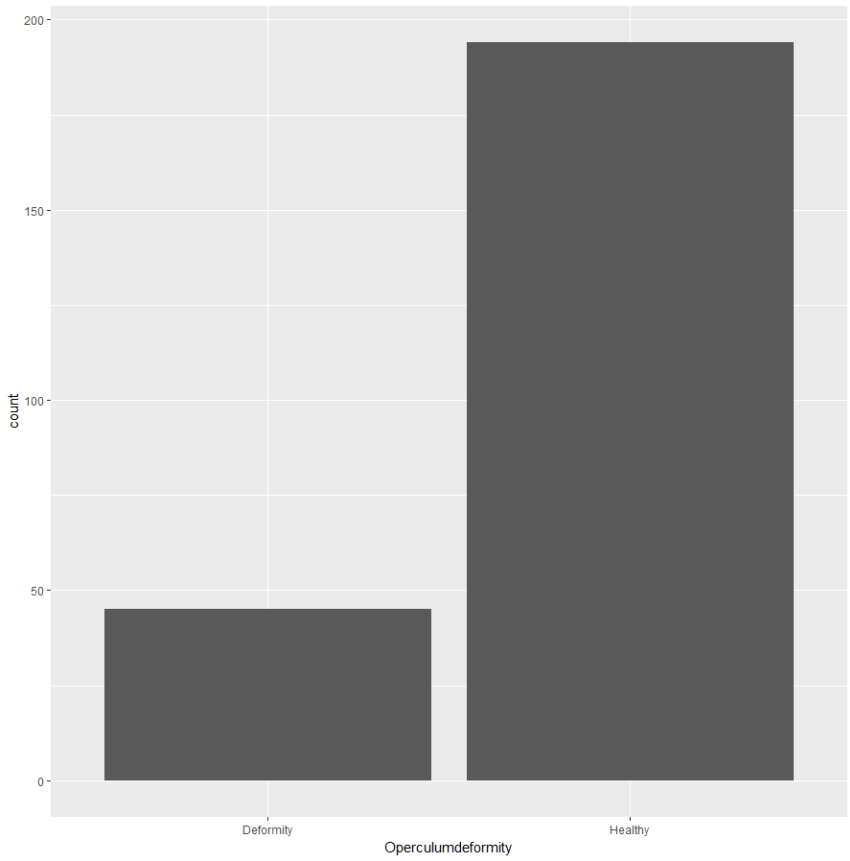

Figure 29: Count data of operculum deformities. N=240, Atlantic salmon exposed to 0.6-, 1.2-, and 2.4 ppm PAA and the control.

Table 7 Raw data of the histopathological Alteration Score.

ID	HAI-score	HAI	Tank	Treatment	Samplingtime	Exposure
457 18 64	3	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 65	6	0-10	A	0 ppm PAA	2 hours	2. Exposure
407 18 171	3	0-10	A	0 ppm PAA	2 hours	2. Exposure
407 18 172	8	0-10	A	0 ppm PAA	2 hours	2. Exposure
407 18 173	3	0-10	A	0 ppm PAA	2 weeks	2. Exposure
407 18 174	3	0-10	A	0 ppm PAA	2 weeks	2. Exposure
407 18 175	2	0-10	A	0 ppm PAA	2 weeks	2. Exposure
407 18 186	6	0-10	A	0 ppm PAA	2 weeks	2. Exposure
407 18 187	4	0-10	A	0 ppm PAA	2 weeks	2. Exposure
407 18 188	3	0-10	A	0 ppm PAA	2 weeks	Exposure

407 18 24	3	0-10	A	0 ppm PAA	2 weeks	1. Exposure
407 18 25	7	0-10	A	0 ppm PAA	2 weeks	1. Exposure
407 18 26	7	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 17 113	6	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 1	3	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 111	1	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 112	4	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 114	4	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 115	2	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 12	5	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 126	7	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 127	4	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 128	5	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 129	5	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 13	2	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 130	6	0-10	A	0 ppm PAA	2 days	1. Exposure
457 18 14	3	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 15	6	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 16	1	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 189	5	0-10	A	0 ppm PAA	2 weeks	2. Exposure
457 18 190	4	0-10	A	0 ppm PAA	2 weeks	2. Exposure
457 18 2	5	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 22	4	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 18 3	2	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 32	5	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 18 33	5	0-10	A	0 ppm PAA	2 weeks	1. Exposure

457 18 34	6	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 18 35	10	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 18 36	3	0-10	A	0 ppm PAA	2 weeks	1. Exposure
457 18 4	7	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 42	3	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 43	6	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 44	3	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 45	1	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 46	3	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 5	2	0-10	A	0 ppm PAA	2 hours	1. Exposure
457 18 52	1	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 53	5	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 54	2	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 55	6	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 56	2	0-10	A	0 ppm PAA	2 hours	2. Exposure
457 18 66	1	0-10	A	0 ppm PAA	2 days	2. Exposure
457 18 72	2	0-10	A	0 ppm PAA	2 days	2. Exposure
457 18 73	4	0-10	A	0 ppm PAA	2 days	2. Exposure
457 18 74	0	0-10	A	0 ppm PAA	2 days	2. Exposure
457 18 75	4	0-10	A	0 ppm PAA	2 days	2. Exposure
457 18 76	2	0-10	A	0 ppm PAA	2 days	2. Exposure
407 18 23	17	11-20	A	0 ppm PAA	2 weeks	1. Exposure
457 18 62	17	11-20	A	0 ppm PAA	2 days	2. Exposure
457 18 63	15	11-20	A	0 ppm PAA	2 days	2. Exposure
509 18 1	2	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 2	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure

509 18 4	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 6	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 7	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 8	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 9	7	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 10	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 11	2	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 13	2	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 14	5	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 17	4	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 19 18	2	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 19	2	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 20	4	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 21	7	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 22	6	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 23	3	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 24	4	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 25	5	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 26	2	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 27	1	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 28	4	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 29	3	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 30	1	0-10	D	0.6 ppm PAA	2 weeks	1. Exposure
509 18 31	2	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 32	4	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 33	2	0-10	D	0.6 ppm PAA	2 hours	2. Exposure

509 18 34	3	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 35	2	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 36	2	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 37	5	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 38	2	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 39	4	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 40	5	0-10	D	0.6 ppm PAA	2 hours	2. Exposure
509 18 41	5	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 42	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 43	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 44	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 45	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 46	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 47	3	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 48	3	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 49	3	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 50	4	0-10	D	0.6 ppm PAA	2 days	2. Exposure
509 18 51	5	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 53	6	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 54	7	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 55	4	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 56	3	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 57	3	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 59	6	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 60	7	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 3	4	11-20	D	0.6 ppm PAA	2 hours	1. Exposure

509 18 5	3	0-10	D	0.6 ppm PAA	2 hours	1. Exposure
509 18 15	3	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 16	1	0-10	D	0.6 ppm PAA	2 days	1. Exposure
509 18 52	3	0-10	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 58	13	11-20	D	0.6 ppm PAA	2 weeks	2. Exposure
509 18 12	27	21-50	D	0.6 ppm PAA	2 days	1. Exposure
457 18 121	6	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 122	5	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 123	7	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 124	3	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 106	2	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 107	5	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 108	3	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 109	3	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 110	4	0-10	E	1.2 ppm PAA	2 days	1. Exposure
457 18 161	2	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 162	3	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 163	2	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 164	2	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 165	5	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 96	4	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 97	2	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 98	3	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 99	5	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 100	1	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 101	6	0-10	E	1.2 ppm PAA	2 hours	1. Exposure

457 18 102	2	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 103	4	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 104	1	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 105	5	0-10	E	1.2 ppm PAA	2 hours	1. Exposure
457 18 156	5	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 157	3	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 158	4	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 17 159	2	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 160	6	0-10	E	1.2 ppm PAA	2 days	2. Exposure
457 18 136	2	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 137	4	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 138	4	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 139	2	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 140	3	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 141	3	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 142	3	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 143	3	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 144	5	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 145	3	0-10	E	1.2 ppm PAA	2 weeks	1. Exposure
457 18 146	3	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 148	2	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 149	3	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 150	2	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 151	2	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 152	3	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 153	2	0-10	E	1.2 ppm PAA	2 hours	2. Exposure

457 18 154	5	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 155	5	0-10	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 166	4	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 167	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 168	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 169	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 170	2	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 181	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 182	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 183	3	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 184	4	0-10	E	1.2 ppm PAA	2 weeks	2. Exposure
457 18 125	15	11-20	E	1.2 ppm PAA	2 days	1. Exposure
457 18 147	14	11-20	E	1.2 ppm PAA	2 hours	2. Exposure
457 18 185	13	11-20	E	1.2 ppm PAA	2 weeks	2. Exposure
40 18 177	5	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
40 18 178	5	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
407 18 176	3	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
407 18 179	5	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
407 18 180	6	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
407 18 27	6	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
407 18 28	2	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 10	1	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 116	5	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 117	8	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 118	5	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 119	4	0-10	F	2.4 ppm PAA	2 days	1. Exposure

457 18 120	4	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 131	3	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 132	7	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 133	5	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 134	4	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 135	6	0-10	F	2.4 ppm PAA	2 days	1. Exposure
457 18 17	2	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 18	4	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 19	5	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 191	6	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
457 18 192	5	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
457 18 194	2	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
457 18 20	5	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 31	5	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 37	6	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 38	8	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 39	7	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 41	1	0-10	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 47	6	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 48	3	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 49	2	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 50	2	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 51	5	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 57	3	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 58	3	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 59	4	0-10	F	2.4 ppm PAA	2 hours	2. Exposure

457 18 6	3	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 60	2	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 61	1	0-10	F	2.4 ppm PAA	2 hours	2. Exposure
457 18 67	5	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 68	4	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 69	6	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 7	2	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 70	2	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 71	4	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 77	4	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 78	3	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 79	3	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 8	6	0-10	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 80	2	0-10	F	2.4 ppm PAA	2 days	2. Exposure
457 18 81	2	0-10	F	2.4 ppm PAA	2 days	2. Exposure
459 18 193	8	0-10	F	2.4 ppm PAA	2 weeks	2. Exposure
407 18 29	16	11-20	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 195	13	11-20	F	2.4 ppm PAA	2 weeks	2. Exposure
457 18 21	15	11-20	F	2.4 ppm PAA	2 hours	1. Exposure
457 18 30	15	11-20	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 40	17	11-20	F	2.4 ppm PAA	2 weeks	1. Exposure
457 18 9	15	11-20	F	2.4 ppm PAA	2 hours	1. Exposure