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The prevalence of *Aeromonas* species in a Norwegian water treatment plant after disinfection

Stian Fantoft Alvestad
Master's in public health science

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Abstract

Background: Microorganisms such as bacteria, viruses and parasites in drinking water may poses a risk to human health. *Proteobacteria* includes the most common pathogens isolated from drinking water. Belonging to this phylum, we find *Aeromonas* spp., which are bacteria that naturally reside in various aquatic environments. The most common clinical presentations of *Aeromonas* infections are diarrhea, wound infections, and bacteremia. Only a few studies have investigated the effect of various hygienic barriers and disinfection treatments on the prevalence of this pathogen in drinking water, and research is scarce in Norwegian settings. Therefore, this study investigates the effect of different treatments on the prevalence of *Aeromonas* spp. in a Norwegian drinking water treatment plant. **Materials and methods:** ten sampling points were tested weekly from September to November 2021 at various sites throughout the treatment plant. All samples were examined through three rounds of dilution, filtrated, and cultivated at 37°C for 20-24 hours on a selective *Aeromonas* medium with ampicillin. The quantified load was expressed in CFU/l with median and range. The *Aeromonas* prevalence was contextualized by descriptive trends of other drinking water quality parameters provided by the treatment plant. Inferential statistics were based on descriptive findings. **Results:** this study observed a great decline in median *Aeromonas* prevalence throughout the treatment process, with a 2.26 $\log^{10}$ reduction from raw water to clean drinking water leaving the treatment plant. Although a slight regrowth in the distribution network was observed, the median load is well below the infective dose reported for the most frequently isolated *Aeromonas* species, *A. hydrophila*. All sampling points appeared to have a declining *Aeromonas* load over the course of the study. Throughout the treatment course, the highest median *Aeromonas* growth was observed after the marble filter, with a 1.37 $\log^{10}$ increase from the sampling point before. The load remained elevated after the biological filter, and the median *Aeromonas* load observed after both filters were significantly higher compared to all sampling points except the raw water. Although a $\log^{10}$ reduction of 1.37 was observed from the samples before and after the ozone chamber, the greatest decline in median *Aeromonas* load was observed after the UV radiation chamber, with a 2.08 $\log^{10}$ reduction from the sampling point before. **Conclusion:** this study suggests that; (i) the environmental conditions such as temperatures and pH might favor *Aeromonas* growth, (ii) UV radiation seems effective in reducing the prevalence of this microorganism, and (iii) the observed *Aeromonas* load in the drinking water distribution may not pose a risk

for human consumption. Nevertheless, more studies in a Norwegian setting are needed, both at different locations and in various seasons throughout the year.

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1. Introduction

1.1 Drinking water

The supply and quality of drinking water accessible to a population are critical determinants of public health (Levallois & Villanueva, 2019). Despite this, just 71% of the world's population has access to safe and clean drinking water, with 785 million people lacking even the most basic level of water service (World Health Organization [WHO], 2019). It is mainly developing regions like Sub-Saharan Africa that have the highest proportion of people lacking access to safely managed drinking water (WHO & United Nations Children's Fund [UNICEF], 2017). However, although Europe and Northern America are among the regions with the highest levels of safely managed drinking water, 16 million people lack access to basic drinking water, and 31 million people lack basic sanitation in this region (WHO, n.d.). Globally, at least 2 billion people are exposed to water contaminated with feces, transmitting diseases like cholera, polio, typhoid, and dysentery. Consequently, 485 000 people are expected to die from diarrhea caused by microbial contamination of drinking water annually (WHO, 2019).

In Norway, the supply and quality of drinking water are good, with 99.3% of the population having access to hygienically safe drinking water, in terms of the indicator bacteria *Escherichia coli (E. coli)* (Statistics Norway, 2020). However, outbreaks of diseases linked to drinking water are reported each year (Norwegian Institute of Public Health [NIPH], 2016). The water quality may differ between Norwegian regions, counties, and municipalities. According to Statistics Norway, the whole population in Stavanger municipality has access to hygienically safe drinking water (Statistics Norway, 2021).

1.2 Drinking water as a determinant of public health

Drinking water can be harmful and considered a public health issue if it contains physical, chemical, or microbiological hazards that are toxic, infectious, allergenic, or cancerogenic to people who consume it or are in contact with it. The sources of contamination could either be of human origins, like products from agricultural or industrial activity, or natural like salt and minerals or organic matter from animals and plants (NIPH, 2016). While some microbiological hazards lead to less severe and self-limiting infections, others are known to cause more severe and even life-threatening conditions (WHO, 2017). The most significant risk is associated with microorganisms that cause food poisoning and infections in the gastrointestinal system. These could be transmitted by consuming contaminated water or eating raw food produced using contaminated water (NIPH, 2016).

1.3 Microorganisms in drinking water

The primary source of pathogenic microorganisms in the drinking water comes from the feces of sick animals or humans. The origin is commonly animals living close to or on water used for drinking water production and nearby sewage pipes. Microorganisms are commonly classified into three main groups: protozoa, viruses, and bacteria. Parasitic protozoa can live in the gut of a human or animal host, where they produce gastrointestinal disease. They are the biggest in size of the three groups. (NIPH, 2016). Viruses are the smallest of these three microorganism groups and contain genetic material that can cause infection in the host. They are primarily species-specific, and only viruses transmitted through the fecal-oral route are considered significant as waterborne infectious agents (NIPH, 2016). Bacteria are single-celled organisms that can survive in every environment, and several of them are known to be pathogenic and cause waterborne diseases (NIPH, 2016). Gram-negative bacteria, and *Proteobacteria* are the most frequently isolated bacteria from drinking water. There are predominantly three Proteobacteria classes: *Alpha, Beta,* and *Gamma* (Vaz-Moreira et al., 2017). The class of *Gammaproteobacteria* includes important water pathogens like *E. coli*, *Salmonella*, and *Vibrio* species that can cause infection in humans (Williams et al., 2010; WHO, 2017). *E. coli* is the most common found intestinal microorganism in the feces of healthy humans. Its presence is thus the most suitable indicator of fecal contamination of drinking water (WHO, 2017). *Aeromonas* spp. are included in the class of *Gammaproteobacteria* in the *Aeromonadales* order, which compromises the *Succinivibrionaceae*- and *Aeromonadaceae* families. The members of the latter are typically connected with aquatic environments and compromise the genus *Tolumonas*, *Oceanimonas*, *Oceanisphaera*, *Zobellella*, and *Aeromonas* (Huys, 2014; Santos & Thompson, 2014).

2. Aim of the study

Although *Aeromonas* spp. are ubiquitous environmental microorganisms, their incidence is worldwide unknown (Gonçalves Pessoa et al., 2019). Preliminary results from a national laboratory-based survey, on *Aeromonas* infections in Norway during 2014-2018, show that cases are reported throughout the years in all Norwegian counties without a clear source of exposure. Although some areas, such as Stavanger, showed the highest number of reported infections, local outbreaks have not been reported [unpublished data]. In their study, Ørmen and Østensvik (2001) identified *Aeromonas* species in almost all of their water samples from various Norwegian water sources, including alpine spring water, alpine rivers with both slow and rapid currents, lowland rivers, lakes, and drinking water sources. This points to the pathogen's widespread distribution and its indigenous character concerning water. Besides this study, the empirical data on *Aeromonas* in a Norwegian setting are limited, and the effect of different treatment methods on the presence of *Aeromonas* in drinking water in Norway has not been investigated yet.

Following these premises, this research project has been proposed by the NIPH in collaboration with IVAR, under the framework of the Norwegian University of Life Sciences (NMBU) Master thesis program in public health science. This study aims to investigate the effect of various treatment steps on the prevalence of *Aeromonas* species in the drinking water treatment plant at Langevatn, Stavanger to fill knowledge gaps on which treatment measure is most effective in reducing the presence of this environmental opportunistic pathogen.

3. Research questions

This study has the following research questions:

1. *What is the prevalence of Aeromonas species along with the water supply system from raw water until reaching the consumers?*
2. *How effective are the different treatment steps for the prevalence of Aeromonas in the Langevatn water treatment facility?*

4. Objective

To answer the research questions, the following objective will be met:

- Quantify and compare the *Aeromonas* load in drinking water samples at different treatment steps at the water treatment plant serving Stavanger municipality

5. Literature review

5.1 *Aeromonas* species

5.1.1 Taxonomy

The genus *Aeromonas* compromises 36 species and is recognized as an emergent human pathogen that can cause various infections in both immunocompetent and immunocompromised hosts, which refers to the functionality and effectiveness of their immune system (Bhowmick & Bhattacharjee, 2018; Fernandez-Bravo & Figueras, 2020). The bacteria are rod-shaped, and the size range from 300-1000 x 1000-3.500 nanometers (nm) (Igbinosa et al., 2012), although the size varies with the different species (Fernandez-Bravo & Figueras, 2020). These bacteria are, in addition, to be Gram-negative, facultatively anaerobic, non-spore-forming, catalase and oxidase-positive, and able to ferment glucose. The genus can be divided into two subcategories based on biochemical characteristics and environmental conditions affecting growth: a) Psychrophilic, non-motile and grows well in temperatures in the range of 22-25°C, b) Mesophilic, motile and grows well in temperatures in the range of 35-37°C (Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019). *Aeromonas* species belonging to the mesophilic group are causing infections like gastroenteritis and septicemia at a higher frequency than species in the psychrophilic group (Gonçalves Pessoa et al., 2019). In their review, Fernandez-Bravo and Figueras (2020) conclude that 98% of all *Aeromonas* species identified in clinical cases belong to one of five species: *A. caviae, A. dhakensis, A. veronii, A. hydrophila,* and *A. media*. Salvat and Ashbolt (2019) argue that, although *A. hydrophila* is the most frequently isolated species, it is neither the dominant species nor pathogenic. They report that *A. dhakensis, A. veronii,* and *A. caviae* are the dominant species in contaminated water and clinical cases.

5.1.2 Epidemiology

Aeromonas has been considered an opportunistic pathogen for a long time, but as mentioned earlier, it can affect both immunocompetent and immunocompromised human hosts. This bacteria is a versatile opportunistic pathogen that can cause a range of infections due to its diverse virulence factors, metabolism, and genetic plasticity, where the most common are: gastroenteritis, wound infections and septicemia (Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019). The main route of infection is through the consumption of contaminated food or water, but it is also transmitted by direct contact with contaminated water environments (Gonçalves Pessoa et al., 2019; Igbinosa et al., 2012) and the incubation period is 1-2 days (Salvat & Ashbolt, 2019).

The global incidence of *Aeromonas* infections is not known. This could be due to the technical issue of incorrectly identifying the genus or incorrectly determining the presence of *Aeromonas* in various outbreaks, in addition to the lack of surveillance system since *Aeromonas* is not notifiable in several countries (Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019). Fernandez-Bravo and Figueras (2020) claim that there could be a geographical variation in the incidence of the genus *Aeromonas*. They make this claim based on studies that showed an annual incidence of *Aeromonas* in California of 10.5 cases per million people in 1998, in addition to an incidence of bacteriemia caused by *Aeromonas* of 0.66 cases per million people in France in 2006, 76 cases per million in Taiwan between 2008-10 and 1.5 cases per million people in England in 2004. Bhowmick and Bhattacharjee (2018) report that the prevalence of *Aeromonas* seems to be higher in developing regions like India, Bangladesh, Vietnam, China, Nigeria, Iran, Libya, Venezuela, Egypt, and Brazil. This is supported by Fernandez-Bravo and Figueras (2020). They report that the geographical variance in the incidence of *Aeromonas* could be due to the hygiene habits in low-resource regions. Considering the favorable growth rate of *Aeromonas* at higher water temperatures, this could also influence uneven geographic occurrence (Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019).

The possibilities to be infected by *Aeromonas* are higher during the summer season due to the rising water temperatures causing bacterial populations to increase. In addition, the prevalence of *Aeromonas*-related diseases is higher during the rainy season with low water salinity than during dry seasons with high water salinity (Bhowmick & Bhattacharjee, 2018; Gonçalves Pessoa et al., 2019). People who have undergone antibiotic treatment also show a higher susceptibility to *Aeromonas* infections afterward (Salvat & Ashbolt, 2019)

5.1.3 Clinical manifestations

5.1.3.1 Gastroenteritis

This pathogen's main site of infection is the gastrointestinal tract, causing fever, nausea, vomiting, and stomach cramps. In addition, diarrhea is a common symptom, and colitis occurs in one-third of the diarrhea cases (Bhowmick & Bhattacharjee, 2018; Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019). *Aeromonas* can cause infection in any part of the colon, although the transverse and ascending sections are the more commonly affected. It can cause intestinal hemorrhage, including bowel obstruction and refractory inflammatory bowel disease (Bhowmick & Bhattacharjee, 2018). Fernandez-Bravo and Figueras (2020) point out in their review that the study of Teunis and Figueras (2016) demonstrates that

Aeromonas should be considered a human enteropathogen on the same basis as *Salmonella* and *Campylobacter*.

The same strains of *Aeromonas* that are known to cause diarrhea have been isolated from food and water in multiple studies (Fernandez-Bravo & Figueras, 2020). The most susceptible groups to these infections are children up to 5 years old, the elderly, and patients with underlying conditions (Salvat & Ashbolt, 2019). The incidence among the pediatric population was presented in Fernandez-Bravo and Figueras (2020) as 2.3% in Taiwan, 13% in Nigeria, and 7.5 and 1.4% in two different Spanish studies. In addition, Ghenghesh et al. (2015) reported a prevalence of 2-35% among children in several Arabic countries, with a mean of 14.5%. Among adults, Fernandez-Bravo and Figueras (2020) present an incidence of *Aeromonas* of 2% in Spain, 6.9% in Hong Kong among immunocompetent people, and 13% in Hong Kong among immunocompromised people. These numbers substantiate the previously mentioned finding about geographical variance in the incidence of infections caused by this bacterium.

For *Aeromonas*-induced gastroenteritis, the following species are responsible for 96% of the cases: *A. caviae* (37.6%), *A. veronii* by *sobria* (27.2%), *A. dhakensis* (16.5%), and *A. hydrophila* (14.5%) (Gonçalves Pessoa et al., 2019). These findings align with Batra et al. (2016), which point out *A. caviae* as the predominant *Aeromonas* isolate from diarrheal stools. However, they point out that there are geographical variations, like the one presented by Ghenghesh et al. (2015), that report *A. caviae* and *A. hydrophila* as the most common isolates related to gastrointestinal diseases in the Arabic region.

5.1.3.2 Wound infections

After the gastrointestinal tracts, wounds are the most common site of infection, particularly on arms and legs. Most cases affect immunocompetent people and are associated with burns, scolding, or natural disasters like hurricanes (Fernandez-Bravo & Figueras, 2020). As mentioned earlier, water contaminated by *Aeromonas* can be a source of infection, most often in wounds that occur while the host is in direct contact with the water environment. Infections are more common in patients who obtained the wound in freshwater than in seawater (Parker & Shaw, 2011). This makes sense since, as mentioned earlier, *Aeromonas* primarily reside in freshwater (Janda & Abbott, 2010).

The infections of soft tissues and the skin caused by *Aeromonas*, which range from mild to severe, include pustular lesions, cellulitis, myonecrosis, septic arthritis, fatal fulminant cellulitis, septic shock, and necrotizing fasciitis (NF) (Batra et al., 2016; Bhowmick &

Bhattacharjee, 2018). NF is a life-threatening condition known as a flesh-eating disease that can cause hypertension, necrosis, and gangrene. However, this is a rare consequence of *Aeromonas* wound infections (Fernandez-Bravo & Figueras, 2020; Igbinosa et al., 2012).

The most isolated species associated with wound infections are *A. hydrophila*. However, only 17-52% of the cases are monomicrobial (Batra et al., 2016). It is also the most frequent species associated with cases of NF, where open wounds have been in direct contact with contaminated water (Bravo et al., 2012).

5.1.3.3 Septicemia

Multiple studies have associated cases of septicemia and/or bacteremia with *Aeromonas* (Fernandez-Bravo & Figueras, 2020). Often used interchangeably, bacteremia refers to the presence of *Aeromonas* in the bloodstream (Oxford University Press, 2021a). In contrast, septicemia refers to the infections caused by *Aeromonas* in the bloodstream (Oxford University Press, 2021b). The symptoms include fever, jaundice, abdominal pain, dyspnea, and diarrhea. In addition, 40-45% of the cases develop septic shock, a life-threatening condition with a mortality rate between 20-50% (Hotchkiss et al., 2016; Janda & Abbott, 2010). Septicemia as a total, however, has a mortality rate of 30% (Fernandez-Bravo & Figueras, 2020).

As discussed in chapter 5.1.2, the incidence of *Aeromonas* bacteremia seems to vary by geographical location, with studies in Taiwan reporting a higher incidence rate than studies in California, England, and France (Fernandez-Bravo & Figueras, 2020). Trying to explain such differences, Salvat and Ashbolt (2019) argue that the incidence of *Aeromonas* bacteremia is higher in Asian countries than the global average, probably due to cirrhosis, an essential underlying condition. According to Fernandez-Bravo and Figueras (2020), the global incidence rate is 0.12 – 3.3%. However, Janda and Abbott (2010) point out that the reported incidence related to *Aeromonas* bacteremia are minimum estimates as many cases are undetected or unreported. In line with the mentioned claim about the seasonal variance of *Aeromonas*, 42-67% of the septicemia cases occur during the summer season (Bhowmick & Bhattacharjee, 2018). Important underlying conditions, in addition to cirrhosis, are diabetes mellitus, hepatobiliary disease, malignancy, chronic liver disease, neoplasia, biliary disease, myleoplastic syndrome, renal- and cardiac issues, thalassemia, and aplastic anemia (Bhowmick & Bhattacharjee, 2018; Fernandez-Bravo & Figueras, 2020). Janda and Abbott (2010) have classified *Aeromonas* septicemia cases into four groups based on population incidence: (1) Immunocompromised people, which represent 80% of the cases, (2) people

with a traumatic experience, which is the group with the second-highest incidence, (3) immunocompetent people and (4) people who went through reconstructive surgery.

Finally, it is possible to have blood infections caused by *Aeromonas* through contaminated catheters and dialysis champers. However, most cases of septicemia caused by this genus seem to arise through transfer from the gastrointestinal tract into the circulatory system. Other possible routes include transferring infected wounds, peritonitis, or biliary disease into the bloodstream (Bhowmick & Bhattacharjee, 2018).

Infections in the blood circulatory system of human hosts are associated with *A. hydrophila*, *A. caviae*, and *A. veronii* by *sobria*. In contrast, *A. jandaei*, *A. schubertii*, and *A. veronii* by *veronii* are known to cause septicemia, according to Bhowmick and Bhattacharjee (2018). However, Fernandez-Bravo and Figueras (2020) report *A. caviae*, *A. veronii*, and *A. dhakensis* as the *Aeromonas* species most frequently associated with septicemia.

5.1.3.4 Other infections

Other less common *Aeromonas* sites of infections have been reported, mainly due to the dissemination of this pathogen from wounds or the gastrointestinal system. These include infections of the respiratory tract, infections of the urinary tract, spontaneous bacterial peritonitis, and meningitis (Batra et al., 2016; Fernandez-Bravo & Figueras, 2020). In addition, cholangitis and infections in the eyes, muscles, and bones have been rarely reported (Bhowmick & Bhattacharjee, 2018; Igbinosa et al., 2012).

5.1.4 Ecology and sources of infection

Aeromonas have been isolated from foods like fruit, vegetables, dairy products, meat, and sausages. They are also associated with activities related to animal husbandry such as ranching, breeding, and aviculture. Moreover, they are indigenous to aquatic environments and have been found in groundwater, surface water, underground water, and seawater, among others, even though they primarily reside in freshwater (Fernandez-Bravo & Figueras, 2020; Gonçalves Pessoa et al., 2019; Igbinosa et al., 2012; Janda & Abbott, 2010). *Aeromonas* spp. has a ubiquitous nature which makes it present in most sources used for drinking water production. It can make up to 1-27% of the total bacterial count in finished drinking water, indicating that drinking water may be a source of infection (Igbinosa et al., 2012; Parker & Shaw, 2011). However, Janda and Abbott (2010) cite a study claiming the risk of humans getting infected by *Aeromonas* through oral ingestion of drinking water to be relatively low, with 7.3 cases per billion people.

Factors favoring the prevalence of *Aeromonas* in drinking water include higher water temperature, turbidity, and low residue chlorine (Salvat & Ashbolt, 2019). They have the highest growth rate in temperatures between 22◦C - 37◦C, although they are adaptable and able to tolerate low temperatures, where some species may grow in the range between 0◦C - 45◦C (Gonçalves Pessoa et al., 2019; Igbinosa et al., 2012). The optimal pH levels for *Aeromonas* growth are between 5.5 and 9, although they tolerate levels down to 4.5 (Igbinosa et al., 2012). Specifically for *A. hydrophila*, an ideal pH ranging from 7 to 9 has been reported (Palumbo et al., 2006).

5.1.5 Antimicrobial resistance determinants

In recent years, the high levels of antibiotic usage have led to the rise of varieties of bacteria that display resistance to these treatment agents. Globally, antimicrobial resistance [AMR] is estimated to contribute to 4.95 million and is a direct cause of 1.27 million deaths annually. (Antimicrobial Resistance Collaborators, 2022). AMR is a genetic-evolutionary response that the genes are responsible for (Bhowmick & Bhattacharjee, 2018; Fernandez-Bravo & Figueras, 2020). Bacteria have inherent survival mechanisms that include the horizontal exchange of genes, some of which are responsible for antimicrobial resistance. *Aeromonas* spp. is indigenous to aquatic environments that often contain discharges from urban and industrialized areas, including resistant bacteria that can exchange genes with nearby *Aeromonas* (Gonçalves Pessoa et al., 2019). Multiple antibiotic resistance (MAR) in *Aeromonas* spp. have been displayed by multiple researchers around the globe, and the resistant strains are capable of disseminating from wastewater into other environments (Fernandez-Bravo & Figueras, 2020; Igbinosa et al., 2012). This is relevant for public health because severe infections and infections among the immunocompromised might need antibiotics to be treated. With resistance to these agents, it might not be possible to treat these infections (Ghenghesh et al., 2015).

5.1.6 Virulence determinants

Aeromonas have a complex virulence profile where multiple factors contribute to the infections caused by this pathogen. These factors fall into three categories; structural components, extracellular products, and secretion systems (Gonçalves Pessoa et al., 2019). The structural components of the bacteria enable the adhesion of the bacteria cell to the host tissue and compromise the defense mechanisms of the host cells, initiating its invasion of it. *Aeromonas* have excreting virulence factors, from different secretion systems, like toxins, either directly into the host cell or into the medium between the cells. An example of this is

the Shiga-toxin causing the inactivation of ribosomes in the host cell, leading to cell death (Fernandez-Bravo & Figueras, 2020). The temperature may also have an impact as studies have shown that clinical isolates of *A. hydrophila* produce more toxins at 37°C compared to 28°C. As the former temperature represents the human body temperature, this may have clinical significance (Igbinosa et al., 2012).

5.2 Water treatment

The greatest strategy to protect against contaminants in drinking water is to reduce their levels before they enter the water treatment facility through extensive water source protection and maintenance; nevertheless, water treatment is also required. (Igbinosa et al., 2012; Rolston & Linnane, 2020). Nearly 10% of the total global burden of the disease could be eliminated by having sufficient and proper water treatment (Prüss-Üstün et al., 2008). This is a process that changes the physical, chemical or microbial quality of the water. The methods used in this process ensure that the water is free of color, odor, taste, corrosion, and infectious agents (NIPH, 2016). The treatment process is adapted to fit the demands and characteristics of the individual water supply system, the raw water quality, and expected consumption (NIPH, 2016).

5.2.1 Overview of hygienic barriers

The term *hygienic barrier* is used when describing how the water supply system can ensure safe and healthy drinking water for the population. To achieve this the barriers must be strong enough that microorganisms are removed, inactivated, or killed. In order to avoid serious implications caused by barrier failure, there should be multiple barriers for different types of contamination that work independently from each other (NIPH, 2016). It is common to differentiate between the *physical process*, that are blocking microorganisms from proceeding in the water supply system or removing them, and disinfection which refers to the inactivation of the microbes. The physical process includes coagulation/flocculation and membrane filtration, while the disinfection process includes chlorination, UV radiation, and ozonation (NIPH, 2016). As mentioned, the first line of defense against microorganisms in drinking water is the protection of the water source. The second line is the physical removal and blockage of the microorganisms. Ideally, the water should be 99.9% free from microorganisms after passing these barriers, however, this is not always the case and that is the reason this process always needs to be followed by disinfection (NIPH, 2016).

5.2.1.1 Physical blocking or removal

5.2.1.1.1 Coagulation and flocculation

In this process, chemicals (coagulants) are added to the water to make small particles, humus, and other substances combined to form larger particles. This includes microorganisms that are often attached to particles in the water (NIPH, 2016). To facilitate this merge of substances, a gentile agitation of the water causes the coagulated particles to move, collide and form even larger entities. Eventually, they reach a size that makes it possible to remove them from the water. This removal is facilitated in one of two ways. One option is sedimentation where the water is passed through a basin causing the particles to move to the bottom where they are removed. Alternatively, flotation involves pumping water, saturated with air, into the bottom of the basin which causes the production of air bubbles. The particles attach to the bubbles, which drives them to the surface of the basin where they are removed (NIPH, 2016). One of the limitations associated with these methods is that a poorly performed coagulation process is difficult to repair in subsequent steps, impairing their effectiveness. Particles that are not coagulated are harder to remove and filter and would make the reliance on disinfection more crucial. It is also dependent on being operational at all times. However, it does not produce any health-limiting by-products (NIPH, 2016). Coagulation and flocculation have been shown effective in physically removing *A. hydrophila* from water originating from surface sources (Casanova & Sobsey, 2015).

5.2.1.1.2 Membrane filtration

Filters used for membrane filtration have openings of less than 5000 nm. This ensures that the dissolved and particulate material is retained on the concentrate side of the membrane, while the water is passing through to the permeate side. The smaller the opening, the better it purifies the water, and openings down to 1-10 nm (nano-membranes) are used. It works well as a hygienic barrier following the coagulation- and flotation process because the larger particles are unable to pass through the membrane openings (NIPH, 2016). As mentioned earlier, the smallest microorganisms are viruses, with a diameter of approx. 20 nm, making them able to pass through most membrane filters except nano-membranes. Bacteria are larger, which makes them unable to pass through membranes with larger openings (NIPH, 2016).

5.2.1.2 Disinfection

5.2.1.2.1 Chlorination

Chlorine is effective in inactivating microorganisms that are harmful to public health, but some bacteria and spores are resistant to them (NIPH, 2016). Chlorine attacks the cell wall and membrane of the bacteria and oxidizes them, so they are destroyed. Then it enters the cell where they cause the destruction of the genetic material and the cell itself. The effect of

chlorine as a disinfectant depends on the concentration of chlorine and contact time. The ideal is to find the magnitude sufficient to kill the bacteria, but low enough to avoid effects on the odor and taste of the water. However, because chlorine will react with other substances as well, the clarity of the water is a co-determinant of the levels of chlorine needed. Higher contents of humus and color in the water demand a higher amount of chlorine. In addition, temperature and pH levels influence the effectiveness of chlorine. Preferably the pH levels should be below 7.5 and the chlorine requirement increased with lower water temperatures (NIPH, 2016). One of the limitations regarding chlorine treatment is that it requires a high degree of operational follow-ups, and it can produce odor and taste as mentioned. However, it is cheap, easily available and it is easy to monitor sufficient chlorine residue (NIPH, 2016).

5.2.1.2.2 UV radiation

Ultraviolet light (UV-light) is electromagnetic radiation with a wavelength of 100-400 nm. The ideal wavelength to inactivate microorganisms lies between 240-280 nm, which all of the lamps used for UV treatment can produce (NIPH, 2016). The UV light inflicts damage on the genetic material and proteins of the microorganisms. This makes them unable to divide in addition to impairing important life processes of the microorganism. This damage can be irreversible or reversible, where the microbial repair process is most often dependent on visible light. Therefore, the water should not be exposed to visible light right after UV radiation. As with chlorination, the effect as a disinfectant is dependent on the dose and duration. The intensity depends on the number and strength of the lamps, in addition to their placements. It also drops the further it travels in water, and the lower the water quality is. A dosage sufficient against most microorganisms, including bacteria, is 30 mJ/ cm^2, although 40 mJ /cm^2 is required for bacterial spores. The duration of the irradiation is dependent on the time the water spends in and the volume of the radiation chamber, in addition to the water velocity (NIPH, 2016). Some limitations of this method include the process which is vulnerable to changes in water quality and volume that may alter the radiation requirement, and in addition, it requires a high degree of technical expertise to design. However, when implemented correctly, it inactivates the microorganisms fast and effectively, and it rarely produces by-products harmful to the public. In addition, it does not alter the taste and odor of the water (NIPH, 2016).

5.2.1.2.3 Ozonation

As with chlorine, ozone (O_3) is also a powerful oxidizing agent. When added to water the O_3 will dissolve and immediately react with the components in the water. It is a very harmful substance to microorganisms, although different microorganisms have different susceptibility

to this substance (NIPH, 2016). It destroys the cell membrane, subsequently causing intracellular leakage and death of the bacterial cells (Thanomsub et al., 2002). As with chlorine and UV radiation, the effect is dependent on the dose and time of the exposure. A dose of 0.2 mg O_3 per liter for 10 minutes is usually sufficient for a 99.9% inactivation of viruses and bacteria, however, for spores 5mg/liter for 10 minutes is required for 99% inactivation. However, as with the other disinfecting methods mentioned, these requirements are dependent on the quality of the water as the O_3 will react with other organic and inorganic materials. The more color and humus the water contains, the higher the required O_3 for sufficient inactivation of microorganisms gets. The pH value of the water should be below 6.5 because O_3 dissolves faster at high pH levels. (NIPH, 2016). One of the limitations of this method is that ozonation of the drinking water results in the formation of a range of by-products like aldehydes, carboxylic acids, and carbonyl compounds. Some of these cause odor and smell, while others affect human health. This treatment also requires sufficient safety measures as O_3 has a toxic gas form at room temperature. However, O_3 has slightly better disinfecting properties than chlorine. In addition, the operation is relatively simple and the substance can be made on-site where there is no need to handle chemicals (NIPH, 2016).

5.2.2 Effectiveness of hygienic barriers on *Aeromonas*

Coagulation and flocculation have been shown effective in physically removing *A. hydrophila* from water originating from surface water sources (Casanova & Sobsey, 2015). This is the most frequently isolated *Aeromonas* species in a study conducting water samples from various Norwegian water sources (Ørmen & Østensvik, 2001). The average size of *Aeromonas* is, as mentioned, 300-1000 x 1000-3.500 nm, although it varies with species (Fernandez-Bravo & Figueras, 2020; Igbinosa et al., 2012). This makes them unable to pass through nano-membranes, and ultra-membranes (10-100 nm openings). However, some micro-membranes (apertures of 100–5000 nm) do not retain this pathogen unless it is part of a larger coagulated particle. Membrane filtration produces no by-products. The major risk is associated with defects or leaks in the membrane, which makes the retention of the microbes ineffective and is hard to detect (NIPH, 2016).

Aeromonas has also been isolated from chlorinated water, suggesting that there might be some resistance present. A residual chlorine concentration (the remaining chlorine levels in the water after initial application) above 0.1 – 0.2 mg/L is the recommended amount to prevent growth (Igbinosa et al., 2012), however, a study by Scoaris et al. (2008) suggests that multiple species of *Aeromonas* resist chlorine concentrations up to six times the

recommended amount. On the other hand, Salvat and Ashbolt (2019) argue that is not impossible to inactivate them, and sufficient residual chlorine is still an important factor in preventing the growth of this pathogen. Specifically, *A. hydrophila* has been shown to be susceptible to chlorination compared to other coliform bacteria (Igbinosa et al., 2012). Some studies have also investigated the effectiveness of UV radiation on this pathogen. Latif-Eugenín et al. (2017) found *Aeromonas* in 7 out of 13 water samples, whereas the 4 samples without *Aeromonas* had been treated with chlorine and UV radiation. In addition, *A hydrophila* has been shown to be susceptible to UV radiation type C (UV-C) (Kaur et al., 2015). There also appears to be a species difference in the effectiveness of O_3 against *Aeromonas*. The bacteria has been detected in water that has previously been treated with O_3 (Figueira et al., 2011), although the species *A. jandaei* and *A. sobria* have been shown to be susceptible to this substance (Ding et al., 2019).

5.3 Drinking water in Norway

5.3.1 Norwegian water supply systems

A Norwegian water supply system normally consists of the water catchment area, the water source, water treatment plant, transport system, and operating routines. The network that makes up the whole system consists of transmission lines from the source to the treatment facility, and branch lines that deliver the water to the consumers. In addition, the system consists of technical components like pumping stations, basins, valves, troughs, and pressure-reducing devices. The systems are built and planned for a low risk of quality failure and interruption of production (NIPH, 2016). There has been an improvement over the last 20-30 years in Norwegian waterworks, much because of economic investments in the waterworks by the government. The majority of the population (90%) receives water that is approved and registered at the Waterworks Register. There are two types of waterworks in Norway, larger facilities that supply 50 or more people, and smaller facilities with 50 or fewer consumers (Hyllestad, 2017). There are 1500 larger facilities in Norway that supply 89% of the Norwegian population (NIPH, 2016).

5.3.1.1 Water sources

In general, the water sources used for drinking water production in Norway holds high quality, and contaminants entering the treatment plants in the first place are limited. This makes the consequences of treatment failures in the plant less detrimental (Igbinosa et al., 2012; NIPH, 2016). According to the data recorded in the Waterwork Register, 10% of the waterworks receive raw water from groundwater sources, while 90% receive their water from surface water sources. The 10 % of the population that is not receiving water from approved

waterworks most likely receives water from private wells and small common facilities (Hyllestad, 2017; NIPH, 2016).

Groundwater sources refer to water sources below ground level. The conditions of the ground will affect the quality of the water, and organic material can be added from discharges into the topsoil, while groundwater near the coast can be affected by seawater. A few metals may affect the taste of the water, but in general, both the taste and odor are good (NIPH, 2016). Groundwater in general is better protected from microbial contamination than surface water. The reason for this is that before the rainwater reaches the pockets underground, it goes through a series of natural purification processes, that reduce the contamination (Norges Geologiske Undersøkelse, 2020).

Surface waters include rivers, ponds, lakes, and streams and are, as mentioned, the most common source of drinking water in Norway. Since it is more prone to be contaminated by pathogenic microorganisms from humans and animals, it must always be treated before being distributed to the public, even though the Norwegian surface sources, in general, have a low level of contamination. There are natural layers of protection that occur before the water leaves for the treatment plant. Lake water quality is naturally improved by the long residence duration of the water by degrading pollutants, being taken up by organisms and sediments (NIPH, 2016). Lagooning is a water treatment method that takes advantage of these mechanisms and has been proposed to reduce the microbial load of *Aeromonas* (Fernandez-Bravo & Figueras, 2020). In addition, in the summertime, a natural temperature stratification occurs in Norwegian lakes. Cold water on the bottom is separated from the warm water on the top of the lake, and the different densities keep them from mixing. This acts as a natural barrier, preventing contaminated water from reaching the cooler water at the bottom of the lake (NIPH, 2016).

5.3.1.2 Monitoring of water quality – microbiological indicators

Norwegian waterworks routinely conduct analyzes of the water to monitor its quality (NIPH, 2016). As mentioned, the presence of *E. coli* is the best indicator of human fecal contamination of the drinking water, and the parameter used for monitoring the quality of the water in the waterworks in Norway (Hyllestad, 2017). However, 99.3% of the population has, as mentioned, access to safe drinking water in terms of the absence of *E. coli* (Statistics Norway, 2020). Another bacteria, *Enterococci*, are commonly found in animal digestive tracts. This pathogen is excreted from animals' feces into the environment, where especially water isolates have been linked to human infections. They are globally used to monitor water

quality (Byappanahalli et al., 2012), and alongside *E. coli*, they are recognized by the European Union and the Norwegian government, as parameter indicators of microbial contamination of water (*The quality of water intended for human consumption*, Council Directive 98/83/EC; Drikkevannsforskriften, 2017). Although *E. coli* and *Enterococci* are the primary indicators of fecal contamination, *Clostridiumperfringens* (*C. perfringens)* is an alternative indicator in environments where the primary indicator bacteria has shown the capacity to grow without fecal contamination (Ashbolt et al., 2001), but only if the water is originating from surface water sources (*The quality of water intended for human consumption*, Council Directive 98/83/EC). *Aeromonas*, however, is not recognized as a microbial indicator for water quality in the Council Directive (*The quality of water intended for human consumption*, Council Directive 98/83/EC).

5.3.1.3 Challenges and improvements

Despite the improvements in the last 20-30 years, there are still some challenges related to the Norwegian water supply systems. The pipeline network is vulnerable, where 1 in 5 networks have not been upgraded in more than 60 years. They are prone to fractures and often lie in the same ditch as sewage pipes which makes it an area of high risk of containment during repairs and leaks. This could lead to outbreaks of gastrointestinal disease (Hyllestad, 2017). In addition, climate change may cause problems to the water supply systems. This is both because the rising temperatures make already fragile waterpipes more prone to leaks (Hyllestad, 2017), and also because the increased frequencies of floods overload sewage pipes, making them more prone to enter the water sources used for drinking water production. Increased water runoff from land areas will also facilitate this problem and potentially increase the demand for the waterworks (NFSA, 2019). The treatment methods used in Norway have changed based on the increasing knowledge about possible infectious agents in the water. Some microorganisms, like *Aeromonas*, have shown the capacity to resist chlorination. As a result, more Waterworks have started to use UV radiation as a method of disinfection, and it surpassed chlorination in 2007/2008 as the most commonly used method for disinfection in Norway (NIPH, 2016).

5.3.2 Framework of laws, regulations, and guidelines

5.3.2.1 Laws

The Public Health Act secures a long-term developmental direction of the society that facilitates the health of the public, and also more equal distribution of health and its determinants. This includes environmental factors that can influence public health and cause disease, where unsafe drinking water is exemplified. Furthermore, The Public Health Act

determines the responsibility of various actors in the society in following through with the provisions of the law, as well as facilitating collaboration between them (Folkehelseloven, 2011, § 1; NFSA, 2020). The municipalities are responsible to have an overview of conditions, within their geographical borders, that have an impact on the population's health (Folkehelseloven, 2011, § 5) and are required to initiate appropriate measures for these challenges (§ 7). The municipalities, therefore, have independent responsibilities in ensuring that their population has access to secure drinking water (NFSA, 2020)

The Food Act secures foods and drinks along the whole production line, to make sure that they are healthy and safe to consume for the population, in addition to safeguarding the environment and ecology. The scope of this law covers the production line of drinking water (Matloven, 2003, §1, §2) and would apply if the water contains substances that are harmful to the public. It sets requirements for the waterworks to make sure the water is safe and healthy, and a duty to report to the supervisory authority when there is suspicion that the water contains health limiting substances or organisms (§5, §6). The supervisory authority is the Norwegian Food Safety Authority (NFSA) (§23)

The foundation of the Act on health and social preparedness is to protect the health of the population during war, crises, and catastrophes. This law requires that operations defined by the law, including waterworks and municipalities, make plans to continue their operation during the defined hard times (Helseberedskapsloven, 2000, §1-1, §1-2, §2-2).

Other relevant laws include the Pollution Control Act, which requires the protection and reduction of contamination of the environment including water (Forurensningsloven, 1981, §1, §6), and the Water Resources Act, which requires a sound use and management of the groundwater and watercourses of the country. Which include both how the public and private entities handle the groundwater and watercourses, and how the management of groundwater and watercourses affects the public (Vannressursloven, 2000, §1, §5, §43a).

5.3.2.2 Drinking water regulations

In 2017 new regulations of drinking water were introduced, with legal basis in the Food Act, the Public Health Act, and the Act on health and social preparedness. In addition, it is in line with the EU Water Framework Directive and Drinking Water Directive (*The quality of water intended for human consumption*, Council Directive 98/83/EC). The administrative and supervisory authority lies with the NFSA (Hyllestad, 2017, NIPH, 2016), while it is the municipality that is the local responsible authority where the Food Act does not apply and in emergencies, following the Public Health Act (NIPH, 2016). It is the owner of the waterworks

that are responsible for meeting the requirements of the regulations. These include that the water supply system must deliver enough safe drinking water to the population, and the water must be clear and free from odor, color, and taste (Hyllestad, 2017; NFSA, 2020). These requirements are stated in the form of limit values for indicator parameters of common threats to the quality of the drinking water. This includes organic material, where the indicator is the color number of the water that should not exceed 20 mg / l platinum unit (Pt). If the color exceeds this level, the water must be pre-treated before chlorination (NIPH, 2016). The regulation states that the treatment facilities must include steps to remove or inactivate microbes if it comes from surface water sources (Drikkevannsforskriften, 2017, §12), and the owner of the waterworks are responsible for consistently sampling and monitoring the water (§ 12).

5.3.2.3 National goals for the water

Based on article 6 in the protocol for water and health, the Norwegian government adopted national goals for water in 2014. The aim is to achieve a sufficient supply of clean drinking water, in addition, to ensure that the sanitarian conditions are acceptable, although these goals are not regulatory. The responsible institution for following through with the goals is the Ministry of Health and Care Services (HOD), and with the NFSA as the central directorate in close collaboration with NIPH (NFSA, 2019). Together with the regulations for drinking water, the national goals provide guidelines for the supply of sufficient and safe drinking water. It requires that the operators of the waterworks in the country need focus on areas that are defined as particularly important. Especially, the goals focus on the mentioned challenge of maintenance and replacement of old and worn pipelines in the supply network. It is often the municipality that is the owner of the waterworks and is therefore required to meet the demands of the drinking water regulations, as well as work towards the national goals for the drinking water (NFSA, 2019).

5.4 Drinking water in Stavanger

Stavanger municipality is the responsible unit for the supply of drinking water within its borders (Stavanger kommune, 2019). The drinking water that goes out to the public is supplied by IVAR IKS (IVAR), a company owned by 12 municipalities that are responsible for water, sewerage, and renovation. Stavanger municipality is both the biggest owner and customer. This entails that IVAR handles the whole supply system including water source protection/selection, treatment, and distribution of the water to the public under the water regulations (IVAR, n. d.; Stavanger kommune, 2019). The water sources used for drinking

water production in Stavanger municipality comes from two surface water sources: *Storavatn* in Gjesdal municipality and *Stølsvatn* in Bjerkreim municipality (Stavanger kommune, 2019). The raw water is treated at the *Langevatn* water treatment plant in Gjesdal municipality. This is one of Norway's largest treatment facilities which supplies 12 municipalities with drinking water, including all inhabitants of Stavanger municipality.

The treatment steps include: (1) Adding ozone to the raw water. The substance will react with the humus and color particles and split them into smaller parts to reduce color, taste, and odor. (2) Carbon dioxide (CO2) is added to reduce the pH level of the water. This will cause the marble in the chamber to dissolve, which will lead to the alkalinization of the water. (3) The next treatment step is to lead the water through a marble filter that removes particles, (4) and pass it through a biofilter where the split color particles remaining are eaten up by bacteria. Subsequent disinfection steps are then initiated to inactivate the microbes with (5) UV radiation and (6) chlorination of the water (IVAR, 2021; Stavanger kommune, 2019). In line with the drinking water regulations, IVAR is obligated to have an active sampling plan for monitoring the water quality in terms of various parameters, including microbial content (Drikkevannsforskriften, 2017, §19). In May 2021, alongside the opening of a new water treatment facility, IVAR introduced a more extensive monitoring program than what was requested by the Norwegian Food Safety Authority and the drinking water regulations. These samplings are taken regularly, at different sampling points, before and after the various hygienic barriers at the treatment facility, according to the drinking water facility.

The treated drinking water is transported through large transmission lines. The mainline goes to the Tjensvoll basins which supply most of the population in Stavanger municipality. In addition, the old Langevann pipeline supplies parts of Stavanger directly and works as a supplement and reserve for the mainline. The process of building a new pipeline from Langevatn to Tjensvoll is underway to increase supply security (Stavanger kommune, 2019).

Even if the system outlined above fails, IVAR is still responsible for supplying water to the municipality, per Helseberedskapsloven (2000, §1-1, §1-2, §2-2). The basins containing treated drinking water have a spare capacity of around 24 hours, which is considered sufficient to be able to repair any cable breaks in the transmission line. Reserve water sources include *Hagavann* in Hå municipality, *Langevatn* in Gjesdal municipality. In addition, *Store Stokkevann* in Stavanger municipality is an emergency source. Groundwater Sources in

Oltedal and Dirdal can be used, in addition to an agreement with the dairy company TINE to use their groundwater source if needed (Stavanger kommune, 2019).

6. Materials and Methods

6.1 Study design

The foundation of any academic research is the research questions and the objectives aimed at answering them. The methodology refers to *how* the study was designed and conducted to fulfill the objectives - and by doing that, hopefully, contribute to illuminating the research questions. Therefore, the decisions made on the study design and process should be based on its suitability to provide us with answers to these questions we are asking in the study (Bui, 2020). In other words, the methodology used in the present study was designed and conducted to quantify and compare the load of *Aeromonas* from drinking water samples at different treatment steps at the Langevatn water treatment facility. The aim is that this would lead to an increased understanding of the prevalence of the microbe throughout the water supply system and what influence different treatment steps might have on this microbiological parameter. The study followed a quantitative, inductive experimental design with three distinct steps: (1) sample collection of drinking water at different sampling points at Langevatn water treatment facility, (2) identification and quantification of *Aeromonas* through microbiological analysis at a laboratory near the water treatment facility, (3) statistical analysis describing and comparing the *Aeromonas* load between different sampling points, based on data received from the laboratory. Steps 1 and 2 were carried out by IVAR and the laboratory, respectively, whereas step 3 was carried out by the present study's author. Other relevant parameters monitored and collected by the drinking water treatment plant have contextualized the *Aeromonas* prevalence at different sampling points. These additional parameters include the heterotrophic plate count [HPC], temperature, pH, adenosine triphosphate [ATP], colour, and turbidity. While the HPC is a parameter measuring the general bacteria population throughout the treatment facility (Health Canada, 2013), the ATP is a variable used to monitor the total microbial contamination of the water (Whalen et al., 2018). The study's design is experimental because it aims to elevate our understanding of the potential causal effect of the various treatment steps on the prevalence of *Aeromonas* in a Norwegian drinking water treatment plant (Lowhorn, 2007).

6.2 Sampling plan

The drinking water treatment plant agreed to assist the current project in conducting sampling for *Aeromonas* spp. detection at the facilities in addition to their established monitoring program. The samples for the current project were taken during the fall of 2021, starting from week 38 until week 49. The sampling plan in this project consisted of two phases. IVAR collected water samples of 1000 milliliters (ml) at all points included in their regular

monitoring program in the study's first phase. These points include, as presented in **Figure 1**: the raw water (SP1), the ozone chamber (SP2), before marble filtration (SP3), after marble filtration (SP4), after biofiltration (SP5), after UV-disinfection (SP6), after chlorination (SP7), and Tjensvoll basin for storage of clean drinking water (SP8). The aim was to get an overview of the variation in the prevalence of *Aeromonas* spp. within the treatment chain. The characterization provided the foundation for the decisions made in the second phase of the sampling.

The sampling plan was adjusted to ensure that all the critical points were assessed based on the first phase results and the project partners' discussion. The deliberation revolved around the best sampling size (1000 ml or 500 ml) and relevant adjustments of sampling points, including the possible inclusion of two additional sampling points at the level of the biofilter (SP9) and marble filter (SP10), as presented in **Figure 1**. After this evaluation, a total of eight samples per week were sent to the laboratory, near the facility, for quantification of the *Aeromonas* spp. load. These eight weekly samples covered: 7 sampling points along the drinking water treatment plant (SP1, SP3, SP4, SP5, SP6, SP7, and SP8) and two sampling points, alternately every two weeks of the backwash water of the bio- and marble filters (SP9 and SP10). All samples, in both phases, were clearly marked with the point of sample and sampling date to identify the origin of the quantified samples in the next step. The complete sampling plan is presented in **Table 1.**

Table 1. Sampling plan for the study

Week	Raw water (SP1)	Grind 1 (SP2)	Before marble filter (SP3)	After marble filter (SP4)	After biofilter (SP5)	Before chlorine treatment (SP6)	Clean drinking water (SP7)	Water Basins (SP8)	Biofilter (SP9)	Marble filter (SP10)
38	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	-	
39	-	-	-	-	-	-	-	-	-	
40	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-
41	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-	500 ml
42	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-
43	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-	500 ml
44	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-
45	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-	500 ml
46	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-
47	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-	500 ml
48	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-
49	500 ml	-	500 ml	500 ml	500 ml	500 ml	500 ml	500 ml	-	500 ml

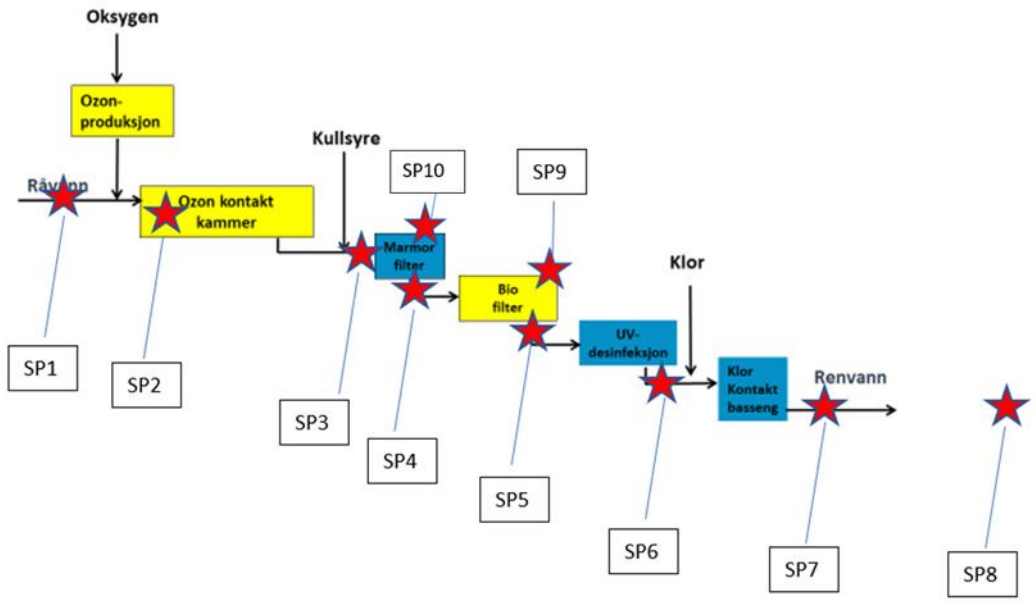

Figure 1. Description of the water treatment process and sampling points (SP) at Langevatn water treatment facility. SP1-SP8 are sampled from the water progressing through the treatment course, while SP9-SP10 are sampled from the backwash water of the filters. This chart of the treatment process included in this figure is adopted from IVAR (n. d.-b).

6.3 Microbiological analysis

All collected water samples were delivered to the laboratory after overnight express shipment at refrigerated temperature (~4°C). The laboratory filtered the water samples through a 0.45µm cellulose ester membrane filter (MerckMillipore), then transferred them to culture dishes. Each sample was filtered with three different volumes (10, 100, 500 mL during the first sampling phase; 1, 10, 100 mL during the second sampling phase) to cover the measuring range of 2 - 10^4 and 10 - $2x10^5$ colony-forming units [CFU]/l, respectively. The filtrated samples were cultivated at 37 ± one °C for 22 ± 2hours on agar plates containing a selective *Aeromonas* medium and ampicillin (thermoscientific Oxoid *Aeromonas* Medium). *Aeromonas* spp. usually form green colonies. The results, expressed in CFU/l, were reported to FHI for data analysis.

6.4 Data collection and analysis

All *Aeromonas* reports sent by the laboratory were collected, stored, and processed to create a dataset to be further analyzed. In addition, the drinking water treatment plant provided a dataset containing other various parameters, such as HPC, temperature, pH, ATP, colour, and turbidity, measured regularly as part of their quality monitoring program. This additional dataset was used to contextualize the *Aeromonas* findings. Relevant parameters from this dataset were sampled at the same site and date as the *Aeromonas* spp. samples provided the

material for data analyses to answer the current study's research questions. Therefore, the next step was to create a clean Excel file with structured tables of the *Aeromonas* spp. load reported by the laboratory, and relevant parameters extracted from the dataset provided by the drinking water treatment plant. The data analysis consisted of two components: *descriptive* analysis and *inferential statistics.*

6.5 Descriptive analysis

The descriptive analysis in the present study aimed to illustrate (i) the trends of *Aeromonas* spp. based on the sampling site, (ii) the trends of *Aeromonas* spp. along the drinking water treatment plant over time and (iii) compare them to the trend of other parameters measured at the same site and time. The descriptive analyses were conducted using several formulas and graphs in Microsoft Excel and SPSS software.

The first step was to measure the *central tendency* of the numbers, or the typical number for each sampling point (Bui, 2020). Because tests show that the data is non-normally distributed and contains outliers, the current study uses the median value to portray the central tendency for each sampling point for better robustness than the mean (Pupovac & Petrovecki, 2011). The normality tests were carried out in SPSS, and the results are shown in **Appendix A.**

The second step was to evaluate the *variability* of the data or the distance in parameter value between each sample from the same site (Bui, 2020). In this study, the evaluation of the variance consists of the *range* and the *median absolute deviations* [MAD]. The *range* refers to the distance between the sample with the highest and lowest parameter value for each sample point. Since the parameter's median value evaluated the central tendency for each sampling point, the MAD was used to evaluate each sampled parameter's distance from the parameter median for each sampling site (Bui, 2020). Both the range and the MAD were calculated using SPSS.

Data visualization is a valuable tool for identifying patterns in data (Larson, 2006). The third step of the descriptive analysis was to create a graphical representation of the data that displayed both the trend over time for each sampling point and the median trend throughout the treatment plant. This study employs line graphs because they provide the best visual representation of the trend of continuous variables with time or a category as the independent variable (JMP, n. d.). As the *Aeromonas* spp. samples from the bio- and marble filter (SP9, SP10) were extracted from backwash water and not the water proceeding through the treatment plant; these sample points will be presented separately in the graphs. In

comparison to the HPC, the quantification of *Aeromonas* is expressed in CFU/ml, as the letter is presented in the specified unit in the raw data provided by the drinking water treatment plant. All logarithmic development trends were calculated using the $\log^{10}$ function of Excel.

6.6 Inferential statistics

Inferential statistics is a set of methods where representative samples of a larger entity are investigated and used to draw conclusions about the whole population that makes up the sample entity from which the samples are extracted. This process is more efficient and realistic than including every individual component of the population in a single study, which is often impossible (Bui, 2020). The present study's inferential statistics aim to draw conclusions about the general *Aeromonas* spp. population in the drinking water moving through Langevatn water treatment facility, based on the samples taken throughout the sampling weeks and the other parameter measures provided by the drinking water treatment plants. Samples obtained in week 49 were excluded from the inferential statistics because the drinking water treatment plant reported deviations from the study protocol regarding sampling procedures, and the laboratory reported issues related to possible sample contamination. Samples from the filters (SP9, SP10) were also excluded from the analyses when these samples were extracted from the backwash water. All inferential statistics were calculated using SPSS. *Spearman's rank correlation coefficient test* was used to measure the bivariate relationship between continuous variables, while the *Wilcoxon rank-sum test* was used to investigate possible significant differences in *Aeromonas* spp. load between sampling points. An alpha level (α) of 0.05 was chosen for both tests. Apparent trends in descriptive statistics served as the foundation for decisions being made on which sites and parameters to examine. The test of normality of the difference between the sampling points is presented in **Appendix B,** while **Appendix C** presents the evaluation of appropriate statistical methods for the inferential analyses.

7. Results

This chapter will present the results in separate undersections. The first section presents the descriptive analysis in three parts: (i) the preliminary characterization of *Aeromonas* in the drinking water treatment plant based on samples from the first phase, (ii) the prevalence of *Aeromonas* along the drinking water treatment plant based on samples from the second phase, and (iii) comparison of *Aeromonas* prevalence with other parameters of water quality, such as HPC, temperature, pH, ATP, colour and turbidity. The section that follows presents the result of the interpretive analyses.

7.1 Preliminary characterization of the drinking water treatment plant towards *Aeromonas* spp. prevalence

The sampling carried out during the first phase (week 38) provided an overview of the variation in the prevalence of *Aeromonas* spp. within the treatment plant. As shown in Table 1, the most considerable load was recorded after the marble- and biofilter (SP4, SP5), with counts of 33,000 and 25,000 CFU/l, respectively. The ozone chamber (SP2) had the lowest value, with a measured load of 2 CFU/l. The highest $\log^{10}$ reduction is shown after UV radiation with a value of 2.7 $\log^{10}$ CFU/l.

Table 2. Characterization of the drinking water treatment plant based on *Aeromonas* spp. load (expressed in CFU/l) in week 38

	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8
CFU/L	2.4×10^3	2	30	3.3×10^4	2.5×10^3	5	23	15
$\log^{10}$ reduction	Ozonation: 1.9				UV radiation: 2.7			

7.2 Prevalence of *Aeromonas* spp. along the drinking water treatment plant

As shown in **Table 3** and **Figure 2**, the median load in the raw water entering the treatment plant (SP1) is 540 CFU/l (MAD = 460), which is reduced to 3 CFU/l (MAD = 0) for the treated drinking water leaving the treatment plant (SP7). There is a slight increase in the average CFU/l in the water basins providing the consumers with drinking water (SP8). The highest median values, together with the raw water (SP1), were detected after the marble filter (SP4) and the biofilter (SP5), with a load of 650 and 360 CFU/l (MAD = 557, 300), respectively. Other than the post-filter and raw water sampling points (SP1, SP4, SP5), all sites had a median load of less than 24 CFU/l. The steepest decline is observed after the UV radiation chamber (SP6).

Table 3. Prevalence of *Aeromonas* spp. (expressed in CFU/l) per each sampling point during weeks 38-49 and basic statistics for each sampling point.

	SP1	SP2	SP3	SP10	SP4	SP9	SP5	SP6	SP7	SP8
Week 38	**2400**	**2**	**30**	**-**	**33000**	**-**	**2500**	**5**	**23**	**15**
Week 40	1000	-	4200		27000	2000000	2400	<3	<3	15
Week 41	95	-	10	530000	19600		1500	<3	3	10
Week 42	215	-	30		2300	270000	770	<3	<3	8
Week 43	20	-	8	650000	570		360	<3	3	8
Week 44	590	-	23		1080	52000	3700	<3	120	15
Week 45	540	-	43	64000	650		225	<3	<3	10
Week 46	20	-	5		450	170000	190	<3	8	30
Week 47	1500	-	10	33000	265		123	<3	13	30
Week 48	80	-	10		93	62000	60	<3	3	25
Week 49	2240	-	427	2000000	80		157	43	17	110
Average	790.91	-	436	655400	7735,27	510800	3135	6.82	18.45	25.09
Median	540	-	23	530000	650	170000	360	3	3	15
Range	2380	-	4195	1967000	32920	1948000	24940	40	117	102
MAD	460	-	13	466000	557	108000	300	.0	.0	7

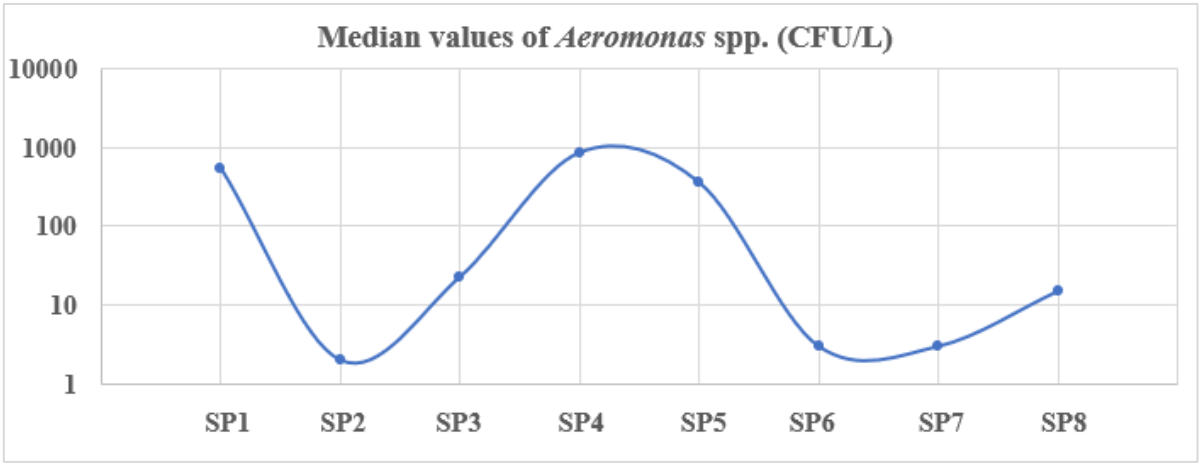

Figure 2. *Aeromonas* spp. median values (11 repeats) expressed in CFU/l for each sampling point along the treatment plant, week 38-49. Note: the value reported for SP2 represents a single sample's result in week 38.

The *Aeromonas* spp. load patterns for each sampling point over time during the study period are depicted in **Figure 3.** There is a general declining tendency for the upstream sampling points (SP1-SP5), which is present in the bio- and marble filter as well (SP9 and SP10). In comparison, the reported downstream values (SP6-SP8) have a more stable development over the weeks. SP2 is not included in this graph as the data consist of one single sample from this site.

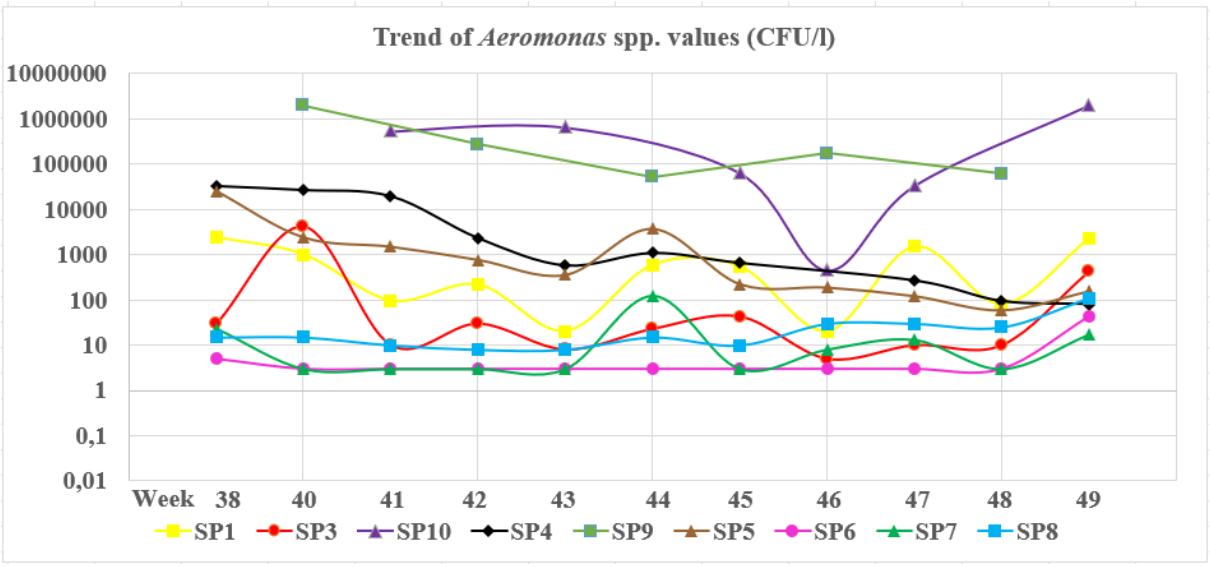

Figure 3. *Aeromonas spp.* (CFU/l) trends over time for each sampling point along the drinking water treatment plant, including marble and biofilters, week 38-49.

7.3 Comparison of *Aeromonas* spp. prevalence with other parameters of water quality

7.3.1 Heterotrophic plate count

The median value of *Aeromonas* spp. follows roughly the same trends as the median value of the HPC throughout the treatment plant, as illustrated in **Figure 4**. There is, however, a slightly steeper increase after the marble filter (SP4) and a slightly steeper decline after the UV filter (SP6) for *Aeromonas* spp. compared to the HPC. The single *Aeromonas* spp. sample of the ozone filter (SP2) also deviates from the median HPC. **Figure 5** shows that the highest percentage of *Aeromonas* spp. over the average HPC is detected in the raw water (SP1) and after the marble filter (SP4), with the lowest percentage after the UV radiation (SP6) and the ozone chamber (SP2).

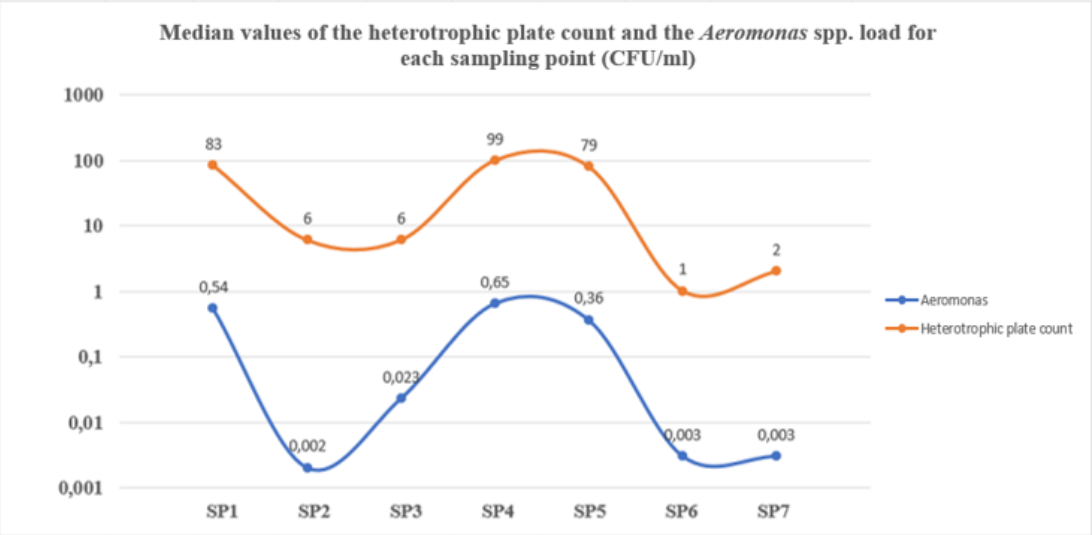

Figure 4. Median values for the load of *Aeromonas* spp. (11 repeats) compared to the median values of the HPC for each sampling point (11 repeats), weeks 38-49. Note that SP8 is omitted because the HPC is not monitored at this site.

Table 4 shows the percentage of HPC identified as *Aeromonas*. Week 38 had some of the largest fractions, of 13.3%, 19.2%, and 25.5%, measured in the raw water (SP1) and after the filters (SP4, SP5), respectively. However, the greatest proportion of *Aeromonas* species is found after the ozone chamber (SP3) in week 40, where 60% of the HPC is made up of *Aeromonas* species.

Table 4. Percentage of HPC identified as *Aeromonas*.

	SP1	SP2	SP3	SP4	SP5	SP6	SP7
Week 38	13.3	0.0	1.5	19.2	25.5	0.0	0.1
Week 40	1.8	-	60.0	9.5	2.0	0.3	0.1
Week 41	0.1	-	0.3	7.7	1.9	0.3	0.0
Week 42	0.2	-	0.6	3.4	1.3	0.0	0.3
Week 43	0.0	-	0.1	0.4	0.4	0.2	0.1
Week 44	0.5	-	0.4	1.4	4.9	0.3	6.0
Week 45	0.7	-	0.3	0.7	0.3	0.3	0.0
Week 46	0.0	-	0.1	0.7	0.2	0.3	0.8
Week 47	1.8	-	0.1	0.3	0.2	0.3	1.3
Week 48	0.1	-	0.1	0.1	0.1	0.3	0.3
Week 49	1.8	-	7.1	0.1	0.3	4.3	1.7
Average	0.9	-	5.6	5.9	3.9	0.1	0.4
Median	0.7	-	0.4	0.7	0.5	0.3	0.4

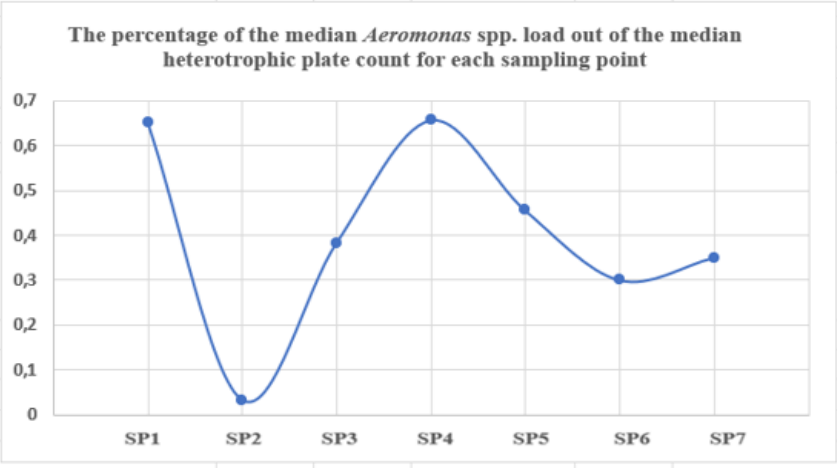

Figure 5. Percentage of the *Aeromonas* spp. (11 repeats) out of the median HPC (11 repeats). Note that SP8 is omitted because the HPC is not monitored at this site.

7.3.2 Water temperature

The water temperature reported by the drinking water treatment plant was measured weekly at the raw water sampling point (SP1). **Figure 6** depicts a continuous decrease in raw water temperature measured in degrees Celsius (°C) across the study period. After the marble- and bio filter (SP4, SP5), the sampling points, on a logarithmic scale, show a similar decreasing trend for *Aeromonas* spp. (**Figure 7)**. However, the other sampling points do not appear to follow this similar trend (**Figure 8**).

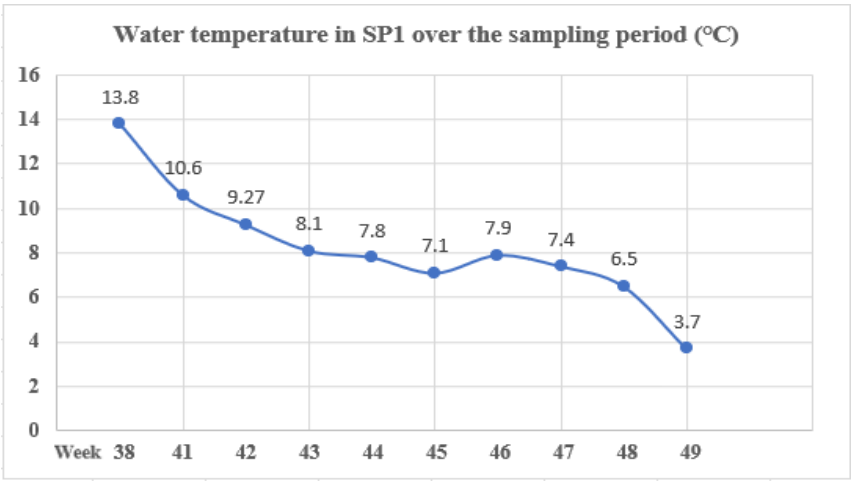

Figure 6. Water temperature measured in the raw water (SP1) throughout the sampling period (expressed in °C).

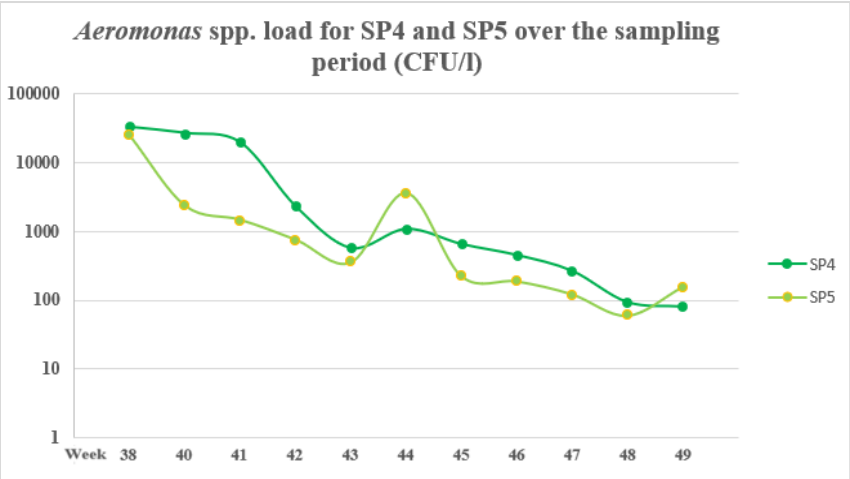

Figure 7. *Aeromonas* spp. load (CFU/l) measured after the marble (SP4)- and biofilter (SP5) over the sampling weeks.

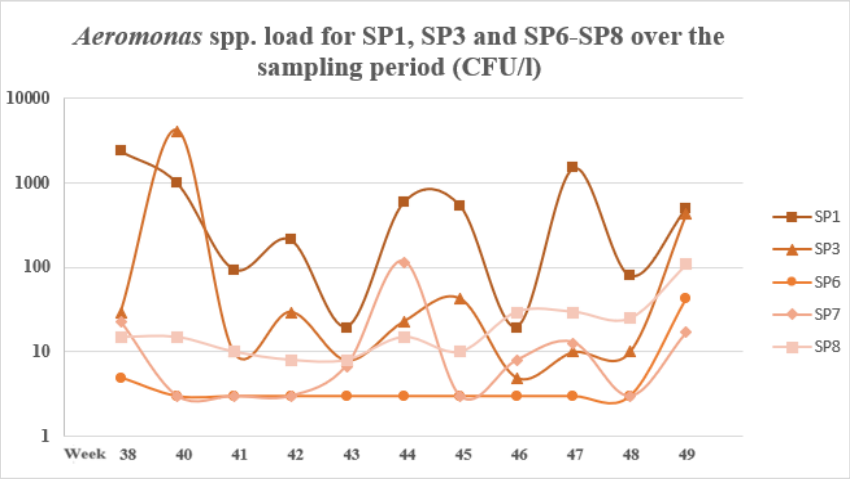

Figure 8. *Aeromonas* spp. load (CFU/l) over the sampling period, measured in the raw water (SP1), after the ozone chamber (SP3), after the UV radiation (SP6), the finished drinking water (SP7), and the water basin (SP8).

7.3.3 pH values

Figure 9 indicates that following the marble filter (SP4), the median pH value observed at the drinking water treatment facility appears to rise and remain higher after the marble filter (SP5). Besides the single sample point measured in the ozone chamber (SP2), the logarithmic development of the median *Aeromonas* spp. load appears to be following the same trend (**Figure 10**). The pH value appears to be stable for each sampling point over the research period (**Figure 11)**, while the *Aeromonas* spp. load shows more variance (**figure 12**).

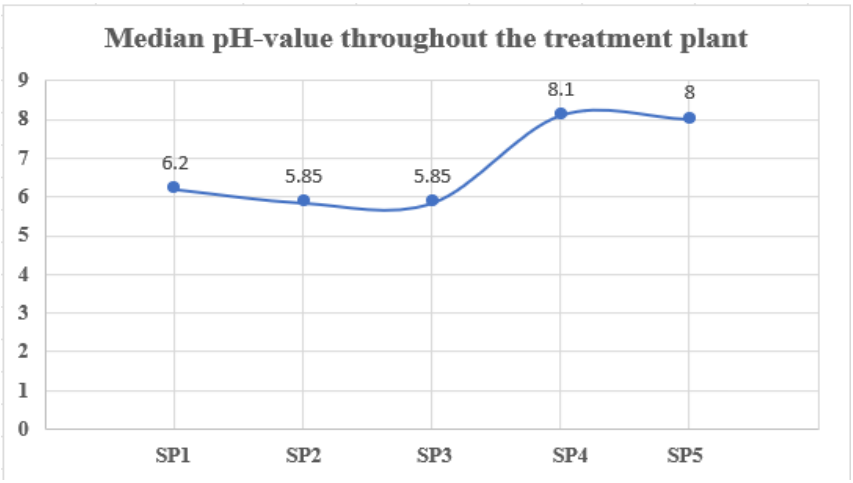

Figure 9. The trend of the median pH values for each sampling point throughout the water treatment facility (6 repeats). Note: the pH level is only monitored at SP1-SP5, with data from weeks 38 and 41-45.

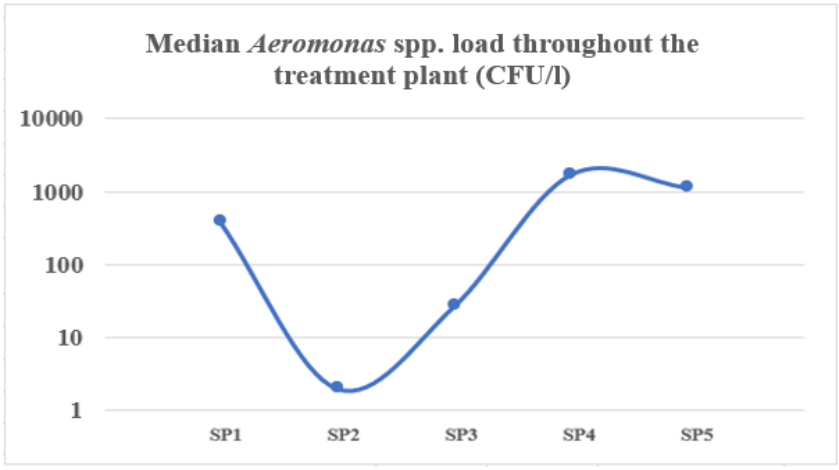

Figure 10. Trend of the median *Aeromonas* spp. values that correspond in site and location with the measured pH values (6 repeats). Note: Only the *Aeromonas* spp. samples corresponding to site and time with the pH-values are utilized for this graph for comparison purposes.

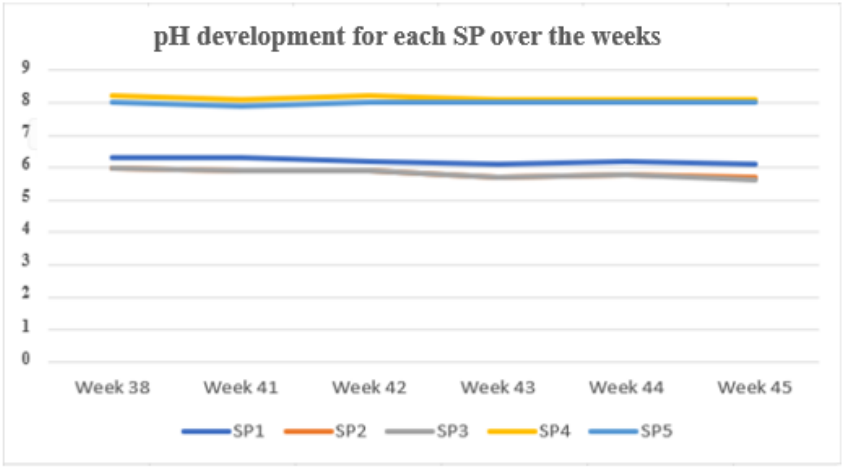

Figure 11. pH values over the sampling period for each sampling point. Note: the pH level is only monitored at SP1-SP5, with data from weeks 38 and 41-45.

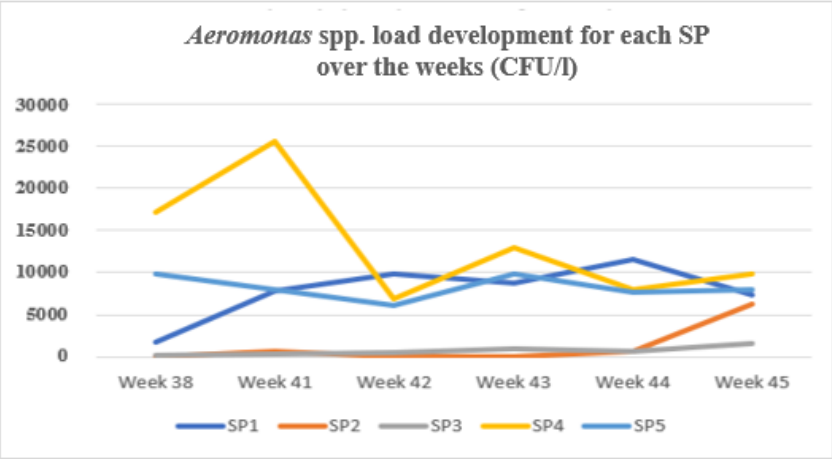

Figure 12. *Aeromonas* spp. load over the weeks for each sampling point. Only samples corresponding in time and site with the measured pH values are used in this graph for comparison purposes.

7.3.4 Other parameters: adenosine triphosphate, turbidity, and colour

In addition to the parameters compared in the above sections, the median values of several other variables have been evaluated. **Figure 13** shows that the median ATP load is clearly highest in the raw water (SP1). Although the median loads at the other sampling points vary, they do not exceed one-fifth of the median load measured at the first sampling point. The median ATP load appears to follow approximately the same logarithmic patterns as the median *Aeromonas* spp. load, as shown in **Figure 2**; the median load for both parameters decreases after the ozone chamber (SP3), with a higher quantity after the marble- and biofilter (SP4, SP5), with a lower load in the finished drinking water (SP7). Unlike the median ATP load, however, the median *Aeromonas* spp. load appears to be higher after the filters (SP4, SP5) than the raw water (SP1).

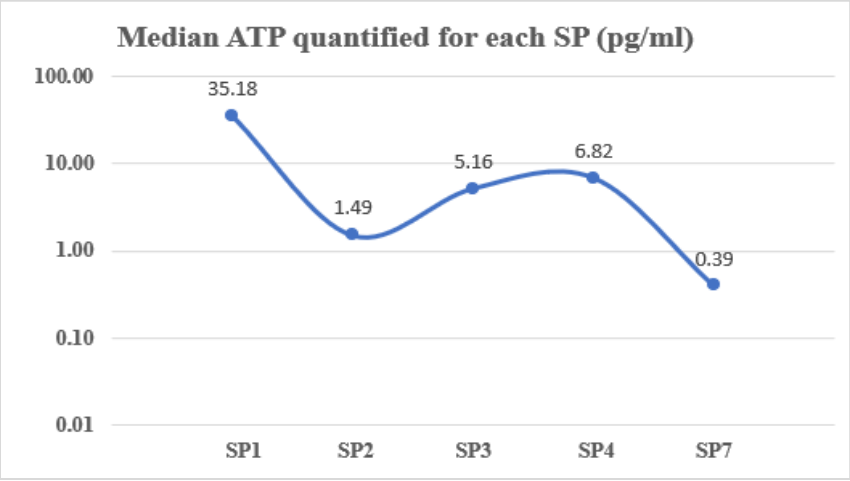

Figure 13. Median ATP quantified in picogram per milliliter (pg/ml) for each sampling point at the drinking water treatment facility (10 repeats). Note: ATP is only monitored at SP1-SP4 and SP7, with data from weeks 38 and 41-49.

Both the median turbidity (**Figure 14**) and the median color (**Figure 15),** observed during the sampling period, shows a decreasing trend throughout the treatment plant; The highest levels measured are in the raw water (SP1), and the lowest levels measured in the finished drinking water (SP7). Unlike the trend of the median, the *Aeromonas* spp. throughout the plant, shown in **Figure 2**, the median turbidity and color do not appear to increase after the marble- and biofilter (SP4, SP5).

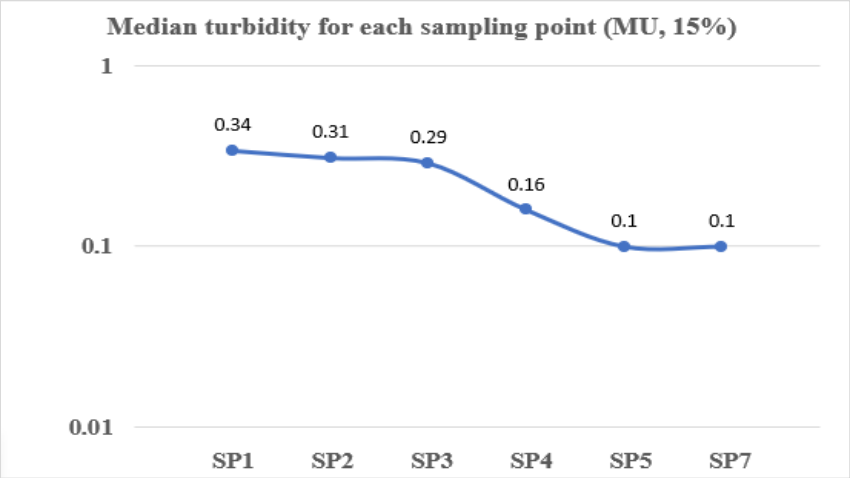

Figure 14. Median turbidity was measured for each sampling point at Langevatn water treatment facility measured in Formazin Nephelometric Unit [FNU] (11 repeats). Note: the turbidity is only monitored at SP1-SP5 and SP7, with data from weeks 38-44 and 46.

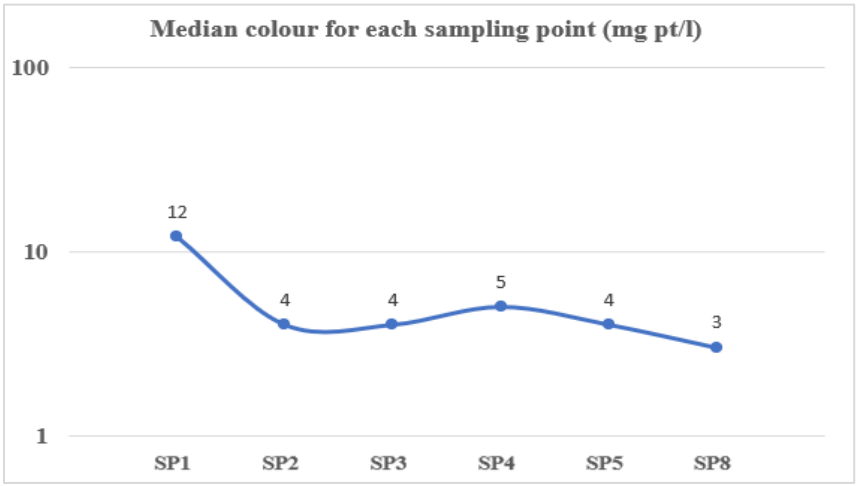

Figure 15. Median colour measured for each sampling point in milligram platina per liter (pt/l) at Langevatn water treatment facility (11 repeats). Note: the turbidity is only monitored at SP1-SP5 and SP8.

7.4 Inferential analyses

7.4.1 Correlation between water temperatures and *Aeromonas* spp. load after the filters

The relationship between raw water temperature (SP1) and both the *Aeromonas* spp. load after the marble filter (SP4) and the biofilter (SP5) appear monotonic, as indicated by **Figures 16 and 17.** The monotonic trend indicates a positive relationship between increased water temperature and *Aeromonas* spp. load after the filters, as shown by the fitted lines in the figures. The raw water temperatures (SP1) and the *Aeromonas* spp. load after the biofilter (SP5) in week 44 does not appear to follow this trend (**Figure 17**).

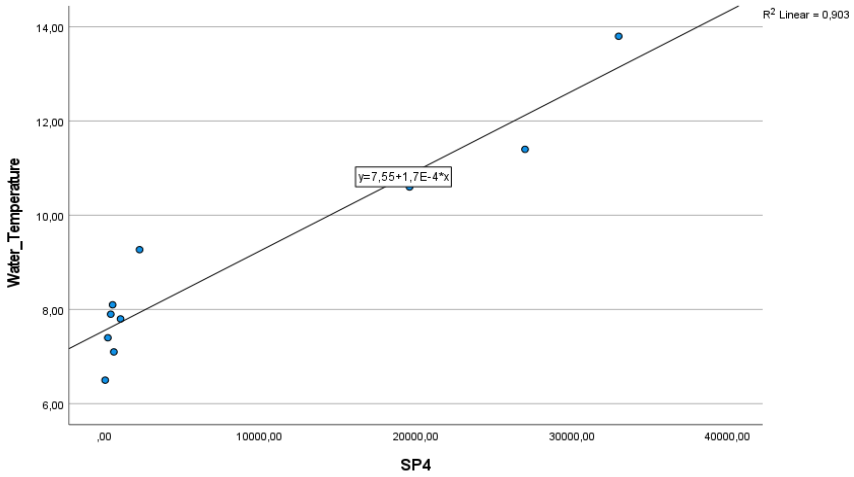

Figure 16. Scatter plot of the relationship between the raw water temperature (SP1) and the *Aeromonas* spp. load after the marble filter (SP4), with fitted line.

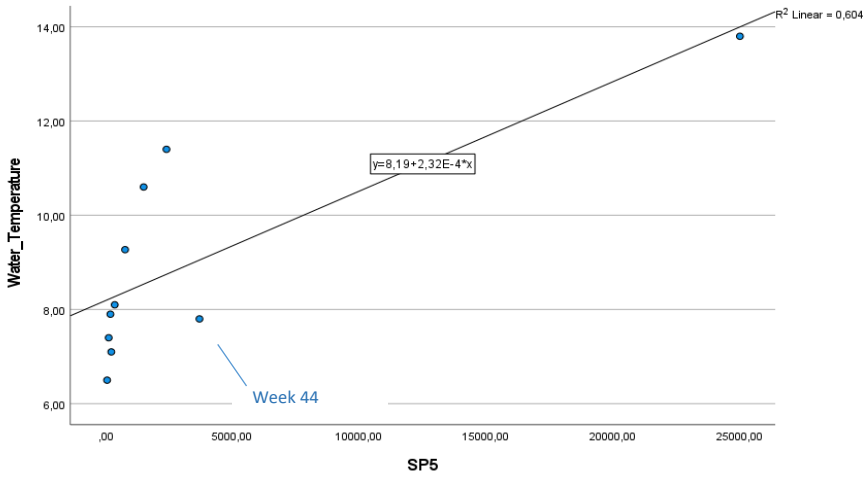

Figure 17. Scatter plot of the relationship between the raw water temperature (SP1) and the *Aeromonas* spp. load after the biofilter (SP5), with fitted line. Week 44 does not appear to follow the positive trend of the other weeks and is identified in the graph.

The results, presented in **Table 5**, show a correlation coefficient of 0.867 between the raw water temperatures (SP1) and the *Aeromonas* spp. load after the marble filter (SP4). The strength of the correlation between the raw water temperature (SP1) and the *Aeromonas* spp.

load after the biofilter (SP5) is slightly less powerful, with a correlation coefficient of 0.770. Both bivariate associations are considered statistically significant, with p-values of 0.001 and 0.009, respectively, below the α of 0.005. Interestingly, the correlation coefficient of the *Aeromonas* spp. load between the post-filter sampling points shows a statistically significant correlation coefficient with a value of 0.915 and a p-value of <0.001.

Table 5. Spearman correlation coefficient analysis, comparing raw water temperatures (SP1) with the *Aeromonas* spp. load after the marble filter (SP4) and the biofilter (SP5).

Variables	Correlation coefficient	P-value (2-tailed)
SP4 – Water Temperature	0.867	0.001
SP5 – Water Temperature	0.770	0.009
SP4 – SP5	0.915	<0.001

7.4.2 Differences between the post-filter samples and the other sampling points

The results, presented in **Table 6**, show that the *Aeromonas* spp. load after the marble filter (SP4) and the biofilter (SP5) are significantly different from the load in all other sampling points, except the raw water (SP1). The highest p-value observed for the significant associations is the relationship between the *Aeromonas* spp. load after the ozone chamber (SP3) and after the biofilter (SP5), with a value of 0.002, well below the α of 005. The relationship between the *Aeromonas* spp. load after the marble filter (SP4) and the raw water (SP1) has a p-value of 0.082, considerably lower than the p-value of 0.256 for the association between the raw water (SP1) and the sampling point after the biofilter (SP5). However, the post-filter samples are the most similar of all the tested associations, with a p-value of 0.364.

Table 6. Wilcoxon rank-sum test result of the association between the *Aeromonas* spp. load after the filters (SP4, SP5) and all other sampling points.

Variables	Wilcoxon W	Z-score	P-value (2-tailed)
SP4 – SP1	82.000	-1.739	0.082
SP4 – SP3	62.000	-3.257	0.001
SP4 – SP5	93.000	-0.907	0.364
SP4 – SP6	55.000	-3.963	<0.001
SP4 – SP7	56.000	-3.754	<0.001
SP4 – SP8	55.000	-3.790	<0.001
SP5 – SP1	90.000	-1.135	0.256
SP5 – SP3	64.000	-3.105	0.002
SP5 – SP6	55.000	-3.963	<0.001
SP5 – SP7	56.000	-3754	<0.001
SP5 – SP8	55.000	-3790	<0.001

8. Discussion

This quantitative, inductive experimental study provides new insight into the impact of various treatment steps on the prevalence of *Aeromonas* spp. in the drinking water treatment plant outside Stavanger, Norway, from a public health perspective. This chapter will discuss the study's findings, contextualized by IVAR's drinking water quality measures, and consider relevant academic literature to answer the research questions. The chapter is structured in four different sections to discuss various aspects of the results: (1) prevalence of *Aeromonas* in the drinking water treatment plant (2) prevalence of *Aeromonas* in the marble- and biofilter (3) efficacy of the drinking water treatment process against *Aeromonas* spp. (4) study limitations.

8.1 Prevalence of *Aeromonas* in the drinking water treatment plant

The median *Aeromonas* spp. load was reduced by 2.26 $\log^{10}$ from the raw water entering the treatment plant (SP1) to the clean drinking water leaving the facility (SP7), with loads of 540 CFU/l (MAD = 466) and 3 CFU/l (MAD = 0), respectively. This indicates that the treatment process as a whole is effective in reducing the pathogen's load. Other notable trends include: (i) a 1.37 $\log^{10}$ reduction in the median *Aeromonas* load from the raw water (SP1) to after the ozone chamber (SP3), (ii) a 1.45 $\log^{10}$ increase from the post-ozone chamber sampling point (SP3) to after the marble filter (SP4), (iii) a 2.08 $\log^{10}$ reduction from the post-biofilter sampling point (SP5) to after the UV radiation (SP6), and (iv) all sampling points show declining trends for the median levels of *Aeromonas* spp. over the sampling period, including the backwash water samples (SP9, SP10).

The highest median *Aeromonas* spp. load was observed in the raw water, after the marble filter, and after the biological filter, with respective quantified loads of 540, 650, 360 CFU/l (MAD = 460, 557, 300). These concentrations are more than ten times greater than the median load of the other sampling points (SP3, SP6, and SP7), which were all below 23 CFU/l and considered significantly lower by the Wilcoxon rank-sum test. These numbers suggest more favorable growth conditions for the pathogen during filtration treatment compared to other hygienic barriers at the facility. Illustrated further, the samples extracted from the backwash water of the filters (SP9, SP10) showed median *Aeromonas* amounts of 530,000 and 170,000 CFU/l (MAD = 466,000, 108,000), respectively.

The median *Aeromonas* spp. load observed in the clean drinking water (SP7) and the water stored at the water basins for distribution to consumers (SP8) was 3 and 15 CFU/l (MAD = 0, 7), respectively. The load is well below the infective dose of *A. hydrophila* 10^{10} CFU, reported by the Canadian authorities (Government of Canada, n. d.). which indicates

that the load of this opportunistic pathogen in the drinking water leaving the water treatment facility and the water distributed from the water basins is considered safe for human consumption.

8.1.1 The decreasing trends of *Aeromonas* prevalence for each sampling site over the study period

All sampling points in this study show a declining trend over the weeks, including the samples from the backwash water (SP9, SP10). The most apparent explanation for this trend is the lowering water temperatures throughout the fall. Previously cited literature states that the ideal temperature for *Aeromonas* growth is 22-35°*C* (Igbinosa et al., 2012) and 35-37°C for mesophilic *Aeromonas* in particular (Fernandez-Bravo & Figueras, 2020). Therefore, as the temperature shifted further away from their ideal temperature range during the sampling period, we may have seen a decrease in the prevalence of this bacteria. The pH value of the water is another crucial environmental parameter impacting *Aeromonas* growth, which prefers a level ranging between 5.5 and 9 (Igbinosa et al., 2012). Nonetheless, the pH level for each sample point remained steady over the weeks. and hence did not appear to be an influencing factor in the decreasing trend in *Aeromonas* over the sampling period.

8.1.2 *Aeromonas* presence throughout the plant concerning other parameters of water quality

The median HPC development throughout the treatment plant follows roughly the same patterns as the median *Aeromonas* load throughout the treatment plant. However, the HPC identified as *Aeromonas* is higher in the raw water (SP1) and after the filter (SP4, SP5) compared to the other sampling points (SP3, SP6-SP8), indicating that the various treatment steps might have a slightly different effect on *Aeromonas* species compared to the general bacterial population. Interestingly, the percentage of *Aeromonas* out of the HPC seems to decrease over the sampling period. The decreasing temperature over the weeks might explain these numbers; The ideal growth temperatures for the general bacteria population vary depending on the species, typically between 4 - 80°C (OpenStax, 2019). Suppose the bacteria population in the drinking water facility is evenly distributed throughout that range. In that case, this might explain the decreasing proportion over the weeks as the temperature moves further away from the ideal growth temperature of *Aeromonas*.

Not many studies have investigated the possible influence of colour and turbidity on the presence of *Aeromonas* in drinking water. Although Salvat and Ashbolt (2019) argue that higher turbidity is associated with more favorable growth conditions for this pathogen, studies investigating this relationship have yielded mixed results; Liu et al. (2019) found no significant correlation between turbidity levels and the prevalence of *Aeromonas* in Chinese

tap-water, while Egorov et al. (2011) discovered that *Aeromonas* had a higher likelihood of being detected in dichotomized samples with 0.5 FNU in comparison of samples with 0.1 FNU. The measured turbidity and colour of the present study, given in FNU and ptg/ml, respectively, show a declining trend throughout the treatment facility, not corresponding to the growth and decline of median *Aeromonas* load throughout the treatment process.

8.1.3 *Aeromonas* reduction after the ozone chamber

Second to the decline observed before (SP5) and after the UV radiation (SP6), the greatest $\log^{10}$ reduction in median *Aeromonas* load is observed between the raw water (SP1) and the water leaving the ozone chamber (SP3) with a $\log^{10}$ reduction of 1.37. In this estimate, the single sample from the ozone chamber (SP2) is not included. As mentioned earlier, when water pathogens come in contact with O_3 in the chamber, the cell membrane is destroyed, causing leakage and cell death (Thanomsub et al., 2002). This could explain why both the median *Aeromonas* load and the median HPC drop from before (SP1) and after (SP3) the ozone chamber. Nevertheless, the decline appears steeper for the median *Aeromonas load*, suggesting that O_3 may be more successful in activating *Aeromonas* than the general bacterial population. According to NIPH (2016), 10 minutes of exposure to 0.2 mg O3/l is enough to inactivate 99 percent of bacteria in drinking water. However, there does not appear to be an agreement upon the sufficient quantity and time of exposure to disinfect *Aeromonas* specifically.

Although limited in numbers, a few studies have sought to investigate the possible effect of O_3 on the prevalence of the pathogen. As mentioned earlier, one study showed that the gas effectively reduces *A. jandei* and *A. sobria* (Ding et al., 2019), while a few others observed a lower load of *A. salmonicida* (Liltved et al., 1995; Wedemeyer & Nelson, 1977). Investigating other species, Thanh Dien et al. (2021) were able to disinfect *A. hydrophila* quite effectively when delivering O_3 in nano-bubbles (NB-O_3). Batagoda et al. (2019) argue that treating the drinking water with NB-O_3 has advantages over traditional O_3 transmission due to increased retention time and higher concentrations. These arguments align with NIPH (2016) 's, claims that the effectiveness of O_3 as a disinfectant is dependent on the time of exposure and concentration.

Concerning other drinking water quality parameters measured in this study, the water temperature measured in the raw water (SP1) represents the temperature throughout (SP2) and after the ozone chamber, according to the treatment facility. Therefore, it is not likely to explain the reduced *Aeromonas* load throughout this treatment step. The pH level of the water

is reduced from 6.2 (SP1) to 5.85 (SP3) throughout the ozone chamber. Igbinosa et al. (2012) claim that the optimal pH level for *Aeromonas* growth is between 5.5 – 9, however, Vivekanandhan et al. (2003) state that the ideal pH level for *A. hydrophila* is between 7 and 9, which differs from Palumbo et al. (2006) results showing *A. hydrophila* growing well at pH as low as 6.5. The reduced pH from SP1 to SP3 could explain the reduction in *Aeromonas* load as the level moves further away from the suggested ideal pH for *A. hydrophila* growth at 7 – 9 (Vivekanandhan et al., 2003). Nevertheless, this suggestion is contingent on species identification of the isolates in the samples before (SP1) and after (SP3) the ozone chamber. Moreover, the pH reduction is modest, and there is a paucity of literature on the sensitivity of *Aeromonas* load in response to pH changes.

The turbidity remains relatively steady from raw water (SP1) to after the ozone chamber (SP3). In contrast, the color drops greatly from 12 mg pt/l to 4 pt/l, but the literature on the relationship between this water quality parameter and *Aeromonas* prevalence is limited. The greatest parameter reduction observed throughout the ozone chamber (SP1-SP3) was the ATP reduction from 35.18 to 5.16. Interestingly, the ATP measured in the ozone chamber (SP2) was 1.49, nearly 24 times lower than the ATP load in the raw water (SP1). Penru et al. (2013) found similar results, where ATP was nearly irradicated in sea water exposed to O_3 levels of 0.38 mg/l. As ATP is a measure of the general microbial activity in the water (Vang, 2013), it appears that the substance effectively reduces the prevalence of organic compounds in the water, with *Aeromonas* being no exception.

8.1.4 *Aeromonas* reduction after the UV radiation

The most substantial reduction in median *Aeromonas* prevalence was observed between the post-biofilter sampling point (SP5) and the water leaving the UV radiation chamber (SP6). A $\log^{10}$ reduction of 2.08 to a load consistently below 3 CFU/l, except in week 49, suggests that the hygienic barrier effectively reduces the pathogen's presence and might be a vital treatment step in the facility. All samples not containing *Aeromonas* isolates in the Latif-Eugenín et al. (2017) study had previously been treated with UV radiation and chlorine. The present study's findings might support that the UV radiation contributed to these results. Other studies investigating the relationship between *Aeromonas* and UV radiation are limited, besides Kaur et al. (2015) showing a possible susceptibility to UV radiation type C among *A. hydrophila* isolates.

Considering other parameters of drinking water quality measured in the present study, the median HPC has a more gradual decline between the sample before (SP5) and after (SP6)

the UV radiation chamber compared to the median *Aeromonas* load. As a result, the fraction of *Aeromonas* over HPC decreased accordingly through the UV chamber treatment step (SP5, SP6). With a lesser $\log^{10}$ reduction of 1.90, these results do not align with Shaban et al. (1997) 's findings that *Aeromonas* survived UV irradiation better than other organisms. However, the last cited study site was the Nile river, with many potential variables that vary from the conditions in a drinking water treatment facility. One of these is the higher turbidity levels in rivers due to contamination fra alges, mud from the soil, and industrial activities (Environment and Natural Resources, n. d.), which according to Salvat and Ashbolt (2019), is influential in *Aeromonas* growth. Nevertheless, as turbidity measures are an established indicator of microbial activity in the water (Buss da Silva et al., 2019), the relationship between this parameter and *Aeromonas* prevalence does not appear unique in a microbial context. The effect of turbidity on *Aeromonas* presence compared to other bacteria are yet to be studied. As the temperature remains steady throughout the treatment plant, it is not likely to be an influential variable in the observed difference in the median *Aeromonas* load between these two sampling points (SP5, SP6). However, the pH level is not measured after the UV radiation chamber (SP6), and it is not possible to rule out that an altered pH level might influence the presence of *Aeromonas* in the water leaving the UV chamber, following the discussion of the relationship between these parameters in chapter 8.1.3.

8.2 Prevalence of *Aeromonas* in the marble and- and biofilter

8.2.1. The backwash water

The samples of the backwash water (SP9, SP10) showed a median *Aeromonas* load substantially greater than all the treatment process samples (SP1-SP8). According to the treatment facility, the biofilter's objective is to physically prevent bacteria and particles from progressing through the water, which is also one of the functions of the marble filter. Therefore, a high load is expected at these sites. Bacteria are retained at the concentrate side of the filters as long as the entity's size is larger than the membrane opening, causing the isolates to accumulate in the filters over time as the water passes by the treatment process (NIPH, 2016).

In the biofilter, microorganisms passing through the filter will gradually accumulate and colonize the filter media. It feeds on organic substances and competes with other microorganisms it comes in contact with (Chaudhary et al., 2003). A larger bacterial load is predicted and desired at this site as bacterial growth in the biological filter is part of its primary function (Chaudhary et al., 2003), which could explain the high load of *Aeromonas*

isolated from the biofilter in the present study (SP9). The fact that *Aeromonas* seems to be a part of the bacterial colony in the water moving through the treatment facility might explain why isolates belonging to this genus have developed as a part of the natural biofilm in the filter. Further, this might also explain why the median *Aeromonas* load is roughly three times higher in the backwash water from the biofilter compared to the backwash water of the marble filter (SP10).

8.2.2 The marble filter

The highest *Aeromonas* growth throughout the treatment plant, with a $\log^{10}$ increase of 1.45, is observed after the marble filter (SP4). Pinto et al. (2012) observed that bacterial communities from several filter media were able to slough off into the water at the permeate side of the filter, causing colonization of the drinking water. These findings could explain why the *Aeromonas* load increases from the sampling point before (SP3) and after the marble filter (SP4) and is significantly higher compared to all other sampling points except the raw water (SP1) and after the marble filter (SP5). This hypothesis is supported by the fact that both the *Aeromonas* load and the HPC increase after the marble filter. However, the existing literature seems to lack in this area which mainly emphasizes the biological filters (Lautenschlager et al., 2014).

A possible slough off from the marble filter might not be the only explanation for the observed increase in median *Aeromonas* load after the marble filter (SP4). The observed fraction of *Aeromonas* spp. over the HPC is higher in the raw water (SP1) and after the marble filter (SP4) compared to all other sampling points (SP3, SP6-SP8). Considering that the bacterial colony in the filter is naturally selected, a possible peeling off from the filter media alone does not look likely to favor *Aeromonas* growth compared to other bacteria in the water. Nevertheless, different bacteria species have various generation times or reproduction rates, partly due to environmental conditions that could benefit some strains while disadvantageous to others (Kaiser, n. d.). Therefore, environmental factors in the various sampling sites could explain the discriminatory higher proportion of *Aeromonas* concerning other bacteria after the marble filter (SP4) compared to the proportion in the other samples (SP3, SP6-SP8).

As shown in Table 5, there is a significant positive correlation between the raw water temperatures (SP1), which are representable for the whole treatment plant, and the *Aeromonas* load after the marble filter (SP4). Interestingly, as the water temperatures decrease throughout the sampling period, it also appears that the proportion of *Aeromonas* load over HPC in the

marble (SP4) appears to decrease over the weeks. Following the discussion in chapter 8.1.1, these data could suggest that the temperature influences the *Aeromonas* load more than the general bacterial population after the marble filter, as the temperatures decrease further away from the preferred growth temperature of *Aeromonas* (Janda & Abbott, 2010).

One valid question to ask at this point, given that temperature is an important environmental factor influencing *Aeromonas* growth, is why the temperature development throughout the weeks is significantly correlated with the median *Aeromonas* load after the marble filter (SP4), but does not appear to follow the trend of most sites (SP1, SP3, SP6-SP8).

Part of the explanation might be statistical; Columb and Atkinson (2015) argue that small sample sizes are less likely to convey true patterns in the population with a high level of variability; hence, the probability of displaying a false negative correlation is higher under such circumstances. Most of the sampling points showing different trends compared to the water temperature in this study (SP3, SP6-SP8) have a median *Aeromonas* load below 23 CFU/l. Therefore, a possible correlation between water temperature and the median *Aeromonas* load at these sites might not be apparent. However, the median *Aeromonas* load in the raw water (SP1) does not follow the decreasing trend of the raw water temperatures despite having a median load of 540 CFU/l. This development indicates that small sample sizes alone cannot explain why only the median *Aeromonas* load in the post-filter samples (SP4, SP5) appears to follow the trend of decreasing water temperatures.

Another explanation might be that other environmental factors are more favorable for *Aeromonas* growth after the marble filter (SP4) compared to other sites throughout the treatment course. When water is filtered using marble stones, calcium from the filter is dissolved into the water, raising the pH level (IVAR, 2021; Skagen, 1993). The median pH value in the sampling point before the marble filter (SP3) had a pH value of 5.85, which raised to 8.1 after the marble filter (SP4). Following the discussion in chapter 8.1.3, the former pH is below- while the latter is within the optimum pH range for *A. hydrophila* growth reported by Palumbo et al. (2006); Vivekanandhan et al. (2003). Therefore, the pH level could contribute to the favorable growth conditions for the bacteria after the marble filter, but this argument is dependent on species identification.

The growth of median *Aeromonas* load compared to HPC might be steeper because the optimum pH level for the general bacterial population varies between taxa in a broad spectrum ranging from 1 – 11.5 (Parker et al., n. d.). As the pH reaches adequate levels for *Aeromonas*, we might observe an increase in the growth of *Aeromonas* and other bacteria that

thrive at this pH. In contrast, other bacteria's growth slows, resulting in a higher proportion of *Aeromonas* over HPC in the post-marble filter sampling point (SP4).

Following the discussion in chapter 8.1.2, the turbidity and color are not likely to explain the increased median *Aeromonas* load observed after the marble filter (SP4). The reason is that both parameters show a declining trend throughout the treatment process.

8.2.3 The biological filter

As with the post-marble filter sampling point (SP4), this study shows that the median *Aeromonas* load in the post-biofilter sampling point (SP5) is significantly higher compared to most sites (SP3, SP6-SP8). The high quantity does not appear to be caused by increased growth, but rather a low $\log^{10}$ reduction of 0.26 from the sampling point before the biofilter (SP4). Considering the high median *Aeromonas* load identified in the biofilter (SP9) it does not necessarily mean that the biofilter is ineffective in holding back the *Aeromonas* load. Despite the backwash water of the biofilter (SP9) containing three times the median *Aeromonas* load compared to the backwash water of the marble filter (SP10), the median *Aeromonas* load after the biofilter (SP5) is lower than the median *Aeromonas* load after the marble filter (SP4). As the literature is raising awareness of a possible off-peeling effect from the biofilter (Lautenschlager et al., 2014; Pinto et al., 2012), these results suggest that a possible slough off from the filter into the water, if apparent, is not higher from biofilter compared to the marble filter.

Most parameters of drinking water quality measured remain relatively stable between both the post-marble filter sampling point (SP4) and the post-biofilter sampling point (SP5): The median pH value is reduced from 8.1 to 8.0, still within the optimal levels for *A. hydrophila* growth (Igbinosa et al., 2012), the water temperature remains the same throughout the treatment plant, and the turbidity and color are reduced by 0.06 FNU and 1 mg pt/l, respectively. These relatively stable conditions might explain the low $\log^{10}$ reduction between the two post-filter sampling points (SP4, SP5) because the environmental factors have a crucial influence on the growth rate of bacteria (Kaiser, n. d.).

The fraction of HPC identified as *Aeromonas* spp. appears to decrease between the sampling point before the biofilter (SP4) and after (SP5), showing a greater reduction in *Aeromonas* spp. compared to the general bacterial population through this hygienic barrier. If, as discussed above, favorable growth conditions facilitate more remarkable *Aeromonas* growth than the HPC between the sampling point before and after the marble filter (SP3, SP4), why does the *Aeromonas* load seem to decrease more than the HPC between the

sampling point before and after the biofilter (SP4, SP5), under relatively similar environmental circumstances?

The discriminatory effect in the development of median *Aeromonas* load and median HPC following the filters (SP4, SP5) could be induced by the nature of the filters. According to Chaudhary et al. (2003), because biofilters are complex structures, it is difficult to explain the biological activities that occur while water passes through them. Further, the last cited authors support this argument by emphasizing that the biological processes depend on the composition of biofilms formed on the filter media. According to the treatment facility, the microbial content in the biofilter media of the present study is naturally selected from the water passing through it. Natural selection causes a relatively random formation of biofilm communities on the surface of the filters, with subsequently unpredictable efficiency in the bidegeneration of specific pollutants (Chaudhary et al., 2003). Based on this discussion, the biofilter in the present study might have a bacterial community that is more effective in causing *Aeromonas* degeneration than the HPC. However, it is impossible to conclude without knowing the exact biological structure of this specific biofilter (Chaudhary et al., 2003). In addition, the performance of the biofilter seems to be affected by the backwashing technique at the treatment facility (Ahmad et al., 1998), the organic loading rate, the design (Boon et al., 1997), and the contact time between the water pathogens and the filter media (Servais et al., 1994). Nevertheless, it is not clear if this altered performance is discriminatory to the biogdegenerative properties of various pathogens. The literature on the marble filter's possible discriminatory effect on various pathogens is limited.

8.3 Efficacy of the drinking water treatment process against *Aeromonas* spp.

8.3.1 *Aeromonas* prevalence in the distribution system

The levels of *Aeromonas* spp. measured in the treated drinking water of the present research (SP7, SP8) is relatively low compared to other relevant studies. A national survey in the Netherlands reported a median load of 200 CFU/l over one year in the clean drinking water leaving the treatment plant (Trouwborst, 1992). A study measuring the presence of *Aeromonas* at various sites in several water distribution systems in the USA identified *Aeromonas* in 130 out of 5,042 samples (2.6%), with a median concentration of 16 CFU/l in the positive samples, ranging from 2 – 8800 CFU/l (Egorov et al., 2011). In a Danish study, Knøchel and Jeppesen (1990) extracted water samples from several drinking water distribution systems, with samples positive for *Aeromonas* having a load ranging from 10 – 400 CFU/l. A study conducted in Sweden reported that out of 122 tap water samples from various distribution systems, 34 samples (28%) contained a load above 100 CFU/l, with a

maximum of 7500 CFU/l (Kühn et al., 1997). Similarly, Krovacek et al. (1992) found a maximum quantity of 8600 CFU/l in positive samples from several Swedish drinking water distribution systems, while Stelzer et al. (1992) measured a maximum quantity of 2400 *Aeromonas*/l in German drinking water supplies.

All the quantities measured in the cited studies are well below the infective dose of 10^{10} CFU reported by the Canadian authorities for *A. hydrophila* (Government of Canada, n. d.). The distance between the observed load and the infective load indicates that the prevalence of the pathogen might not pose a public health risk across various distribution systems and geographical locations. However, according to Janda and Abbott (2010), the risk of humans becoming infected by this pathogen through contaminated water is relatively low, citing a study reporting 7.3 cases per billion people through oral intake.

8.3.2 *Aeromonas* regrowth in the distribution system

The *Aeromonas* growth from the clean drinking water leaving the treatment facility (SP7) to the water basins (SP8) is minimal; with a $\log^{10}$ rise of 0.69, the load remains relatively stable. The increase, however, does not appear to be unique; according to Holmes et al. (1996), the typical median load of *Aeromonas* is 10 – 10,000 CFU/l in drinking water leaving treatment facilities and 10 – 1,000,000 CFU/l in the distribution system, suggesting that regrowth is observed in other water distribution systems. This idea is supported by Havelaar et al. (1990), who observed regrowth of *Aeromonas* in 16 out of 20 Dutch water distribution systems measured. Sartory et al. (n. d.) argue that regrowth of *Aeromonas* in distributed treated drinking water is expected because the pathogen is native to fresh water, even in water considered hygienically safe in terms of indicator organisms and contains low levels of nutrients. This argument could explain why a slight regrowth was observed in the current study despite the drinking water in Stavanger municipality being considered hygienically safe in terms of indicator organisms. (Statistics Norway, 2021).

8.3.3 Variables influencing the presence of *Aeromonas* in the distribution system

Supported by the cited literature in the previous sections of this chapter, Sartory et al. (n. d.) claim that *Aeromonas* are regular inhabitants of drinking water distribution networks. There are, however, limited data on the factors influencing the presence. Existing data suggest that treated water with higher pollution might cause a higher regrowth than hygienically safe drinking water (Sartory et al., n. d.). This literature could explain why we only observed a slight regrowth from the clean drinking water leaving the plant (SP7) to the water basins

(SP8) in a drinking water supply system that is considered hygienically safe (Statistics Norway, 2021).

Sartory et al. (n. d.) argue that higher *Aeromonas* populations in the distribution systems occur during warmer months. Kühn et al. (1997) support this idea, measuring the highest *Aeromonas* load in Swedish tap-water samples from August, the month with the highest average water temperature in Sweden (Worlddata.info, n. d.). Another study giving grounds to this claim was conducted by Gavriel et al. (1998), examining the incidence of mesophilic *Aeromonas* in a water distribution system in Scotland. The researchers discovered a seasonal trend, with a higher frequency of isolations occurring during the summer and with water temperatures above 12°C. These findings correspond with previously cited literature, stating that the ideal temperature for mesophilic *Aeromonas* growth is 35-37°C (Fernandez-Bravo & Figueras, 2020). Considering that the raw water temperature (SP1) is representative of all other sampling points in the study, the low regrowth of *Aeromonas* from the drinking water exiting the treatment facility (SP7) to the water basins (SP8) could be explained by the fact that raw water temperatures in this study (SP1) did not exceed 13.8°C.

As mentioned, there is not a consensus on the effectiveness of residual chlorine in the distribution network on the occurrence of *Aeromonas* spp. However, the recommended levels are between 0.1 – 0.2 mg/l (Igbinosa et al., 2012). A Spanish study isolated *Aeromonas* spp. from distribution networks with a chlorine level above the guidelines (Pablos et al., 2009), while a Lebanese study identified *Aeromonas* spp. at a chlorine level of 0.4 mg/l (Tokajian & Hashwa, 2004). According to Scoaris et al. (2008), the pathogen can resist chlorine concentrations up to six times the recommended levels of 0.1 – 0.2 mg/l. However, residual chlorine is still an essential factor in preventing the growth of the bacteria, and especially *A. hydrophila* is more susceptible to chlorine than other coliform bacteria, a common parameter of drinking water quality (Igbinosa et al., 2012; Li & Liu, 2019; Salvat & Ashbolt, 2019). According to an Italian study, the efficacy of chlorination as an *Aeromonas* disinfectant in the water distribution system may be influenced by water temperature, being two to three times more effective in winter temperatures of 5°C compared to summer temperatures of 20°C (Sisti et al., 1998). This possible relationship is strengthened further by Australian research discovering that the mean *Aeromonas* load followed the mean water temperature when the water was either free from chlorine or had a level less than 0.3 mg/l (Burke et al., 1984). Holmes et al. (1996) argue that the probability of *Aeromonas* occurrence increases greatly in water with a temperature above 14°C and chlorine levels below 0.1 mg/l. The added chlorine

in the chamber is lowered from 0.3 mg/l to 0.05 mg/l after 30 minutes of contact time, according to the drinking water facility, and the highest temperature measured was 13.8°C. These findings suggesting that the risk of *Aeromonas* regrowth might be higher in warmer months when the temperfuature exceeds 14°C due to the low chlorine concentrations (Holmes et al., 1996).

In their study, Havelaar et al. (1990) discovered that the highest level of *Aeromonas* was detected in the distribution network of drinking water, leaving a treatment facility with a groundwater source. Further, a study reported that the density of *Aeromonas*- and the rate of biofilm formation in the drinking water were correlated when the water was originating from groundwater sources (Sartory et al., n. d.). In contrast, the water treatment facility of the present study receives its raw water from surface water sources (Stavanger kommune, 2019). However, the literature on this possible connection is limited. It is impossible to draw any conclusions on whether it could contribute to the low levels of *Aeromonas* observed in the water basin (SP8).

8.4 Study strengths and limitations

The research project has several strengths. Primarily, to the best of our knowledge, this is the first study to investigate the prevalence of *Aeromonas* species along the course of a drinking water facility in Norway, and therefore contributing to the fulfillment of a knowledge gap of potential public health relevance, following the literature review in chapter 5. Another strength is that the samples were taken from all sampling points included in the established water quality monitoring program at the treatment facility, providing a thorough description on the prevalence of *Aeromonas* along the process from the raw water until reaching the consumers. Finally, this study is a collaboration of several organizations consisting of engineers, microbiologists, and public health scientists, providing a multidisciplinary perspective on the research question, the process, and the results.

This study also has somelimitations. Because of the *Aeromonas* species' strong seasonality indicated by several studies (Batra et al., 2016; Bhowmick & Bhattacharjee, 2018; Gonçalves Pessoa et al., 2019), one of the study's primary limitations is that the sampling phase was solely undertaken in the fall and early winter. All of the latter cited studies indicate that the prevalence and the risk of human infections are higher in the summer season with an increased risk of human infections. The present study showed the measured median *Aeromonas* load in the distribution system (SP7, SP8) may not pose a public health risk,

according to the infectious dose for *A. hydrophila* reported by the Government of Canada (n. d.).

Another limitation of this study is that few repeats for each sampling point were used to calculate the central tendency of the various parameters. With a maximum of 11 unique samples for each site, the calculation of the central tendency might show a higher level of variability- and is less likely to convey the authentic patterns in the population (Columb & Atkinson, 2015). Krithikadatta (2014) argues that small sample sizes are an essential factor causing non-normally distributed data. Therefore, the few repeats might have contributed to the non-normality of the data (**Appendix A** and **Appendix B)**, resulting in the need to use non-parametric statistics and the median as a measure of central tendency (Pupovac & Petrovecki, 2011), as described in **Appendix C**. Additional samples from each location could potentially have strengthened the data, which would increase the likelihood of capturing the true tendencies of the population in the statistical analyses (Baldi & Moore, 2018).

In continuation of the discussion of the paragraph above, this study had to make decisions based on time and budget. One example was the decision to weekly alternate between which filter to sample (SP9 SP10) and leave out the ozone chamber sampling point (SP2) after the characterization period. The results were fewer repeats, with the possible consequences discussed above.

8.5 Suggestions and recommendations

In light of the results of our analysis, further research studies filling research gaps and improving our understanding of the prevalence of *Aeromonas* spp. in Norwegian drinking water treatment plants are needed. These further investigations could consider extending the study period to include different seasons, particularly focusing on the summer months. In addition, there is a paucity of data on the prevalence of *Aeromonas* spp. in Norwegian drinking water settings, therefore it could be useful to investigate the prevalence of *Aeromonas* in different treatment facilities and geographical regions throughout the country to further evaluate the relevance of such opportunistic pathogen.

Another recommendation is regarding species identification. Indeed, further studies focusing on species identification of *Aeromonas* in Norwegian drinking water treatment plants, especially at other sites of the treatment course, could help us to better comprehend the public health risk connected with the presence of this bacteria. In addition, further studies focusing on the infectious dose by species, both in immunocompetent and immunocompromised individuals, are needed to better understand the public health impact of

such infections. Indeed the infectious dose reported by the Government of Canada (n. d.) is solely for *A. hydrophila*. Although it is the most common species, it is not the only species compromising the genus *Aeromonas.* Further microbiological characterization such as analysis of AMR and virulence of isolated strains could enrich discussion from the public health perspective.

9. Conclusion

This study provides new scientific insights on this opportunistic pathogen and its presence in drinking water treatment plants taking into account the public health perspective. Three major conclusions can be made from this study.

Due to the significantly increased median load seen in this study after the marble filter (SP4) compared to all other sample points except the raw water (SP3, SP6-SP8), the first conclusion is that the marble filter may be associated with *Aeromonas* growth. Although the load decreases from before (SP4) and after (SP5) the biofilter, it remains significantly higher than the above-mentioned sampling points (SP3, SP6-SP8). One explanation might be that the pH values observed after the filter (SP4, SP5) are higher compared to the water sampled in the preceding sampling points (SP1-SP3), which is within the optimal growth conditions for *A. hydrophila* (Palumbo et al., 2006). However, while the pH value remained stable for each sampling point throughout the sampling period, the *Aeromonas* showed variance within each sampling site, indicating that increased pH levels might not be the sole explanation for the growth of this pathogen. However, more research studies in Norwegian settings are needed before the findings can be generalized, encompassing different water systems and periods.

The second conclusion is that UV radiation appears to reduce the median *Aeromonas* load in the treatment plant, with the greatest $\log^{10}$ decline, of 2.08, observed in the treatment process occurring between the sample sites before (SP5) and after (SP6) the radiation chamber. The irradiated water consistently had an *Aeromonas* load below 3 CFU/L, except for an outlier in week 49 (Table 3), which highlight that the UV radiation is an important factor in reducing the prevalence of this pathogen in the treatment facility.

Finally, the third conclusion is that the median *Aeromonas* load observed in the treated water (SP7, SP8) is greatly lower than the infectious dose reported in the literature (Government of Canada (n. d.). Although the quality of the drinking water is considered good and safe for human consumption, additional studies focusing on *Aeromonas*'s infectious dose for both immunocompetent and immunocompromised individuals, as well as microbiological characterization of *Aeromonas* species isolated along the treatment plant could provide useful information in further assessing the public health risk associated with this opportunistic pathogen.

10. References

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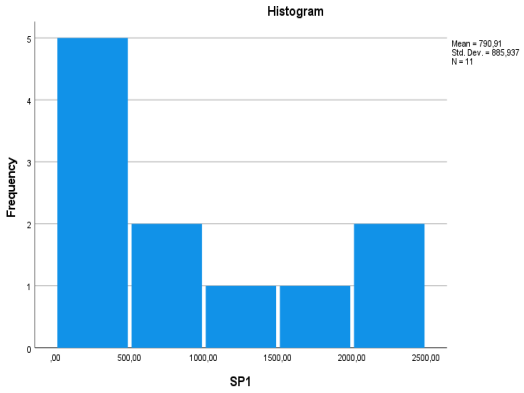
APPENDIX A

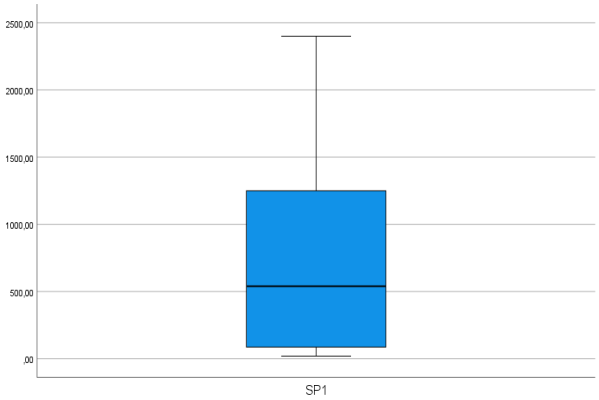
Test for normality for each sampling point

	Kolmogorow – Smirnov[K-G]	Shapiro-Wilk [S-W]	Skewness	Kurtosis	Outliers
SP1	0.122	0.20	1,010189	-0,393144	0
SP3	<0.001	<0.001	3,261322	10,715042	2
SP10	0.151	0.116	1,611386	2,800169	1
SP4	<0.001	<0.001	1,395788	0,294723	2
SP9	0.005	0.002	2,174134	4,771751	1
SP5	<0.001	<0.001	3,171526	10,264119	1
SP6	<0.001	<0.001	3,301879	10,926299	2
SP7	<0.001	<0.001	3,084739	9,827235	1
SP8	<0.001	<0.001	2,869696	8,784894	1

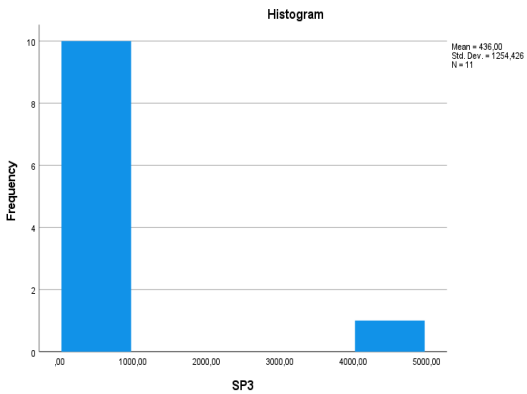
Note. Several parameters of normality measured in SPSS. Reference value for K-G and S-W: P > 0.05. Reference value for skewness and kurtosis: -3 – +3 and -10 – +10, respectively (Brown, 2006). Number of outliers is based on the box plots presented below.

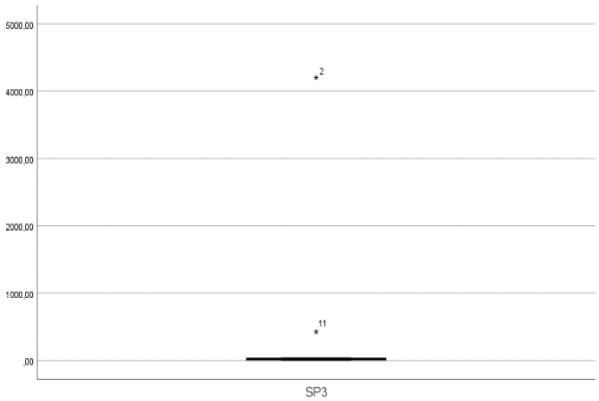
SP1



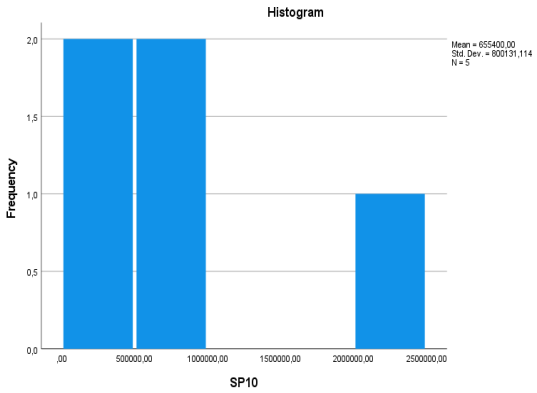


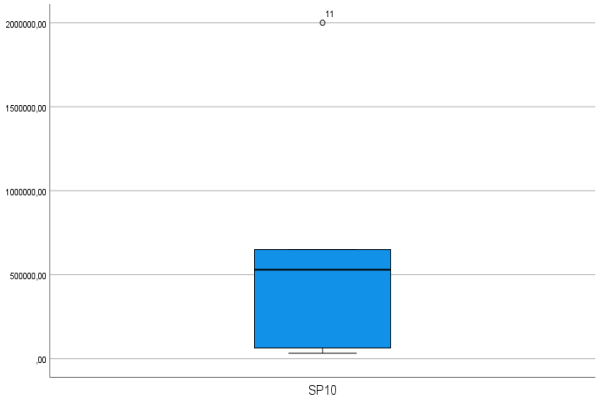
SP3



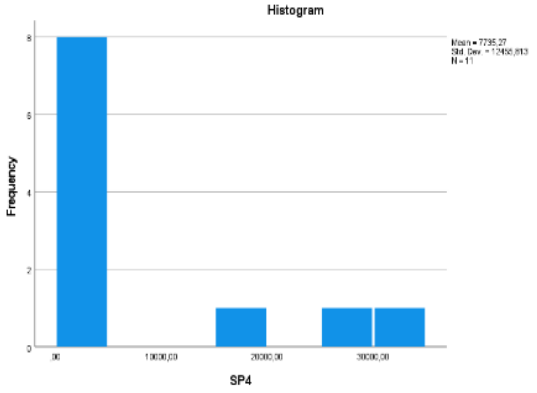


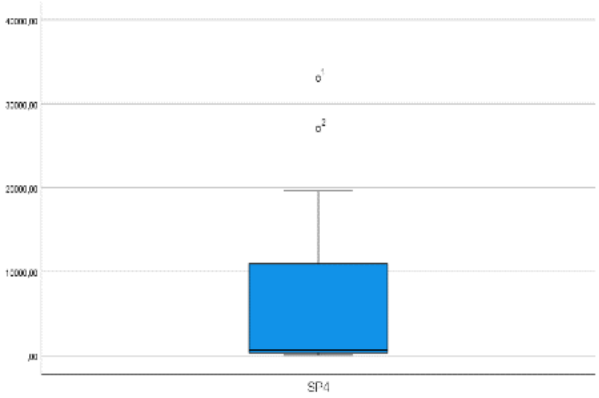
SP10


Histogram
Mean = 655400,00
Std. Dev. = 800131,114
N = 5
Frequency
SP10

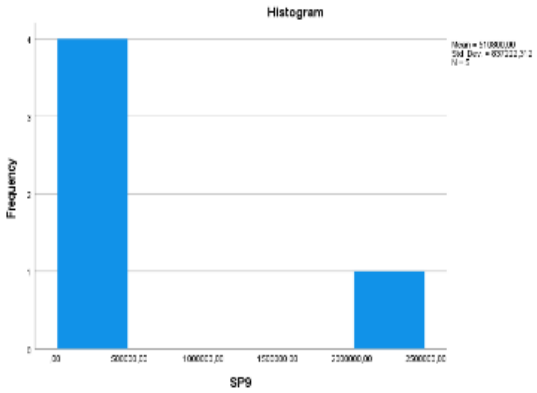

SP10

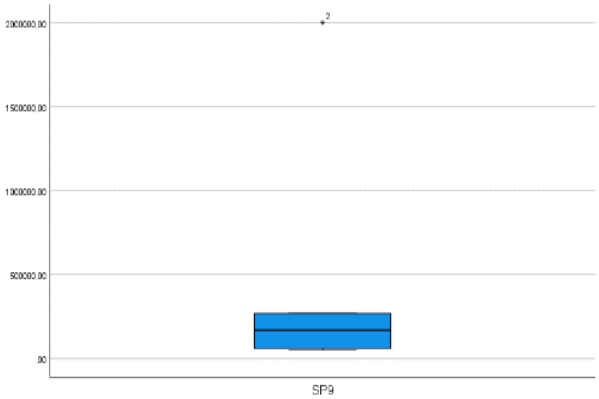
SP4


Histogram
Frequency
SP4

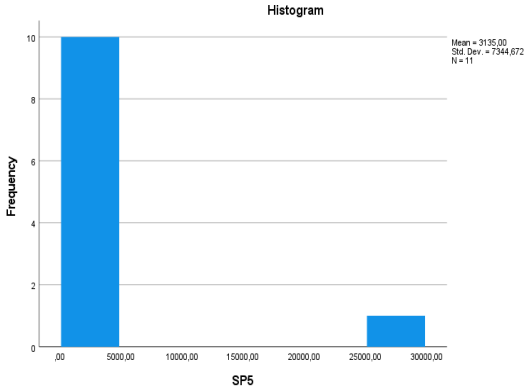

SP4

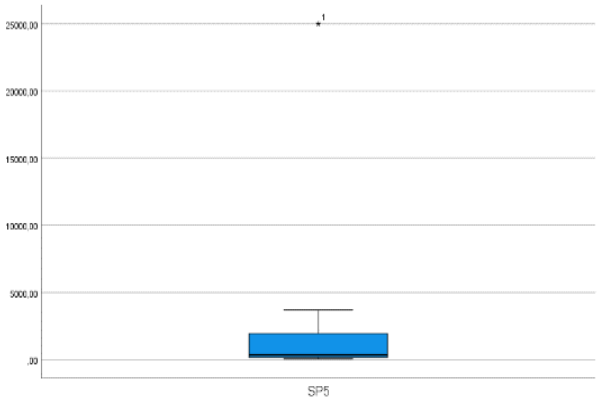
SP9


Histogram
Frequency
SP9

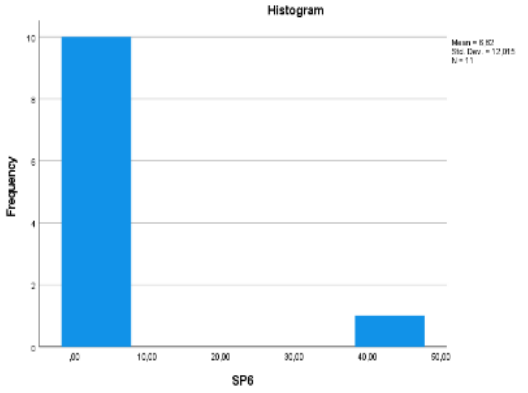

SP9

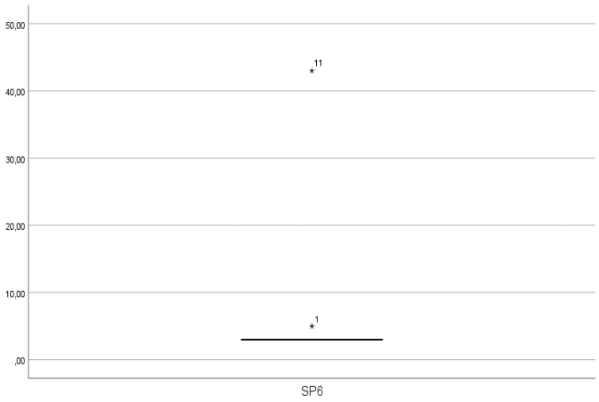
SP5



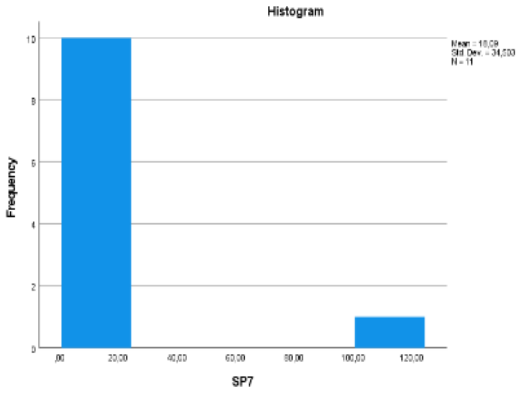


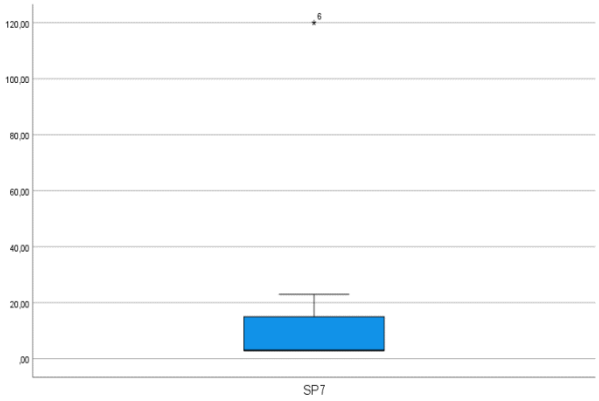
SP6



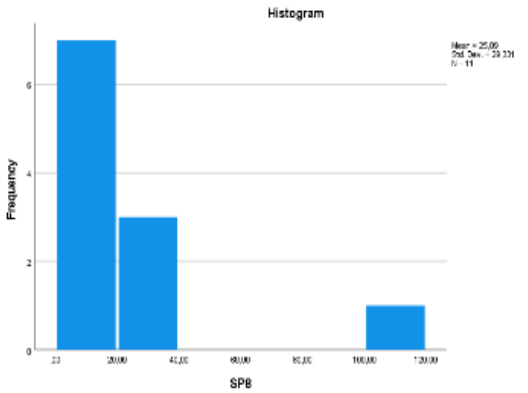


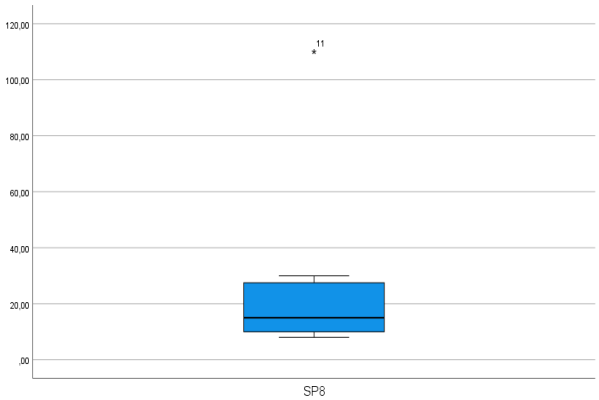
SP7





SP8





APPENDIX B

Test for normality of difference between sampling points

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
SP4SP1	,379	10	<,001	,703	10	<,001
SP4SP3	,382	10	<,001	,696	10	<,001
SP4SP5	,351	10	<,001	,731	10	,002
SP4SP6	,385	10	<,001	,689	10	<,001
SP4SP7	,385	10	<,001	,688	10	<,001
SP4SP8	,385	10	<,001	,689	10	<,001
SP5SP1	,382	10	<,001	,524	10	<,001
SP5SP3	,376	10	<,001	,517	10	<,001
SP5SP6	,386	10	<,001	,491	10	<,001
SP5SP7	,391	10	<,001	,489	10	<,001
SP5SP8	,386	10	<,001	,492	10	<,001

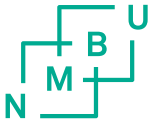
a. Lilliefors Significance Correction

APPENDIX C

Arguments for statistical tests

The most common test for a bivariate relationship between continuous variables is the *Pearson correlation coefficient,* which assumes a linear relationship and that the variables are non-normally distributed (Schober et al., 2018). The test for normality below shows that *Aeromonas* spp. samples are not normally distributed **(Appendix A),** and the relationship between the variables looks to be non-linear (**Figure 15, 16)**. It does, however, appear to be monotonic (a constant positive or negative relationship), which meets the criteria for a *Spearman correlation coefficient* test because both variables are measured at a numerical level (Statistical Solutions, n. d.). Therefore, the latter statistical method was chosen to investigate a possible relationship between the *Aeromonas* spp. load in the bio- and marble filter (SP4, SP5), and the water temperature measured in the raw water (SP1).

Second, to investigate whether the *Aeromonas* spp. load after the filters (SP4, SP5) are significantly higher compared to the other sampling points throughout the treatment plant. A suitable statistical test would be the *two-sample t-test* which investigates the difference between two groups of continuous measures and compares the post-filter sampling points with the other sampling points at the plant (Ford, 2017). However, the tests presented in **Appendix B** show that the differences in *Aeromonas* spp. load between the sampling points are not normally distributed, which is a requirement for a two-sample t-test (Ford, 2017). As an alternative, the present study used the *Wilcoxon rank-sum test*, which does not assume any known data distribution (Ford, 2017).



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway