Universidade do Algarve

Faculdade de Ciências e Tecnologia

Dissertação de Mestrado em Aquacultura e Pescas

2019/2020

Effect of low pressure and medium pressure UV radiation on Atlantic salmon pathogens' inactivation

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Trabalho efetuado sob a orientação de:

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Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Universidade do Algarve, 22 de Setembro de 2020

Dedico este trabalho aos meus pais, José Rui Guerreiro e Margarida Matos, como símbolo de gratidão por todo o amor, esforço e dedicação que recebi ao longo de todos os meus anos de estudo. Muito obrigado!

Resumo

A produção de salmão do Atlântico (*Salmo salar*) tem estagnado nos últimos anos devido à redução de novas concessões, ao aumento de casos de doenças e limitações na eficácia de fármacos. Certas doenças são tratadas e/ou prevenidas por meios como a vacinação, uso de antibióticos e peixes de limpeza, mas existe também uma estratégia diferente e complementar que passa pela desinfeção da água via ozono e/ou radiação ultravioleta (UV). Porém existe pouca informação relativamente às doses de UV necessárias para inativar vírus e bactérias que afetam a produção de salmão. Por esse motivo, este estudo teve como objetivos averiguar o impacto de UV de baixa pressão ("low pressure", LP) e UV de média pressão ("médium pressure", MP) nos microrganismos "infectious pancreatic necrosis virus" (IPNV), *Moritella viscosa* e *Yersinia ruckeri*. Foi determinada a dose de UV necessária para atingir 99,9 % (3 log) de inativação de cada microrganismo por LP e MP, foram encontradas as diferenças entre inativação por LP e MP e foram comparadas as reações a UV entre vírus e bactérias. Cada microrganismo foi colocado numa solução de água salgada artificial. A concentração viral/bacteriana foi quantificada, processo após o qual uma alíquota foi exposta a uma dose de UV (entre 5 doses previamente definidas) emitida por uma lâmpada LP ou MP contida dentro de um aparelho de feixe colimado ("Collimated beam apparatus", CBA). Após exposição à luz UV, a concentração viral/bacteriana foi de novo quantificada. Os resultados indicaram que o IPNV, a *M. viscosa* e a *Y. ruckeri* sofreram uma inativação de 99,9 % por LP UV com as seguintes respetivas doses de UV: 200, 15, 10 mJ/cm^2. Os mesmos patogénicos foram inativados de igual forma por MP UV pelas respetivas doses de UV: 56,4, 4,6 e 11,3 mJ/cm^2. As bactérias estudadas demonstraram menos resistência do que o vírus face à radiação de LP e MP UV. Isto deve-se ao facto de terem diferentes estruturas externas, tamanhos, genomas e mecanismos de reativação. MP UV atingiu inativação do IPNV e da *M. viscosa* com doses de UV mais baixas do que as emitidas por LP UV e destacou-se pela capacidade de atingir certas proteínas e enzimas capazes de reativar o vírus e bactérias estudadas.

Palavras chave: Salmão do Atlântico; UV de baixa pressão; UV de alta pressão; Aparelho de feixe colimado; Vírus; Bactéria.

Abstract

The production of Atlantic salmon (*Salmo salar*) in Norway has flattened in the past years due to the reduction of new licenses, diverse pathogen outbreaks and treatment limitations. Some diseases have been treated with vaccines, antibiotics and cleaner fish but a complementary approach involves water treatment by ozonation and/or ultra-violet (UV) radiation. However, few studies have determined the UV doses necessary to inactivate Atlantic salmon pathogens. This work aimed to study the effect of low pressure (LP) UV radiation and medium pressure (MP) UV on infectious pancreatic necrosis virus (IPNV), *Moritella viscosa* and *Yersinia ruckeri* by (i) determining the UV dose necessary to achieve a 99.9% inactivation (3 log) of each pathogen, (ii) assessing inactivation differences between LP and MP UV and (iii) finding similarities and differences among viral and bacterial pathogens inactivation. Each microorganism was diluted in artificial seawater, quantified, exposed to an UV dose (out of 5 doses) emitted either by a LP or MP UV lamp inside a collimated beam apparatus (CBA) and quantified after exposure to UV. Results showed that 99.9 % inactivation of IPNV, *M. viscosa* and *Y. ruckeri* required 200, 15 and 10 mJ/cm^2 respectively by LP UV. The same pathogens were 99.9 % inactivated with 56.4, 4.6 and 11.3 mJ/cm^2 respectively by MP UV. Overall, bacteria species demonstrated being less resistant to LP and MP UV than IPNV. This difference resulted from their different external structures, sizes, genome compositions and repair mechanisms. MP UV was able to inactivate IPNV and *M. viscosa* with lower doses than LP UV, even though being less energy efficient. The main advantage of MP over LP UV was its ability to emit wavelengths that are absorbed by repair proteins and enzymes, thus avoiding pathogens reactivation.

Keywords: Atlantic salmon; Low pressure UV; Medium pressure UV; Collimated beam apparatus; Virus; Bacteria.

Table of Contents

Index of Figures

Index of Tables

1. Introduction

1.1 Overview on the salmon industry

The salmon farming industry is one of the most complex aquaculture business and has evolved substantially during the last 20 years (Mowi, 2020). Its annual production has increased worldwide from 1.4 million tons in 2010 to over 2.6 million tons in 2020 and is expected to surpass 3.0 million tons by 2022 (Ernst & Young AS, 2019). The four world's largest Salmon producers are Norwegian companies: MOWI, Lerøy Seafood ASA, Cermaq Norway and SalMar Farming AS. In addition, 95% of the total production comes from Norway, Chile, Canada and UK (Scotland). Other producing countries are Denmark (Faroe Islands), France, Australia, Ireland and United States of America (Figure 1).

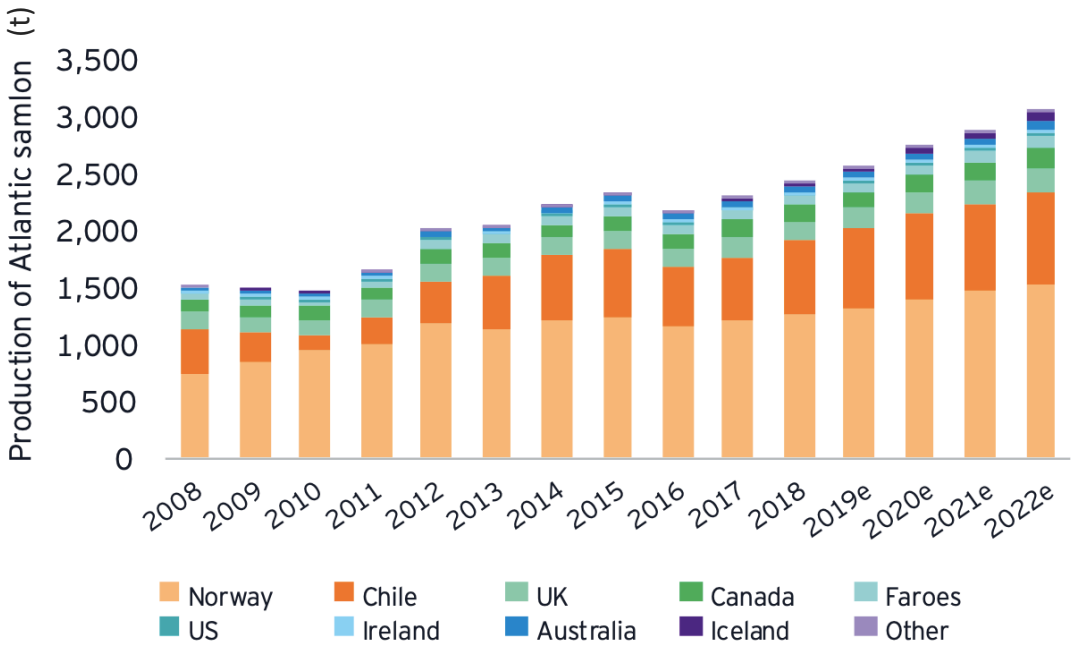

Figure 1 – World production (in thousand tonnes) per country of Atlantic salmon from 2008 to date and prospect until 2022 (Ernst & Young AS, 2019)

The average price of Norwegian gutted Atlantic salmon has also risen significantly over the last years, from around 3.80 € per kg in 2012 to an average of 6 € per kg in 2019 (Mowi, 2020). Behind this increase is a higher demand and a flattened production due to an increase of pathogens occurrence, treatment limitations and reduction of new licenses (by governments such as in Norway) for production in net pens (Ernst & Young AS, 2019). To get around the reduction of new licenses for salmon

production in sea cages, many companies have increased their production in recirculated aquaculture systems (RAS) but also in semi-closed systems (SCS).

Even though Norwegian salmon farms being equipped with some of the most advanced technologies within all aquaculture branches (Ernst & Young AS, 2019; Mowi, 2020) there are still many environmental concerns that tarnish this industry image. The most problematic issues are nutrients run-off from sea cages to the surrounding areas, fish escapes that alter the genetic diversity of wild salmon populations and the high risk of spreading parasites and pathogenic bacteria and viruses to wild populations or other farms when transporting fish from inland facilities to sea cages or through escapes (Fischer et al., 2017; Krkošek, 2017). Many of these problems are still being addressed through research and Norwegian companies are committed to solve these problems in order to obtain more licenses for new production sites and to keep up with the growing demand (Mowi, 2020). For example, Hauge Aqua is developing a new semi-closed system, called "the egg" (Figure 2), that has an egg shape and the water is filtered mechanically and disinfected before being pumped inside. This concept minimizes the entry of organic particles as well as fish escapes and pathogens (sea lice, pathogenic virus and bacteria) that otherwise would increase the risk of disease outbreaks.

Figure 2 – Hauge Aqua's new Egg closed containment aquaculture system. Retrieved from https://haugeaqua.com/technology/egget

1.2 Water Quality-Disease relationship on commercial fish farms

Water quality has a direct impact on fish health and a good water quality will lead to less disease outbreaks related to pathogens (Hjeltnes et al., 2019). There is a

natural balance between pathogens, their hosts and environmental factors. These can occur more frequently when water quality drops, thus damaging the host immune system and reducing the fish's resistance against the disease (Johansen et al., 2011; Krkošek, 2017). Also, the risk of disease outbreak can be boosted by an increase in the stocking density of fish (Johansen et al., 2011).

Water quality in sea cages is dependent on environmental factors, which is also related to the placement of the sea cage (more or less exposed to currents; Johannesen et al., 2020). On the other hand, SCS and RAS allow to better control the interaction between farmed fish and the environment since the inlet water is pumped from 40-60 meters depth in order to avoid warmer waters and sea lice that are usually present in surface waters (Rud et al., 2017). Besides, RAS are equipped with different equipment capable of performing water filtration, disinfection, aeration, CO2 removal, among other functionalities in order to allow up to 99.6% water recirculation (Bregnballe, 2015; Munang'andu et al., 2016).

However, RAS and SCS are not entirely free of pathogens and diseases. Pathogens outbreaks have occurred in RAS due to the poor quality of the intake water and sometimes due to the mismanagement of the water quality within the farm (Hjeltnes et al., 2019). Pumping deep waters also increases the risk of introducing upwelling particles that are reservoirs of pathogenic bacteria such as *Vibrio spp.* and *Moritela viscosa* (Rud et al., 2017). According to the Fish Health Report 2018, pathogenic organisms are difficult to eradicate from recirculated systems once they form colonies in biofilters and in areas of difficult access that cannot be cleaned or disinfected (Hjeltnes et al., 2019).

1.3 Disease transmission between farmed and wild Atlantic salmon

Pathogens such as parasites, viruses and bacteria are shared between wild and farmed salmon (Fischer et al., 2017). Therefore, salmon farms located in wild salmon habitats or near its migration route have a higher chance of contamination, diseases outbreaks and large mortality events. Furthermore, salmon produced in net pens are much more exposed to spill-over (disease transmission from wild to farmed fish) since

the water that runs through the nets is not treated and dependent on tides (Behringer et al., 2020; Nylund et al., 2019). A spill-back (disease transmission from farmed to wild) effect is also often and other farms in the region can get contaminated and suffer high mortality events (Krkošek, 2017).

For instance, ISAV appeared in farmed salmon after a mutation in one of its variants transmitted from wild salmonids, followed by transmission between farms due to wild salmon transit and other farming activities (Nylund et al., 2019).

1.4 Diseases in Atlantic salmon farming in Norway

Over the last years many disease outbreaks related to pathogenic bacteria, viruses and parasites have occurred. These had a serious impact on the production of Atlantic salmon in Norway since they were responsible for a mortality of 14.7% in 2018 (Hjeltnes et al., 2019). Since this specie is first produced in freshwater and then transferred into saltwater it is prone to catch specific diseases from both environments in each stage of production.

1.4.1 Parasite related diseases

Sea lice are ectoparasitic copepods that belong to the genera *Lepeophtheirus*, *Caligus* and *Neoparamoeba*, and are commonly found in wild and farmed *S. salar*. The two most common species are *Lepeophtheirus salmonis* in the Atlantic Ocean and *Caligus rogercresseyi* in the Pacific Ocean (Aaen et al., 2015). These parasites feed on the mucus, skin and blood of salmonids, damaging fish health and causing large economic losses to the salmon industry (Nekouei et al., 2018). In order to combat sea lice plagues, salmon companies use parasiticides and lumpfish for treatment and mechanical filters for prevention in their farms (Cerbule & Godfroid, 2020; Imsland et al., 2014). Overall, lumpfish remain an efficient way to remove sea lice from Atlantic salmons (Imsland et al., 2014). However, the parasiticides efficacy has declined due to the gain in resistance of the most common parasites species to chemical treatments after many years of intensive treatments (Cerbule & Godfroid, 2020; Helgesen et al., 2015).

1.4.2 Virus related diseases

Virus remain a huge challenge for salmon farmers to prevent and treat. Some of the viruses that affect the health of *Salmo salar* are the infectious salmon anemia virus (ISAV), the heart and skeletal muscle inflammation virus (HSMI), the infectious pancreatic necrosis virus (IPNV) and the salmon alphavirus (SAV), among others (Hjeltnes et al., 2019). Vaccination programs are in place against some diseases, such as pancreas disease and IPNV (Mowi, 2020). Nonetheless some do not have treatment yet or are difficult to eradicate due to the fact that some viruses can be carried in adults reproductive organs (milt), eggs or feces (Hjeltnes et al., 2019; Nylund et al., 2019).

IPNV targets mainly juvenile and post-smolts Atlantic salmon (Hjeltnes et al., 2019). Infected individuals usually swim at the surface or at the outlet water, head up or on their side and hyperventilating. Furthermore, this virus causes severe necrosis and an extensive and progressive destruction of the pancreatic cells. Over time, the liver suffers from necrosis (Figure 3), the whole abdomen becomes swollen and the dorsal fin tips rot. The virulence and mortality rate of IPNV depend on the strain and environmental factors and can vary from 10-20% in low stocking density to 70% in high stocking density (Nylund et al., 2015). IPNV strains can be transmitted vertically from broodstock to the eggs and horizontally (Nylund et al., 2015). It is known that the main reservoirs of this pathogen are farmed salmon that carry the virus but are immune to it, and farmed salmon that carry IPNV but are not immune and show clear symptoms of IPNV disease (Nylund et al., 2015). Detection can be done based on clinical signs, histopathology, identification by PCR methods and cell cultures (CHSE-214 cells) (Mutoloki et al., 2016). The only preventive measure in place against IPNV is vaccination in the juvenile phase of Atlantic salmon. A selective breeding program also contributed for a high degree of IPNV resistance in Atlantic salmon produced by Norwegian companies (Hjeltnes et al., 2019). However, IPNV outbreaks are still susceptible of happening since 19 outbreaks were detected during 2018 in freshwater hatcheries and in marine sites (Hjeltnes et al., 2019).

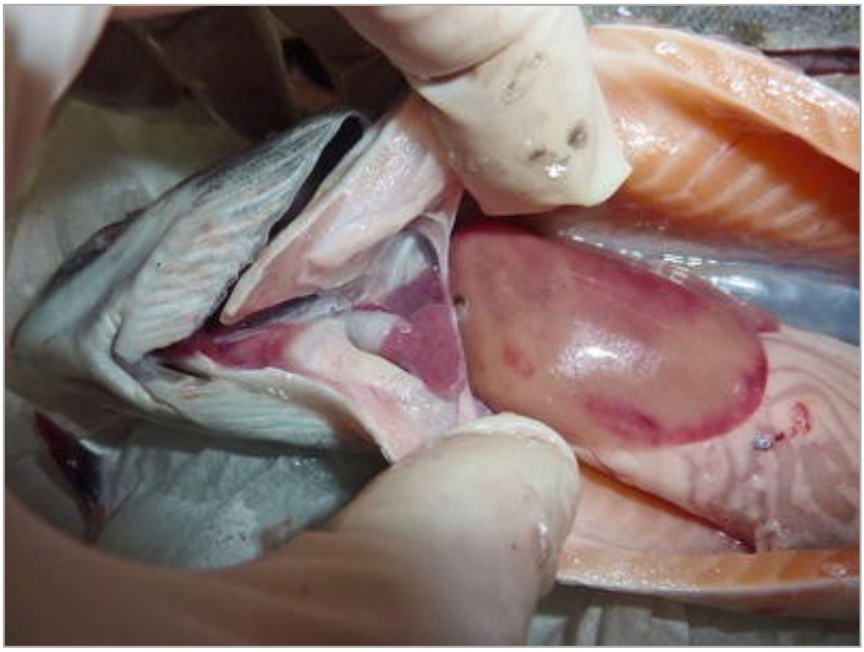

Figure 2 – Atlantic salmon presenting clinical signs related to infection by IPNV: pale heart and liver with hemorrhages. Retrieved from Bruno et al., 2013.

1.4.3 Bacteria related diseases

Some of the most important Gram-negative pathogens are from the genera *Yersinia*, *Aeromonas*, *Moritella*, *Tenacibaculum* and *Vibrio*, while the important Gram-positive bacteria are from the genera *Streptococcus, Mycobacterium and Renibacterium*.

The Gram-negative bacteria *Moritella viscosa* is one of the pathogens responsible for the winter-ulcer disease in farmed *Salmo salar* (Figure 4A). Two different types of winter-ulcer disease can be expected depending on the strain of *M. viscosa* that causes the infection and are denominated by “typical” or “atypical” winter ulcer (Hjeltnes et al., 2019). However, the last one (also known as tenacibaculosis) is less common and mostly related to infections caused by *Tenacibaculum spp.* (Hjeltnes et al., 2019). Fish are more susceptible to contract winter-ulcer disease during the sea phase production during the autumn and winter months, even though it can occur during the whole year (Nylund et al., 2015). Symptoms of the “typical” winter-ulcer on Atlantic salmon are related to external infections such as large and chronic ulcers on the skin, septicemia, fin rot, tissue necrosis but also to internal infections such as small hemorrhages on certain internal organs (Nylund et al., 2015). Mortality is usually

moderate to high but the disease leaves clear marks of ulcer that reduce the quality of the fish at harvest (Bruno et al., 2013). It is common to find other bacteria on the skin ulcers, such as *Tenacibaculum spp.* and *Vibrio wodanis* (Hjeltnes et al., 2019). According to Hjerde et al. (2015) *V. wodanis* inhibits the growth of *M. viscosa*, and it is believed that it could have a major role in retarding ulcers formation. This disease can be transmitted from infected to healthy Atlantic salmons and the infectious can be accelerated if the skin and mucus layer present some sort of injury (Bruno et al., 2013; Nylund et al., 2015). The majority of farmed Atlantic salmon in Norway are vaccinated against the "typical" winter-ulcer disease, however *M. viscosa* outbreaks can still occur and are treated with antibiotics, even though its variable efficacity (Bruno et al., 2013; Hjeltnes et al., 2019). Unfortunately, there is no vaccine against the "atypical" winter-ulcer disease (Hjeltnes et al., 2019).

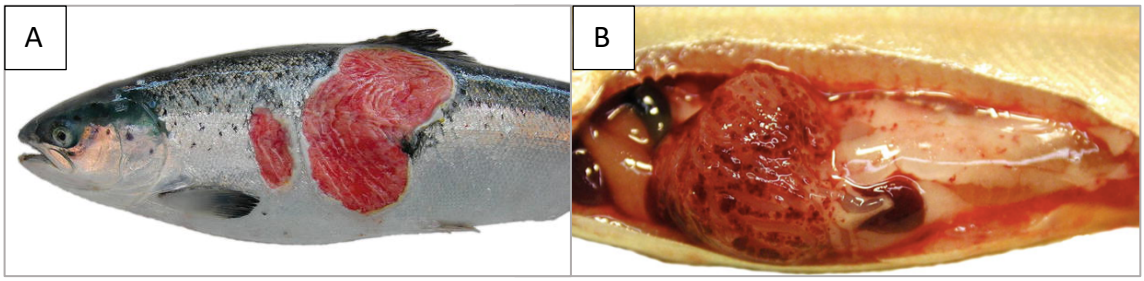

Figure 3 – Winter ulcers in farmed Atlantic salmon (A) and signs of enteric redmouth disease (B) in internal organs of a farmed rainbow trout. Retrieved from Bruno et al., 2013.

Another major disease in salmon farming is yersiniosis (also known as enteric redmouth disease; Figure 3B), caused by *Yersinia ruckeri* infection. Out of different strains of this bacteria found in Norway (serotype O1, O2 and O5) serotype O1 has been the most problematic during the last 20 years (Hjeltnes el al., 2019). It can occur during the juvenile freshwater phase but also after fish transfer to sea cages, due to stress related to handling or treatments against sea lice (Hjeltnes et al., 2019). *Y. ruckeri* was also isolated in the recent years from broodstock, cleaner fish (lump fish) and wild salmon but also from salmons in the smolt phase in RAS (Hjeltnes et al., 2019). The first clinical sign of infection is septicemia on the gills, then homorrhages on the mouth, eyes and in internal organs, exophthalmia, enteritis and septicemia on the skin (Bruno et al., 2013). *Y. ruckeri* is present in feces excreted from infected fish and can therefore be transmitted in freshwater and seawater since it survives for long periods of time (Bruno

et al., 2013; Nylund et al., 2015). In order to prevent contamination and disease outbreaks salmon farms rely on vaccination and disinfection of the eggs in hatcheries (Hjeltnes et al., 2019; Nylund et al., 2015). However, fish have to be medicated with antibiotics in case of a serious yersiniosis outbreak (Nylund et al., 2015).

1.5 Prevention and control of diseases in aquaculture

Disease management is one of the biggest concerns and constraints to the salmon industry (Ernst & Young AS, 2019). To combat this issue, breeding programs were created with the aim of obtaining new generations of Atlantic salmon more resistant to diverse pathogens encountered in the past years of production (Hjeltnes et al., 2019; Yáñez et al., 2014). Companies have invested in juvenile vaccination against several diseases such as vibriosis, winter-ulcers, furunculosis, yersiniosis, IPNV, ISAV, pancreas disease, among others (Hjeltnes et al., 2019; Mowi, 2020; Nylund et al., 2015; Sommerset et al., 2005). In hatcheries, broodstocks are regularly screened using laboratory test kits in order to identify and remove contaminated fish that could vertically contaminate eggs (Munang'andu et al., 2016). However, farmed Atlantic salmon are still prone to be infected by microorganisms and sometimes carry one or more diseases, both in freshwater and seawater farms (Hjeltnes et al., 2019).

After adapting from freshwater to seawater conditions, the majority of Atlantic salmon are transferred to sea cages which cannot avoid the entrance of bacterial and viral microorganisms. A more recent approach consists in growing salmon in RAS. These systems are equipped with different types of filters that are capable of removing organic matter such as feces, large particles, uneaten feed, that could decrease water quality (Bregnballe, 2015). However, it was mentioned previously that pathogens can enter the system through infected fish, inlet water and other sources. They are difficult to eradicate from RAS after establishing themselves in the biofilter or other areas that are difficult to clean, even though disinfection methods being applied (Hjeltnes et al., 2019).

Disinfection methods are a key component in water quality and pathogens control. The disinfection technologies most used in aquaculture are ozonation and ultra-violet radiation (UV), but this work will only focus on UV further on.

1.6 Water disinfection by ultraviolet light

Water disinfection by ultraviolet light (UV) has been used in sewage treatments, pool disinfection, in specific industries effluents but also to manage marine and freshwater aquaculture effluents. UV radiation ranges between 100 and 400 nm and can be divided into four categories: far UV or vacuum UV (these wavelengths are only propagated in a vacuum; 100-200 nm), UV (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). However, lamps emitting UV wavelengths have shown the most efficient disinfection rate, since they target microorganism's nucleic acids (DNA and RNA) peak absorbance at 260 nm, and damage enzymes and proteins that absorb UV light at wavelengths below 240 nm (Beck et al., 2015; Gerba, 2015; Rastogi et al., 2010).

Three main technologies are commercially available: standard low pressure (LP), amalgam low pressure and medium pressure (MP) UV emitting lamps (Fischer et al., 2006; Rastogi et al., 2010; Schalk et al., 2005). Each one of these lamps contains mercury, either in a free state or attached to the lamp tube's surface (Schalk et al., 2005).

The way these lamps works is based on electrical excitation and ionization levels of the mercury within the lamp. Power is first applied to the lamp electrodes. This generates an electrical arc out of the ionized gas as temperature increases inside the lamp. The wavelengths emitted afterwards depend on the quantity of mercury and pressure inside the lamp (Aquafine, 2017).

A standard low pressure lamp emits a single peak at 254 nm (monochromatic) and operates at around 45 °C. Approximately 35-40 % of the electrical input results in a 254 nm wavelength, which is close to the 260 nm peak absorbance of nucleic acids, thus inducing damage on DNA and RNA. Working pressures (1-10 Pa) and power density per unit length of lamp arc are the lowest when compared with the other two types of lamps. Out of the three lamps, LP lamps require less cleaning maintenance (Aquafine, 2017).

Amalgam low pressure lamps are monochromatic at 254 nm like standard LP lamps. However, they contain another element that reacts with mercury to operate at

higher temperatures (100 °C) and higher pressures, what results in an increase of power density about 1.5 to 4 times of a standard LP lamp per unit length of lamp arc. About 30-35 % of the electrical input can be transcribed in a 254 nm peak. A disadvantage of lamps reaching high temperatures is that they are more susceptible to fouling and require more maintenance (Aquafine, 2017).

Medium pressure lamps are polychromatic, which means that they are capable of emitting different wavelengths, between 200 and 280 nm. This type of lamp operates at temperatures between 800 and 900 °C, which results in high pressures inside the lamp. Furthermore, the power density per unit arc length of this lamp is around ten times superior to LP lamps. Nevertheless, only 15-18 % of the electrical input result in a 254 nm peak. Filters running MP lamps usually have automated cleaning systems due to the frequent maintenance required (Aquafine, 2017).

The UV dose (or fluence) for water disinfection depends on several factors, such as particle size and concentration in the water, the microorganisms presence in the system, UV Transmittance in the water (UVT), exposure time, water flowrate and the UV intensity (Bolton, 2005; Bolton et al., 2015). UVT determines how much UV light passes through a water sample compared to the amount of UV light that passes through a pure water sample (Bolton et al., 2001). Its value is expressed in percentage and vary over time and from site depending on the amount of organics and solids that absorb part of the UV radiation that is emitted by the UV filter (Bolton et al., 2001). The UVT value can be determined using a spectrophotometer by measuring the absorbance of a water sample at 254 nm (LP) or the mean of the absorbances from 200 to 280 nm (MP), in a 10 mm quartz cell, and comparing it to the absorbance of a distilled water sample. Higher UVT values are desirable for an efficient disinfection by UV filters (Astuti & Rongjing, 2016).

Therefore, the use of UV in RAS has to be properly studied and sized to maximize the disinfection rate. It is also desirable to monitor water quality frequently to predict the UV intensity for the minimal UV dosage required to maintain the pathogenic microorganisms' concentrations as low as possible (Shang & Leung, 2007).

1.7 Effect of low pressure UV radiation on bacteria and viruses

As mentioned previously, low pressure UV lamps emit a monochromatic wavelength at 254 nm that is absorbed by the pathogens' DNA/RNA and damages the nucleic acids by altering the nucleotide base pairing (Abdallah et al., 2012; Rodriguez et al., 2014). According to Kim et al. (2017), UV radiation is capable of damaging bacteria outer membrane leading to bacterial inactivation.

Several UV photoproducts can be produced and affect bacteria survival, such as pyrimidine dimers (6-4 pyrimidine-pyrimidone and cyclobutane pyrimidine) (Goodsell, 2001). Pyrimidine dimers are lesions that alter the DNA chain, specially thymine (T) bases and uracil (U) bases in RNA that further lead to problems in transcription and replication (Goodsell, 2001; Ravanat and Douki, 2016; Santos et al., 2013). However, the formation of pyrimidine dimers can be reversed by repair mechanisms such as photoreactivation and dark repair (Goosen and Moolenaar, 2008). In photoreactivation, photolyase is an enzyme capable of recognizing a pyrimidine dimer in the DNA chain and uses the energy from UV-A radiation to bind to the thymine dimer, thus breaking the T-T bases and converting them back to their original bases (Coohill and Sagripanti, 2008). In dark repair, enzymes are able to cut the pyrimidine dimer out of the DNA chain and close the gap with undimerized thymine (Coohill and Sagripanti, 2008).

UV radiation causes damage to the viruses' genome and structure. Viruses carrying a high molecular weight DNA/RNA are easier to inactivate than those with a low molecular weight. Furthermore, viruses with a double-stranded DNA genome are more difficult to inactivate than single-stranded DNA genome since there are more repair enzymes within the host cell to repair the damaged sections of the double-stranded DNA genome, using the nondamaged strand as a template (Rodriguez et al., 2014).

1.8 Effect of medium pressure UV radiation on bacteria and viruses

The major advantage of MP UV radiation is that it prevents photoreactivation of bacteria and repair mechanisms in viruses (Gerba, 2015). While low pressure UV lamps are only capable of emitting a 254 nm wavelength, close to the maximum absorbance of DNA (260 nm), medium pressure lamps emit a wide range of wavelengths between 200 and 300 nm that not only damage the pathogens' DNA and RNA but also other cellular components such as proteins (repair enzymes), amino acids, lipids and other small molecules that absorb UV radiation below 240 nm (Beck et al., 2015; Gerba, 2015). For example, a microorganism loses the ability to multiply itself when polymerase enzymes are denatured due to the absorption of a specific wavelength and cannot repair the nucleic acids after photolyase enzymes being damaged (Kalisvaart, 2004).

1.9 Objectives

This work aimed to study the effect of UV radiation (low pressure and medium pressure UV lamp) on Atlantic salmon viral and bacterial pathogens (IPNV, *Moritella viscosa* and *Yersinia ruckeri*) by (i) determining the UV dose necessary to achieve a 99.9% inactivation (3 log) of each pathogen, (ii) assessing inactivation differences between low and medium pressure UV lamps for the same microorganism, (iii) finding similarities among viral and bacterial pathogens inactivation.

2. Materials and methods

2.1 Experimental design

This experiment was performed at the laboratories of Nofima, in Tromsø (Norway) using two different collimated beam apparatus (CBA). IPNV, *Moritella viscosa* and *Yersinia ruckeri* as well as *E. coli* for quality control were obtained from previous isolation works. All species were exposed individually to 3 pairs of UV tests, under a LP UV lamp and a MP UV lamp, on different days (Figure 5).

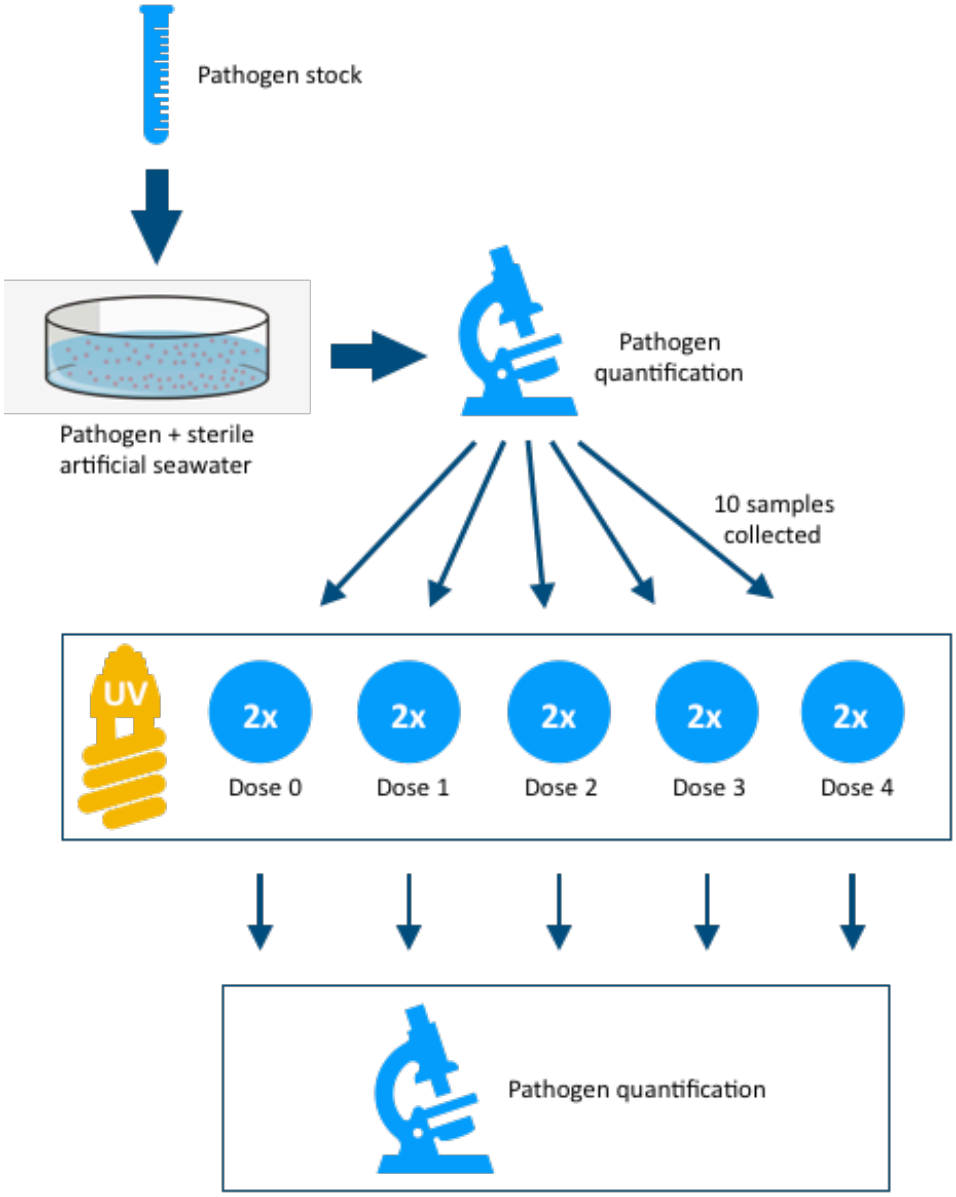

Figure 4 – Summarized steps of a UV test using a CBA with a low pressure/medium pressure UV lamp

Each test consisted in adding a specie of bacteria or virus to sterile artificial seawater at 30 ppt, then determining its concentration. This step was followed by collecting 5 pairs of samples from the previous solution and exposing each pair to a UV dose out of five doses. After UV exposure the pathogen concentration was determined in each sample, according to the same quantification method performed before UV exposure.

From the 3rd of February to the 13th of March 2020, I participated in each phase of the first two replicate tests for each microorganism, which took place at Nofima and consisted in sampling, culturing, exposing to UV and quantifying each microorganism. However, due to the COVID-19 pandemic situation I had to return to Portugal and Vasco Mota continued the other replicate tests that were planned. All the statistics and data analysis were made by me.

2.2 Samples preparation for UV tests

2.2.1 Artificial seawater

Sea Salt from Aquaforest (Marine aquarium products manufacturer, Poland) was used to prepare the artificial seawater at 30 ppt, using 30.0 g of Sea Salt were diluted in one liter of distilled water. Then, this solution was autoclaved, stirred with a magnetic stirrer overnight and stored at 12 °C for further use.

2.2.2 *Escherichia coli*

E. coli CCUG 3274 was chosen as a control, not to be compared to the other pathogens but as a quality control of the methods put into practice in the present work. Several strains of *E. coli* have been the subject of many studies and their resistance to LP and MP UV radiation has been described (Malayeri et al., 2016).

The tube containing *E. coli* was defrosted and a sterile inoculating loop was used to inoculate a Lysogeny broth medium 3% NaCl (LB-medium; preparation described in Annex A). The LB-medium inoculated with *E. coli* was then stirred overnight inside an incubator at 30 °C. After incubating for 24 hours, the optical density was $OD_{600} = 6.0$. A dilution was carried in order to obtain an $OD_{600} = 1$, which is known to be approximately

equivalent to a concentration of 10^9 CFU/mL. This solution was then centrifuged twice at 3000 G for 10 minutes at room temperature, and the dilution steps were performed the same way as done for *M. viscosa and Y. ruckeri*. The concentration of *E. coli* before the UV tests was estimated at 10^6 CFU/mL. This process was repeated for each LP and MP UV test.

2.2.3 IPNV

The IPNV used in this study was isolated from the pylorus of an infected dead Atlantic salmon in 2010. It had been frozen until this experiment at -80 °C in serum to be preserved for a long period. The virus concentration was estimated around 10^7 $TCID_{50}$/mL.

Each pair of tests required 500 mL of artificial seawater containing IPNV: 250 mL for LP and 250 mL for MP. Therefore, 5 mL of IPNV stock were defrosted an hour prior to the UV tests and introduced in sterile artificial seawater at 30 ppt by a ten times dilution, resulting in a 50 mL sample of artificial seawater and IPNV around 10^6 $TCID_{50}$/mL. Afterwards, the absorbance of the sample was measured in a spectrophotometer at 254 nm to calculate the UVT value. Since this parameter was lower than 90 % (less than 90 % of UV light passed through the water sample, (Bolton et al., 2001), the sample had to be diluted ten times in order to increase its UVT value, ending in a 500 mL of artificial seawater and IPNV around 10^5 $TCID_{50}$/mL. The absorbance of the sample was measured a second time and the corresponding UVT value was superior to 90 %. These steps were repeated for run 1, 2 and 3 of LP UV and for run 1 and 2 of MP UV.

2.2.4 *Moritella viscosa*

Moritella viscosa LFI 5006/2 (serial number at Nofima AS) was isolated from Atlantic salmon in 2014. Since then, it had been frozen at -80 °C with glycerol to be properly preserved.

M. viscosa was defrosted four days in advance each pair of UV tests since it had to be grown in two medias. First, a sterile inoculating loop was used to inoculate a blood

agar 3 % NaCl plate (first growth medium; preparation described in Annex C). This plate was then incubated for 2 days at 12 °C. After the incubation period, the blood agar plate was mostly covered with bacteria colonies. Using a sterile inoculating loop, 4 colonies from the plate were removed and placed in a flask containing 10 mL of fluid marine broth 3 % NaCl (second growth medium; preparation described in Annex C). This solution was then shaken over the next 48 hours on an orbital shaker (KS501 digital, IKA, Germany) and the optical density (OD) was monitored until OD_{600} = 1 (optical density; estimation of 10^9 CFU/mL). At this point *M. viscosa* was ready to be introduced in sterile artificial seawater.

On the day of the UV tests, 500 mL of sample containing *M. viscosa* were needed per pair of tests: 250 mL for LP and 250 mL for MP. Therefore, 5 mL of fluid marine broth 3% NaCl and *M. viscosa* around 10^9 CFU/mL were centrifuged twice at 3000 G for 10 minutes at 5 °C. The purpose of both centrifugations was to separate the bacteria from the marine broth 3% NaCl solution that was causing a cloudiness effect on the sample and lowering the UVT value. The bacteria pellet was afterwards resuspended in 500 mL of artificial seawater at 30 ppt, leading to a bacterial concentration around 10^7 CFU/mL. Lastly, a ten times dilution had to be performed to increase the UVT value of the sample, which resulted in a final *M. viscosa* concentration around 10^6 CFU/mL.
This process was repeated for run 1, 2 and 3 of LP UV and for run 1, 2 and 3 of MP UV.

2.2.5 *Yersinia ruckeri*

Yersinia ruckeri Serotype O1 was isolated from the head kidney of Atlantic salmon in February 2015 by the Norwegian Veterinary Institute located in Harstad (Norway). Since then, it had been frozen at -80 °C with glycerol to be properly preserved. This pathogen was grown using the same mediums and culture methods as mentioned above for *Moritella viscosa* (mediums preparation described in Annex C). When OD_{550} = 1, the bacterial concentration of the sample was estimated around 10^9 CFU/mL. This pathogen solution was also centrifuged twice at 3000 G for 10 minutes at room temperature, and the dilution steps were performed the same way as for *M. viscosa*. The *Y. ruckeri* concentration before the UV tests was estimated at 10^6 CFU/mL. This process was repeated for run 1, 2 and 3 of LP UV and for run 1, 2 and 3 of MP UV.

2.3 Low pressure and medium pressure UV tests

A CBA is designed to irradiate microorganisms in a water sample with a fixed UV intensity, for a certain period of time. The longer the exposure time, the higher will be the UV dose. Part of the output of the UV lamp is channeled through a collimating tube (Figure 6) to hit the sample from the top to cover the whole area of the petri dish (Reed & Muench, 1938). The only characteristic that differs among LP and MP UV CBA's is the lamp.

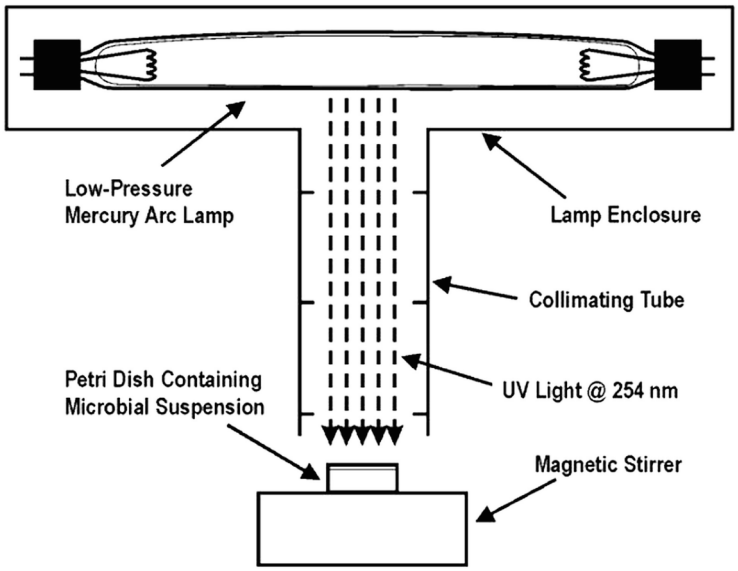

Figure 5 – Design of a low pressure UV collimated beam apparatus. Retrieved from Bolton et al. (2015).

The UV tests were conducted with two CBA's provided by Atlantium (UV sterilizer manufacturer; Israel). One was equipped with a low pressure UV lamp and the other with a medium pressure lamp. Both were calibrated by Atlantium engineers before the UV tests using a calibrated radiometer and detector (USB4000, Ocean Optics, USA) to obtain the UV intensity value. This value was later used to calculate UV doses for each pathogen.

2.4 Experimental procedure and UV doses

The procedure was the same for each pathogen, independently of exposing to LP or MP UV radiation. After preparing the pathogen sample from the pathogen stock and correcting its UVT value, the necessary volume for quantification was collected. Then 20 mL were taken from the sample and placed on a 55x14.2 mm sterile petri dish along with a 12x4.5 mm stir bar to homogenize the sample during the exposure time (Figure 7A). The petri dish was then placed in a larger petri dish filled with ice (preventing the culture from warming up during the UV radiation) on top of a magnetic stirrer inside the CBA. The CBA door was closed to contain the UV radiation inside. After the exposure time (corresponding to the UV dose tested), the CBA shutter closed and microorganisms in the sample were quantified.

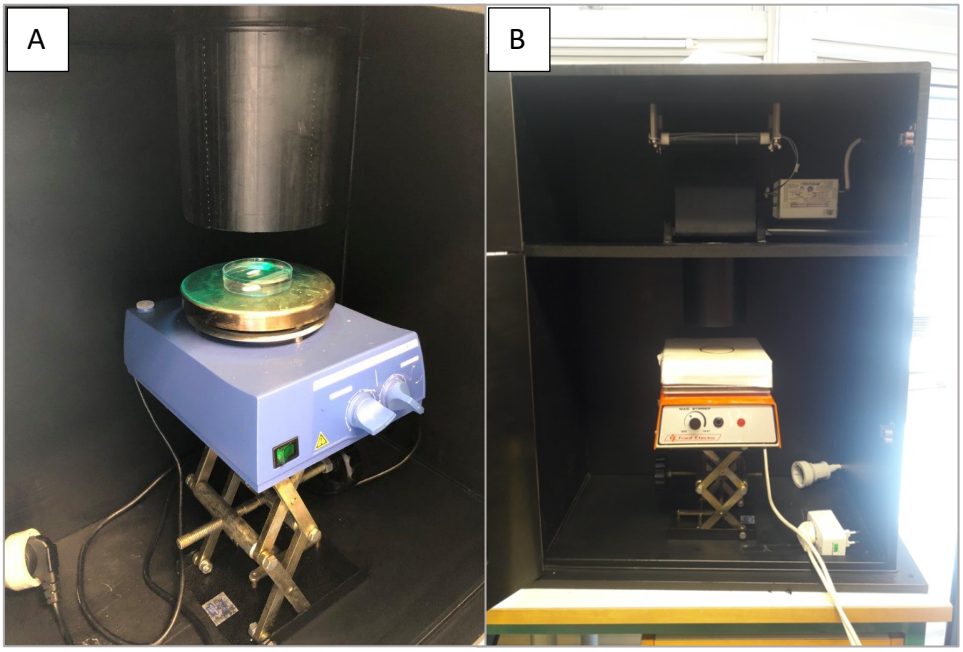

Figure 6 – Water sample containing IPNV being exposed to UV radiation emitted by a collimated beam apparatus equipped with a low pressure UV lamp (A and B; Photo A taken by Vasco Mota, Nofima AS).

A new 55 mm petri dish was used with new 20 mL of sample for each UV dose and each replicate. All samples were kept on ice during the preparations, inside the CBA's and after the tests while waiting for the quantification process.

Tested UV doses are presented in Tables 1 and 2 for each pathogen and UV technology. These were chosen prior to the UV tests based on data from the literature.

Table 1 – UV doses (mJ/cm^2) in low pressure UV for IPNV, *M. viscosa*, *Y. ruckeri* and *E. coli*.

Low Pressure UV doses (mJ/cm^2)											
Pathogen	Run	Dose 0		Dose 1		Dose 2		Dose 3		Dose 4	
		A	B	A	B	A	B	A	B	A	B
IPNV	1	0	0	50.0	50.0	100.0	100.0	150.0	150.0	200.0	200.0
	2	0	0	50.0	50.0	100.0	100.0	150.0	150.0	200.0	200.0
	3	0	-	50.0	-	100.0	-	150.0	-	200.0	-
M. viscosa	1	0	0	25.0	25.0	40.0	40.0	55.0	55.0	75.0	75.0
	2	0	0	15.0	15.0	25.0	25.0	40.0	40.0	55.0	55.0
	3	0	0	15.0	15.0	25.0	25.0	40.0	40.0	55.0	55.0
Y. ruckeri	1	0	0	10.0	10.0	20.0	20.0	30.0	30.0	40.0	40.0
	2	0	0	3.0	3.0	5.0	5.0	10.0	10.0	20.0	20.0
	3	0	0	3.0	3.0	5.0	5.0	10.0	10.0	20.0	20.0
E. coli	1	0	0	1.0	1.0	3.0	3.0	6.0	6.0	10.0	10.0
	2	0	0	3.0	3.0	6.0	6.0	10.0	10	20	20

Table 2 – UV doses (mJ/cm^2) in medium pressure UV for IPNV, *M. viscosa*, *Y. ruckeri* and *E. coli*.

Medium Pressure UV doses (mJ/cm^2)											
Pathogen	Run	Dose 0		Dose 1		Dose 2		Dose 3		Dose 4	
		A	B	A	B	A	B	A	B	A	B
IPNV	1	0	0	20.4	20.4	34.0	34.0	45.3	45.3	56.6	56.6
	2	0	0	20.2	20.2	33.7	33,.7	44.9	44.9	56.2	56.2
M. viscosa	1	0	0	11.4	11.4	22.7	22.7	45.5	45.5	68.2	68.2
	2	0	0	4.6	4.6	11.5	11.5	22.9	22.9	45.8	45.8
	3	0	0	4.6	4.6	11.4	11.4	22.8	22.8	45.6	45.6
Y. ruckeri	1	0	0	4.4	4.4	11.1	11.1	22.2	22.2	44.3	44.3
	2	0	0	2.3	2.3	4.6	4.6	11.5	11.5	23.0	23.0
	3	0	0	2.3	2.3	4.6	4.6	11.5	11.5	23.0	23.0
E. coli	1	0	0	1.0	1.0	3.0	3.0	5.0	5.0	7.0	7.0
	2	0	0	2.3	2.3	4.6	4.6	6.9	6.9	9.2	9.2

2.5 Pathogens quantification

2.5.1 IPNV quantification

IPNV concentration in artificial seawater was determined by end point titration on 96-well plates with Chinook salmon embryo cells (CHSE cells; host cells to be infected with the virus). It required culturing CHSE cells a few weeks ahead the UV tests (methods described in Annex B) to have viable cells to prepare the 96-Well plates (methods described in Annex B) two days before IPNV quantification (incubation period). When quantifying, cells in each well were inoculated with the virus at different concentrations (10-fold dilutions) from row A to G. The inoculated plates were then incubated at 20 °C with 5 % CO_2 for 7 days. After this period each plate was examined on a microscope and visible cytopathic effect in a well determined that the virus was present (Figure 8).

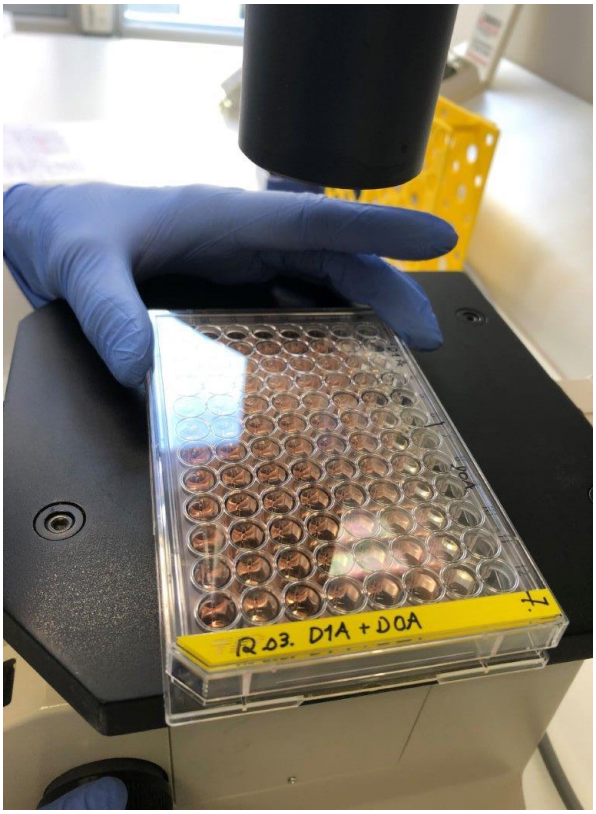

Figure 7 – Observation at a 96-wells plate via microscope to detect cytopathic effect on the CHSE cells. Photo taken by Vasco Mota, Nofima AS.

2.5.2 *Moritella viscosa* and *Yersinia ruckeri*

M. viscosa and *Y. ruckeri* were quantified separately, before and after UV exposure on marine agar 3% NaCl plates (Annex C), following several 10-fold dilutions (Figure 9). The plates were then incubated for a week at 12 °C to ensure that all bacteria had grown for plate reading.

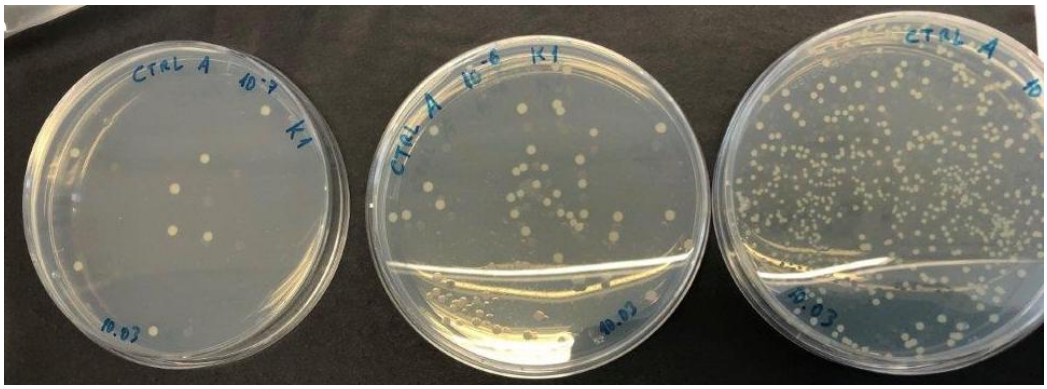

Figure 8 – Colonies of *Moritella viscosa* on marine agar 3% NaCl plates. Photo taken by Vasco Mota, Nofima AS.

2.5.3 *Escherichia coli*

The concentration of *E. coli* was determined by plating several 10-fold dilutions on plate count agar (PCA) 3% NaCl plates (Annex A). The plates were then incubated for a week at 12 °C to ensure that all bacteria had grown for plate reading.

2.6 Calculations

2.6.1 UV doses and exposure time: low pressure UV

Equation 1 allows to calculate the UV dose. However, the UV doses were chosen based on previous studies (Liltved et al., 2006; Liltved & Landfald, 1996; Liltved et al., 1995; Øye & Rimstad, 2001). Therefore Equation 1 was rewritten as equation 2 to obtain the respective exposure times (t).

$$\text{Equation 1:} \quad D = E_I P_f (1 - R) \frac{L\,(1 - 10^{-ad})}{(d - L)\,ad \ln 10)} t$$

$$\text{Equation 2:} \quad t = \frac{D}{E_I P_f (1 - R) \dfrac{L\,(1 - 10^{-ad})}{(d - L)\,ad \ln 10)}}$$

D = UV dose (mJ/cm^2)

E_I = Average UV irradiation (mW/ cm^2)

P_f = Petri Factor (unitless)

R = Reflectance at the air-water interface at 254 nm (unitless)

L = Distance from the lamp centerline to the surface of the petri dish (cm)

d = Depth of the petri dish (cm)

a = UV absorption coefficient of the testing solution containing the pathogen at 254 nm (1/cm)

t = Exposure time (s)

2.6.2 UV doses and exposure time: medium pressure UV

As mentioned before, MP UV emits a wide range of wavelengths between 200 and 280 nm. Therefore, Equation 3 takes that interval into account and was used to obtain the exposure times corresponding to the UV doses to be tested.

$$\text{Equation 3:} \quad t = \frac{D}{\sum_{i=200}^{280} E_{I_i} P_f (1 - R) \dfrac{L\,(1 - 10^{-a_i d})}{(d - L)\,a_i d \ln 10)}}$$

2.6.3 Processing CBA results of bacteria

When reading plates, only those containing 20 to 200 colonies of bacteria were considered. These values were then converted to bacterial concentration, measured in Colony-forming unit per milliliter (CFU/mL). A logarithmic transformation ($\log_{10}(x)$) of the bacterial concentration values allowed to calculate the log inactivation resulting of each UV dose.

2.6.4 Processing CBA results of viruses

The viral concentration of IPNV was assessed by end point titration on 96-well plates. Results were expressed in fifty-percent tissue culture infective dose ($TCID_{50}$/mL) after applying the Reed-Muench method (Malayeri et al., 2016). This measure of virus titer quantifies the amount of virus that produce a cytopathic effect to 50% of the host cells. $TCID_{50}$/mL values were then transformed to logarithmic values using the $\log_{10}(x)$ function in order to calculate the log inactivation specific to each UV dose.

2.7 Statistics

All statistical analyses were performed using SPSS Statistics (version 16.0, IBM, USA). Data from groups that did not achieve normality was transformed using the square root function. Independent-samples T tests were performed to assess the reaction of each pathogen to UV radiation emitted between the LP and the MP UV lamp. When normality was not achieved a Mann-Whitney U test was performed. Differences of UV doses among pathogens for LP or MP UV were analyzed by a One-Way ANOVA, followed by a Tukey test. When data normality was not achieved, a Kruskal-Wallis H test was done and several Mann-Whitney U tests were performed to find differences between pathogens inactivation. A significance level of $\alpha=0.05$ was used for all tests. All figures and tables shown in the results section present means and respective standard deviations.

3. Results

3.1 Pathogens response to low pressure and medium pressure UV

3.1.1 *Escherichia coli* (control microorganism)

A statistical significant difference was found in LP and MP UV doses that achieved 2.5-3.5 log inactivation of *E. coli* ($t(5) = 6.17$, $p = 0.002$). A 2.5-3.5 log inactivation of *E. coli* resulted from 6-8.1 mJ/cm^2 under a MP UV lamp, meanwhile 3.5 log inactivation was obtained by 20 mJ/cm^2 under a LP UV lamp (Figure 10).

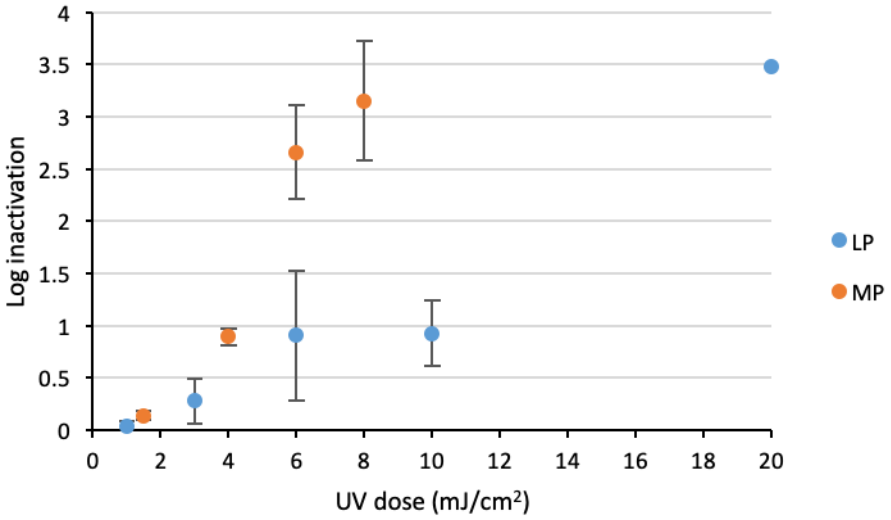

Figure 9 – Log inactivation of *Escherichia coli* resulting of different UV doses, under low pressure and medium pressure UV

An UV dose of 6 mJ/cm^2 achieved two different outcomes depending on the UV technology used. A 0.91 ±0.62 log inactivation was reached by the LP UV lamp (Table 3), while the MP UV lamp resulted in a 2.66 ± 0.45 log inactivation (Table 4).

Table 3 – *Escherichia coli* response to UV doses emitted by the LP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Low pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	1.53E+06	1.73E+06		
1.0	4	8.95E+05	8.54E+04	0.04	0.04
3.0	13	6.89E+05	1.33E+05	0.28	0.21
6.0	26	2.60E+05	1.64E+05	0.91	0.62
10.0	43	1.30E+05	7.16E+04	0.93	0.31
20.0	85	5.73E+02	2.52E+01	3.47	0.00

Table 4 – *Escherichia coli* response to UV doses emitted by the MP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Medium pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	9.51E+05	1.19E+05		
1.65	1	6.89E+05	5.03E+04	0.14	0.05
3.80	2	1.23E+05	2.23E+04	0.89	0.07
6.00	3	3.44E+03	4.06E+03	2.66	0.45
8.10	4	1.49E+03	1.83E+03	3.15	0.57

Table 3 and 4 point that an exposure of 4 seconds under the MP UV and 85 seconds under the LP UV were sufficient to reduce 99.9% of *E. coli*.

3.1.2 IPNV

A difference was found in LP and MP UV doses that led to a 2.5-3.5 log inactivation of IPNV ($t(6) = 5.348$, $p = 0.002$; Figure 11). MP UV required a lower dose than LP UV to achieve the same log inactivation value. For instance, a 2.1-3.0 log

reduction (99.9 %) of IPNV was achieved using 200 mJ/cm^2 in LP UV and 1.92-3.0 log inactivation resulted from 56.4 mJ/cm^2 in MP UV

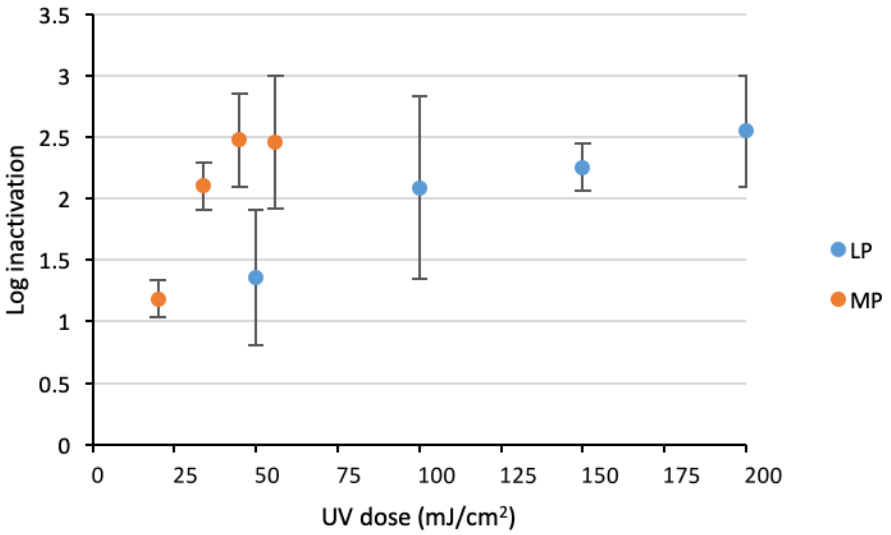

Figure 10 – Log inactivation of IPNV resulting of different UV doses, under low pressure and medium pressure UV.

The obtained results show that an exposure time of 855 seconds under a LP UV lamp and 25 seconds under a MP UV lamp (Table 5 and 6) allowed to obtain a 99.9 % reduction of IPNV.

Table 5 – IPNV response to UV doses emitted by the LP lamp trough the evolution of the respective concentrations ($TCID_{50}$/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Low pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	$TCID_{50}$/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	3.39E+05	1.86E+05		
50	214	2.09E+04	2.06E+04	1.35	0.55
100	428	4.91E+03	4.63E+03	2.09	0.74
150	641	1.76E+03	7.39E+02	2.25	0.19
200	855	1.21E+03	1.15E+03	2.55	0.45

Table 6 – IPNV response to UV doses emitted by the MP lamp trough the evolution of the respective concentrations ($TCID_{50}$/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Medium pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	$TCID_{50}$/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	4.88E+05	2.87E+05		
20.3	9	3.03E+04	1.36E+04	1.18	0.15
33.85	15	3.70E+03	1.54E+03	2.10	0.19
45.1	20	1.72E+03	9.61E+02	2.48	0.38
56.4	25	2.06E+03	1.21E+03	2.46	0.54

3.1.3 *Moritella viscosa*

UV doses that led to 2.5-3.5 log inactivation of *M. viscosa* are significantly different among LP and MP UV ($t187$) = 3.245, p = 0.004). The exponential phase in the inactivation of *M. viscosa* was not recorded since the UV doses tested were too high (Figure 12).

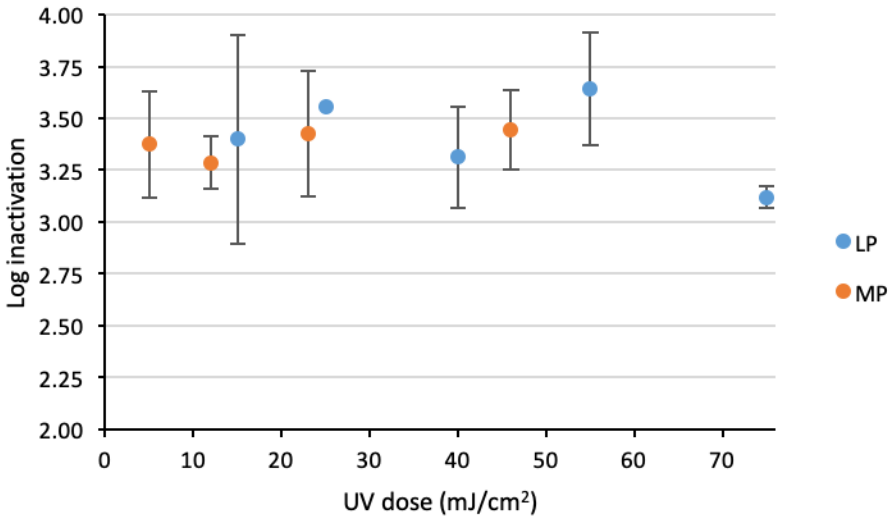

Figure 11 – Log inactivation of *Moritella viscosa* resulting of different UV doses, under low pressure and medium pressure UV.

Several doses reached the same inactivation level of *M. viscosa*. Therefore, an exposure time of 65 seconds under a LP UV (Table 7) and 2 seconds under a MP UV (Table 8) was enough to achieve 2.5 to 3.5 log inactivation.

Table 7 – *Moritella viscosa* response to UV doses emitted by the LP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Low pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	1.57E+06	3.34E+05		
15	65	6.58E+02	6.26E+02	3.40	0.50
25	107	3.40E+02		3.55	
40	172	8.65E+02	5.18E+02	3.31	0.24
55	236	3.40E+02	1.86E+02	3.64	0.27
75	322	1.33E+03	1.59E+02	3.12	0.05

Table 8 – *Moritella viscosa* response to UV doses emitted by the MP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Medium pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	1.46E+06	4.26E+05		
4.60	2	5.47E+02	1.95E+02	3.37	0.25
11.45	5	7.34E+02	1.84E+02	3.28	0.13
22.80	10	5.52E+02	2.44E+02	3.43	0.30
45.46	20	5.48E+02	3.43E+02	3.44	0.19

3.1.4 *Yersinia ruckeri*

No significant differences were found between LP UV doses and MP UV doses that enabled 2.5-3.5 log inactivation of *Y. ruckeri* ($U = 38$, $p = 0.586$). Both LP and MP

seem to have a similar effect on the inactivation of this pathogen (Figure 13).

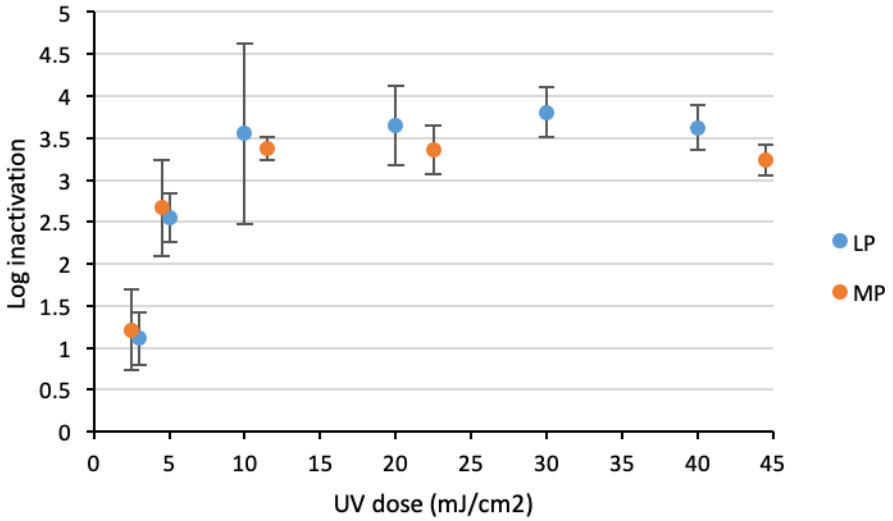

Figure 12 – Log inactivation of *Yersinia ruckeri* resulting of different UV doses, under low pressure and medium pressure UV.

This pathogen was inactivated by 99.9 % after being under the LP UV for 43 seconds (10 mJ/cm^2) and under the MP UV for 5 seconds (11.3 mJ/cm^2; Table 9 and 10).

Table 9 – *Yersinia ruckeri* response to UV doses emitted by the LP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Low pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	2.99E+06	1.54E+06		
3	65	1.60E+05	3.06E+04	1.11	0.31
5	107	8.45E+03	5.62E+03	2.55	0.28
10	43	8.83E+02	9.51E+02	3.55	1.08
20	86	5.83E+02	2.70E+02	3.65	0.48
30	128	5.73E+02	3.64E+02	3.80	0.30
40	171	8.48E+02	4.99E+02	3.62	0.27

Table 10 – *Yersinia ruckeri* response to UV doses emitted by the MP lamp trough the evolution of the respective concentrations (CFU/mL mean and standard deviation) and log inactivation values (mean and standard deviation)

Medium pressure UV					
UV dose (mJ/cm^2)	Exposure time (s)	CFU/mL		Log inactivation	
		Mean	STD	Mean	STD
0	0	2.83E+06	1.24E+06		
2.3	1	3.20E+05	2.48E+05	1.21	0.48
4.5	2	7.91E+03	7.52E+03	2.67	0.57
11.3	5	1.15E+03	4.14E+02	3.37	0.13
22.6	10	1.38E+03	8.10E+02	3.36	0.29
44.3	20	9.05E+02	3.68E+02	3.24	0.18

3.2 Pathogen's reaction to low pressure UV

An analysis of variances (ANOVA) showed that there was a significant difference among doses in LP UV that resulted in 2.5-3.5 log inactivation of IPNV, *M. viscosa* and *Y. ruckeri* ($F(2,13) = 32.029$, $p < 0.001$; Figure 14). Further analysis conducted by a post hoc Tukey test revealed that there was no statistically significant difference between doses that let to 2.5-3.5 log inactivation of *M. viscosa* and *Y. ruckeri* ($p = 0.062$). However IPNV needed different doses from *M. viscosa* and *Y. ruckeri* to reach the same range of inactivation (respectively $p < 0.001$ and $p < 0.001$). A much higher dose was needed to inactivate 99.9% (3 log) of IPNV (200 mJ/cm^2) when compared with the dose necessary to achieve the same inactivation rate of *M. viscosa* and *Y. ruckeri* (10-15 mJ/cm^2).

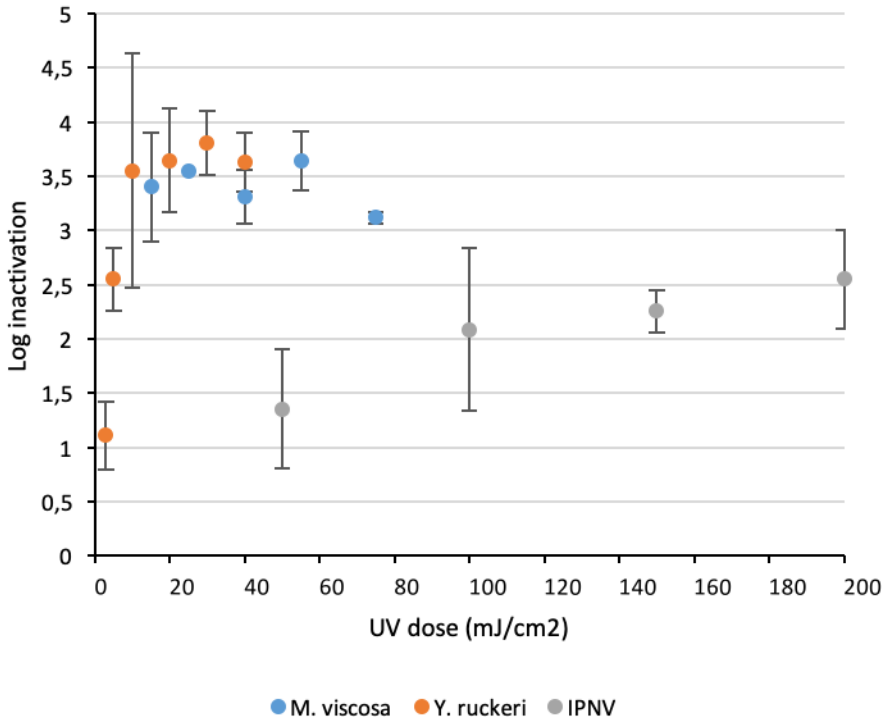

Figure 13 – IPNV, *Moritella viscosa* and *Yersinia ruckeri* reduction resulting from LP UV exposure

3.3 Pathogen's reaction to medium pressure UV

A Kruskal-Wallis H test showed that there was a statistically significant difference among doses in MP UV that resulted in 2.5-3.5 log inactivation of IPNV, *M. viscosa* and *Y. ruckeri* ($\chi^2(2) = 8.370$, $p = 0.015$; Figure 15). *M. viscosa* and *Y. ruckeri* surpassed 99.9% inactivation with no significant difference among MP UV doses ($U = 73$, $p = 0.579$). However a significant difference was found between MP UV doses resulting in 2.5-3.5 log inactivation of IPNV and *M. viscosa* ($U = 46$, $p = 0.023$), but also between IPNV and *Y. ruckeri* ($U = 48$, $p = 0.001$). As in LP, IPNV also required higher doses under MP UV when compared to the other pathogens tested to achieve the same inactivation level.

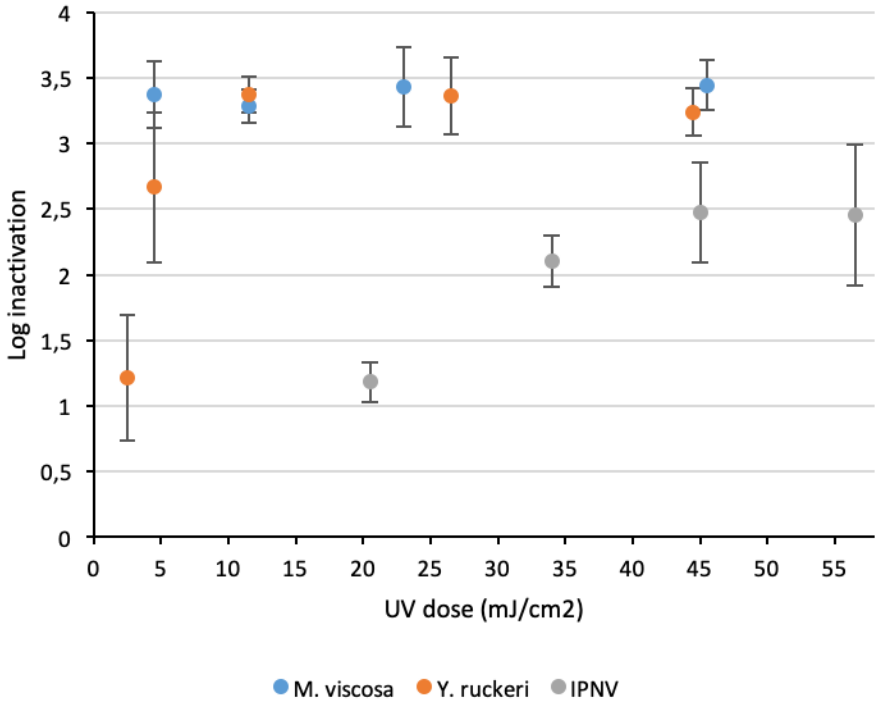

Figure 14 – IPNV, *Moritella viscosa* and *Yersinia ruckeri* reduction resulting from MP UV exposure

4. Discussion

There are many works published about the use of UV after ozonation to reduce and inactivate pathogens from aquaculture effluents in recirculated systems. However, very few focuses specifically on the use of UV radiation to inactivate the pathogens tested in this study.

Depending on the pathogen, large variations in UV doses for 3 log inactivation were found between the present work and previous similar studies, but also among other studies (Lichi et al., 2018; Liltved et al., 2006). The variations can be explained by differences in the experimental design, in the procedures during UV exposure and pathogens handling (Liltved et al., 2006). Factors such as storage temperature of the pathogens stock, quantification methods and respective host cells, water quality, salinity, temperature during the trials (before, during and after UV exposure), aggregation to particles or surfaces (before, during and after UV exposure) and different subspecies of IPNV are part of the reason for such variations (Bolton et al., 2015; Bolton & Linden, 2003). Furthermore, the collimated beam apparatus must be calibrated correctly before the trials and several parameters must be measured to be incorporated in the UV doses calculations that influence directly the end results. Besides, the standardization methods to determine the (UV Dose) in Bench-Scale UV Experiments was only published in 2003 by Bolton and Linden, later edited in 2015 (Bolton et al., 2015). Therefore, calculations in studies published prior that year may differ and promote differences in results among authors.

Control microorganism

E. coli CCUG 3274 has not been exposed to UV radiation in other studies but many other strains have (Malayeri et al., 2016). Nevertheless, its response to LP UV resembles to results obtained in similar studies. For instance, Wu et al. (2005), Quek & Hu (2008) and Chatterly & Linden (2010) obtained a 3 log inactivation of different *E. coli* strains (ATCC 11303, ATCC 700891 and ATCC 29425) using a LP UV lamp emitting doses between 9 and 20 mJ/cm^2. Besides that, the UV dose that led to 99.9 % inactivation of *E. coli* CCUG 3274 under MP UV is similar to doses used by Quek & Hu (2008) and Guo

et al. (2009), who obtained the same inactivation percentage of *E. coli* (ATCC 15597, ATCC 700891, NBIMB 9481, CGMCC 1.3373) with doses between 7.3 and 9.6 mJ/cm^2. The fact that the UV doses for 99.9 % inactivation of *E. coli* CCUG 3274 under MP and LP lamps fit the results obtained by other authors suggests that the methodology performed was correct and valid for the other pathogens.

Bacteria and virus response to LP and MP UV

Infectious pancreatic necrosis virus is one of the most studied viruses associated with salmon farming. The few studies focusing on IPNV resistance to UV radiation were conducted with similar methodologies. The results obtained in this work involving the use of LP UV for IPNV inactivation are in line with the findings of Liltved et al. (2006), who obtained a 3 log inactivation of IPNV in filtered seawater by applying an UV dose of 246 mJ/cm^2 under a LP UV lamp, and a 2 log inactivation (99 %) by applying an UV dose of 165 mJ/cm^2. Besides that, Liltved et al. (1995) obtained different results in a previous work using brackish water, where 122 mJ/cm^2 were necessary to get a 3 log inactivation of the same virus. Øye and Rimstad (2001) also tested IPNV resistance using a LP UV, however in freshwater and determined 118.8 mJ/cm^2 as the necessary dose to obtain 99.9 % inactivation.

The first approach to the effect of medium pressure UV on IPNV was performed by Atlantium Technologies Ltd researchers (Lichi et al., 2018). In their study, a 3 log inactivation of this pathogen was achieved after exposure to a dose of 80 mJ/cm^2. However in this work, the same inactivation rate was reached only once by the MP UV with a lower dose (56.4 mJ/cm^2), even though the respective mean of log inactivation being only 2.46. Nonetheless, IPNV inactivation was more efficient by MP UV. Lichi et al. (2018) verified MP UV lamps being 2.2-8.6 times more efficient than LP UV lamps when inactivating this virus, even though being less energy efficient.

The exterior of IPNV is composed by an icosahedral shape capsid made from a single capsid protein that covers two linear double-stranded RNA segments (dsRNA), one between 3.1 to 3.6 kbp and the other 2.8 to 3.3 kbp, that encode viral and structural proteins depending on the IPNV strain (Delmas et al., 2019; Mutoloki & Evensen, 2011).

The fact that high UV doses in LP UV were necessary to inactivate 99.9 % of IPNV suggests that its dsRNA was protected from ultra-violet photons by the capsid protein that does not absorb wavelength near 254 nm. However, MP UV wavelengths are absorbed by proteins and enzymes and were able to damage the capsid, thus disrupting the dsRNA kept inside with a lower UV dose than the one emitted by the LP UV. This way, the repair enzymes of IPNV were also affected and RNA repair was avoided. Nonetheless, viruses can use the host cell enzymes (from the cells used for quantification of viable viruses) after UV exposure to repair their damaged nucleic acids (Gerba et al., 2002; Liltved et al., 2006).

The presence of functional groups, amino acids and the sequence and composition of nucleotides also determine the susceptibility of changes in the DNA/RNA (pyrimidine dimers; Mayer et al., 2015). According to Wigginton & Kohn (2012) the modifications in capsid proteins derived from UV radiation make it impossible for the virus to the bind to the host cell and cause direct damage to the nucleic acids.

The nucleotide composition and genome size of bacteria is a determining factor to its susceptibility to absorb UV photons and being modified with UV photoproducts (Nocker et al., 2018; Pullerits et al., 2020). For instance, *Y. ruckeri* has a DNA sequence of 3.7 Mbp and 47 % of G-C bonds (Kumar et al., 2015) while *M. viscosa* has a DNA sequence around 5 Mbp and 42.5 % G-C bonds in its DNA chain (Benediktsdóttir et al., 2000; Lunder et al., 2000).

Nonetheless their differences in DNA size and composition, the results showed that *M. viscosa* is as resistant (15 mJ/cm^2 for 3.40±0.50 log inactivation) as *Yersinia ruckeri* (10 mJ/cm^2 for 3.55±1.08 log inactivation) under LP UV radiation. In this study, *M. viscosa* was exposed for the first time to UV radiation and results demonstrated that it required higher doses for 99.9 % inactivation than other common pathogenic bacteria to Atlantic salmon and cleaner fish such as *Aeromonas salmonicida* (3.1 mJ/cm^2) and *Vibrio anguillarum* (1.5 mJ/cm^2; Hjeltnes et al., 2019; Liltved & Landfald, 1996). Regarding the use of MP UV, the resistance of *M. viscosa* (4.6 mJ/cm^2 for 3.37±0.25 log inactivation) was also similar to *Y. ruckeri* (11.3 mJ/cm^2 for 3.37±0.13 log inactivation).

Previously, (Liltved & Landfald, 1996) obtained 99.9 % inactivation of *Y. ruckeri* with a lower dose of 4.9 mJ/cm^2 emitted by a LP UV.

Y. ruckeri inactivation by LP and MP was similar, which leads to two different hypotheses. Either its repair mechanisms were ineffective after exposure to LP UV or they were not damaged by MP UV. However, further investigation would be needed to better understand which components of this bacteria are more affected during LP and MP UV radiation.

Although the reaction of both bacteria being similar after LP UV radiation, the dose necessary to achieve 99.9 % inactivation of *Y. ruckeri* corresponded to 67 % the dose responsible for the same inactivation level of *M. viscosa*. An hypothesis behind this result might be that *M. viscosa* repair mechanisms were more active than those of *Y. ruckeri* when reverting the UV photoproducts in the modified DNA. On the other hand, the fact that *M. viscosa* required a dose corresponding to 41 % the dose that achieved 3 log inactivation of *Y. ruckeri* under MP UV might be explained by their different DNA size and composition. *M. viscosa* DNA is more prone to being modified with T-T pairs than *Y. ruckeri* after UV exposed. Furthermore, MP UV wavelengths are absorbed by repair proteins, lipids and outer membrane proteins that further increased cell damage and avoided repair inside the cell. However, it is not possible to mention which proteins involved in cell repair mechanisms were damaged after UV exposure.

Bacteria resistance to UV radiation also depends on wall thickness and cell size. For instance, *Y. ruckeri* is more susceptible of being hit with UV photons than IPNV, since being much larger in size (0.75 µm in diameter and 1-3 µm in length; (Kumar et al., 2015; Mayer et al., 2015)). Besides damaging proteins, UVC disinfection also affects lipids membranes that contain lipopolysaccharides and constitute part of the bacterial outer membrane (Santos, Moreirinha, et al., 2013). According to Abdallah et al. (2012) the outer membrane proteins play an important role in the host infection, during cellular and physiological mechanisms and in adaptation to extreme conditions. The synthesis of these proteins can be enhanced by an environmental stress such as UV radiation. First, the production of non-essential proteins and cell division is interrupted to concentrate efforts in synthetizing stress proteins that will play a major role in cell repair

(Abdallah et al., 2012). The formation of these proteins was not assessed in *Y. ruckeri* and *M. viscosa* but could provide useful knowledge about how repair mechanisms are responsible for the survival of these microorganisms after disinfection with LP UV.

The Norwegian Department of fisheries (Nærings- og fiskeridepartementet) is responsible for regulating aquaculture activities in Norway. Regulation 1997-02-20 no. 192 sets 25 mJ/cm^2 as the minimum UV dose for disinfection of influent and effluent waters in land-based facilities. In ideal conditions of UV disinfection (high UVT value) it guarantees a 3.43-3.55 and 3.36-3.65 log inactivation of *M. viscosa* and *Y. ruckeri* respectively, depending on the type of UV installed (LP or MP). Many other pathogenic bacteria (not tested in this study) are also inactivated from 99.9 % to 99.99% (Malayeri et al., 2016). However, this UV dose is less efficient against IPNV since it does not reach 99 % inactivation in LP and MP only achieves 99 % inactivation (1.18 log inactivation at 20.3 mJ/cm^2). Besides that, running an UV filter that generates doses high as 250 mJ/cm^2 in such ideal conditions over 24/24 h is costly, but the cost becomes greater in land-based facilities where higher UV doses are required, predicting fouling around the UV lamps and shading effect by small particles that escape the filtration processes and reduce the UVT value (Aquafine, 2017).

As mentioned previously, pathogens genomes have been studied over the last decades and it is known that proteins and nucleic acids are damaged by UV radiation (Wigginton & Kohn, 2012). However, there is a lack of knowledge concerning exactly which proteins and structures of IPNV, *M. viscosa*, *Y. ruckeri* and other Atlantic salmon pathogenic microorganisms are the most affected and which are important to repair mechanisms. Similar studies used fluorescent dyes in flow cytometry to assess the effect of UV disinfection on cell physiology and properties such as membrane integrity, membrane potential, enzyme activity, metabolic performance, DNA damage, among others (Díaz et al., 2010; Kim et al., 2017; Schenk et al., 2011). This approach could be applied to understand which elements of Atlantic salmon pathogens are the most affected by UV disinfection and provide more advanced knowledge and accurate UV doses to fish farms.

5. Conclusions

Bacteria species (*M. viscosa* and *Y. ruckeri*) demonstrated being less resistant to LP and MP UV than virus species (IPNV). Inactivation of 99.9 % for IPNV, *M. viscosa* and *Y. ruckeri* required 200, 15 and 10 mJ/cm^2 respectively, using the LP UV. The fact that IPNV required higher doses (regardless of the UV technology) than bacteria species to reach the same inactivation level used resulted from their different external structures (capsid proteins in viruses and outer membrane proteins in bacteria), nucleic acids repair mechanisms, size and genome composition (such as molecular weight and G-C content).

The main advantage of MP over LP UV is its ability to emit wavelengths that are absorbed by repair proteins and enzymes. This absorption modifies their structure, thus avoiding pathogens reactivation and limiting host infection. IPNV and *M. viscosa* were 99.9 % inactivated with lower doses than LP UV: 56.4 and 4.6 mJ/cm^2 respectively.

Despite inactivation differences between virus and bacteria species, different responses were also obtained among bacteria species. While *M. viscosa* reacted differently to LP and MP UV, no difference was obtained for *Y. ruckeri* after exposure to these technologies.

Suggestions for future studies:

Future works involving Atlantic salmon pathogenic bacteria and virus's resistance to UV could resort to flow cytometry (as performed in similar studies) to identify which inner and outer mechanisms are responsible for the studied microorganism's inactivation and reactivation. This technology could help determine which *Y. ruckeri* mechanisms were responsible for the same inactivation pattern obtained after exposure to LP and MP UV and help understanding why MP UV did not affect this pathogen.

In an upcoming study, microorganisms with similar structures and genomes could be exposed to LP and MP UV to determine possible similar inactivation patterns

that could help predict the response of other similar pathogens to UV, whose UV dose for inactivation is yet to be determined.

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References

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K., & Horsberg, T. E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology*, *31*(2), 72–81.

Abdallah, F., Lagha, R., Ellafi, A., Namane, A., Rousselle, J.-C., Lenormand, P., & Kallel, H. (2012). Identification of outer membrane proteins altered in response to UVC-radiation in *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. *Indian Journal of Microbiology*, *52*(4), 660–665.

Aquafine. (2017). *UV lamp technologies*. Retrived from https://www.aquafineuv.com/cms-portals/aqua_com/cms/static/resources/AquafineUVLampTechnologies.pdf

Astuti, M. P., & Rongjing, X. (2016). Application of ultraviolet (UV) technology on water dechlorination: Influence of UV fluence dose and UV transmittance on chlorine removal efficiency. *Journal of Environmental Engineering and Waste Management*, *1*(1), 55–63.

Beck, S. E., Wright, H. B., Hargy, T. M., Larason, T. C., & Linden, K. G. (2015). Action spectra for validation of pathogen disinfection in medium-pressure ultraviolet (UV) systems. *Water Research*, *70*, 27–37.

Behringer, D. C., Wood, C. L., Krkošek, M., & Bushek, D. (2020). Disease in fisheries and aquaculture. *Marine Disease Ecology*, 183.

Benediktsdóttir, E., Verdonck, L., Spröer, C., Helgason, S., & Swings, J. (2000). Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographical locations: A proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *50*(2), 479–488.

Bolton, J. R. (2005). Fundamentals of ultraviolet treatment: Terms and definitions do matter! *Water Conditioning and Purification International*, 24.

Bolton, J. R., Beck, S. E., & Linden, K. G. (2015). Protocol for the determination of fluence (UV dose) using a low-pressure or low-pressure high-output UV lamp in bench-scale collimated beam ultraviolet experiments. *IUVA News*, *17*(1), 11–16.

Bolton, J. R., & Linden, K. G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering*, *129*(3), 209–215.

Bolton, J. R., Stefan, M. I., Cushing, R. S., & Mackey, E. (2001). The importance of water absorbance/transmittance on the efficiency of ultraviolet disinfection reactors. *Proceedings of the First International Congress on Ultraviolet Technologies, Washington, DC, June*.

Bregnballe, J. (2015). *A guide to recirculation aquaculture. Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation*.

Bruno, D. W., Noguera, P. A., & Poppe, T. T. (2013). *A colour atlas of salmonid diseases* (Vol. 91). Springer Science & Business Media.

Cerbule, K., & Godfroid, J. (2020). Salmon louse (*Lepeophtheirus salmonis* (Krøyer)) control methods and efficacy in atlantic salmon (*Salmo salar* (Linnaeus)) aquaculture: A literature review. *Fishes*, *5*(2), 11.

Chatterley, C., & Linden, K. (2010). Demonstration and evaluation of germicidal UV-LEDs for point-of-use water disinfection. *Journal of Water and Health*, *8*(3), 479–486.

Coohill, T. P., & Sagripanti, J. (2008). Overview of the inactivation by 254 nm ultraviolet radiation of bacteria with particular relevance to biodefense. *Photochemistry and Photobiology*, *84*(5), 1084–1090.

Delmas, B., Attoui, H., Ghosh, S., Malik, Y. S., Mundt, E., & Vakharia, V. N. (2019). ICTV virus taxonomy profile: Birnaviridae. *Journal of General Virology*, *100*(1), 5–6.

Díaz, M., Herrero, M., García, L. A., & Quirós, C. (2010). Application of flow cytometry to industrial microbial bioprocesses. *Biochemical Engineering Journal*, *48*(3), 385–407.

Ernst & Young AS. (2019). *The Norwegian Aquaculture Analysis*. Retrieved from ey.com/no

Fischer, C., Guttormsen, A. G., & Smith, M. D. (2017). Disease risk and market structure in salmon aquaculture. *Water Economics and Policy*, *3*(02).

Fischer, J., Rudkowski, J. B., Riepe, D., & Sief, R. (2006). *Amalgam low pressure mercury UV lamp*. Google Patents.

Gerba, C. P. (2015). Disinfection. *Environmental Microbiology*, 645–662.

Gerba, C. P., Gramos, D. M., & Nwachuku, N. (2002). Comparative inactivation of enteroviruses and adenovirus 2 by UV light. *Applied and Environmental Microbiology*, *68*(10), 5167–5169.

Goodsell, D. S. (2001). The molecular perspective: ultraviolet light and pyrimidine dimers. *Stem Cells*, *19*(4), 348–349.

Goosen, N., & Moolenaar, G. F. (2008). Repair of UV damage in bacteria. *DNA Repair*, *7*(3), 353–379.

Guo, M., Hu, H., Bolton, J. R., & El-Din, M. G. (2009). Comparison of low-and medium-pressure ultraviolet lamps: Photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research*, *43*(3), 815–821.

Helgesen, K. O., Romstad, H., Aaen, S. M., & Horsberg, T. E. (2015). First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. *Aquaculture Reports*, *1*, 37–42.

Hjeltnes, B.; Bang-Jensen, B.; Bornø, G.; Haukaas, A.; Walde, C. (2019). Fish health report 2018. *Norwegian Veterinary Institute: Norwegian*.

Hjerde, E., Karlsen, C., Sørum, H., Parkhill, J., Willassen, N. P., & Thomson, N. R. (2015). Co-cultivation and transcriptome sequencing of two co-existing fish pathogens *Moritella viscosa* and *Aliivibrio wodanis*. *BMC Genomics*, *16*(1), 447.

Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Foss, A., Vikingstad, E., & Elvegård, T. A. (2014). The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *424*, 18–23.

Johannesen, Á., Patursson, Ø., Kristmundsson, J., Dam, S. P., & Klebert, P. (2020). How caged salmon respond to waves depends on time of day and currents. *PeerJ*, *8*.

Johansen, L.-H., Jensen, I., Mikkelsen, H., Bjørn, P.-A., Jansen, P. A., & Bergh, Ø. (2011). Disease interaction and pathogens exchange between wild and farmed

fish populations with special reference to Norway. *Aquaculture*, *315*(3–4), 167–186.

Kalisvaart, B. F. (2004). Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Science and Technology*, *50*(6), 337–344.

Kim, D.-K., Kim, S.-J., & Kang, D.-H. (2017). Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts. *Food Research International*, *97*, 280–287.

Krkošek, M. (2017). Population biology of infectious diseases shared by wild and farmed fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(4), 620–628.

Kumar, G., Menanteau-Ledouble, S., Saleh, M., & El-Matbouli, M. (2015). *Yersinia ruckeri*, the causative agent of enteric redmouth disease in fish. *Veterinary Research*, *46*(1), 103.

Lichi, T., Kertser, M., & Rozenberg, Y. (2018). *System and method for inactivation of infectious pancreatic necrosis virus (IPNV) using medium pressure ultraviolet (UV) light* (Patent No. US10029926B2). Google Patents.

Liltved, H, & Landfald, B. (1996). Influence of liquid holding recovery and photoreactivation on survival of ultraviolet-irradiated fish pathogenic bacteria. *Water Research*, *30*(5), 1109–1114.

Liltved, H, Vogelsang, C., Modahl, I., & Dannevig, B. H. (2006). High resistance of fish pathogenic viruses to UV irradiation and ozonated seawater. *Aquacultural Engineering*, *34*(2), 72–82.

Liltved, Helge, Hektoen, H., & Efraimsen, H. (1995). Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. *Aquacultural Engineering*, *14*(2), 107–122.

Lunder, T., Sørum, H., Holstad, G., Steigerwalt, A. G., Mowinckel, P., & Brenner, D. J. (2000). Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with "winter ulcer." *International Journal of Systematic and Evolutionary Microbiology*, *50*(2), 427–450.

Malayeri, A. H., Mohseni, M., Cairns, B., Bolton, J. R., Chevrefils, G., Caron, E., Barbeau, B., Wright, H., & Linden, K. G. (2016). Fluence (UV dose) required to achieve incremental log inactivation of bacteria, protozoa, viruses and algae. *IUVA News*, *18*(3), 4–6.

Mayer, B. K., Yang, Y., Gerrity, D. W., & Abbaszadegan, M. (2015). The impact of capsid proteins on virus removal and inactivation during water treatment processes. *Microbiology Insights*, *8*, MBI-S31441.

Mowi. (2020). *Mowi Salmon Farming Industry Handbook*. https://mowi.com/investors/resources/

Munang'andu, H. M., Mutoloki, S., & Evensen, Ø. (2016). Prevention and control of viral diseases in aquaculture. *Aquaculture Virology*, 77–93.

Mutoloki, S., & Evensen, Ø. (2011). Sequence similarities of the capsid gene of Chilean and European isolates of infectious pancreatic necrosis virus point towards a common origin. *Journal of General Virology*, *92*(7), 1721–1726.

Mutoloki, S., Jøssund, T. B., Ritchie, G., Munang'andu, H. M., & Evensen, Ø. (2016). Infectious pancreatic necrosis virus causing clinical and subclinical infections in atlantic salmon have different genetic fingerprints. *Frontiers in Microbiology*, *7*, 1393.

Nekouei, O., Vanderstichel, R., Thakur, K., Arriagada, G., Patanasatienkul, T., Whittaker, P., Milligan, B., Stewardson, L., & Revie, C. W. (2018). Association between sea lice (*Lepeophtheirus salmonis*) infestation on Atlantic salmon farms and wild Pacific salmon in Muchalat Inlet, Canada. *Scientific Reports*, *8*(1), 1–11.

Nocker, A., Shah, M., Dannenmann, B., Schulze-Osthoff, K., Wingender, J., & Probst, A. J. (2018). Assessment of UV-C-induced water disinfection by differential PCR-based quantification of bacterial DNA damage. *Journal of Microbiological Methods*, *149*, 89–95.

Nylund, A, Karlsen, C. R., Good, C., Jørgensen, S. M., Plarre, H., Isaksen, T. E., Handeland, S., Wollseth, K., & Ottem, K. F. (2015). *Review of microparasites that could represent a future problem for production of salmonids in closed or semi-closed containment systems*.

Nylund, Are, Brattespe, J., Plarre, H., Kambestad, M., & Karlsen, M. (2019). Wild and farmed salmon (*Salmo salar*) as reservoirs for infectious salmon anaemia virus, and the importance of horizontal-and vertical transmission. *PloS One*, *14*(4).

Øye, A. K., & Rimstad, E. (2001). Inactivation of infectious salmon anaemia virus, viral haemorrhagic septicaemia virus and infectious pancreatic necrosis virus in water using UVC irradiation. *Diseases of Aquatic Organisms*, *48*(1), 1–5.

Pullerits, K., Ahlinder, J., Holmer, L., Salomonsson, E., Öhrman, C., Jacobsson, K., Dryselius, R., Forsman, M., Paul, C. J., & Rådström, P. (2020). Impact of UV irradiation at full scale on bacterial communities in drinking water. *Npj Clean Water*, *3*(1), 1–10.

Quek, P. H., & Hu, J. (2008). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology & Biotechnology*, *35*(6), 533.

Rastogi, R. P., Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, *2010*.

Ravanat, J.-L., & Douki, T. (2016). UV and ionizing radiations induced DNA damage, differences and similarities. *Radiation Physics and Chemistry*, *128*, 92–102.

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology*, *27*(3), 493–497.

Rodriguez, R. A., Bounty, S., Beck, S., Chan, C., McGuire, C., & Linden, K. G. (2014). Photoreactivation of bacteriophages after UV disinfection: Role of genome structure and impacts of UV source. *Water Research*, *55*, 143–149.

Rud, I., Kolarevic, J., Holan, A. B., Berget, I., Calabrese, S., & Terjesen, B. F. (2017). Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. *Aquacultural Engineering*, *78*, 50–62.

Santos, A. L., Moreirinha, C., Lopes, D., Esteves, A. C., Henriques, I., Almeida, A., Domingues, M. R. M., Delgadillo, I., Correia, A., & Cunha, A. (2013). Effects of UV radiation on the lipids and proteins of bacteria studied by mid-infrared spectroscopy. *Environmental Science & Technology*, *47*(12), 6306–6315.

Santos, A. L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N. C. M., Almeida, A., Correia, A., & Cunha, Â. (2013). Wavelength dependence of biological damage induced by UV radiation on bacteria. *Archives of Microbiology*, *195*(1), 63–74.

Schalk, S., Adam, V., Arnold, E., Brieden, K., Voronov, A., & Witzke, H.-D. (2005). UV-lamps for disinfection and advanced oxidation-lamp types, technologies and applications. *IUVA News*, *8*(1), 32–37.

Schenk, M., Raffellini, S., Guerrero, S., Blanco, G. A., & Alzamora, S. M. (2011). Inactivation of *Escherichia coli*, *Listeria innocua* and *Saccharomyces cerevisiae* by UV-C light: study of cell injury by flow cytometry. *LWT-Food Science and Technology*, *44*(1), 191–198.

Shang, C., & Leung, C. Y. (2007). Factors influencing UV transmittance in chemically-enhanced primary treatment effluents. *World Congress on Ozone and Ultraviolet Technologies, IUVA-IOA, Los Angeles, CA, USA*.

Sommerset, I., Krossøy, B., Biering, E., & Frost, P. (2005). Vaccines for fish aquaculture. *Expert Review of Vaccines*, *4*, 89–101.

Wigginton, K. R., & Kohn, T. (2012). Virus disinfection mechanisms: the role of virus composition, structure, and function. *Current Opinion in Virology*, *2*(1), 84–89.

Wu, Y., Clevenger, T., & Deng, B. (2005). Impacts of goethite particles on UV disinfection of drinking water. *Applied and Environmental Microbiology*, *71*(7), 4140–4143.

Yáñez, J. M., Houston, R. D., & Newman, S. (2014). Genetics and genomics of disease resistance in salmonid species. *Frontiers in Genetics*, *5*, 415.

Annexes

A. *Escherichia coli* growth mediums preparation

1) Lysogeny broth 3% NaCl:

12.5 g of Lysogeny Broth (miller; L3522, Sigma-Aldrich, USA) and 10 g of sodium chloride (1.06404.1000, Emsure, USA) were diluted in 500 mL of distilled water. The solution was then autoclaved and divided in flasks. The flasks were stored at 30 °C until being inoculated with *E. coli*.

2) Plate count agar 3 % NaCl plates (PCA):

12.25 g of PCA (CM0325, Oxoid, England) and 21.0 g of sodium chloride (1.06404.1000, Emsure, USA) were diluted in 700 mL of distilled water, then autoclaved. After cooling down the liquid medium was poured in sterile 92x16 mm petri dishes and kept at 30 °C until being used.

B. Culturing CHSE cells for 96-Well plates preparation

1) First culture of Chinook salmon cells:

The frozen tube containing CHSE cells (CHSE-214, LOT 00/F/031, ECACC, England) was taken out from the liquid nitrogen container and placed in a tube with 70 % ethanol to prevent any contamination. After completely thawed, 1 mL was pipetted and placed in a small culture flask with 9 mL of MEM (Minimum Essential Medium; Ref. 21090-022, Gibco, USA). Afterwards, the cell culture flask was incubated at 20 °C with 5 % CO_2 for one week.

2) Subculturing Chinook salmon cells:

In order to multiply cells and maintain them healthy and alive over time, their nutrient medium needed to be replaced once a week. The new growth medium was composed by 10 % FBS (Fetal bovine serum, LOT:BCBQ7890V, Sigma-Aldrich, USA) and 90 % MEM supplemented with L-glutamine (Ref. 25030-024, Gibco, USA), NEAA (non-

essential amino acids; Ref. 11140-035, Gibco, USA), Hepes (Ref. 15630-056, Gibco, USA) and Pen/Strep (Penicillin-Streptomycin, P4333, Sigma-Aldrich, USA). The proportions of MEM supplemented are 5 mL of L-glutamine, 10 mL of Hepes, 5 mL of NEEA and 1 mL of Pen/Strep per 500 mL of MEM.

In order to prepare a new sterile cell culture flask with the new growth medium, 4 mL of FBS were added to 46 mL of MEM supplemented. This culture flask was placed in the incubator at 20 °C for 30 minutes for the temperature of the new medium to match the temperature of the old cells flask and avoid a thermal shock.

Therefore, the new growth medium was prepared half an hour in advance before detaching old cells from a previous culture flask. When making the new growth medium, 4 mL of FBS were added to 46 mL of MEM (Minimum Essential Medium; Ref. 21090-022, Gibco, USA) supplemented with L-glutamine (Ref. 25030-024, Gibco, USA), NEAA (non-essential amino acids; Ref. 11140-035, Gibco, USA), Hepes (Ref. 15630-056, Gibco, USA) and Pen/Strep (Penicillin-Streptomycin, P4333, Sigma-Aldrich, USA) to a new sterile cell culture flask. Then it incubated for 30 minutes at 20 °C with 5 % CO_2 for the temperature to match the temperature in the old cells flask and avoid a thermal shock.

Then, a one-week old culture containing a monolayer of CHSE cells was taken from the incubator. The old growth medium was discarded and the cells monolayer was washed with 5 mL of PBS (diluted ten times in distilled water; Ref. 14200-166, Gibco, USA). The PBS was discarded right after and 2 mL of 0.25 % trypsin-EDTA (Ref. 25200-056, Gibco, USA) were added and also discarded right away (this step was repeated twice). Then 1 mL of trypsin was added. At this point CHSE cells started detaching from the bottom of the culture flask. Cells were then gathered by washing with 2 mL from the new growth medium, pipetted and placed in the cell culture flask containing the new growth medium. The new culture flask was labeled (CHSE, n+1, date) and incubated for a week, at 20 °C with 5 % CO_2. This process was performed weekly in order to maintain the cells alive, therefore making them available for the UV tests and to repeat the tests if needed.

3) 96-Well plates preparation ("cell plates"):

Each plate required 20mL (+2 mL) of new growth medium. Therefore, a solution was prepared in a cell culture flask with 1.76 mL of FBS and 20.24 mL of MEM supplemented with L-glutamine, NEAA, Hepes and Pen/Strep. 2mL of growth medium were placed in a tube to later wash and gather CHSE cells from a precious established culture. Both the plate and the tube were incubated for 30 minutes at 20 °C with 5 % CO_2.

Then, one-week old CHSE cells would be detached from the bottom of their culture flask by the same method mentioned in the chapter b) and placed in a disposable 100mL reservoir together with the new growth medium. Using an 8-channel pipette, each well of the 96-Well plate was filled with 200 µL from the previous solution containing CHSE cells. Every plate was labelled and placed in an incubator with 5 % CO_2 for 2 days, at 20 °C.

C. *Moritella viscosa*/*Yersinia ruckeri* growth mediums preparation

1) Blood agar 3 % NaCl plates:

28.0 g of Blood Agar (CM0271, Oxoid, England) and 17.5 g of sodium chloride (1.06404.1000, Emsure, USA) were diluted in an erlenmeyer containing 700 mL of distilled water. After that the solution was autoclaved and after cooling down 35 mL of full blood were added. The solution was then plated in sterile 92x16 mm petri dishes. These plates were stored at 12 °C until being used.

2) Fluid marine broth 3 % NaCl:

18.7 g of Marine Broth (279110, Difco, USA) and 5.0 g of sodium chloride (1.06404.1000, Emsure, USA) were diluted in an erlenmeyer containing 500 mL of distilled water. The solution was boiled, then filtrated in a Büchner flask with a Büchner funnel and a 90 mm glass microfibers filter (Cat No 1822 090, Whatman, USA). The filtered solution was autoclaved and divided in flasks, filling each with 10 mL. The flasks were stored at 12 °C until being inoculated with *M. viscosa*.

3) Marine agar 3 % NaCl plates:

These plates were made by diluting 37.4 g of Marine Broth (279110, Difco, USA) and 7.0 g of sodium chloride (1.06404.1000, Emsure, USA) in an erlenmeyer containing 700 mL of distilled water. This step was followed by boiling the solution and filtrating it in a Büchner flask, with a Büchner funnel and a 90 mm glass microfibers filter (Cat No 1822 090, Whatman, USA). After that, 10.08 g of Bacto Agar (214010, Difco, USA) were diluted in the previous solution, autoclaving it afterwards. After autoclaving, the solution was poured into sterile 92x16 mm petri dishes as soon as the temperature cooled down around 40 °C. These plates were then stored at 12 °C until being used.