UNITED NATIONS UNIVERSITY
Fisheries Training Programme

unuftp.is Final Project 2016

STUDIES OF EXPERIMENTAL INFECTIONS OF ARCTIC CHAR (*Salvelinus alpinus* L.) WITH *Aeromonas salmonicida* subsp. *achromogenes*

Nguyen Thi Thuy Giang
Nha Trang University
N$_0$ 2 Nguyen Dinh Chieu, Nha Trang, Khanh Hoa, Vietnam
E-mail: giangntt@ntu.edu.vn

Supervisor:
Bjarnheidur K. Gudmundsdottir
Faculty of Medicine, School of Health Sciences, University of Iceland
Læknagardur, Vatnsmyrarvegur 6, 101 Reykjavik.
E-mail: bjarngud@hi.is

ABSTRACT

A gradually increased mortality caused by atypical furunculosis induced by *Aeromonas salmonicida* subsp. *achromogenes* (Asa) in vaccinated Arctic char (*Salvelinus alpinus*, L.) farmed in Iceland has been reported. Therefore, resistance to atypical furunculosis has been included in the breeding aims for Arctic char in Iceland. Selection of genetic disease resistance and evaluation of vaccine efficacy requires reliable and applicable challenge methods. The aims of the present study were to develop an effective and reliable method for challenge of Arctic char with Asa, and to describe the gross pathology of atypical furunculosis in Arctic char. First a cohabitation challenge was performed where injection infected donor fish was cohabitated with vaccinated and unvaccinated fish. Different numbers (12, 18 & 20) of donor fish infected with Asa, strain Keldur265-87, were introduced into 4 tanks (2 replicates), once at water temperature 8^{o}C and twice at 12^{o}C. An immersion challenge was performed by bathing 200 unvaccinated char in a 120L of a suspension of Asa, strain F131-16 (10^6 CFU/mL) at 13^{o}C. Moribund and dead fish were collected for pathological examinations. Disease signs included haemorrhages of fins, tail, skin, muscle and internal organs, pale skin and gill colour, loss of appetite and gaping. The cohabitation challenge induced very weak disease transmission under the conditions applied in this study and vaccinated fish were fully protected. The survival estimates were significantly elevated when the water temperature was increased (p= 0.015). Immersion challenge of char resulted in high and acute mortalities. The cumulative mortality reached 98.6 % (± 2.61) in 17 days with a mean day to death of 7.13 ± 0.23. Virulence of strain F131-16 to char was found to be superior to that of strain Keldur265-87. Strain F131-16 was recently isolated from vaccinated char with atypical furunculosis, but strain Keldur265-87, originating from diseased Atlantic salmon (*Salmo salar* L.), has been kept frozen for 3 decades, but occasionally passaged (injected into fish and re-isolated from the head kidney of dead fish) in fish. An interesting next step would be to try a cohabitation challenge of char with strain F131-16.

This paper should be cited as:
Nguyen, T.T.G. 2018. *Studies of experimental infection of Arctic char (*Salvelinus alpinus *L.) with* Aeromonas samonicida *subsp.* Achromogenes*.* Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/giang16prf.pdf

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

1.1 Statement of the problem

1.1.1 In Vietnam

The aquaculture sector has been considered as one of the key economic sectors of Vietnam. In 2015, the total aquaculture production was 3.53 million tons, which valued at 6573 million $US and ranked of the 5th highest export earners (VASEP, 2015). However, disease outbreaks, resulting in high mortalities and serious losses, are a significant constraint to the further development of this industry (Ferguson *et al.*, 2001; Crumlish *et al.*, 2010; Halls & Johns, 2013). Motile *Aeromonas* septicaemia (MAS) is considered as a major threat to aquaculture in Vietnam, where the main aetiological agent is identified as *Aeromonas hydrophila* (Ly *et al.*, 2009; Crumlish *et al.*, 2010; Gudmundsdottir & Bjornsdottir, 2017). Severe losses caused by MAS have been documented in cultured catfish as the outbreaks frequently occur in larger fish that are at or near market size and it spreads rapidly throughout the population with mortality ranging from 20-80% (Ly *et al.*, 2009). Moreover, MAS induces high mortalities in many other farmed warm water fishes. Most fish and many aquatic invertebrates are susceptible to disease caused by *A. hydrophila*. The bacterium also causes disease in amphibians, reptiles, birds and mammals, including humans (Cipriano *et al.*, 1984; Cipriano, 2001; Janda & Abbott, 2010). The strains of the species are a heterogeneous group that show a variety of pathological characteristics, depending on the host and virulence properties of the infecting strain. This complicates the development of effective protective measures such as vaccination (Janda & Abbott, 2010; Gudmundsdottir & Bjornsdottir, 2017).

Public concerns have been raised regarding the use of antibiotics and chemicals to control disease outbreaks in aquaculture, as bacterial resistance is a severe health and environment risk (WHO, 2016). Hence, environmentally friendly disease prevention methods, which do not hamper fish health, are a key to a successful and sustainable aquaculture. Vaccination is considered to be an environmentally friendly prophylactic method to reduce the losses caused by disease outbreaks. Vaccination has become not only a preventive method against various pathogens in aquaculture, improving fish farming, but has also lowered the use of antibiotics dramatically (Hastein *et al.*, 2005; Sommerset *et al.*, 2005; Brudeseth *et al.*, 2013). However, no licensed vaccine against MAS is available in Vietnam hitherto. The first commercial fish vaccine was licensed in Vietnam in 2013, which is an injection vaccine developed to protect pangasius (*Pangasius hypophthalmus* Sauvage) against white spot disease induced by the bacterium *Edwardsiella ictaluri* (Pharmaq, 2013). Vaccination can, however, not be applied for crustaceans like shrimp, as they do not have immune memory (Rowley & Pope, 2012).

To accomplish a sustainable growth of aquaculture in Vietnam, the development of prophylactic methods, including vaccination and improvement of disease resistance is essential. Thanks to a finding of genetic associations to disease resistance in aquatic organisms by Drangsholt *et al.* (2011), selection of genetic traits related to disease resistance may lead to enhanced prophylaxis in aquaculture. Thus, disease resistance should be included in the breeding aims in aquaculture. *A. salmonicida* and *A. hydrophyla*, belong to the same genus and share many virulence properties (Gudmundsdottir & Bjornsdottir, 2017). The project that is about to start in Iceland with the aim to reduce susceptibility of Arctic char (*Salvelinus alpinus*, L.) to atypical furunculosis caused by *Aeromonas salmonicida* subsp. *achromogenes* via genetic selection, can be of great importance to

the Vietnam's aquaculture. Furthermore, the development of suitable challenge models to evaluate vaccine efficacy may be of importance for the development of vaccines for Vietnamese aquaculture.

1.1.2 In Iceland

Furunculosis causes severe septicaemia and high rates of mortality in salmonid fish such as Arctic char. In Iceland, A. *salmonicida* subsp. *achromogenes* causing atypical furunculosis is endemic in the Icelandic waters (Gudmundsdottir & Magnadottir, 1997). Although Arctic char farmed in Iceland are vaccinated against the disease, increasing mortalities of Arctic char due to atypical furunculosis just before they reach market size, have been (Figure 1). In other words, the vaccine induced protection of the char appears to decrease gradually with time. This may contribute to reduced resistance in the late stages of the on-growing phase of Arctic char.

Although the heritability of resistance to atypical furunculosis caused by *A. salmonicida* subsp. *achromogenes* has not been estimated in salmonids, results of preliminary studies suggest that it may be possible to select for increased resistance to this disease in Arctic char (Drangsholt *et al.*, 2011; Yanez *et al.*, 2014). For this reason, a project has been started in Iceland, which aims to reduce susceptibility of Arctic char to atypical furunculosis caused by *A. salmonicida* subsp. *achromogenes* by selection of the trait of higher resistance to the disease for the Icelandic char breeding program carried out by Holar University, Iceland. Moreover, there may be a genetic correlation between resistance to different diseases (Odegard *et al.*, 2007) and, therefore, selection for increased resistance to one disease may improve the overall resistance of the fish.

1.2 Project objectives

Challenge methods that reflect natural infection are a prerequisite for evaluation of vaccine efficacy and selection of disease resistant fish families. The aims of this study were to develop a method to challenge Arctic char with *A. salmonicida subsp. achromogenes*, which mimic the natural routes of the infection, and to describe the gross pathology of atypical furunculosis in Arctic char.

2 LITERATURE REVIEW

2.1 Overview of Arctic char farming

Arctic char is a cold-water fish species in the family *Salmonidae*, commonly found around the Arctic waters, hence the name Arctic char. The species was first ordered in the genus *Salmo* before moved into the genus *Salvelinu*s. The genus name *Salvelinus* is from German "Saibling" - little salmon. The fish species is well-known as the most northerly of all freshwater fish species and native to Austria, Canada, Britain, Ireland, Faroe Islands, Finland, France, Germany, Greenland, Iceland, Ireland, Italy, Norway, Russia, Scotland, Sweden, Switzerland, the United States (Brunner *et al.*, 2001; Jonsson & Jonsson, 2001; Wilson *et al.*, 2004; Maitland *et al.*, 2007; Freyhof & Kottelat, 2008; Shikano *et al.*, 2015).

Arctic char is known for both anadromous and landlocked resident population (Jonsson & Jonsson, 2001), for its ability to grow well at low temperatures and for being tolerant of high stocking densities, which makes them an ideal candidate for intensive farming in Nordic countries (Heasman & Black, 1998; Jobling *et al.*, 1998; Sæther *et al.*, 2013). Successful breeding programs have been carried out in Iceland and Sweden so that the interest for farming of Arctic char is increasing (Nilsson *et al.*, 2010; Brannas *et al.*, 2011). Certification of Arctic char as sustainably produced may guarantee a further improvement of the marketing opportunities (Eriksson *et al.*, 2010; Arnason *et al.*, 2015). Farming of Arctic char is relatively recent in Iceland, Canada, Sweden, Norway, Finland, Estonia, West Virginia, British Isles and Ireland (Summerfelt *et al.*, 2004; Maitland *et al.*, 2007; Pickova *et al.*, 2007; Skybakmoen *et al.*, 2009; Eriksson *et al.*, 2010; Paisley *et al.*, 2010; Brannas *et al.*, 2011; Hermansen & Troell, 2012; Sæther *et al.*, 2013). Iceland is the largest exporter of farmed Arctic char in the world. The global annual production of Arctic char in aquaculture is approximately 6000 - 10000 tones with half the total production originating from Iceland (Hermansen & Troell, 2012).

Wild Arctic char can be found in lakes all around Iceland (Freyhof & Kottelat, 2008) and it is the most common and widespread salmonid fish farmed in Iceland (Paisley *et al.*, 2010; Hermansen & Troell, 2012). In recent years, the production capacity of farms has increased and about 22 farms produced Arctic char in the year 2016. Twenty one land-based farms use flow through systems and one farm has cages in a brackish water lagoon (Sæther *et al.*, 2013). An important step in the development of Arctic char farming in Iceland was taken when a governmental breeding program was initiated in 1992. Production of Icelandic Arctic char has increased from about 500 tonnes in 1995 up to 3,500 tonnes in 2012 and is expected to exceed 4.000 tons in 2016 (www.fisheries.is/aquaculture/species/arctic-char/).

Infectious disease in Arctic char can be caused by a number of pathogenic species that belong to the bacteria, fungi, protozoan, metazoan, or crustacean groups (Kristmundsson & Richter, 2009; Kristmundsson *et al.*, 2010; Blasco-Costa *et al.*, 2014). The main infectious disease in Icelandic char farming is atypical furunculosis induced by the bacterium *A. salmonicida* subsp. *achromogenes* (Gudmundsdottir & Bjornsdottir, 2017). Other diseases that have caused problems are bacterial kidney disease (BKD), caused by the bacterium *Renibacterium salmoninarum* (Jonsdottir *et al.*, 1998); proliferative kidney disease, caused by the myxozoan parasite *Tetracapsuloides bryosalmonae* (Kristmundsson *et al.*, 2010); and winter ulcer disease, caused by the bacterium *Moritella viscosa* (Gudmundsdottir & Bjornsdottir, 2007). Regarding virus

infections, Iceland is unique in the world as aquaculture is free from viral diseases (Hastein *et al.*, 2001).

Most of the char farmed in Iceland are vaccinated against atypical furunculosis, using a commercial vaccine based on a different subspecies of the bacterium, or *A. salmonicida* subsp. *salmonicida.* The protection was found to be good, but in the recent years the mortality of vaccinated fish that is reaching the market size has being increasing (Figure 1).

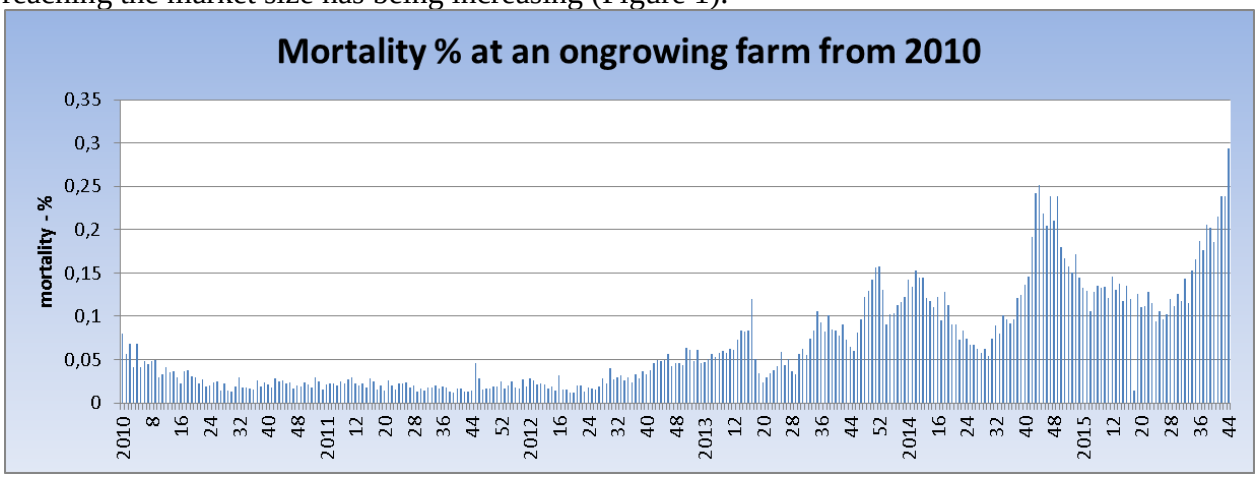


Figure 1. Weekly mortality (%) of vaccinated Arctic char (0.1-1.6 kg) caused by *A. salmonicida* subsp. *achromogenes* at an on-growing fish farm in Iceland. By courtesy of Heiddis Smaradottir.

Hence, for selective breeding programmes, considered as the key for sustainable aquaculture (Olesen *et al.*, 2003), improved resistance to diseases is crucial, along with other renowned objectives such as faster growth, later sexual maturity or better flesh quality. In Iceland, selective breeding programmes exist for Atlantic salmon and Arctic char as well as Atlantic cod (http://www.fisheries.is/aquaculture/slective-breeding).

2.2 Furunculosis, a serious disease in aquaculture

Furunculosis is a worldwide serious disease problem occurring in many species of cultured and wild fish, both in fresh water and sea water. The culprit of the disease is classified as the bacterial species *A. salmonicida* (Austin, 2011; Cipriano & Austin, 2011; Austin & Austin, 2012; Menanteau-Ledouble *et al.*, 2016; Gudmundsdottir & Bjornsdottir, 2017). High mortalities caused by *A. salmonicida* have been reported, especially from areas in the northern hemisphere (Wiklund & Bylund, 1993; Gudmundsdottir, 1998; Wiklund & Dalsgaard, 1998; Gudmundsdottir & Bjornsdottir, 2007; Austin, 2011), such as in salmonids species (Harmon *et al.*, 1991; Magnadottir & Gudmundsdottir, 1992; O'Brien *et al.*, 1994; Perez *et al.*, 1996; Gunnlaugsdottir & Gudmundsdottir, 1997; Wiklund & Dalsgaard, 1998), Atlantic cod (*Gadus morhua* L.) (Magnadottir *et al.*, 2002), Atlantic halibut (*Hippoglossus hippoglossus* L.) (Hjeltnes *et al.*, 1995; Gudmundsdottir *et al.*, 2003b), spotted wolffish (*Anarhichas minor* O.) (Foss *et al.*, 2004), common wolffish (*Anarhichas lupus* L.) (Hellberg *et al.*, 1996), turbot (*Scophthalmus maximus* L.) (Gudmundsdottir *et al.*, 2003b; Bjornsdottir *et al.*, 2005), goldfish (*Carassius auratus* L.) (Mawdesley-Thomas, 1969; Irianto *et al.*, 2003), European grayling (*Thymallus thymallus* L.) (Pylkko *et al.*, 2005) and flounder (*Platichthys flesus* L.) (Wiklund and Dalsgaard, 1998).

There are two forms of furunculosis declared. Furunculosis caused by strains identified as *A. salmonicida* subsp. *salmonicida* is named as the classical one and has been reported mainly in salmonids worldwide, except for Australia, Chile and New Zealand (Gudmundsdottir & Bjornsdottir, 2017). While atypical furunculosis is caused by *A. salmonicida* subsp. *achromogenes*, whose infections have been found in farmed and wild fish, salmonid species and many others around the world except for New Zealand (Austin, 2011; Cipriano & Austin, 2011; Austin & Austin, 2012; Gudmundsdottir & Bjornsdottir, 2017). Furunculosis is the most serious bacterial disease to Arctic char who are susceptible to both forms, especially when fish are under stress conditions such as poor water quality, high temperature and low oxygen (Johnston, 2002). In Iceland, classical furunculosis, caused by *A. salmonicida* subsp. *salmonicida*, was first detected in June 1995, but the pathogen was not identified in aquaculture after 1997 (Gudmundsdottir & Bjornsdottir, 2017). However, atypical furunculosis caused by *A. salmonicida* subsp. *achromogenes* is an endemic disease in the Icelandic aquaculture (Gudmundsdottir & Magnadottir, 1997; Gudmundsdottir, 1998). Also, *A. salmonicida* subsp. *achromogenes* has been isolated from infected wild fish (Wiklund & Dalsgaard, 1998).

Furunculosis caused by *A. salmonicida* subsp. *salmonicida* is a systemic disease which can occur in four forms: peracute, acute, subacute or chronic (Gudmundsdottir & Bjornsdottir, 2017). Peracute disease is common in young fish that die without external symptoms other than skin darkening and exophthalmos. Fish that survive often develop lesions with hyperaemia and haemorrhages, and gill congestion. Bacteria are found in anterior kidney, gills, spleen and myocardium. Necrosis in cardiac tissue may occur. Disease progresses rapidly without obvious host responses (Cipriano & Austin, 2011). Acute furunculosis which causes high mortalities is clearly evident by septicaemia. Fish may die rapidly without detectable pathology except for general reddening at the base of the fins. Hyperaemia occurs in all serous membranes. The spleen is often enlarged, and red. Kidneys become soft and friable or liquefied. The liver can be pale with haemorrhages or mottled due to focal necrosis. Skin lesions may develop, showing ectodermal red spots and point-like bleedings at the base of the fins and along the sides of the belly or distinguishing furuncles. Septicaemia, haemorrhages, necrosis, myocardial and renal degeneration are observed (Gudmundsdottir & Bjornsdottir, 2017). Subacute and chronic forms of furunculosis are more common in older fish, which often survive and recover. Infected fish have darkened skin and loss of appetite. Lethargy and furuncles are common. The gross signs of subacute furunculosis is quite similar to the acute form. Whereas general visceral congestion and peritonitis are found in the chronic form (Gudmundsdottir & Bjornsdottir, 2017). Fish that survive from epizootics can become carries that may spread the disease (Menanteau-Ledouble *et al.,* 2016). According to Gudmundsdottir and Bjornsdottir (2017), the disease course of atypical furunculosis is comparable to that of classical furunculosis, but damages in fish effected by classical furunculosis are more severe than in those affected by atypical furunculosis. The ulcers induced by classical furunculosis extend deeper into the musculature, liquefactive necrosis is more prominent, and skin haemorrhages are more intensive compared to fish infected with atypical furunculosis.

No evidence of vertical transmission of furunculosis has been confirmed. Disease transmission is rapid through horizontal routes, either by physical contact, likely entering hosts orally or through injuries to the skin, or by shedding the bacterium from the faeces of infected individuals and from the bodies of dead fish into the water environment. The bacteria are easily spread from tank to tank by contaminated water, and on equipment and clothing that are not disinfected. Carriers including

the survivors from epizootics can be the origins of the disease outbreak. The disease can also be spread by sea lice and possibly via fish-eating birds, such as gulls and cormorants. Transmission can also occur on the surface of eggs that have not been disinfected (Cipriano & Austin, 2011; Gudmundsdottir & Bjornsdottir, 2017).

2.3 *A. salmonicida*, a fish threatening pathogen

A. salmonicida are descrided as Gram negative and non-motile rods (0.3 - 1.0 µm diameter and 1.0-3.5 µm long), which belong to *Aeromonadaceae* family, *Aeromonadales* order and Gammaproteobacteria class (Austin, 2011). Aeromonads are cytochrome oxidase positive, fermentative, facultative anaerobes and resistant to Vibriostat O/129. (Dalsgaard *et al.,* 1998; Gudmundsdottir, 1998; Gudmundsdottir & Bjornsdottir, 2017).

The species can be divided into five subspecies including a typical *A. salmonicida* subsp. *salmonicida* (causing typical furunculosis) and four atypical subspecies, namely as *achromogenes*, *masoucida*, *pectinolytica*, and *smithia* (causing atypical furunculosis and other fish diseases). *A. salmonicida* subsp. *pectinolytica* is the only subspecies that is not reported to be fish pathogenic. The strains of typical *A. salmonicida* subsp. *salmonicida* is considered homogeneous while atypical strains are phenotypically and genotypically heterogeneous.

The optimal growth temperature of *A. salmonicida* is 22–25°C and most strains do not grow at 37°C. The colonies on tryptone soya agar (TSA) or brain heart infusion agar (BHIA) after 2-5-day incubation are circular, raised, friable and of a variable size. *A. salmonicida* has a protein surface A-layer protein (VapA), which is multifunctional (for example bacterial hydrophobicity and auto-agglutination) and an important virulence factor. For this reason, incubation at 15-20°C is recommended, because temperatures above 20°C may enhance loss of the A-layer (Gudmundsdottir & Bjornsdottir, 2017).

Typical *A. salmonicida* strains generally produce a water-soluble brown pigment when grown in tryptone media. Atypical *A. salmonicida* strains can produce brown pigment as well, however production is slower, and there are strains that do not produce the pigment (Dalsgaard *et al.,* 1998). Pigment production, which may correlate with virulence, is regulated by quorum sensing (Schwenteit *et al.,* 2011a).

2.4 Control and prevention of furunculosis

In the last 10–20 years, fish vaccination against common bacterial infections has become well-established and a key prophylactic measure for a successful sustainable aquaculture (Magnadottir, 2010). The emergence of vaccines has significantly reduced the use of antibiotics in aquaculture (Brudeseth *et al.,* 2013). Vaccines are administrated by injection via the i.p. or intramuscular (i.m.) route, or immersion/bath in diluted vaccine suspensions or oral vaccination through feed (Lillehaug, 2014). Each method has advantages and disadvantages with respect to the level of protection, side-effects, practical and cost effectiveness, however, in general, the level and duration of efficacy are highest with the injection method (Gudmundsdottir & Bjornsdottir, 2007; Brudeseth *et al.,* 2013).

Vaccination is the main prevention method against furunculosis in fish farmed in Europe and America (Sommerset *et al.,* 2005; Brudeseth *et al.,* 2013). The effectiveness against typical furunculosis is reported in a range from acceptable to very good protection (Hastein *et al.,* 2005). Vaccination has significantly reduced furunculosis outbreaks (Brudeseth *et al.,* 2013) and antibiotics usages in aquaculture (Gudmundsdottir & Bjornsdottir, 2007; Magnadottir, 2010). Commercial oil-adjuvant vaccines against furunculosis produced from typical *A. salmonicida* subsp. *salmonicida* strains have been available since the 1990s (Wiklund & Dalsgaard, 1998; Gudmundsdottir & Bjornsdottir, 2007). All available commercial furunculosis vaccines are still limited to salmonid species. Furunculosis vaccines can induce cross protection against some atypical *A. salmonicida* infections also in some non-salmonid species. However, vaccines for non-salmonid fish are currently not commercially available (Gudmundsdottir & Bjornsdottir, 2007).

In Iceland, atypical furunculosis is the common disease form affecting the aquaculture. The first vaccine against furunculosis in salmonids was licensed in Iceland in 1990. The vaccine was an autogenous bacterin prepared from *A. salmonicida subsp. achromogenes.* It induced good protection against atypical furunculosis of Atlantic salmon (Gudmundsdottir *et al.* 1996). A study published in 1997, showed that an oil-adjuvant furunculosis vaccine induced cross protection against *A. salmonicida* subsp. *achromogenes* infections in Atlantic salmon (Gudmundsdottir & Gudmundsdottir,1997), also in some non-salmonid species. Following commercial furunculosis vaccines, both monovalent and polyvalent vaccines, were licenced in Iceland and used in aquaculture (Gudmundsdottir & Bjornsdottir, 2007). They were found to protect salmonids well against atypical furunculosis, until recently that there are increasing mortalities in Arctic char hit by atypical furunculosis, just before they reach market size (Figure 1). In other words, for some reasons, the immunity of the Icelandic Arctic char appears to decrease gradually with time from vaccination or the virulence mechanisms of the infecting strain has changed. Furunculosis can affect fish throughout their lifespan, however, injectable oil-based vaccines cannot be used to vaccinate fish in its first developmental stages, due to small size (Mulero *et al.,* 2007). As oil-adjuvants can induce side effects, including adhesions that may affect the gonads, the vaccines cannot be used to protect broodstock fish (Gudmundsdottir & Bjornsdottir, 2007). Besides, the possibilities to protect offspring by parental vaccination is limited (Sommerset *et al.,* 2005; Magnadottir, 2006; Magnadottir, 2010). For this reason, there is a need for alternative environmentally save prophylactic methods, including selective breeding for disease resistant fish and feed supplemented with probiotics, prebiotics and/or immunostimulants.

Various supplements can not only improve fish growth and health, but also combat bacterial infections (Magnadottir, 2010; Cordero *et al.,* 2014; Menanteau-Ledouble *et al.,* 2016; Gudmundsdottir & Bjornsdottir, 2017). Hence, the interest in using them as a means of controlling infections has increased in recent years. Robertson *et al.* (2000) found that *Carnobacterium* sp., isolated from the intestine of Atlantic salmon, was strong antagonistic to bacterial pathogens including *A. salmonicida*. Similarly, lactic acid bacteria (LAB) isolated from fish intestine, such as *Lactococcus lactis, Lactobacillus plantarum,* and *Lactobacillus fermentum,* or from sediment, namely *Lactobacillus pentosus, Pediococcus pentosaceus, Enterococcus mundtii*, demonstrated their probiotic effects to inhibit adhesion of several fish pathogens (*Aeromonas hydrophila, A. salmonicida, Yersinia ruckeri and Vibrio anguillarum*) to fish mucus and surface (Balcazar *et al.,* 2008; Sica *et al.,* 2012). Enriched feed with immunostimulants, for example mannanoligosaccharide (MOS) and beta-glucans significantly improved fish growth and enhanced resistance to *A. salmonicida* infection (Falco *et al.,* 2012; Miest *et al.,* 2012; Pionnier *et al.,* 2013;

Rodriguez-Estrada *et al.,* 2013). In addition, natural dietary supplements have been explored for the prevention and treatment of disease in aquatic organisms. The usage of therapeutic dietary supplements in humans, for example garlic extract, has been practiced. Feed containing garlic extract (0.5% and 1.0%) increased resistance of rainbow trout to *A. salmonicida* (Breyer *et al.,* 2015).
Selective breeding has been applied to control infections by *A. salmonicida* in several countries (Gjedrem, 2000; Menanteau-Ledouble *et al.,* 2016). In salmonids, resistance to typical furunculosis is hereditary (Drangsholt *et al.,* 2011; Yanez *et al.,* 2014), therefore it may be possible to select for improvement of disease resistance (Zhang *et al.,* 2011). Although no estimation of the heritability of resistance to atypical furunculosis caused by *A. salmonicida* subsp. *achromogenes* in salmonids has been published, an effective selection may reduce the susceptibility of Arctic char to atypical furunculosis caused by *A. salmonicida* subsp. *achromogenes* in Iceland.

2.5 Different experimental challenge methods of salmonids with *A. salmonicida*

One of the important criteria in evaluation of candidate vaccines is survival of vaccinated fish (Relative Percent Survival, RPS) compared to the unvaccinated controls following infection challenge trials (Gudmundsdottir & Bjornsdottir, 2007). However, the challenge method may influence the outcome of the trial (Nordmo, 1997; Nordmo & Ramstad, 1997). Hence for avoiding biased evaluation of vaccine-induced protection, an effective and reliable challenge method is a basic requirement. An optimal challenge model for testing vaccine efficacies should closely mimic the natural infection, induce pathological features and cause at least 70% morbidity or mortality in control fish (Nordmo, 1997; Nordmo & Ramstad, 1999). Administration of live bacteria with the purpose of causing disease in fish can be performed by injection, or by waterborne infection (cohabitation, immersion/bath).

Today, in a battle against furunculosis and atypical disease form, vaccination, which has been available since the 1990s, is the main preventive measure for salmonids, though, their efficiency has been uncertain and dependent on the characteristics of the infective strain (Gudmundsdottir & Bjornsdottir, 2007). Protection of furunculosis vaccines is often tested by experimental challenges in which fish are exposed to virulent bacteria either by injection or by waterborne infection (immersion or cohabitation) (Nordmo & Ramstad, 1997; Chettri *et al.,* 2015). The latest challenge method by a tail fin infection was recently recommended by Marana *et al.* (2016), by which live bacteria are introduced in the tail fin epidermis distant from the vaccine injection site (peritoneal cavity), by use of a multi-needle device. This method appears to mimic the natural infection route in aquaculture settings where bacteria gain access to fish through lesions (tail biting). Small skin lesions were made by a multi-puncture device containing 10 needles on the upper part of the caudal fin at which the bacteria were layered for 60 seconds. The bacteria was spread via the ulcers resulting in a systemic infection (Marana *et al.,* 2015; Marana *et al.,* 2016). The injection challenge (i.p.or i.m. injection) is a reliable and reproducible way to challenge individuals with a uniform precise dosage of microorganisms, but it fails to reflect the natural mode of infection. In addition, this method does not allow immune mechanisms located on the surface of the fish to respond; and the bacteria which are injected at the vaccination site, may be deactivated by local inflammatory reactions, thereby the systemic protection induced by vaccination can be affected. This may lead to a high survival of challenged fish, without reflecting general systemic immunity, which can protect the vaccinated fish against natural exposure to the pathogen. Cohabitation (donor fish i.p. injected with the pathogen cohabited with test fish) and immersion/bath challenge methods more

closely resemble the natural route of infection but may be less effective in inducing the disease than the injection methods (Nordmo & Ramstad, 1997; Nordmo *et al.,* 1998; Chettri *et al.,* 2015). It is suggested that immersion or cohabitation challenge model should be used for testing of efficacy of furunculosis vaccines as these models best mimic a natural infection and are applicable for following challenges in field and selective breeding programmes (Nordmo *et al.,* 1998). A waterborne exposure will enable the fish to demonstrate its total immune mechanisms located both externally and internally (Nordmo & Ramstad, 1997). Similarly, Chettri *et al.* (2015) reported that cobihabitation challenge is the best method for evaluation of injectable vaccine efficacies.

For selection of Arctic char families with higher resistance to atypical furunculosis caused by A. *salmonicida* subsp. *achromogenes*, both laboratory and field challenge trials in vaccinated and non-vaccinated fish need to be carried out. However, most published evaluations of the efficiency of furunculosis vaccines in salmonids are based on the evaluation of protection against infection by by A. *salmonicida* subsp. *salmonicida*. No studies involving cohabitation challenge of Arctic char with *A. salmonicida* subsp. *achromogenes* have been reported.

2.6 Other diseases caused by aeromonads in aquaculture

Other than *A. salmonicida* is the non-motile species of the aeromonads causing furunculosis of fish. There are several motile *Aeromonas* species that are potentially zoonotic pathogens causing diseases in aquaculture. These include *A. hydrophila, A. caviae*, *A. veronii* biovar *sobria*, *A. bestiarum, A. dhakensis, A. sobria, A. allosaccharophila,* and *A. encheleia* are potentially zoonotic pathogens causing diseases in aquaculture. The best known species is *A. hydrophila* that causes diseases such as motile *Aeromonas* septicaemia (MAS) and tail-fin rot (Janda & Abbott, 2010; Austin, 2011; Cipriano & Austin, 2011; Austin & Austin, 2012; Stratev & Odeyemi, 2016b; Gudmundsdottir & Bjornsdottir, 2017).

Epizootics of MAS have occurred in a wide range of freshwater, brackish and marine fish species, both farmed and wild fish (Roberts, 2012; Lio-Po *et al.,* 2014), for example Vietnamese striped catfish (pangasius catfish) (*Pangasius hypophthalmus* Sauvage) (Ly *et al.,* 2009; Crumlish *et al.,* 2010; Sirimanapong *et al.,* 2014), channel catfish (*Ictalurus punctatus*) (Hossain *et al.,* 2014), Chinese 'ya-fish' (*Schizothorax prenanti*) (Zheng *et al.,* 2016), (Sahoo *et al.,* 2008), common carp (*Cyprinus carpio* L.) (Wang *et al.,* 2015), sunshine bass (*M. chrysops x M. saxatilis*) (a hybrid striped bass between the striped bass (*Morone saxatilis*) and the white bass (*M. chrysops*) (Schrader *et al.,* 2013), European eels (*Anguilla anguilla*), striped sea catfish (*Plotosus anguillaris*), barramundi (*Lates calcarifer*), spotted grouper (*Epinephelus megachir*), rohu (*Labeo rohita*), blue tilapia (*Serotherodon nilotica*), Atlantic salmon (Salmo salar), rain-bow trout (*Oncorhynchus mykiss*), gizzard shad (*Dorosoma cepedianum*), perch (*Perca fluviatilis*)(Austin & Austin, 2012). High mortalities due to MAS have been reported (Janda and Abbott, 2010; Cipriano and Austin, 2011; Colston *et al.,* 2014), especially of farmed pangasius catfish (*Pangasius hypophthalmus* S.) cultured in Vietnam (Ly *et al.,* 2009; Phu *et al.,* 2015) and channel catfish (*Ictalurus punctatus*) farmed in the Southeastern United State (Hossain *et al.,* 2014; Zhang *et al.,* 2016). Because of the common presence of these motile aeromonads in the normal intestinal microflora of healthy fish and in the aquatic environmental microflora, stress is often considered as a trigger to the outbreak of MAS. The infection can be horizontally spread through contaminated water, carrier fish, external parasites, equipment and clothing (Austin & Austin, 2012).

Clinical signs may vary according to fish species but septicaemia in acute MAS can be fatal with no clinical signs (Lio-Po *et al.,* 2014). The disease is commonly characterized by hemorrhagic septicaemia, haemorrhages, swollen abdomen, red mouth, ulceration, abscesses, ascites, anaemia and damage (such as generalized liquefaction) to the internal organs (notably kidney and liver) and musculature, and scale protrusion (Cipriano, 2001; Austin & Austin, 2012). MAS causes diverse pathologic conditions that include acute, chronic, and covert infections. The disease can induce systemic infection or be restricted to skin lesions or latently infected fish. Severity of disease is influenced by a number of interrelated factors, including bacterial virulence, stressors and the physiological condition of the host (Cipriano & Austin, 2011; Gudmundsdottir & Bjornsdottir, 2017). In the acute form of disease, a fatal septicaemia may occur so rapidly that fish die before any gross signs of disease are evident. Infected fish may show exophthalmia, reddening of the skin, necrosis of the skin, fins and oral cavity, and an accumulation of fluid in the scale pockets. Distended abdomen may be observed as a result of an edema. The haemorrhaged gills and dermal ulcers may develop but most severe pathological changes are seen in systemic infected fish. Histopathologically, necrosis in the kidney, liver, spleen and heart may occur. Degeneration and haemorrhages are common in the epithelium of intestine heart and interstitial tissues of organs. Bacteria have been isolated from the eyes, liver and kidneys. Chronic MAS manifest visible dermal ulcers with focal necrosis, haemorrhage and inflammation. However, lesions and necrosis are not common in most visceral organs.

A. hydrophila (the synonyms including *A. formicans* and *A. liquefaciens*) is the main aetiological agent of motile *Aeromonas* septicaemia (MAS) (Austin, 2011). Although usually considered as an opportunistic pathogen, *A. hydrophila* can also emerge as a primary pathogen, causing outbreaks in fish farms with high mortality rates and severe economic losses to the aquaculture industry worldwide (Pridgeon & Klesius, 2011). In contrast to *A. salmonicida*, *A. hydrophila* strains are capable of growth at 37°C and are motile by single polar flagella. Some isolates have also been determined to produce diffusible brown pigments, as does *A. salmonicida*.

A. hydrophila has two subspecies, *hydrophila* (Seshadri *et al.,* 2006) and *ranae* (Huys *et al.,* 2003), and a third subspecies, *decolorationis*, has been proposed (Ren *et al.,* 2006), however, no infections caused by the subspecies *decolorationis*, have been reported (Gudmundsdottir & Bjornsdottir, 2017). Furthermore, *A. hydrophila* belongs to three DNA hybridization groups (HG). The genome of the *A. hydrophila* type strain ATCC 7966T and genes encoding virulence related mechanisms were described by Seshadri *et al.* (2006). Analysis revealed that the strain has broad metabolic capabilities and numerous putative virulence genes and systems, which allows it to survive and adapt to diverse ecosystems and hosts (Gudmundsdottir & Bjornsdottir, 2017).

MAS in fish is controlled by antibiotics, however the resistance of *A. hydrophila* is continuously reported (Stratev & Odeyemi, 2016b). *A. hydrophila* possesses many enzymes and mechanisms (Chen *et al.,* 2012), which are potential for horizontal gene transfer. Many strains resist a wide range of antibiotics (namely β-lactam antibiotics, trimethorpim and trimethoprim-sulfamethoxazole, aminoglycosides, third-generation cephalosporins, lincosamides, macrolides, nitrofurans, fluoroquinolones, sulfonamides, tetracyclines, chloramphenicol and rifampicin) and bactericidal agents (Guz & Kozinska, 2004; Saavedra *et al.,* 2004; Belem-Costa & Cyrino, 2006; Adanir & Turutoglu, 2007; Kaskhedikar & Chhabra, 2010; Daood, 2012; Odeyemi *et al.,* 2012; Jeeva *et al.,* 2013; Laith & Najiah, 2013; Samal *et al.,* 2014). Vaccination against *A. hydrophila* is

problematic due to strain variation and the different nature of various hosts (Austin & Austin, 2012; Gudmundsdottir & Bjornsdottir, 2017).

Antibiotics are commonly used for control of bacterial infections in aquaculture (Rico *et al.,* 2013). However, the prophylactic use of antibiotics can result in emergence and development of microbial resistance and unacceptable levels of antibiotic residues in fish tissues and the environment. Also, resistant bacteria could be transmitted to human through the food chain that could result in failure of therapy of diseases (Stratev & Odeyemi, 2016a). Due to the development of antibiotic resistance in bacterial pathogens, other environmentally alternatives for disease control and prevention are essential in aquaculture.

3 MATERIALS AND METHODS

3.1 Experimental fish

Fish from the Arctic char breeding program of Holar University College were used for the experiment. The fish were reared in fresh water and free of the pathogen. Prior to the experiment, the fish were kept in 3000 L tanks at 4 °C with daily feeding ad libitum with pelleted feed from the feed producer Laxa, varying in size depending on the size of the fish.

Fish were individually tagged with Passive Integrated Transponder (PIT) tags. The fish were anaesthetized by 5-phenoxy ethanol (50 mg/L, Lifsgledi ehf.) before vaccination and treatment. During the experiments the fish were fed to appetite with commercial pellets (Laxa, Iceland). The fish experiments were approved and performed according to the Icelandic Animal Research Authority (approval no. 2016-11-02, reference MAST1611356)

3.2 Vaccination and transportation of fish to Verid

The commercial vaccine applied was Alpha Ject 3000 (Pharmaq AS, Norway), which contains dead cells of *A. salmonicida* subsp. *salmonicida* and *V. anguillarum* serogroups O1 and O2a in unspecified concentrations emulsified in a paraffin oil adjuvant.

A total of 150 fish were vaccinated by injection via the intraperitoneal (i.p.) route with 0.1mL/fish. Two weeks later, all vaccinated fish and 300 unvaccinated fish of the same size were moved to Verid research station in Saudarkrokur, Iceland. There they were allowed to acclimate to 15-18ppt salinity and 8-9˚C by raising the water temperature and salinity over 5 days before bacterial exposure.

3.3 Bacteria used in experimental challenges

The bacterium used in the experimental challenges was *A. salmonicida* subsp. *achromogenes.* Strain Keldur265-87, isolated in Iceland from atypical furunculosis diseased Atlantic salmon (Gudmundsdottir *et al.,* 1990), was used to inject donor fish in the cohabitation challenge trials. Strain F131-16, isolated from diseased vaccinated Arctic char in Iceland, was used in the immersion challenge experiment. Stock cultures were stored in tryptone soya broth (TSB, Oxoid)

+ l0 % glycerol at -20°C. Bacteria were routinely cultured on brain heart infusion agar (BHI, Oxoid + 2 % Oxoid bacteriological agar No. 1) at 15°C. There are many publications where strain Keldur265-87 has been used, and therefore it was selected for the experiment (Gudmundsdottir *et al.*, 1990; Gudmundsdottir, 1996; 1997; Gudmundsdottir *et al.*, 2003a; Schwenteit *et al.*, 2011b; Schwenteit *et al.*, 2014). Strain F131-16 is a new isolate from an epidemic on a char farm (Figure 1), which is currently being whole genome sequenced (B.K. Gudmundsdottir, personal communication). The number of colony forming units (CFU) in bacterial suspensions was estimated by spreading tenfold dilutions on BHIA plates. Colonies were counted following incubation at 15 °C for 3 days.

3.4 Experimental challenges by cohabitation and immersion

Arctic char was challenged by: (1) cohabitation where a number of char i.p. injected with a bacterial dose of 10^4 to 10^5 CFU of strain Keldur 265-87 (donor fish) were introduced into four experimental tanks containing 30 vaccinated and 30 unvaccinated fish each. The bacterial concentration used was based on a previous study where Arctic char (with the mean weight of 30 g) were i.p. injected with the same bacterial strain (Keldur265-87) and the infection resulted in fifty percent lethal dose (LD_{50}) of 1.6 * 10^3 CFU/fish (Arnadottir *et al.*, 2009); and (2) immersion of 200 unvaccinated char in a suspension containing 10^6 CFU of strain F131-16/mL of aerated brackish water (15ppt salinity, 13^{o}C) for 2h. The fish were distributed to 4 tanks (50 fish per tank) following immersion and cultivated at 13°C in continuously running water with 15ppt salinity (Table 1).

The oxygen concentration, temperature, salinity and mortality in each tank were recorded by inspection daily throughout the experiments. Dead and moribund fish were instantly removed and collected for bacteriological examination. The gross pathological changes of dead and moribund fish were described. The causes of death due to atypical furunculosis was confirmed by re-isolation of the bacterium from the head kidney of dead fish. Bacterial colonies were characterized as *A. salminicida*, using an agglutination test kit (Mono - Aqua AS, Bionor, Norway).

Table 1. An overview of setup in cohabitation and immersion challenges of Arctic char with *A. salmonicida* subsp. *Achromogenes*

Strain/challenge method	Introduction of donor fish/ days post vaccination	Number of donor fish/ tank	Number of vaccinated fish per tank	Number of unvaccinated fish per tank	Water temperature
(1) Keldur265-87 / cohabitation	1st/23	12/1 & 2 18/3 & 4	30	30	8^{o}C
	2nd/36	18/1&2 12/3&4	30	30	12^{o}C
	3rd/57*	20/1,2,3&4	30	30	12^{o}C
(2) F131-16 / immersion				50	13^{o}C

*Strain Keldur265-87 isolated from dead donor fish in the 2nd introduction (fish passaged) was used to inject the donor fish.

3.4.1 Cohabitation challenge

As cohabitation challenge of Arctic char with *A. salmonicida* subsp. *achromogenes* has not previously been performed, the experiment is based on preceding publications of cohabitation and immersion challenges of Atlantic salmon and Arctic char with *A. salmonicida* (Lødemel *et al.,* 2001; Arnadottir *et al.,* 2009; Drangsholt *et al.,* 2011).
Donor fish were introduced into fish tanks 23, 36, and 57 days post vaccination, 2 replicates were of each tank. Different percentage of infected fish was tested and also different temperature of the rearing water in order to find the optimal challenge conditions (Table 1).

Due to low mortalities observed in the experimental tanks, more donor fish were introduced to the tanks on day 36 from vaccination and the rearing water temperature was gradually increased from 8° to 12°C (Table 1).

Due to the low disease transmission induced by the 1st and 2nd introductions of donor fish, the 3rd introduction was performed 57 days post vaccination (Table 1). In this challenge the bacterium had been passaged one time in Arctic char before injection of the donors.

The experiment was terminated by killing the fish with an overdose of anaesthetization 60 days post the initiation of the cohabitation challenges.

3.4.2 Immersion challenge

An immersion challenge of 200 Arctic char was performed in a plastic bucket containing 120 L of the bacterial suspension (Table 1) During immersion, the water salinity, temperature and oxygen saturation were maintained around 15ppt, 13°C and >80%, respectively. After exposure, 50 fish were transferred to each of 4 tanks containing 1000 liters of continuously running 13°C warm water with 15ppt salinity. A tank with the same volume of water containing 50 uninfected fish served as a control.

3.5 Statistical analysis

Kaplan-Meier survival analysis (Matthews & Farewell, 1985) was performed, using the R software to estimate the likelihood of survival/mortality. The cumulative probability of mortality/survival at the end of the experiment was calculated with a 95% degree of confidence (Therneau & Grambsch, 2000; Goel *et al.,* 2010; Therneau, 2015; R-Core-Team, 2016). A chi-square test was used to analyze the significance of differences in the cumulative survival estimate between experimental groups.

Microsoft Excel 2016 was used for statistical analysis, graphs and tables. Student t-test was used to compare the MDDs of different groups. The criterion for significance was set at $P<0.05$.
The mean days to death (MDD) was calculated using the following formula (Bjornsdottir *et al.,* 2005; Arnadottir *et al.,* 2009).

MDD = [Σ (number of mortalities x number of day post-challenge)] / total number of mortalities

4 RESULTS

4.1 Atypical furunculosis induced in Arctic char donors by i.p. injection of *A. salmonicida* subsp. *achromogenes*

A. salmonicida subsp. *achromogenes***,** Keldur265-87**,** caused infection and mortality in i.p. injected Arctic char donors in a temperature dependent manner (Table 2, Figure 2 & Figure 3). Significantly higher and more acute mortalities were recorded in the donors at temperature 12°C than 8°C (t-test p-value=8.28335E-07) (Figure 3). Passage of the bacterium in Arctic char resulted in more rapid mortality (Figure 4 & Table 2). Compared to the group injected with higher dose (injection 2: 10^5 CFU/fish), i.p. injection with a passaged bacterium (dose 10^4), the death appeared drastically earlier (MDD = 3.17 (± 0.34), p-value = 7.88E-05) (Table 2) and the survival was considerably lower (χ^2= 3615 on 1 degrees of freedom, χ^2 test p-value= 0).

Table 2. A, Mean days to death (MDD) of Arctic char donors following i.p. injection with *A. salmonicida* subsp. *achromogenes* Keldur265-87; B, Significance of differences between MDD of the three treatment groups. The criterion for significance was set at P<0.05.

A.

Treatment	Mean day to Death (MDD)					
	Tank 1	Tank 2	Tank 3	Tank 4	Average	SD
Injection 1 10^4 CFU/fish	8.44	9.00	7.79	6.76	8.00	0.96
Injection 2 10^5 CFU/fish	3.95	4.18	3.83	4.00	3.99	0.14
Injection 3 10^4 CFU/fish*	3.68	3.0	3.0	3.0	3.17	0.34

*Strain Keldur265-87 isolated from dead donor fish in the 2nd introduction (fish passaged) was used to inject the donor fish.

B.

Comparison of treatment groups	P-value*
Injection 1 & 2	0.000170852
Injection 2 & 3	0.004420559
Injection 1 & 3	7.88507E-05
Injection 1 & 2+3	8.28335E-07

*t-Test: Two-Sample Assuming Equal Variances

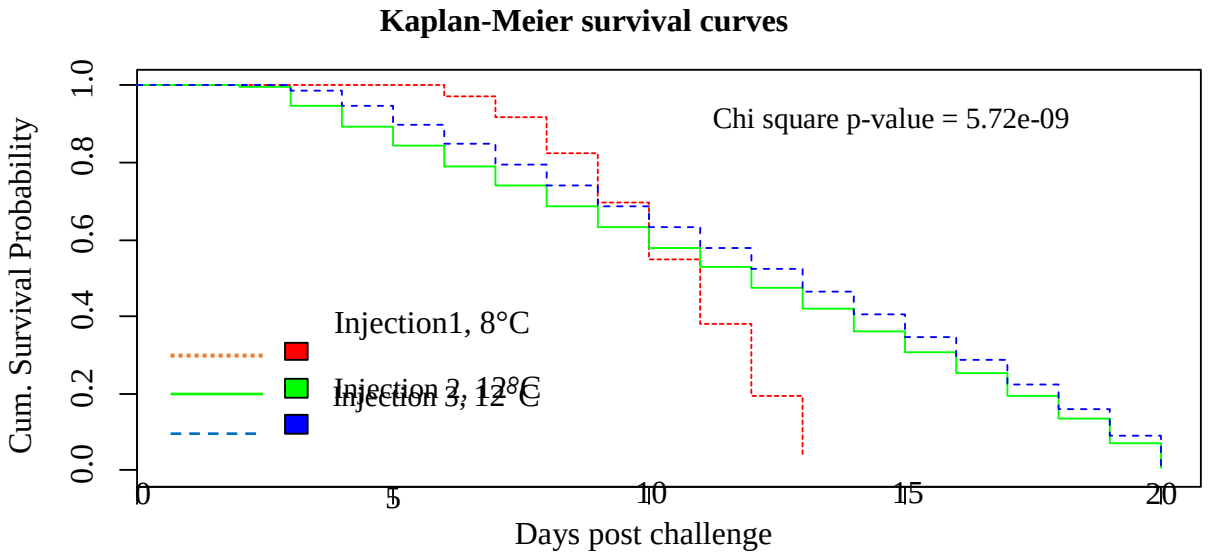


Figure 2. Kaplan–Meier estimates of survival of Arctic char donors i.p. injected with *A. salmonicida* subsp. *achromogenes*, Keldur265-87. The injected donor fish were reared at 8°C, red line; or 12°C, blue and green lines.

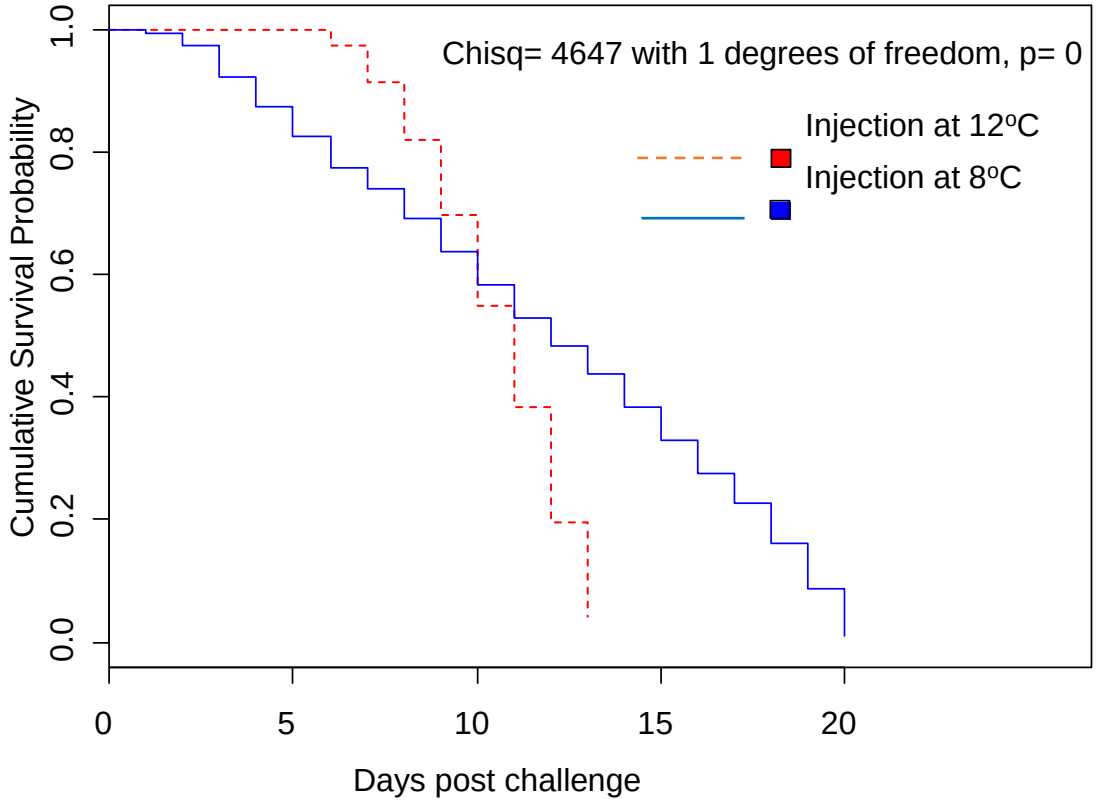


Figure 3. Kaplan–Meier estimates of survival of Arctic char donors i.p injected with *A. salmonicida subsp. achromogenes*, Keldur265-87. The injected donor fish were reared at 8°C, red line; or 12°C, blue line.

There was a significant difference in survival curves of donor fish injected at temperature 8°C and 12°C (χ^2 test p-value= 0) (Figure 3).

Injection of Arctic char donors with *A. salmonicida* subsp. *achromogenes*, Keldur265-87 (10^4 -10^5 CFU/fish) that were reared in water temperature at 8°C to 12°C resulted in very high mortalities (> 80%) (Figure 4).

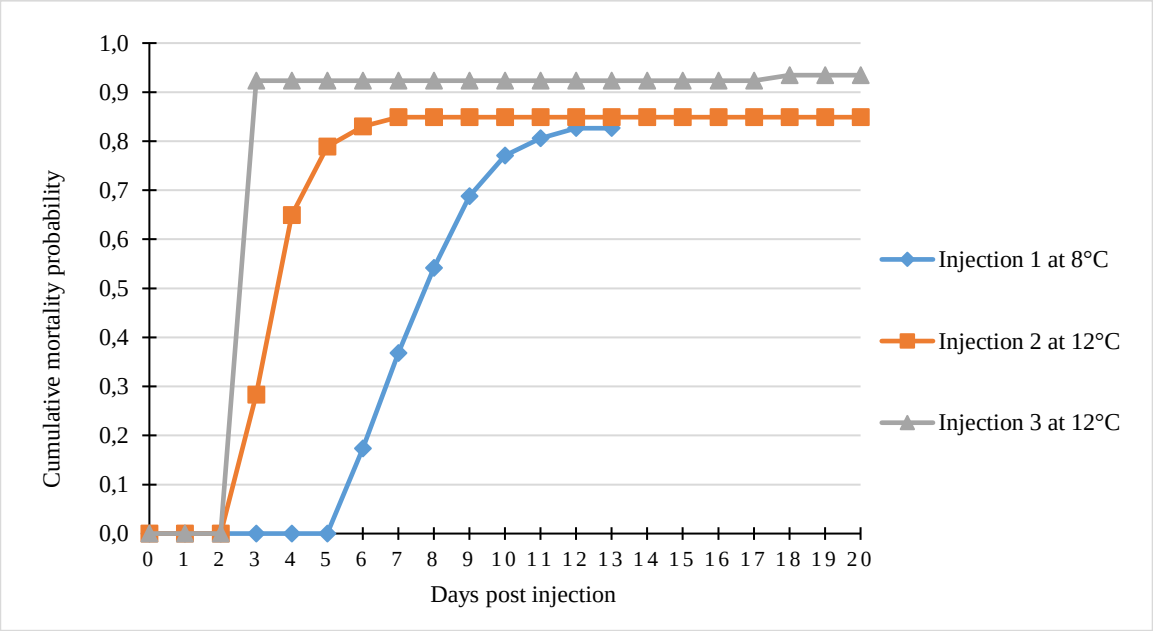


Figure 4. Percentage cumulative mortality (%) of Arctic char donors i.p. injected with A. salmonicida subsp. achromogenes, Keldur265-87. The injected donor fish were reared at 8°C, blue line; or 12°C, orange and grey lines.

The macroscopic clinical signs in infected Arctic char were similar to those in salmonid species described in previous publications, but histopathological examination was not performed (Gunnlaugsdottir & Gudmundsdottir, 1997; Arnadottir *et al.*, 2009; Cipriano & Austin, 2011; Austin & Austin, 2012; Gudmundsdottir & Bjornsdottir, 2017). The fish that died rapidly was without detectable pathology, except for general haemorrhage at the base of the fins and pale skin colour (Figure 5). Fish that survived longer developed clinical signs including bleeding in eyes, mouth, on the head and fins (Figure 6). Figure 7 shows common signs of moribund and dead fish, which is gaping and with exophthalmos (protruding eyes). Fish that was gaping with pale and congested gills was common (Figure 8). A bleeding anus and ectodermal red spots were also frequent (Figure 9). A disease sign observed was point-like bleedings along the sides of the belly (Figure 10). Skin ulcers were sometimes seen on fish that died from the infection 12 days from infection or later (Figure 11 and Figure 12). Fin and tail rot was a common sign (Figure 12). Loss of appetite presented as empty stomach and intestine was also a general disease sign along with very pale liver, enlarged and reddish spleen and hyperaemia at the abdominal wall (muscle) (Figure 13). In more severe cases ascites in the body cavity, often with generalized haemorrhages, were observed (Figure 14).

Figure 5. A, Fish that died at day 5 post challenge with haemorrhage at the base of tail and fins and pale skin colour. B, infected fish that died without clinical signs 4 days post challenge.

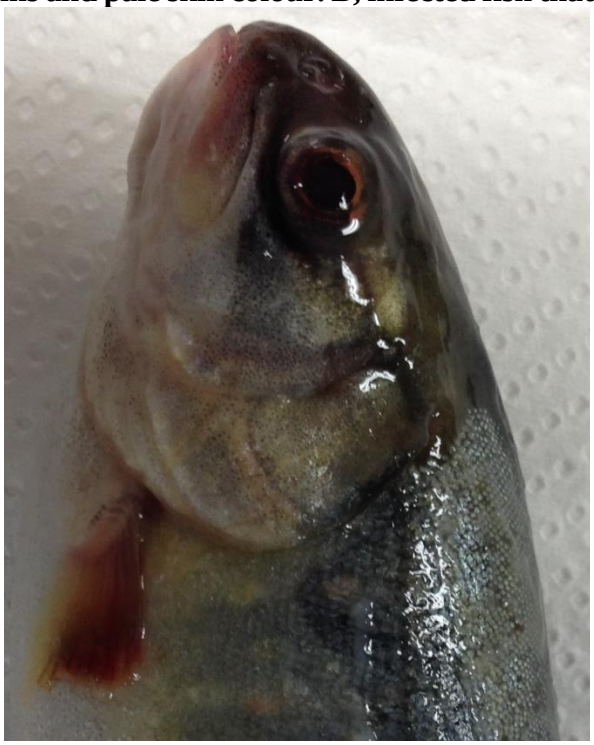


Figure 6. Fish that died 7 days post challenge with haemorrhage in the eyes, mouth and fins.

Figure 7. A fish that died 5 days post infection, which is gaping and with exophthalmos (protruding eyes).

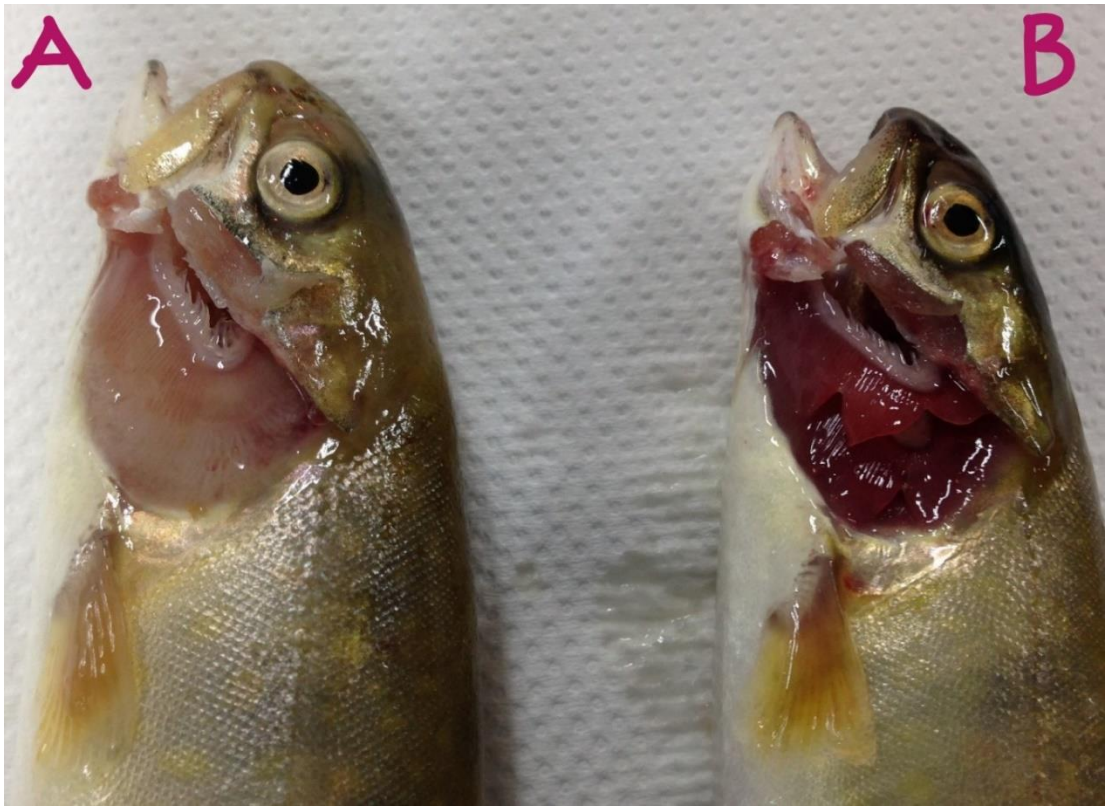


Figure 8. A, fish died 5 days post infection with pale and congested gills; B, fish with normal gills.

Figure 9. Fish died 5 days post infection with haemorrhage in fins and at fin bases and a bleeding anus. The skin colour is pale.

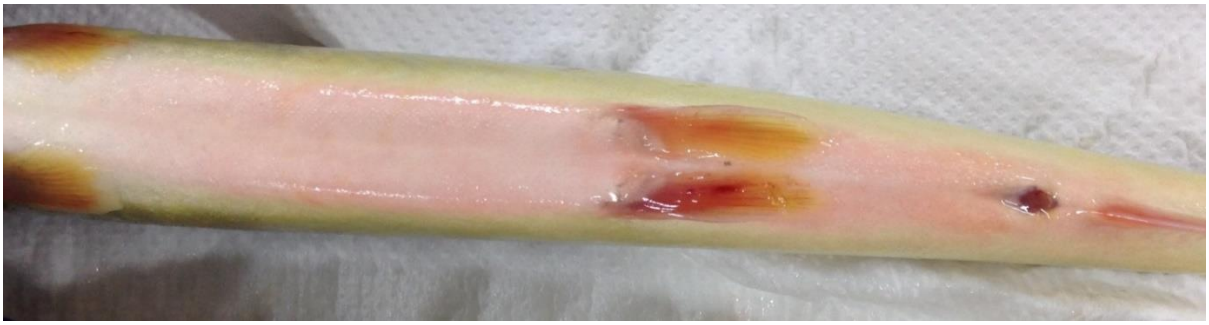


Figure 10. Fish died 5 days post infection with a red belly and pale skin colour

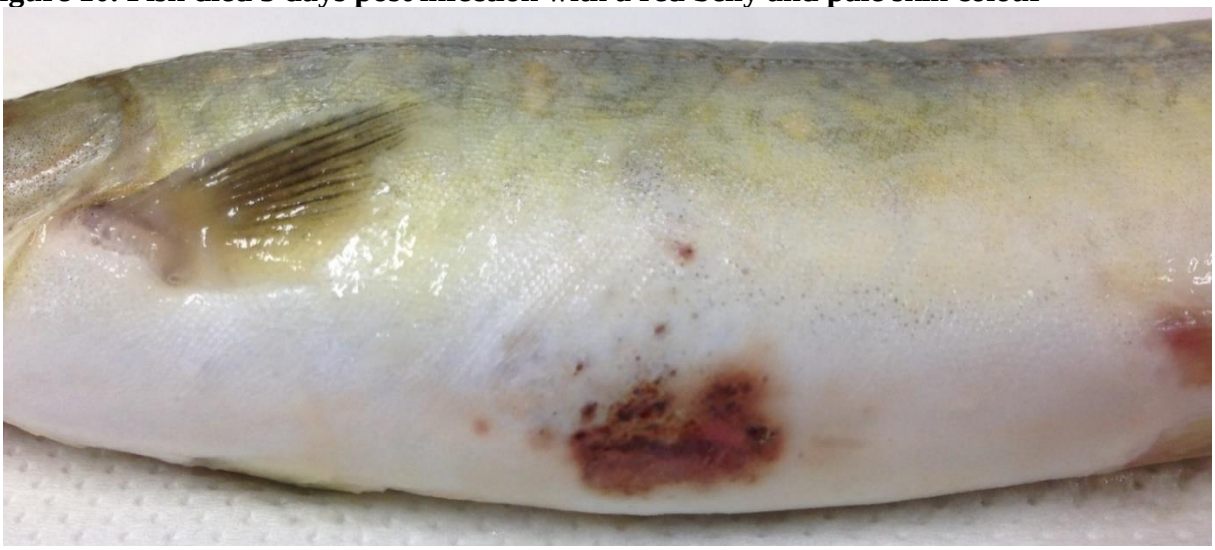


Figure 11. A fish died 12 days post infection with a typical skin lesion and pale skin colour.

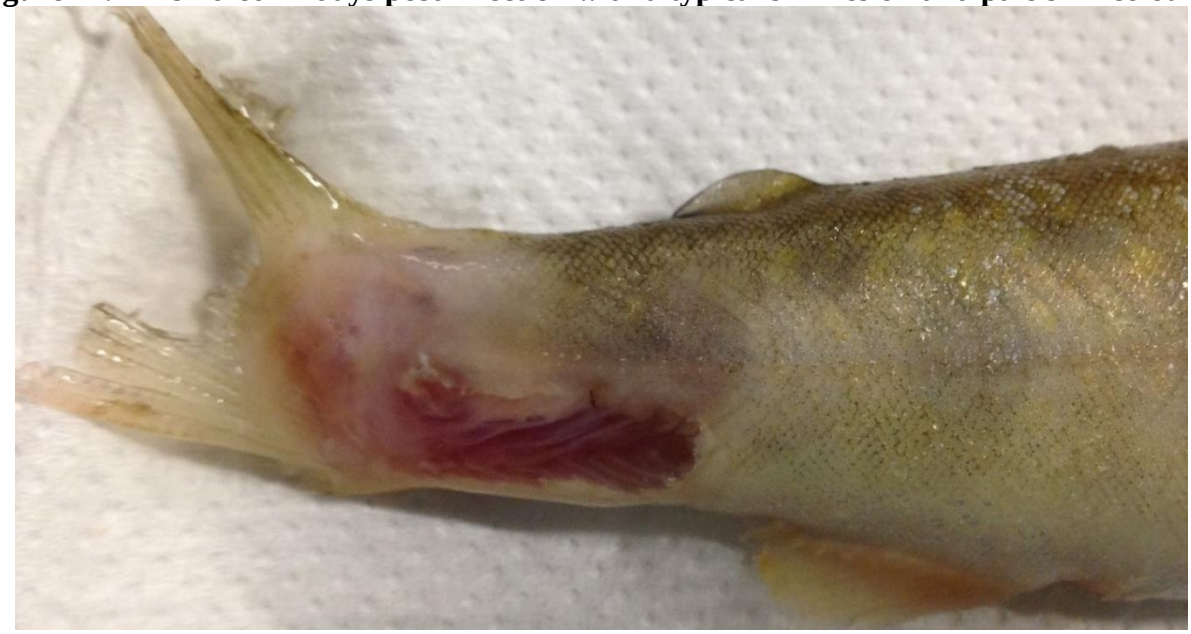


Figure 12. A fish died at the 18 days post infection with a dorsal ulcer and tail rot.

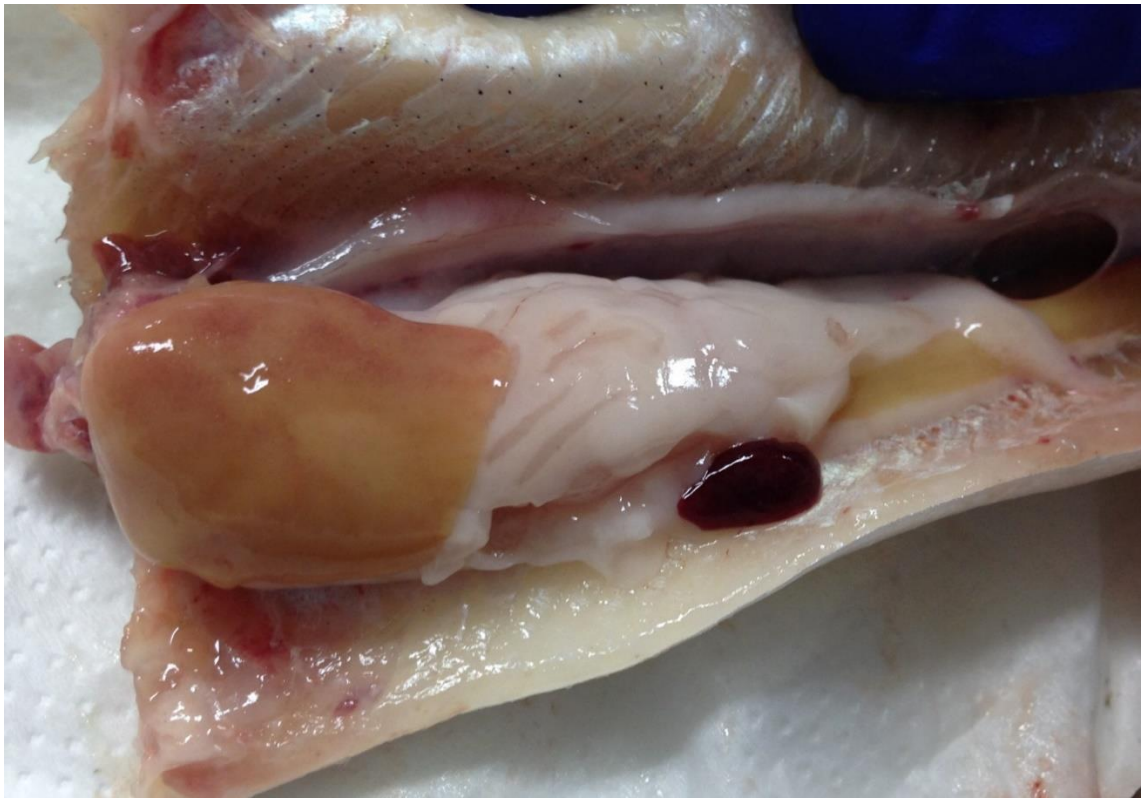


Figure 13. A fish died 5 days post infection with pale liver, reddish and enlarged spleen, and an empty stomach.

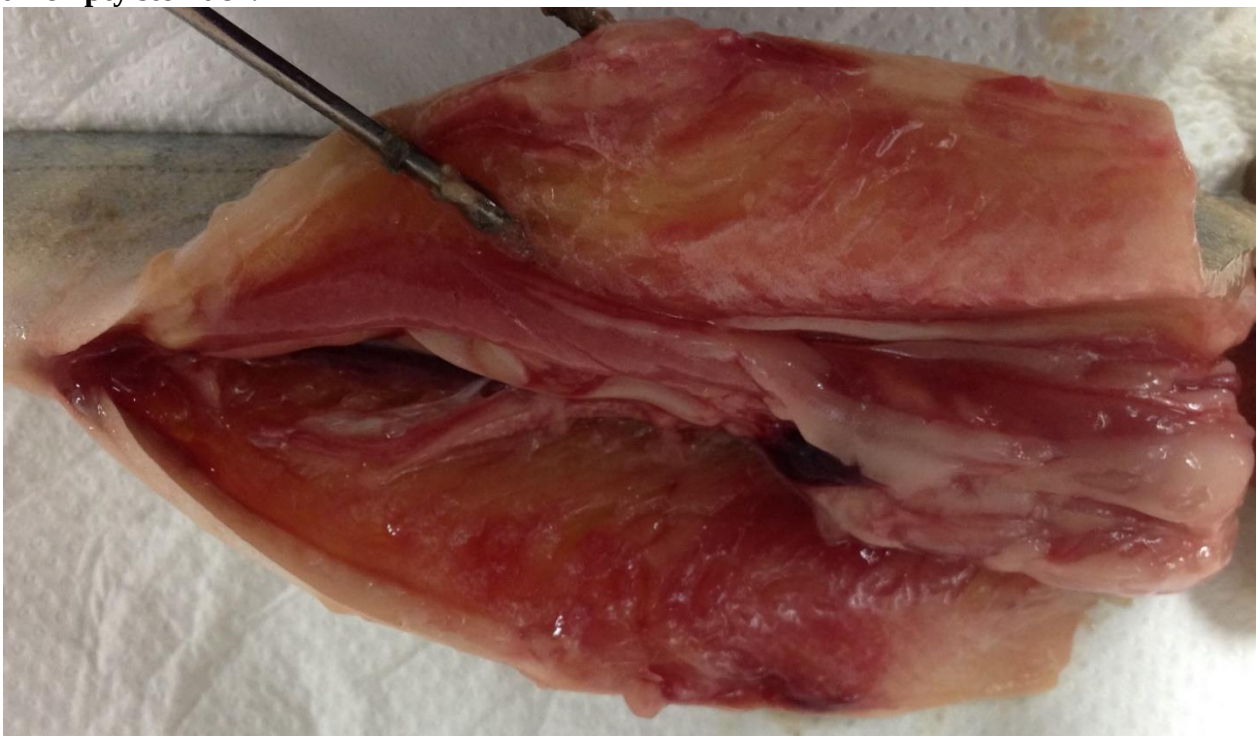


Figure 14. A fish died 7 days post infection with haemorrhage in belly muscle and intestines.

The pathogen *A. salmonicida* was re-isolated from the head kidney of all infected fish on BHIA and serologically identified as *A. salmonicida*, using MONO-As kit (BIONOR AS) (Figure 15 & Figure 16).

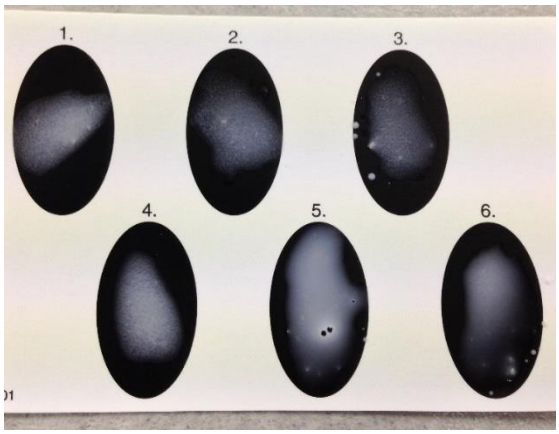


Figure 15. A serological agglutination test. No 1, 2, 3, 4 & 6 are identified as *A. salmonicida* (positive agglutination). No 5 is the negative control without agglutination.

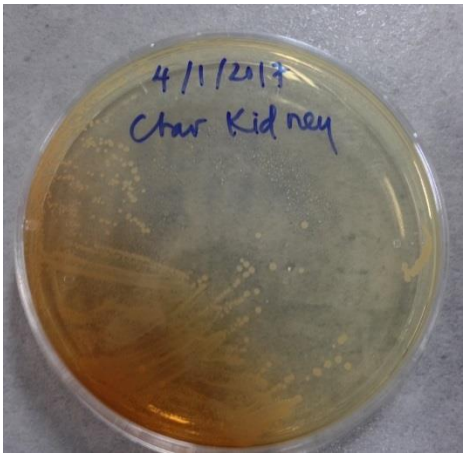


Figure 16. *A. salmonicida* subsp. *achromogenes*, Keldur265-87, producing brow pigment after 3 days cultivation on BHIA at 15°C.

4.2 Challenges of Arctic char with *A. salmonicida* subsp. *achromogenes*

4.2.1 Cohabitation challenge of Arctic char with A. salmonicida subsp. achromogenes, Keldur265-87

The results of the cohabitation challenge showed that this method induced very weak disease transmission of atypical furunculosis in Arctic char under the conditions applied in this study. Few deaths occurred in unvaccinated char and vaccinated char vas fully protected (
Table 3).
The mortality in unvaccinated char occurred and increased when the water temperature was increased from 8°C to 12°C (Figure 17, Figure 18).

Table 3. Cumulative mortality (%) of Arctic char challenged with *A. salmonicida* subsp. *achromogenes*. Strain Keldur265-87 was used for the cohabitation challenge and strain F131-16 for the immersion challenge. The mortalities were calculated at 25 days post cohabitation challenge and 17 days post immersion challenge.

Challenge type	Number of donor fish/ tank	Temp.	Unvaccinated	Vaccinated
		(°C)	Cum. mort.	Cum. mort.
Co-habitation N=120 vaccinated; 120 unvaccinated	12/1 & 2	8°C	0	0
	18/3 & 4	8°C	0	0
	18/1&2	12°C	1.70%	0
	12/3&4	12°C	5.17%	0
	20/1&2	12°C	4.36%	0
	20/3&4	12°C	0	
Immersion		13°C	98.62%	

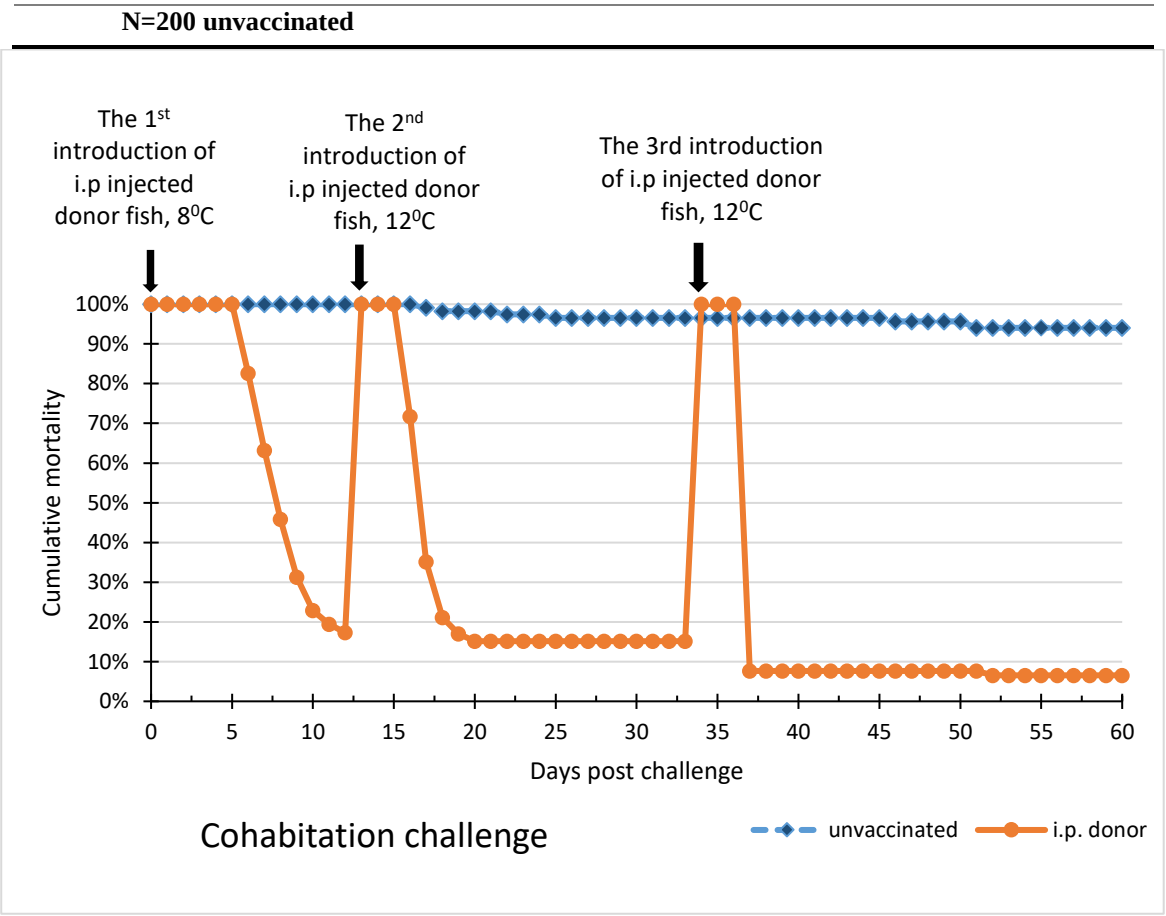


Figure 17. Unvaccinated Arctic char following a cohabitation challenge with three introductions of donor fish i.p injected with *A. salmonicida* subsp. *achromogenes*, strain Keldur265-87.

The survival estimates were influenced by the introduction of infected donors, as introduction of 18, 12 and 20 char donors caused a significantly lower survival probabilities compared to another with 12, 18 and 20 infected donors (Figure 18).

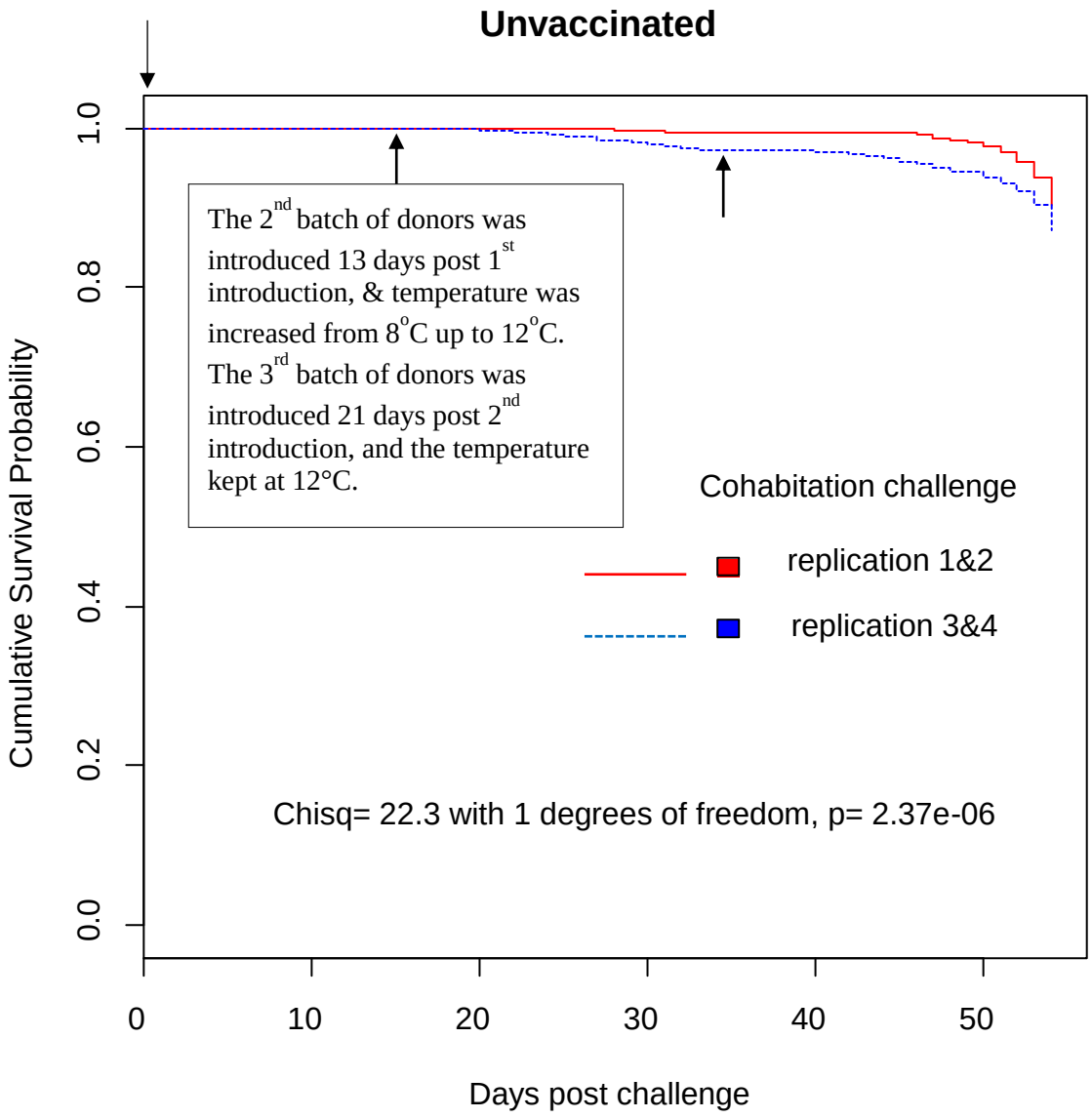


Figure 18. Kaplan–Meier survival estimates of unvaccinated Arctic char challenged by cohabitation with char donors i.p injected with A. *salmonicida* subsp. *achromogenes*, Keldur265-87. The arrows show the introduction of donor fish. The fish were reared at 8°C, but the temperature was elevated to 12°C at the time of the 2nd introduction of donors.

4.2.2 Immersion challenge of Arctic char with A. salmonicida subsp. achromogenes, strain F131-16.

Challenge of Arctic char with *A. salmonicida* subsp. *achromogenes*, strain F131-16, by immersion resulted in very high mortalities in all 4 replication tanks (Figure 19).

The cumulative survival reached 98.6 % (± 2.61) in 17 days and the MDD were 7.13 ± 0.23 days.

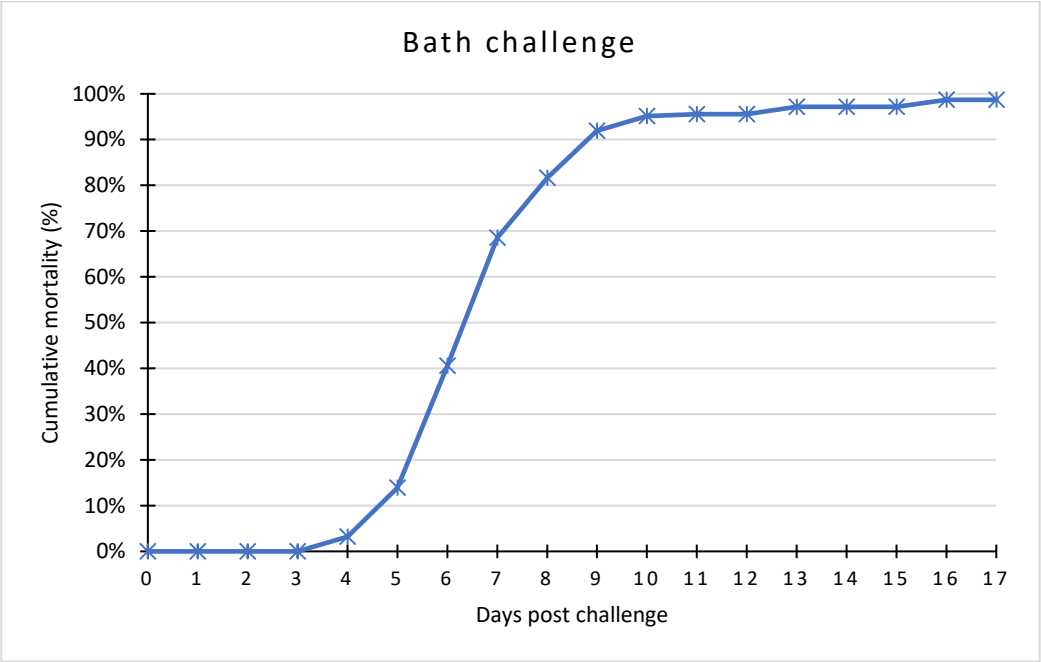


Figure 19. Cumulative mortality (%) of Arctic char challenged by immersion with *A. salmonicida* subsp. *achromogenes*, strain F131-16.

The mortality induced by immersion challenge of Arctic char with *A. salmonicida* subsp. *achromogenes*, strain F131-16, was significantly higher than that caused by cohabitation with donor char i.p injected with *A. salmonicida* subsp. *achromogenes*, strain Keldur265-87 (χ^2 test p-value < 0.05) (Figure 20).

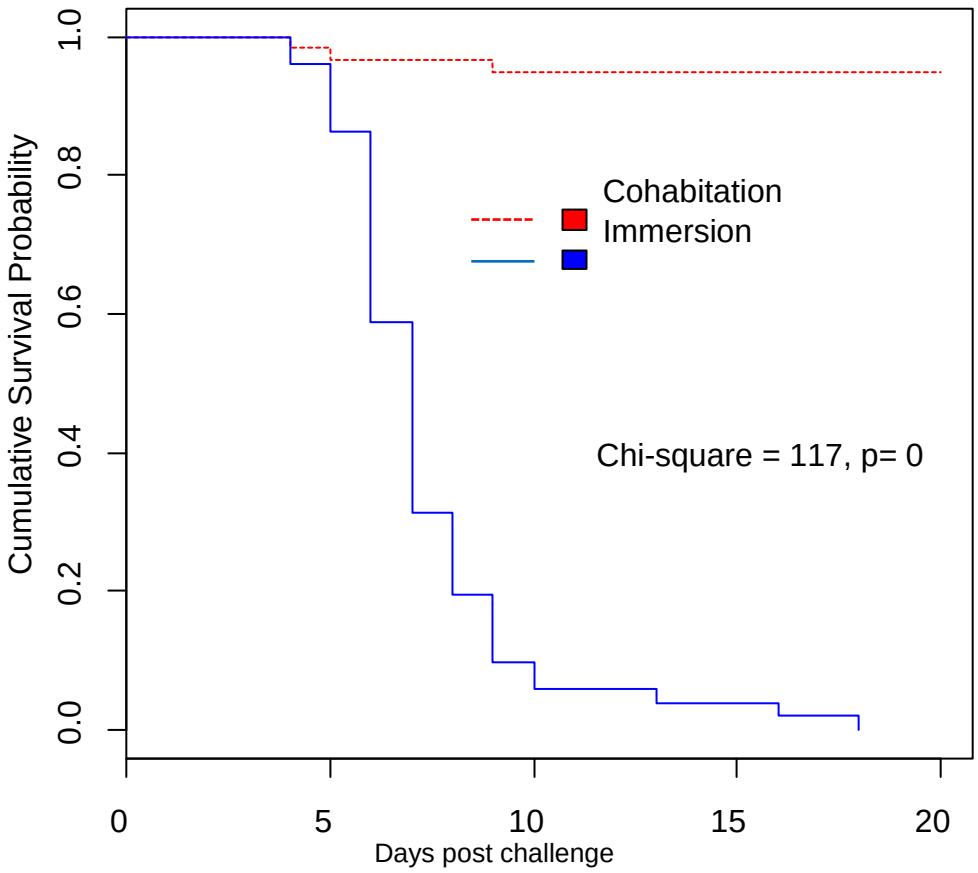


Figure 20. The survival estimates of cohabitation and bath challenges of Arctic char with *A. salmonicida* subsp. *achromogenes*.

5 DISCUSSION

Bacterial pathogenesis is complex and involves many bacterial as well as environmental factors. An infection will only take place where pathogen and host are available in an environment that favours a disease (Gudmundsdottir, 1998). Moreover, the clinical and pathological features of atypical furunculosis of fish induced by *A. salmonicida* subsp. *achromogenes* can vary depending on the interaction between the host and the bacterium (Pylkko *et al.*, 2005). In this study, two atypical *A. salmonicida* strains (Keldur265-87 and F131-16) were both reproducibly virulent and lethal to Arctic char by challenge methods via waterborne route (immersion and cohabitation) or by injection. Pathological signs were more visible at the later stages of the infection. External clinical signs often include loss of appetite, features of an acute septicaemia like haemorrhage at fin bases and anus, anaemia resulting in very pale colour skin, gaping, fin rot and exophthalmos. The gills were often pale. Internal features like hyperaemia of serosal surfaces, haemorrhages in internal organs and mucosa and pale liver, enlarged spleen are frequently detected. Endothelial cells that are anchored on an underlying basement membrane line blood vessel lumens with a thin sheet-like structure, which main structural protein often is type IV collagen (Xu & Shi, 2014). It has been shown that AsaP1, an exoproteinase and a main virulence factor of *A. salmonicida* subsp. *achromogenes*, specifically cleaves type IV collagen (Gudmundsdottir *et al.*, 1990; Arnadottir *et*

al., 2009). This may explain the signs of anaemia, haemorrhages and bleedings observed on diseased char. Nevertheless, these pathological signs are common to many bacterial infections of fish (Gudmundsdottir & Bjornsdottir, 2017). In the acute phase, the char even died without any disease signs (Figure 5B). The development of skin ulcers or lesions which are pathognomonic for "furunculosis" were only detected 12 days post challenge or later and rarely appeared. The gross pathological characteristics of atypical furunculosis observed in Arctic char were comparable to those described for turbot (*Scophthalmus maximus* L.) (Bjornsdottir *et al.*, 2005), but cod infected by *A. salmonicida* subsp. *achromogenes* has been shown to develop different pathology, characterised by granuloma formation (Magnadottir *et al.*, 2002). Pylkko and co-workers (2005) described pathology of grayling infected by atypical *A. salmonicida,* which was represented by disease signs that differ from those obtained in this study. The atypical *A. salmonicida* strains isolated from infected grayling lack production of the AsaP1 proteinase, which may explain the difference as well as different host responses (Gudmundsdottir et al, 2003). As *A. salmonicida* subsp. *achromogenes* infect various fish species both in fresh-water and marine environment, infections are a threat to natural fish stocks as well as aquaculture. Fish surviving from epizootics can become carriers that may spread the disease (Gudmundsdottir & Bjornsdottir, 2017). In our study, two injected donor fish who belonged to the 1st introduction still survived after 60 days post challenge without abnormal performance. But a bacterium was re-isolated from the head kidney of these fish, which was identified as *A. salmonicida*. Thus, these donor char are carriers that are able to spread the disease.

No publication has described experimental infection of Arctic char caused by *A. salmonicida* subsp. *achromogenes* by a cohabitation challenge method. Injection administration of the bacterium has been preferred in experimental infection challenges to effectively control the infection in fish (Gudmundsdottir *et al.*, 2003b; Arnadottir *et al.*, 2009; Schwenteit *et al.*, 2013a; Schwenteit *et al.*, 2013b; Schwenteit *et al.*, 2015). Injection challenge (i.p. or i.m.) is considered as a reliable and reproducible way to challenge individuals with a uniform precise dosage of virulent bacteria (Nordmo & Ramstad, 1997; Chettri *et al.*, 2015; Marana *et al.*, 2015; Marana *et al.*, 2016). However, challenge by injection does not represent the natural way of disease transmission, as it evades surface associated immune protection of the host and it is an impracticable application for field trials.

The very low and slow mortality in non-vaccinated Arctic char observed in the cohabitation challenge performed in this study showed that the cohabitation may not be an effective experimental infection method to infect Arctic char with *A. salmonicida* subsp. *achromogenes*, strain Keldur265-87 under the conditions applied. No mortality of vaccinated Arctic char occurred in this study. As the mortality of the unvaccinated fish was very low (5.17% or lower), the protection induced by the vaccine cannot be evaluated (Gudmundsdottir & Bjornsdottir, 2007). Moreover, the mortalities of vaccinated char related to atypical furunculosis have often occurred after 6 months post vaccination or later (Figure 1). The survival estimates of test groups challenged at low temperature (8^{o}C) were significantly lower than those of groups at higher temperature (12^{o}C) ($p < 0.05$). Hence, this may affect the result of cohabitation challenge models in the field where stable environmental condition is difficult to maintain.

There are many factors that can affect the results of a cohabitation challenge, such as the nature of the bacterial strain used, salinity and temperature of the rearing water, number of donor fish added, fish density, material in tanks and tubes, which bacteria may adhere to, and more (Nordmo *et al.*,

1998; Nordmo & Ramstad, 1999; Coquet *et al.*, 2002; Chettri *et al.*, 2015). Cohabitation challenges do resemble the natural route of infection and are not very laborious, so it would be of interest to perform another experiment where some factors would be changed. These could for example include the strain used to infect the donor fish, fish density, salinity and temperature. As mortality was found to be temperature dependent in this study, it could be reasoned to perform the challenge at the highest temperature that is applicable.

Immersion (bath) challenge is another method that well reflects the natural route of infection, but is like the cohabitation reported to be less effective in causing the disease than the injection method (Nordmo & Ramstad, 1997; Nordmo *et al.*, 1998). The present study showed that immersion in a suspension containing 10^6 CFU/ml of *A. salmonicida* subsp. *achromogenes*, strain F131-16, successfully caused an infection of atypical furunculosis in Arctic char. This strain was recently isolated from vaccinated Arctic char suffering from atypical furunculosis on a fish farm in Iceland (Sigridur Hjartardottir, personal communication). Immersion for 2h in a suspension containing 10^6 CFU/ml of the bacterium in water at a temperature of 13°C and 15 ppt salinity resulted in very high cumulative mortality (98.62% in 17 days) in non-vaccinated Arctic char. The results of the immersion challenge show that it is an effective challenge method for Arctic char.

A study by Arnadottir *et al.* (2009) reported that strain Keldur265-87 killed 60% of Arctic char (30 g) immersed in a bacterial dose of 5 *10^8 CFU/ml for 1h. It is very hard to compare challenge experiments performed under different conditions, but the results obtained in this study and Arnadottir's and co-workers may indicate that strain F131-16 is more virulent for char than strain Keldur265-87. Hence, it may be of interest to try to perform cohabitation challenge of char, using strain F132-16

6 CONCLUSION

A. salmonicida subsp. *achromogenes*, strains Keldur265-87 and F131-16, induced reproducibly atypical furunculosis and mortalities in Arctic char challenged by injection, immersion or cohabitation methods. Gross pathological signs mainly included general external and internal anaemia, haemorrhages and bleedings, which are typical signs of infection by this bacterium. Skin ulcers or lesions were visible at late infection stages but rarely seen. Disease carriers without any signs of disease were observed.

Infection of char by *A. salmonicida* subsp. *achromogenes* was found to be temperature dependent, as disease transmission and development was more prominent at 12°C than 8°C.

A. salmonicida subsp. *achromogenes*, strain F131-16, was found to be more virulent than strain Keldur-265-87. It is recommended that strain F131-16 will be applied in the upcoming challenge experiments.

The cohabitation challenge performed in this study did not induce high enough mortalities to be used in further experiments. The immersion challenge method mimics natural infection. It was found to be an effective method, which may be applied to challenge vaccinated and unvaccinated char belonging to different families aiming for selection of disease resistant fish families.

LIST OF REFERENCES

Adanir, D. O. R., & Turutoglu, H. (2007). Isolation and antibiotic susceptibility of *Aeromonas hydrophila* in a carp (*Cyprunus carpio*) hatchery farm. *Bulletin of the Veterinary Institute in Pulawy, 51*, 361-364.

Arnadottir, H., Hvanndal, I., Andresdottir, V., Burr, S. E., Frey, J., & Gudmundsdottir, B. K. (2009). The AsaP1 Peptidase of *Aeromonas salmonicida subsp. achromogenes* Is a Highly Conserved Deuterolysin Metalloprotease (Family M35) and a Major Virulence Factor. *Journal of Bacteriology, 191*(1), 403-410. doi: 10.1128/jb.00847-08

Arnason, J., Carlberg, H., Brannas, E., Thorarensen, H., Sigurgeirsson, O., & Bjornsdottir, R. (2015). Profitable Arctic charr farming in the Nordic countries – Testing of different diets for Arctic charr under practical conditions. Nordic Innovation Project No. P11081. *Nordic Innovation publication 2015:01* (pp. 55). Oslo, Norway: Nordic Innovation.

Austin, B. (2011). Taxonomy of bacterial fish pathogens. *Veterinary Research, 42*(1), 20-20. doi: 10.1186/1297-9716-42-20

Austin, B., & Austin, D. A. (2012). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish* (5th ed.). Dordrecht: Springer Science & Business Media Dordrecht.

Balcazar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J. L., & Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture, 278*(1-4), 188-191. doi: 10.1016/j.aquaculture.2008.03.014

Belem-Costa, A., & Cyrino, J. E. P. (2006). Antibiotic resistence of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). *Scientia Agricola, 63*, 281-284.

Bjornsdottir, B., Gudmundsdottir, S., Bambir, S. H., & Gudmundsdottir, B. K. (2005). Experimental infection of turbot, *Scophthalmus maximus* L., by *Aeromonas salmonicida subsp. achromogenes* and evaluation of cross protection induced by a furunculosis vaccine. *Journal of Fish Diseases, 28*(3), 181-188. doi: 10.1111/j.1365-2761.2005.00617.x

Blasco-Costa, I., Faltynkova, A., Georgieva, S., Skírnisson, K., Scholz, T., & Kostadinova, A. (2014). Fish pathogens near the Arctic Circle: molecular, morphological and ecological evidence for unexpected diversity of Diplostomum (Digenea: diplostomidae) in Iceland. *International Journal for Parasitology, 44*(10), 703-715. doi: http://dx.doi.org/10.1016/j.ijpara.2014.04.009

Brannas, E., Larsson, S., Sæther, B. S., Siikavuopio, S. I., Thorarensen, H., Henrik Jeuthe, & Sigurgeirsson, O. (2011). *Arctic Charr Farming Production of Juveniles; a Manual*. Umeå, Swedish University of Agricultural Sciences Report. Department of Wildlife, Fish, and Environmental Studies.

Breyer, K. E., Getchell, R. G., Cornwell, E. R., Wooster, G. A., Ketola, H. G., & Bowser, P. R. (2015). Efficacy of an Extract from Garlic, *Allium sativum*, Against Infection with the Furunculosis Bacterium, *Aeromonas salmonicida*, in Rainbow Trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society, 46*(3), 273-282. doi: 10.1111/jwas.12195

Brudeseth, B. E., Wiulsrød, R., Fredriksen, B. N., Lindmo, K., Løkling, K.-E., Bordevik, M., Steine, N., Klevan, A., & Gravningen, K. (2013). Status and future perspectives of vaccines for industrialised fin-fish farming. *Fish & Shellfish Immunology, 35*(6), 1759-1768. doi: http://dx.doi.org/10.1016/j.fsi.2013.05.029

Brunner, P. C., Douglas, M. R., Osinov, A., Wilson, C. C., & Bernatchez, L. (2001). Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution, 55*(3), 573-586. doi: Doi 10.1554/0014-3820(2001)055[0573:Hpoacs]2.0.Co;2

Chen, P.-L., Ko, W.-C., & Wu, C.-J. (2012). Complexity of β-lactamases among clinical *Aeromonas* isolates and its clinical implications. *Journal of Microbiology, Immunology and Infection, 45*(6), 398-403. doi: http://dx.doi.org/10.1016/j.jmii.2012.08.008

Chettri, J. K., Skov, J., Jaafar, R. M., Krossøy, B., Kania, P. W., Dalsgaard, I., & Buchmann, K. (2015). Comparative evaluation of infection methods and environmental factors on challenge success: *Aeromonas salmonicida* infection in vaccinated rainbow trout. *Fish & Shellfish Immunology, 44*(2), 485-495. doi: http://dx.doi.org/10.1016/j.fsi.2015.03.003

Cipriano, R. C., Bullock, G. L., & Pyle, S. (1984). *Aeromonas hydrophila* and motile aeromonad septicemias of fish. *US Fish & Wildlife Publications.* (pp. 134): .

Cipriano, R. C. (2001). *Aeromonas hydrophila* and motile aeromonad septicemias of fish *Fish Disease Leaflet* (Revision - 2001 ed., pp. 25).

Cipriano, R. C., & Austin, B. (2011). Furunculosis and other aeromonad diseases. In Woo, P. T. K. & Bruno, D. W. (Eds.), *Fish diseases and disorders: viral, bacterial and fungal, Volume 3: viral, bacterial and fungal infections* (2nd ed., Vol. 3, pp. 930): CAB International.

Coquet, L., Cosette, P., Junter, G.-A., Beucher, E., Saiter, J.-M., & Jouenne, T. (2002). Adhesion of Yersinia ruckeri to fish farm materials: influence of cell and material surface properties. *Colloids and Surfaces B: Biointerfaces, 26*(4), 373-378. doi: http://dx.doi.org/10.1016/S0927-7765(02)00023-1

Cordero, H., Esteban, M. Á., & Cuesta, A. (2014). Use of Probiotic Bacteria against Bacterial and Viral Infections in Shellfish and Fish Aquaculture. In Hernandez-Vergara, M. (Ed.), *Sustainable Aquaculture Techniques*: InTech.

Crumlish, M., Thanh, P. C., Koesling, J., Tung, V. T., & Gravningen, K. (2010). Experimental challenge studies in Vietnamese catfish, *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. *Journal of Fish Diseases, 33*(9), 717-722. doi: 10.1111/j.1365-2761.2010.01173.x

Dalsgaard, I., Gudmundsdottir, B. K., Helgason, S., Hoie, S., Thoresen, O. F., Wichardt, U. P., & Wiklund, T. (1998). Identification of atypical *Aeromonas salmonicida*: inter-laboratory evaluation and harmonization of methods. *Journal of Applied Microbiology, 84*. doi: 10.1046/j.1365-2672.1998.00435.x

Daood, N. (2012). Isolation and Antibiotic Susceptibility of *Aeromonas spp*. From Freshwater Fish Farm and Farmed Carp (Dam of 16 Tishreen, Lattakia). *Journal of Basic Sciences, 28*(1), 27-39.

Drangsholt, T. M., Gjerde, B., Odegard, J., Finne-Fridell, F., Evensen, O., & Bentsen, H. B. (2011). Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). *Heredity (Edinb), 107*(5), 471-477. doi: 10.1038/hdy.2011.34

Eriksson, L. O., Alanara, A., Nilsson, J., & Brannas, E. (2010). The Arctic charr story: development of subarctic freshwater fish farming in Sweden. *Hydrobiologia, 650*(1), 265-274. doi: 10.1007/s10750-010-0248-1

Falco, A., Frost, P., Miest, J., Pionnier, N., Irnazarow, I., & Hoole, D. (2012). Reduced inflammatory response to *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio* L.) fed with beta-glucan supplements. *Fish & Shellfish Immunology, 32*(6), 1051-1057. doi: 10.1016/j.fsi.2012.02.028

Ferguson, H. W., Turnbull, J. F., Shinn, A., Thompson, K., Dung, T. T., & Crumlish, M. (2001). Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. *Journal of Fish Diseases, 24*(9), 509-513. doi: 10.1046/j.1365-2761.2001.00308.x

Foss, A., Imsland, A. K., Falk-Petersen, I. B., & Oiestad, V. (2004). A review of the culture potential of spotted wolffish *Anarhichas minor* Olafsen. *Reviews in Fish Biology and Fisheries, 14*(2), 277-294. doi: 10.1007/s11160-004-8360-9

Freyhof, J., & Kottelat, M. (2008). *Salvelinus alpinus*. *The IUCN Red List of Threatened Species 2008: e.T19877A9102572* [January 2017] <http://www.iucnredlist.org/details/classify/19877/0>

Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquaculture Research, 31*(1), 25-33. doi: 10.1046/j.1365-2109.2000.00389.x

Goel, M. K., Khanna, P., & Kishore, J. (2010). Understanding survival analysis: Kaplan-Meier estimate. *International Journal of Ayurveda Research, 1*(4), 274-278. doi: 10.4103/0974-7788.76794

Gudmundsdottir, B. K., Hastings, T. S., & Ellis, A. E. (1990). Isolation of a new toxic protease from a strain of *Aeromonas salmonicida* subspecies *achromogenes*. *Diseases of Aquatic Organisms, 9*(3), 199-208. doi: 10.3354/dao009199

Gudmundsdottir, B. K. (1996). Comparison of extracellular proteases produced by Aeromonas salmonicida strains, isolated from various fish species. *Journal of Applied Bacteriology, 80*(1), 105-113. doi: DOI 10.1111/j.1365-2672.1996.tb03196.x

Gudmundsdottir, B. K. (1997). *Aeromonas salmonicida subsp. achromogenes. A study of extracellular virulence factors and immunity in Atlantic salmon (Salmo salar L).* Ph.D. Thesis, University of Iceland, Reykjavik.

Gudmundsdottir, B. K., & Magnadottir, B. (1997). Protection of Atlantic salmon (*Salmo salar* L.) against an experimental infection of *Aeromonas salmonicida ssp. achromogenes*. *Fish & Shellfish Immunology, 7*(1), 55-69. doi: http://dx.doi.org/10.1006/fsim.1996.0065

Gudmundsdottir, B. K. (1998). Infections by atypical strains of the bacterium *Aeromonas salmonicida*. *Icelandic agricultural sciences, 12*, 61-72.

Gudmundsdottir, B. K., Hvanndal, I., Bjornsdottir, B., & Wagner, U. (2003a). Analysis of exotoxins produced by atypical isolates of *Aeromonas salmonicida*, by enzymatic and serological methods. *Journal of Fish Diseases, 26*(1), 15-29. doi: DOI 10.1046/j.1365-2761.2003.00433.x

Gudmundsdottir, B. K., & Bjornsdottir, B. (2017). *Aeromonas spp*. (*A. salmonicida* and *A. hydrophila*). In Woo, P. T. K. & Cipriano, R. C. (Eds.), *Fish Viruses and Bacteria: Pathobiology and Protection* (1 ed.). England: CABI, Nosworthy Way, Wallingford, Oxfordshire.

Gudmundsdottir, B. K., & Bjornsdottir, B. (2007). Vaccination against atypical furunculosis and winter ulcer disease of fish. *Vaccine, 25*(30), 5512-5523. doi: http://dx.doi.org/10.1016/j.vaccine.2007.02.009

Gudmundsdottir, S., Lange, S., Magnadottir, B., & Gudmundsdottir, B. K. (2003b). Protection against atypical furunculosis in Atlantic halibut, *Hippoglossus hippoglossus* (L.); comparison of a commercial furunculosis vaccine and an autogenous vaccine. *Journal of Fish Diseases, 26*(6), 331-338.

Gunnlaugsdottir, B., & Gudmundsdottir, B. K. (1997). Pathogenicity of atypical *Aeromonas salmonicida* in Atlantic salmon compared with protease production. *Journal of Applied Microbiology, 83*(5), 542-551. doi: 10.1046/j.1365-2672.1997.00247.x

Guz, L., & Kozinska, A. (2004). Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L.). *Bull Vet Inst Pulawy, 48*, 391-395.

Halls, A. S., & Johns, M. (2013). Assessment of the vulnerability of the Mekong Delta Pangasius catfish industry to development and climate change in the Lower Mekong Basin. *Report prepared for the Sustainable Fisheries Partnership* (pp. 95).

Harmon, P. R., Knox, D. E., Cornick, J. W., Zwicker, B. M., & Olivier, G. (1991). Atypical furunculosis in Atlantic salmon. *Bulletin of the Aquaculture Association of Canada* (No. 3), 67-68.

Hastein, T., Gudding, R., & Evensen, O. (2005). Bacterial vaccines for fish--an update of the current situation worldwide. *Developments in Biologicals, 121*, 55-74.

Hastein, T., Hellstrøm, A., Jonsson, G., Olesen, N. J., & Parnanen, E. R.-. (2001). Surveillance of Fish Diseases in the Nordic Countries. *Acta Veterinaria Scandinavica, 42*(1), S43. doi: 10.1186/1751-0147-42-s1-s43

Heasman, M. S., & Black, K. D. (1998). The potential of Arctic charr, *Salvelinus alpinus* (L.), for mariculture. *Aquaculture Research, 29*(1), 67-76. doi: 10.1046/j.1365-2109.1998.00929.x

Hellberg, H., Moksness, E., & Hoie, S. (1996). Infection with atypical *Aeromonas salmonicida* in farmed common wolffish, *Anarhichas lupus* L. *Journal of Fish Diseases, 19*(4), 329-332. doi: DOI 10.1111/j.1365-2761.1996.tb00711.x

Hermansen, O., & Troell, M. (2012). Aquaculture in the Arctic–a review. *Marine Ecology Progress Series, 373*, 265-273.

Hjeltnes, B., Bergh, Ø., Wergeland, H., & Holm, J. C. (1995). Susceptibility of Atlantic cod *Gadus morhua*, halibut *Hippoglossus hippoglossus* and wrasse (Labridae) to *Aeromonas salmonicida subsp. salmonicida* and the possibility of transmission of furunculosis from farmed salmon *Salmo salar* to marine fish. *Diseases of Aquatic Organisms, 23*(1), 25-31.

Hossain, M. J., Sun, D., McGarey, D. J., Wrenn, S., Alexander, L. M., Martino, M. E., Xing, Y., Terhune, J. S., & Liles, M. R. (2014). An Asian Origin of Virulent *Aeromonas hydrophila* Responsible for Disease Epidemics in United States-Farmed Catfish. *mBio, 5*(3). doi: 10.1128/mBio.00848-14

Irianto, A., Robertson, P. A. W., & Austin, B. (2003). Oral administration of formalin-inactivated cells of *Aeromonas hydrophila* A3-51 controls infection by atypical *A. salmonicida* in goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases, 26*(2), 117-120. doi: 10.1046/j.1365-2761.2003.00439.x

Janda, J. M., & Abbott, S. L. (2010). The Genus Aeromonas: Taxonomy, Pathogenicity, and Infection. *Clinical Microbiology Reviews, 23*(1), 35-73. doi: 10.1128/cmr.00039-09

Jeeva, S., Lekshmi, N. P., Brindha, J. R., & Vasudevan, A. (2013). Studies on antibiotic subsceptibility of *Aeromonas hydrophila I*solated from gold fish (*Carassius auratus*). *International Journal of Current Microbiology and Applied Sciences, 2*(12), 7-13.

Jobling, M., Tveiten, H., & Hatlen, B. (1998). Review Cultivation of Arctic charr: an update. *Aquaculture International, 6*(3), 181-196. doi: 10.1023/a:1009246509657

Johnston, G. (2002). *Arctic Charr Aquaculture*: Fishing News Books, Blackwell Publishing.

Jonsdottir, H., Malmquist, H. J., Snorrason, S. S., Gudbergsson, G., & Gudmundsdottir, S. (1998). Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland. *Journal of Fish Biology, 53*(2), 322-339. doi: 10.1111/j.1095-8649.1998.tb00984.x

Jonsson, B., & Jonsson, N. (2001). Polymorphism and speciation in Arctic charr. *Journal of Fish Biology, 58*(3), 605-638. doi: 10.1111/j.1095-8649.2001.tb00518.x

Kaskhedikar, M., & Chhabra, D. (2010). Multiple drug resistance in *Aeromonas hydrophila* isolates of fish. *Food Microbiology, 28*, 157-168.

Kristmundsson, A., & Richter, S. H. (2009). Parasites of resident arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta*, in two lakes in Iceland. *Icelandic agricultural sciences, 22*, 5-18.

Kristmundsson, A., Antonsson, T., & Arnason, F. (2010). First record of proliferative kidney disease in Iceland. *Bulletin Of The European Association Of Fish Pathologists, 30*(1), 35-40.

Laith, A., & Najiah, M. (2013). *Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). *Journal of Aquaculture Research & Development, 5*(215). doi: 10.4172/2155-9546.1000215

Lillehaug, A. (2014). Vaccination Strategies and Procedures. In Gudding, R., Lillehaug, A. & Evensen, Ø. (Eds.), *Fish Vaccination* (pp. 140-152). UK: John Wiley & Sons, Ltd.

Lio-Po, G. D., Lim, L. S., Woo, P., & Bruno, D. (2014). Infectious diseases of warmwater fish in fresh water. In Woo, P. & Bruno, D. (Eds.), *Diseases and disorders of finfish in cage culture.* (2 ed., pp. 193-253). Wallingford, UK: CABI Publishing.

Lødemel, J. B., Mayhew, T. M., Myklebust, R., Olsen, R. E., Espelid, S., & Ringø, E. (2001). Effect of three dietary oils on disease susceptibility in Arctic charr (*Salvelinus alpinus L*.) during cohabitant challenge with *Aeromonas salmonicida ssp. salmonicida*. *Aquaculture Research, 32*(12), 935-945. doi: 10.1046/j.1365-2109.2001.00621.x

Ly, L. T. T., Nguyen, D. N., Vo, P. H., & Doan, C. V. (2009). Hemorrhage Disease of Cultured Tra Catfish (*Pangasianodon hypophthalmus*) in Mekong Delta (Vietnam). *The Israeli Journal of Aquaculture - Bamidgeh, 61*(3), 215-224. doi: http://hdl.handle.net/10524/19290

Magnadottir, B., & Gudmundsdottir, B. K. (1992). A comparison of total and specific immunoglobulin levels in healthy Atlantic salmon (*Salmo salar* L.) and in salmon naturally infected with *Aeromonas salmonicida* subsp. *achromogenes*. *Veterinary Immunology and Immunopathology, 32*(1), 179-189. doi: http://dx.doi.org/10.1016/0165-2427(92)90078-5

Magnadottir, B. (2010). Immunological Control of Fish Diseases. *Marine Biotechnology, 12*(4), 361-379. doi: 10.1007/s10126-010-9279-x

Magnadottir, B., Bambir, S. H., Gudmundsdottir, B. K., Pilstrom, L., & Helgason, S. (2002). Atypical *Aeromonas salmonicida* infection in naturally and experimentally infected cod, *Gadus morhua* L. *Journal of Fish Diseases, 25*(10), 583-597. doi: 10.1046/j.1365-2761.2002.00407.x

Magnadottir, B. (2006). Innate immunity of fish (overview). *Fish & Shellfish Immunology, 20*(2), 137-151. doi: http://dx.doi.org/10.1016/j.fsi.2004.09.006

Maitland, P. S., Winfield, I. J., McCarthy, I. D., & Igoe, F. (2007). The status of Arctic charr *Salvelinus alpinus* in Britain and Ireland. *Ecology of Freshwater Fish, 16*(1), 6-19. doi: 10.1111/j.1600-0633.2006.00167.x

Marana, M. H., Skov, J., Chettri, J. K., Krossøy, B., Dalsgaard, I., Kania, P. W., & Buchmann, K. (2015). *A novel challenge method with aeromonas salmonicida in rainbow trout for evaluation of furunculosis vaccines.* Paper presented at the 17th International Conference on Diseases of Fish and Shellfish, 2015.

Marana, M. H., Chettri, J. K., Skov, J., Dalsgaard, I., Kania, P. W., & Buchmann, K. (2016). A New Furunculosis Challenge Method for Evaluation of Vaccine Efficacy in Rainbow Trout. *Open Journal of Immunology, Vol.06No.04*, 12. doi: 10.4236/oji.2016.64014

Mawdesley-Thomas, L. E. (1969). Furunculosis in the Goldfish *Carassius auratus* (L.). *Journal of Fish Biology, 1*(1), 19-23. doi: 10.1111/j.1095-8649.1969.tb03840.x

Menanteau-Ledouble, S., Kumar, G., Saleh, M., & El-Matbouli, M. (2016). *Aeromonas salmonicida:* updates on an old acquaintance. *Diseases of Aquatic Organisms, 120*(1), 49-68. doi: 10.3354/dao03006

Miest, J. J., Falco, A., Pionnier, N. P. M., Frost, P., Irnazarow, I., Williams, G. T., & Hoole, D. (2012). The influence of dietary beta-glucan, PAMP exposure and *Aeromonas salmonicida* on apoptosis modulation in common carp (*Cyprinus carpio*). *Fish & Shellfish Immunology, 33*(4), 846-856. doi: 10.1016/j.fsi.2012.07.014

Mulero, I., García-Ayala, A., Meseguer, J., & Mulero, V. (2007). Maternal transfer of immunity and ontogeny of autologous immunocompetence of fish: A minireview. *Aquaculture, 268*(1–4), 244-250. doi: http://dx.doi.org/10.1016/j.aquaculture.2007.04.046

Nilsson, J., Brannas, E., & Eriksson, L.-O. (2010). The Swedish Arctic charr breeding programme. *Hydrobiologia, 650*(1), 275-282. doi: 10.1007/s10750-010-0232-9

Nordmo, R. (1997). Strengths and weaknesses of different challenge methods. *Developments in Biological Standardization, 90*, 303-309.

Nordmo, R., & Ramstad, A. (1997). Comparison of different challenge methods to evaluate the efficacy of furunculosis vaccines in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases, 20*(2), 119-126. doi: 10.1046/j.1365-2761.1997.d01-114.x

Nordmo, R., Ramstad, A., & Holth Riseth, J. M. (1998). Induction of experimental furunculosis in heterogenous test populations of Atlantic salmon (*Salmo salar* L.) by use of a cohabitation method. *Aquaculture, 162*(1–2), 11-21. doi: http://dx.doi.org/10.1016/S0044-8486(97)00308-6

Nordmo, R., & Ramstad, A. (1999). Variables affecting the challenge pressure of *Aeromonas salmonicida* and *Vibrio salmonicida* in Atlantic salmon (*Salmo salar* L.). *Aquaculture, 171*(1–2), 1-12. doi: http://dx.doi.org/10.1016/S0044-8486(98)00426-8

O'Brien, D., Mooney, J., Ryan, D., Powell, E., Hiney, M., Smith, P. R., & Powell, R. (1994). Detection of *Aeromonas salmonicida*, causal agent of furunculosis in salmonid fish, from the tank effluent of hatchery-reared Atlantic salmon smolts. *Applied and Environmental Microbiology, 60*(10), 3874-3877.

Odegard, J., Olesen, I., Gjerde, B., & Klemetsdal, G. (2007). Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). *Aquaculture, 271*(1-4), 173-177. doi: 10.1016/j.aquaculture.2007.06.006

Odeyemi, O. A., Asmat, A., & Usup, G. (2012). Antibiotics resistance and putative virulence factors of *Aeromonas hydrophila* isolated from estuary. *The Journal of Microbiology, Biotechnology and Food Sciences, 1*(6), 1339.

Olesen, I., Gjedrem, T., Bentsen, H. B., Gjerde, B., & Rye, M. (2003). Breeding Programs for Sustainable Aquaculture. *Journal of Applied Aquaculture, 13*(3-4), 179-204. doi: 10.1300/J028v13n03_01

Paisley, L. G., Ariel, E., Lyngstad, T., Jónsson, G., Vennerström, P., Hellström, A., & Østergaard, P. (2010). An Overview of Aquaculture in the Nordic Countries. *Journal of the World Aquaculture Society, 41*(1), 1-17. doi: 10.1111/j.1749-7345.2009.00309.x

Perez, M., Fernandez, A., Rodriguez, L., & Nieto, T. (1996). Differential susceptibility to furunculosis of turbot and rainbow trout and release of the furunculosis agent from furunculosis-affected fish. *Diseases of Aquatic Organisms, 26*(2), 133-137.

Pharmaq. (2013). The first ever vaccine for pangasius. The first ever licensed fish vaccine in Vietnam. [December, 2016] < https://www.pharmaq.no/updates/the-first-/>

Phu, T. M., Phuong, N. T., Scippo, M.-L., & Dalsgaard, A. (2015). Quality of Antimicrobial Products Used in Striped Catfish (*Pangasianodon hypophthalmus*) Aquaculture in Vietnam. *PLOS ONE, 10*(4), e0124267. doi: 10.1371/journal.pone.0124267

Pickova, J., Brannas, E., & Andersson, T. (2007). Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. *Aquaculture International, 15*(3), 305-311. doi: 10.1007/s10499-007-9084-3

Pionnier, N., Falco, A., Miest, J., Frost, P., Irnazarow, I., Shrive, A., & Hoole, D. (2013). Dietary beta-glucan stimulate complement and C-reactive protein acute phase responses in common carp (*Cyprinus carpio*) during an *Aeromonas salmonicida* infection. *Fish & Shellfish Immunology, 34*(3), 819-831. doi: 10.1016/j.fsi.2012.12.017

Pridgeon, J. W., & Klesius, P. H. (2011). Molecular identification and virulence of three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama (USA) in 2009. *Diseases of Aquatic Organisms, 94*(3), 249-253.

Pylkko, P., Pohjanvirta, T., Madetoja, J., & Pelkonen, S. (2005). Characterisation of atypical *Aeromonas salmonicida* infection in Arctic charr *Salvelinus alpinus* and European grayling

Thymallus thymallus. *Diseases of Aquatic Organisms, 66*(2), 121-128. doi: Doi 10.3354/Dao066121

R-Core-Team (Producer). (2016). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Retrieved from https://www.R-project.org/.

Rico, A., Phu, T. M., Satapornvanit, K., Min, J., Shahabuddin, A. M., Henriksson, P. J. G., Murray, F. J., Little, D. C., Dalsgaard, A., & Van den Brink, P. J. (2013). Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture, 412–413*, 231-243. doi: http://dx.doi.org/10.1016/j.aquaculture.2013.07.028

Roberts, R. J. (2012). The Bacteriology of Teleosts *Fish Pathology* (pp. 339-382): Wiley-Blackwell.

Robertson, P. A. W., O'Dowd, C., Burrells, C., Williams, P., & Austin, B. (2000). Use of *Carnobacterium sp* as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Aquaculture, 185*(3-4), 235-243. doi: Doi 10.1016/S0044-8486(99)00349-X

Rodriguez-Estrada, U., Satoh, S., Haga, Y., Fushimi, H., & Sweetman, J. (2013). Effects of Inactivated Enterococcus faecalis and Mannan Oligosaccharide and Their Combination on Growth, Immunity, and Disease Protection in Rainbow Trout. *North American Journal of Aquaculture, 75*(3), 416-428. doi: 10.1080/15222055.2013.799620

Rowley, A. F., & Pope, E. C. (2012). Vaccines and crustacean aquaculture—A mechanistic exploration. *Aquaculture, 334–337*, 1-11. doi: http://dx.doi.org/10.1016/j.aquaculture.2011.12.011

Saavedra, M. J., Guedes-Novais, S., Alves, A., Rema, P., Tacao, M., Correia, A., & Martinez-Murcia, A. (2004). Resistance to beta-lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (*Oncorhynchus mykiss*). *International Microbiology, 7*(3), 207-211.

Sæther, B.-S., Siikavuopio, S. I., Thorarensen, H., & Brannas, E. (2013). Status of Arctic charr (*Salvelinus alpinus*) farming in Norway, Sweden and Iceland. *Journal of Ichthyology, 53*(10), 833-839. doi: 10.1134/s0032945213100081

Sahoo, P. K., Mahapatra, K. D., Saha, J. N., Barat, A., Sahoo, M., Mohanty, B. R., Gjerde, B., Ødegård, J., Rye, M., & Salte, R. (2008). Family association between immune parameters and resistance to *Aeromonas hydrophila* infection in the Indian major carp, Labeo rohita. *Fish & Shellfish Immunology, 25*(1–2), 163-169. doi: http://dx.doi.org/10.1016/j.fsi.2008.04.003

Samal, S. K., Das, B. K., & Pal, B. B. (2014). Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish. *International Journal of Current Microbiology and Applied Sciences, 3*(12), 259-267.

Schrader, K. K., Harries, M. D., & Darwish, A. M. (2013). In vitro comparisons of the inhibitory activity of florfenicol, copper sulphate and potassium permanganate towards *Aeromonas hydrophila* and *Flavobacterium columnare*. *Aquaculture Research, 44*(2), 212-219. doi: 10.1111/j.1365-2109.2011.03024.x

Schwenteit, J., Gram, L., Nielsen, K. F., Fridjonsson, O. H., Bornscheuer, U. T., Givskov, M., & Gudmundsdottir, B. K. (2011a). Quorum sensing in *Aeromonas salmonicida subsp. achromogenes* and the effect of the autoinducer synthase AsaI on bacterial virulence. *Veterinary Microbiology, 147*(3–4), 389-397. doi: http://dx.doi.org/10.1016/j.vetmic.2010.07.020

Schwenteit, J., Gram, L., Nielsen, K. F., Fridjonsson, O. H., Bornscheuer, U. T., Givskov, M., & Gudmundsdottir, B. K. (2011b). Quorum sensing in *Aeromonas salmonicida* subsp *achromogenes* and the effect of the autoinducer synthase AsaI on bacterial virulence. *Veterinary Microbiology, 147*(3-4), 389-397. doi: 10.1016/j.vetmic.2010.07.020

Schwenteit, J., Bogdanovic, X., Fridjonsson, O. H., Aevarsson, A., Bornscheuer, U. T., Hinrichs, W., & Gudmundsdottir, B. K. (2013a). Toxoid construction of AsaP1, a lethal toxic

aspzincin metalloendopeptidase of *Aeromonas salmonicida subsp achromogenes*, and studies of its activity and processing. *Veterinary Microbiology, 162*(2-4), 687-694. doi: 10.1016/j.vetmic.2012.09.015

Schwenteit, J. M., Breithaupt, A., Teifke, J. P., Koppang, E. O., Bornscheuer, U. T., Fischer, U., & Gudmundsdottir, B. K. (2013b). Innate and adaptive immune responses of Arctic charr (*Salvelinus alpinus*, L.) during infection with *Aeromonas salmonicida subsp. achromogenes* and the effect of the AsaP1 toxin. *Fish & Shellfish Immunology, 35*(3), 866-873. doi: http://dx.doi.org/10.1016/j.fsi.2013.06.024

Schwenteit, J. M., Weber, B., Milton, D. L., Bornscheuer, U. T., & Gudmundsdottir, B. K. (2015). Construction of *Aeromonas salmonicida subsp. achromogenes* AsaP1-toxoid strains and study of their ability to induce immunity in Arctic char, *Salvelinus alpinus* L. *Journal of Fish Diseases, 38*(10), 891-900. doi: 10.1111/jfd.12303

Seshadri, R., Joseph, S. W., Chopra, A. K., Sha, J., Shaw, J., Graf, J., Haft, D., Wu, M., Ren, Q., Rosovitz, M. J., Madupu, R., Tallon, L., Kim, M., Jin, S., Vuong, H., Stine, O. C., Ali, A., Horneman, A. J., & Heidelberg, J. F. (2006). Genome Sequence of *Aeromonas hydrophila* ATCC 7966T: Jack of All Trades. *Journal of Bacteriology, 188*(23), 8272-8282. doi: 10.1128/jb.00621-06

Shikano, T., Jarvinen, A., Marjamaki, P., Kahilainen, K. K., & Merila, J. (2015). Genetic Variability and Structuring of Arctic Charr (*Salvelinus alpinus*) Populations in Northern Fennoscandia. *PLOS ONE, 10*(10), e0140344. doi: 10.1371/journal.pone.0140344

Sica, M. G., Brugnoni, L. I., Marucci, P. L., & Cubitto, M. A. (2012). Characterization of probiotic properties of lactic acid bacteria isolated from an estuarine environment for application in rainbow trout (*Oncorhynchus mykiss*, Walbaum) farming. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 101*(4), 869-879. doi: 10.1007/s10482-012-9703-5

Sirimanapong, W., Thompson, K. D., Kledmanee, K., Thaijongrak, P., Collet, B., Ooi, E. L., & Adams, A. (2014). Optimisation and standardisation of functional immune assays for striped catfish (*Pangasianodon hypophthalmus*) to compare their immune response to live and heat killed *Aeromonas hydrophila* as models of infection and vaccination. *Fish & Shellfish Immunology, 40*(2), 374-383. doi: http://dx.doi.org/10.1016/j.fsi.2014.07.021

Skybakmoen, S., Siikavuopio, S. I., & Saether, B. S. (2009). Coldwater RAS in an Arctic charr farm in Northern Norway. *Aquacultural Engineering, 41*(2), 114-121. doi: 10.1016/j.aquaeng.2009.06.007

Sommerset, I., Krossøy, B., Biering, E., & Frost, P. (2005). Vaccines for fish in aquaculture. *Expert Review of Vaccines, 4*(1), 89-101. doi: 10.1586/14760584.4.1.89

Stratev, D., & Odeyemi, O. A. (2016a). Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. *Journal of Infection and Public Health, 9*(5), 535-544. doi: http://dx.doi.org/10.1016/j.jiph.2015.10.006

Stratev, D., & Odeyemi, O. A. (2016b). An overview of motile *Aeromonas* septicaemia management. *Aquaculture International*, 1-11. doi: 10.1007/s10499-016-0100-3

Summerfelt, S. T., Wilton, G., Roberts, D., Rimmer, T., & Fonkalsrud, K. (2004). Developments in recirculating systems for Arctic char culture in North America. *Aquacultural Engineering, 30*(1-2), 31-71. doi: 10.1016/j.aquaeng.2003.09.001

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. New York: Springer.

Therneau, T. M. (2015). A Package for Survival Analysis in S, Version 2.38 *https://CRAN.R-project.org/package=survival*.

VASEP. (2015). *Overview of aquaculture in Vietnam*. Vietnam Association of Seafood Exporters and Producers. [December 2016] <http://vasep.com.vn/1192/OneContent/tong-quan-nganh.htm.>

Wang, J.-L., Meng, X.-l., Lu, R.-h., Wu, C., Luo, Y.-T., Yan, X., Li, X.-J., Kong, X.-H., & Nie, G.-X. (2015). Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.). *Aquaculture, 435*, 293-300. doi: http://dx.doi.org/10.1016/j.aquaculture.2014.10.004

WHO. (2016). Antimicrobial resistance (Fact sheet). Retrieved 16th December, from World Health Organization http://www.who.int/mediacentre/factsheets/fs194/en/

Wiklund, T., & Bylund, G. (1993). Skin ulcer disease of flounder *Platichthys flesus* in the northern Baltic Sea. *Diseases of Aquatic Organisms, 17*(3), 165-174.

Wiklund, T., & Dalsgaard, I. (1998). Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review. *Diseases of Aquatic Organisms, 32*(1), 49-69.

Wilson, A. J., Gíslason, D., Skúlason, S., Snorrason, S. S., Adams, C. E., Alexander, G., Danzmann, R. G., & Ferguson, M. M. (2004). Population genetic structure of Arctic charr, *Salvelinus alpinus* from northwest Europe on large and small spatial scales. *Molecular Ecology, 13*(5), 1129-1142. doi: 10.1111/j.1365-294X.2004.02149.x

Xu, J. Y., & Shi, G. P. (2014). Vascular wall extracellular matrix proteins and vascular diseases. *Biochimica Et Biophysica Acta-Molecular Basis of Disease, 1842*(11), 2106-2119. doi: 10.1016/j.bbadis.2014.07.008

Yanez, J. M., Houston, R. D., & Newman, S. (2014). Genetics and genomics of disease resistance in salmonid species. *Frontiers in Genetics, 5*(415). doi: 10.3389/fgene.2014.00415

Zhang, D., Xu, D.-H., & Shoemaker, C. (2016). Experimental induction of motile Aeromonas septicemia in channel catfish (*Ictalurus punctatus*) by waterborne challenge with virulent *Aeromonas hydrophila*. *Aquaculture Reports, 3*, 18-23. doi: http://dx.doi.org/10.1016/j.aqrep.2015.11.003

Zhang, Z. B., Niu, C. J., Storset, A., Bogwald, J., & Dalmo, R. A. (2011). Comparison of *Aeromonas salmonicida* resistant and susceptible salmon families: A high immune response is beneficial for the survival against *Aeromonas salmonicida* challenge. *Fish & Shellfish Immunology, 31*(1), 1-9. doi: 10.1016/j.fsi.2010.12.019

Zheng, Q., Wu, Y., Xu, H., Wang, H., Tang, H., Xia, X., & Feng, J. (2016). Immune responses to *Aeromonas hydrophila* infection in *Schizothorax prenanti* fed with oxidized konjac glucomannan and its acidolysis products. *Fish & Shellfish Immunology, 49*, 260-267. doi: http://dx.doi.org/10.1016/j.fsi.2015.12.042

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to all those who provided me the possibility to complete this report.

My heartfelt appreciation goes to my supervisor, Bjarnheidur K. Gudmundsdottir for her amazing guidance, patience and support.

I am really grateful to the management team of the UNU-FTP: Tumi Tomasson, Thor Asgeirsson, Sigridur Kr. Ingvarsdottir and Mary Frances Davidson for granting me the chance to attend the training programme and for their guidance.

I would like to express special thanks to Helgi Thorarensen for his guidance and for letting me kill tons of "baby chars" of his project for my experiments.

Many thanks to all the staff from the Aquaculture and Biology Department of Holar University College, for their help and friendship during my stay in the beautiful place, Saudarkrokur.

A special gratitude I give to my family for their love and support from a distance.

And finally, thanks to the UNU-FTP fellows for their support and friendship. It was great knowing you all.

APPENDIX

Appendix 1: Cohabitation challenges of Arctic char with *A. salmonicida subsp. achromogene* Keldur265-87

Cohabitation challenge with *A. salmonicida subsp. achromogene* in Arctic char																																			
1st challenge			**20% cohabitation**																				**30% cohabitation**													
Date	Dpc		injected fish					vaccinated						unvaccinated								injected fish				vaccinated				unvaccinated				Control	
			tank 1		tank 2			tank 1			tank 2			tank 1			tank 2					tank 3		tank 4		tank 3		tank 4		tank 3		tank 4		vac	unvac
		Dpi	L	D	L	D	C	L	D	C	L	D	C	L	D	C	L	D	C	dpi	Date	L	D	L	D	L	D	L	D	L	D	L	D	L	D
09-Dec	0	0	12	0	12	0		30	0		29	0		29	0		30	0		0	09-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
10-Dec	1	1	12	0	12	0		30	0		29	0		29	0		30	0		1	10-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
11-Dec	2	2	12	0	12	0		30	0		29	0		29	0		30	0		2	11-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
12-Dec	3	3	12	0	12	0		30	0		29	0		29	0		30	0		3	12-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
13-Dec	4	4	12	0	12	0		30	0		29	0		29	0		30	0		4	13-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
14-Dec	5	5	12	0	12	0		30	0		29	0		29	0		30	0		5	14-Dec	18	0	18	0	29	0	28	0	30	0	30	0	30	30
15-Dec	6	6	12	0	12	1		30	0		29	0		29	0		30	0		6	15-Dec	18	2	18	9	29	0	28	0	30	0	30	0	30	30
16-Dec	7	7	12	3	11	1		30	0		29	0		29	0		30	0		7	16-Dec	16	4	9	4	29	0	28	0	30	0	30	0	30	30
17-Dec	8	8	9	2	10	1		30	0		29	0		29	0		30	0		8	17-Dec	12	5	5	3	29	0	28	0	30	0	30	0	30	30
18-Dec	9	9	7	2	9	3		30	0		29	0		29	0		30	0		9	18-Dec	7	2	2	1	29	0	28	0	30	0	30	0	30	30
19-Dec	10	10	5	1	6	3		30	0		29	0		29	0		30	0		10	19-Dec	5	0	1	0	29	0	28	0	30	0	30	0	30	30
20-Dec	11	11	4	1	3	0		30	0		29	0		29	0		30	0		11	20-Dec	5	1	1	0	29	0	28	0	30	0	30	0	30	30
21-Dec	12	12	3	0	3	1		30	0		29	0		29	0		30	0		12	21-Dec	4	0	1	0	29	0	28	0	30	0	30	0	30	30
22-Dec	13	13	3	0	2	0		30	0		29	0		29	0		30	0		13	22-Dec	4	0	1	0	29	0	28	0	30	0	30	0	30	30
T				9		10			0			0			0			0					14		17		0		0		0		0		

This paper should be cited as:
Nguyen, T.T.G. 2018. *Studies of experimental infection of Arctic char (*Salvelinus alpinus *L.) with* Aeromonas samonicida *subsp.* Achromogenes. Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/giang16prf.pdf

2nd challenge			30% cohabitation																		20% cohabitation													
			injected fish					vaccinated					unvaccinated									injected fish				vaccinated				unvaccinated				Control	
Date		Dpi	tank 1		tank 2			tank 1			tank 2			tank 1			tank 2				Date	tank 3		tank 4		tank 3		tank 4		tank 3		tank 4		vac	unvac
			L	D	L	D	C	L	D	C	L	D	C	L	D	C	L	D	C			L	D	L	D	L	D	L	D	L	D	L	D	L	D
22-Dec	13	0	21	0	20	0		30	0		29	0		29	0		30	0	0	0	22-Dec	16	0	13	0	29	0	28	0	30	0	30	0	30	30
23-Dec	14	1	21	0	20	0	1	30	0		29	0	2	29	0		30	0	4	1	23-Dec	16	0	13	0	29	0	28	0	30	0	30	0	30	30
24-Dec	15	2	21	0	19	0	0	30	0		27	0		29	0		26	0	0	2	24-Dec	16	0	13	0	29	0	28	0	30	0	30	0	30	30
25-Dec	16	3	21	5	19	4		30	0		27	0		29	0		26	0	0	3	25-Dec	16	6	13	4	29	0	28	0	30	0	30	0	30	30
26-Dec	17	4	16	11	15	7		30	0		27	0		29	0		26	0	0	4	26-Dec	10	3	9	5	29	0	28	0	30	0	30	1	30	30
27-Dec	18	5	5	2	8	5		30	0		27	0		29	0		26	0	0	5	27-Dec	7	2	4	1	29	0	28	0	30	1	29	0	30	30
28-Dec	19	6	3	1	3	1		30	0		27	0		29	0		26	0	0	6	28-Dec	5	1	3	0	29	0	28	0	29	0	29	0	30	30
29-Dec	20	7	2	0	2	0		30	0		27	0		29	0		26	0	0	7	29-Dec	4	0	3	1	29	0	28	0	29	0	29	0	30	30
30-Dec	21	8	2	0	2	0		30	0		27	0		29	0		26	0	0	8	30-Dec	4	0	2	0	29	0	28	0	29	0	29	0	30	30
31-Dec	22	9	2	0	2	0		30	0		27	0		29	0		26	0	0	9	31-Dec	4	0	1	0	29	0	28	0	29	0	29	1	30	30
1-Jan	23	10	2	0	2	0		30	0		27	0		29	0		26	0	0	10	1-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
2-Jan	24	11	2	0	2	0		30	0		27	0		29	0		26	0	0	11	2-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
3-Jan	25	12	2	0	2	0		30	0		27	0		29	1		26	0	0	12	3-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
4-Jan	26	13	2	0	2	0		30	0		27	0		28	0		26	0	0	13	4-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
5-Jan	27	14	2	0	2	0		30	0		27	0		28	0		26	0	0	14	5-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
6-Jan	28	15	2	0	2	0		30	0		27	0		28	0		26	0	0	15	6-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
7-Jan	29	16	2	0	2	0		30	0		27	0		28	0		26	0	0	16	7-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
8-Jan	30	17	2	0	2	0		30	0		27	0		28	0		26	0	0	17	8-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
9-Jan	31	18	2	0	2	0		30	0		27	0		28	0		26	0	0	18	9-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
10-Jan	32	19	2	0	2	0		30	0		27	0		28	0		26	0	0	19	10-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
11-Jan	33	20	2	0	2	0		30	0		27	0		28	0		26	0	0	20	11-Jan	4	0	1	0	29	0	28	0	29	0	28	0	30	30
T				19		17	1		0			0	2		1			0	4				12		11		0		0		1		2		

3rd challenge			33,3% cohabitation																		33,3% cohabitation														
			injected fish					vaccinated						unvaccinated							injected fish				vaccinated				unvaccinated				Control	
Date			tank 1		tank 2			tank 1			tank 2			tank 1			tank 2					tank 3		tank 4		tank 3		tank 4		tank 3		tank 4		vac	unvac
	dpc	Dpi	L	D	L	D	C	L	D	C	L	D	C	L	D	C	L	D	C	dpi	Date	L	D	L	D	L	D	L	D	L	D	L	D	L	D
12-Jan	34	0	22	0	22	0		30	0		27	0		28	0		26	0		0	12-Jan	24	0	21	0	29	0	28	0	29	0	28	0	30	30
13-Jan	35	1	22	0	22	0		30	0		27	0		28	0		26	0		1	13-Jan	24	0	21	0	29	0	28	0	29	0	28	0	30	30
14-Jan	36	2	22	0	22	0		30	0		27	0		28	0		26	0		2	14-Jan	24	0	21	0	29	0	28	0	29	0	28	0	30	30
15-Jan	37	3	22	21	22	22		30	0		27	0		28	0		26	0		3	15-Jan	24	20	21	19	29	0	28	0	29	0	28	0	30	30
16-Jan	38	4	1	0	0	0		30	0	1	27	0		28	0		26	0		4	16-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
17-Jan	39	5	1	0	0	0		29	0		27	0		28	0		26	0		5	17-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
18-Jan	40	6	1	0	0	0		29	0		27	0		28	0		26	0		6	18-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
19-Jan	41	7	1	0	0	0		29	0		27	0		28	0		26	0		7	19-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
20-Jan	42	8	1	0	0	0		29	0		27	0		28	0		26	0		8	20-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
21-Jan	43	9	1	0	0	0		29	0		27	0		28	0		26	0		9	21-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
22-Jan	44	10	1	0	0	0		29	0		27	0		28	0	1	26	0		10	22-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
23-Jan	45	11	1	0	0	0		29	0		27	0		27	0		26	0		11	23-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
24-Jan	46	12	1	0	0	0		29	0		27	0		27	1		26	0		12	24-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
25-Jan	47	13	1	0	0	0		29	0		27	0		26	0		26	0		13	25-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
26-Jan	48	14	1	0	0	0		29	0		27	0		26	0		26	0		14	26-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
27-Jan	49	15	1	0	0	0		29	0		27	0		26	0		26	0		15	27-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
28-Jan	50	16	1	0	0	0		29	0		27	0		26	0		26	0		16	28-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
29-Jan	51	17	1	0	0	0		29	0		27	0		26	1		26	0		17	29-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
30-Jan	52	18	1	1	0	0		29	0		27	0		25	0		26	0		18	30-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
31-Jan	53	19	0	0	0	0		29	0		27	0		25	0		26	0		19	31-Jan	4	0	2	0	29	0	28	0	29	0	28	0	30	30
1-Feb	54	20	0	0	0	0		29	0		27	0		25	0		26	0		20	1-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
2-Feb	55	21	0	0	0	0		29	0		27	0		25	0		26	0		21	2-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
3-Feb	56	22	0	0	0	0		29	0		27	0		25	0		26	0		22	3-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
4-Feb	57	23	0	0	0	0		29	0		27	0		25	0		26	0		23	4-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
5-Feb	58	24	0	0	0	0		29	0		27	0		25	0		26	0		24	5-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
6-Feb	59	25	0	0	0	0		29	0		27	0		25	0		26	0		25	6-Feb	4	0	2	0	29	0	28	0	29	0	28	0	30	30
T				22		22			0			0			2			0					20		19		0		0		0		0		

Appendix 2: Immersion challenge of unvaccinated Arctic char with *A. salmonicida subsp. achromogene* F131-16

Tank	Date	Dpc	Death	Live	%death	%live	Cum.Sur	Cum.Mor	Tank	Date	Dpc	Death	Live	%death	%live	Cum.Sur	Cum.Mor
1	10-Feb	0	0	36	0.00	1.00	1.00	0.00	3	10-Feb	0	0	53	0.00	1.00	1.00	0.00
1	11-Feb	1	0	36	0.00	1.00	1.00	0.00	3	11-Feb	1	0	53	0.00	1.00	1.00	0.00
1	12-Feb	2	0	36	0.00	1.00	1.00	0.00	3	12-Feb	2	0	53	0.00	1.00	1.00	0.00
1	13-Feb	3	0	36	0.00	1.00	1.00	0.00	3	13-Feb	3	0	53	0.00	1.00	1.00	0.00
1	14-Feb	4	2	36	0.06	0.94	0.94	0.06	3	14-Feb	4	1	53	0.02	0.98	0.98	0.02
1	15-Feb	5	3	34	0.09	0.91	0.86	0.14	3	15-Feb	5	4	52	0.08	0.92	0.91	0.09
1	16-Feb	6	8	31	0.26	0.74	0.64	0.36	3	16-Feb	6	13	48	0.27	0.73	0.66	0.34
1	17-Feb	7	10	23	0.43	0.57	0.36	0.64	3	17-Feb	7	14	35	0.40	0.60	0.40	0.60
1	18-Feb	8	7	13	0.54	0.46	0.17	0.83	3	18-Feb	8	11	21	0.52	0.48	0.19	0.81
1	19-Feb	9	3	6	0.50	0.50	0.08	0.92	3	19-Feb	9	6	10	0.60	0.40	0.08	0.92
1	20-Feb	10	2	3	0.67	0.33	0.03	0.97	3	20-Feb	10	2	4	0.50	0.50	0.04	0.96
1	21-Feb	11	0	1	0.00	1.00	0.03	0.97	3	21-Feb	11	0	2	0.00	1.00	0.04	0.96
1	22-Feb	12	0	1	0.00	1.00	0.03	0.97	3	22-Feb	12	0	2	0.00	1.00	0.04	0.96
1	23-Feb	13	1	1	1.00	0.00	0.00	1.00	3	23-Feb	13	1	2	0.50	0.50	0.02	0.98
1	24-Feb	14	0	0	0.00	1.00	0.00	1.00	3	24-Feb	14	0	1	0.00	1.00	0.02	0.98
2	10-Feb	0	0	61	0.00	1.00	1.00	0.00	3	25-Feb	15	0	1	0.00	1.00	0.02	0.98
2	11-Feb	1	0	61	0.00	1.00	1.00	0.00	3	26-Feb	16	1	1	1.00	0.00	0.00	1.00
2	12-Feb	2	0	61	0.00	1.00	1.00	0.00	4	10-Feb	0	0	51	0.00	1.00	1.00	0.00
2	13-Feb	3	0	61	0.00	1.00	1.00	0.00	4	11-Feb	1	0	51	0.00	1.00	1.00	0.00
2	14-Feb	4	2	61	0.03	0.97	0.97	0.03	4	12-Feb	2	0	51	0.00	1.00	1.00	0.00
2	15-Feb	5	8	59	0.14	0.86	0.84	0.16	4	13-Feb	3	0	51	0.00	1.00	1.00	0.00
2	16-Feb	6	17	51	0.33	0.67	0.56	0.44	4	14-Feb	4	1	51	0.02	0.98	0.98	0.02
2	17-Feb	7	17	34	0.50	0.50	0.28	0.72	4	15-Feb	5	7	50	0.14	0.86	0.84	0.16
2	18-Feb	8	5	17	0.29	0.71	0.20	0.80	4	16-Feb	6	16	43	0.37	0.63	0.53	0.47
2	19-Feb	9	7	12	0.58	0.42	0.08	0.92	4	17-Feb	7	15	27	0.56	0.44	0.24	0.76
2	20-Feb	10	1	5	0.20	0.80	0.07	0.93	4	18-Feb	8	2	12	0.17	0.83	0.20	0.80
2	21-Feb	11	1	4	0.25	0.75	0.05	0.95	4	19-Feb	9	5	10	0.50	0.50	0.10	0.90
2	22-Feb	12	0	3	0.00	1.00	0.05	0.95	4	20-Feb	10	1	5	0.20	0.80	0.08	0.92
2	23-Feb	13	1	3	0.33	0.67	0.03	0.97	4	21-Feb	11	0	4	0.00	1.00	0.08	0.92

2	24-Feb	14	0	2	0.00	1.00	0.03	0.97	4	22-Feb	12	0	4	0.00	1.00	0.08	0.92
2	25-Feb	15	0	2	0.00	1.00	0.03	0.97	4	23-Feb	13	0	4	0.00	1.00	0.08	0.92
2	26-Feb	16	1	1	1.00	0.00	0.00	1.00	4	24-Feb	14	0	4	0.00	1.00	0.08	0.92
2	27-Feb	17	1	0	1.00	0.00	0.00	1.00	4	25-Feb	15	0	4	0.00	1.00	0.08	0.92
									4	26-Feb	16	1	3	0.33	0.67	0.05	0.95
									4	27-Feb	17	0	3	0.00	1.00	0.05	0.95

Appendix 3: Analysis the difference in mean days to death between i.p. injections

t-Test: Two-Sample Assuming Equal Variances

	Inject 1	*Inject 2*
Mean	7.998716153	3.989293086
Variance	0.923131399	0.020410392
Observations	4	4
Pooled Variance	0.471770895	
Hypothesized Mean Difference	0	
df	6	
t Stat	8.255270533	
P(T<=t) one-tail	8.54258E-05	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.000170852	
t Critical two-tail	2.446911851	

	Inject 1	*Inject 3*
Mean	7.998716153	3.170454545
Variance	0.923131399	0.116219008
Observations	4	4
Pooled Variance	0.519675203	
Hypothesized Mean Difference	0	
df	6	
t Stat	9.471958656	
P(T<=t) one-tail	3.94254E-05	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	7.88507E-05	
t Critical two-tail	2.446911851	

	Inject 2	*Inject 3*
Mean	3.989293086	3.170454545
Variance	0.020410392	0.116219008
Observations	4	4
Pooled Variance	0.0683147	
Hypothesized Mean Difference	0	
df	6	
t Stat	4.43053518	
P(T<=t) one-tail	0.00221028	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.004420559	

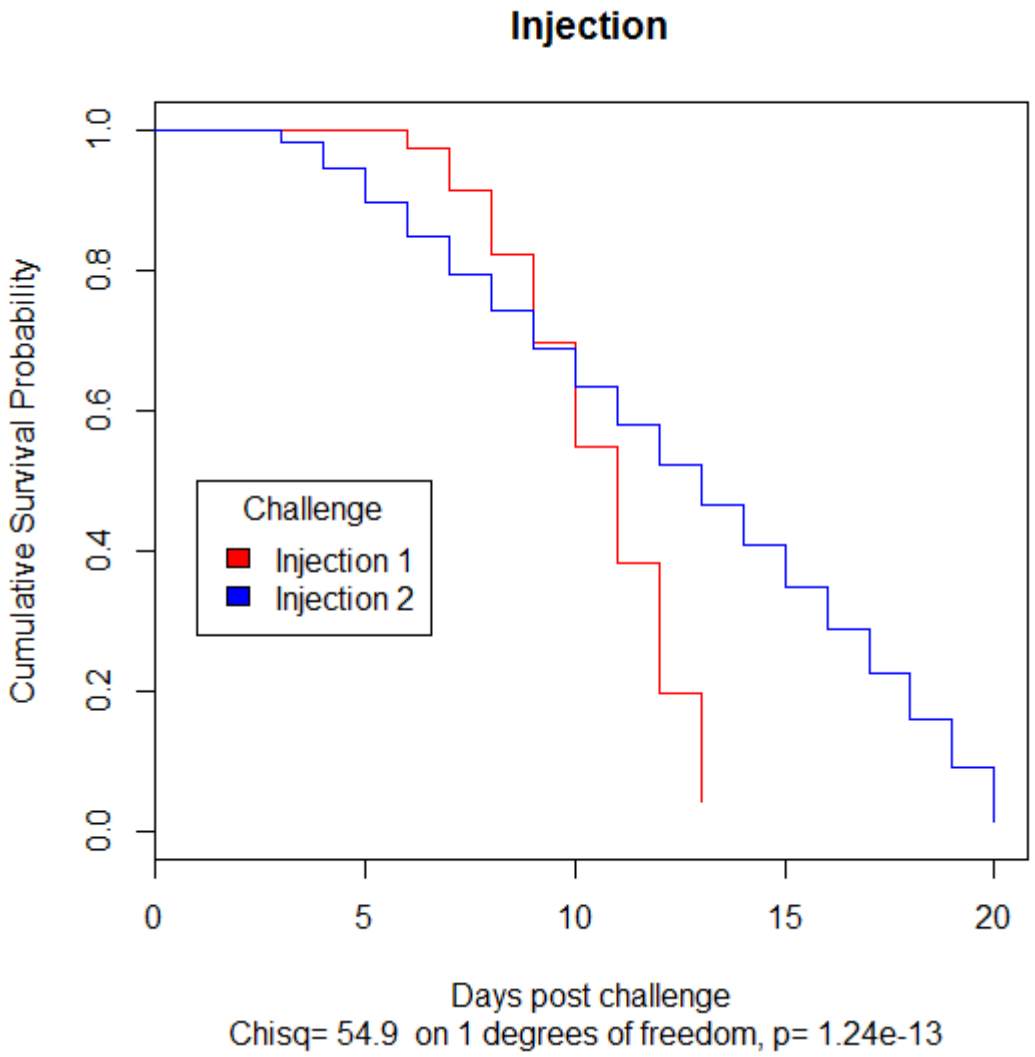
This paper should be cited as:
Nguyen, T.T.G. 2018. *Studies of experimental infection of Arctic char (*Salvelinus alpinus *L.) with* Aeromonas samonicida *subsp.* Achromogenes*.* Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/giang16prf.pdf

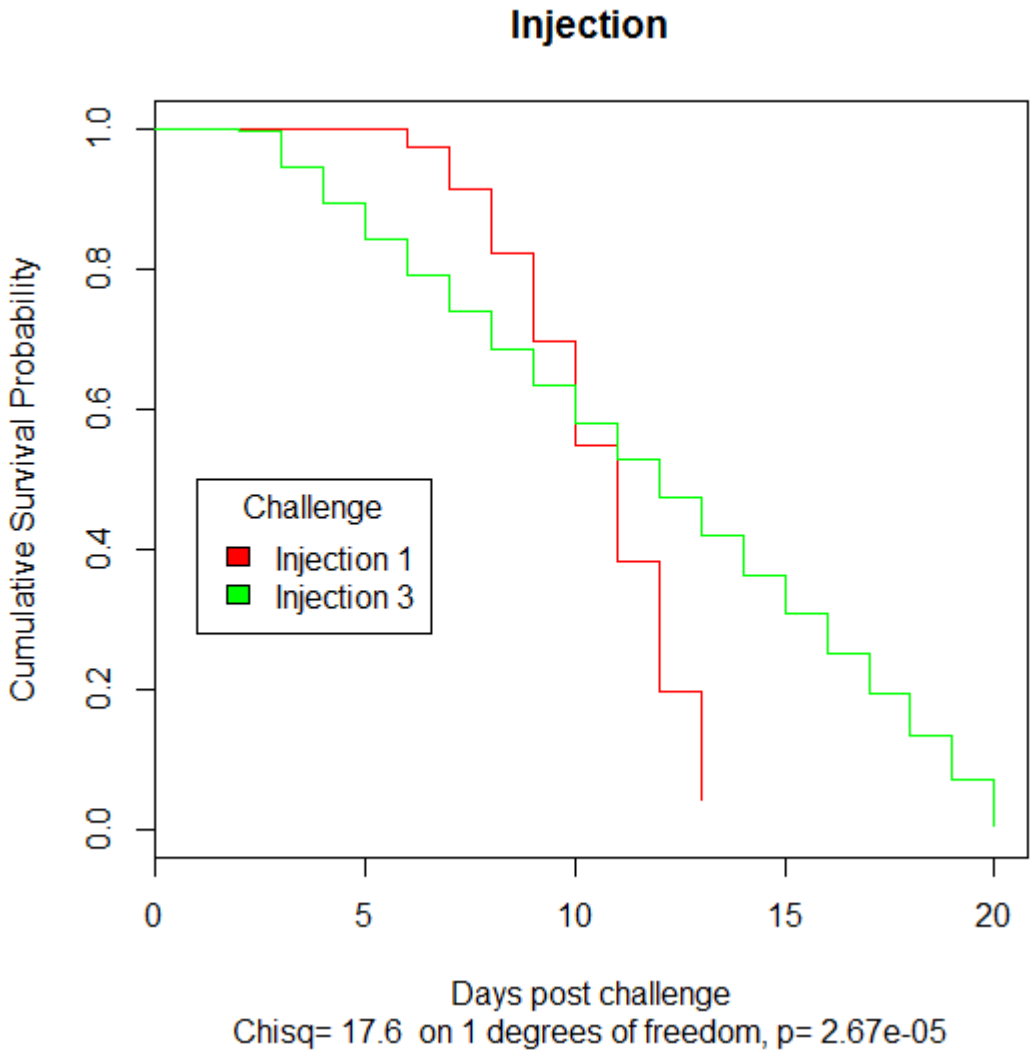
t Critical two-tail	2.446911851

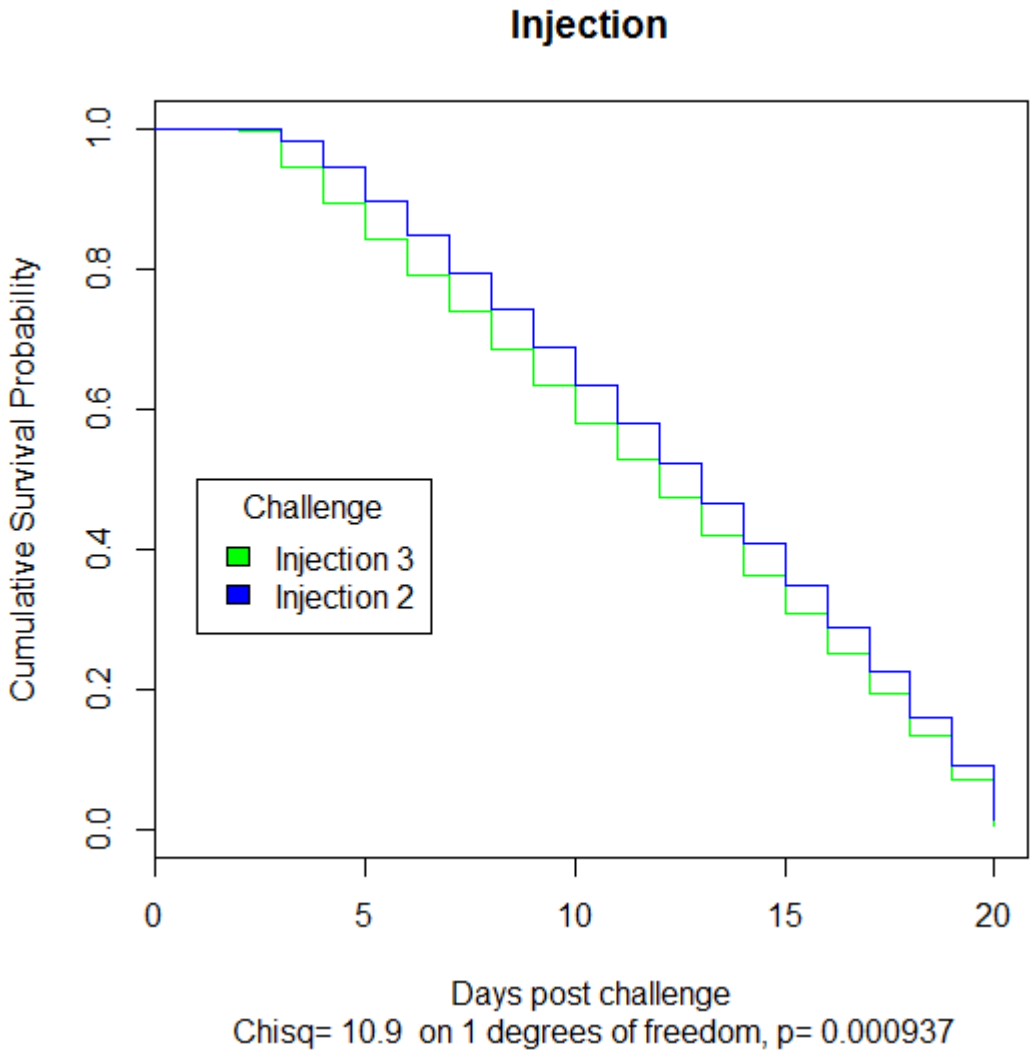
t-Test: Two-Sample Assuming Equal Variances

	12°C	*13°C*
Mean	11.94444444	7.131367173
Variance	6.675925927	0.055505247
Observations	3	4
Pooled Variance	2.703673519	
Hypothesized Mean Difference	0	
df	5	
t Stat	3.832545446	
P(T<=t) one-tail	0.006107991	
t Critical one-tail	2.015048373	
P(T<=t) two-tail	0.012215982	
t Critical two-tail	2.570581836	

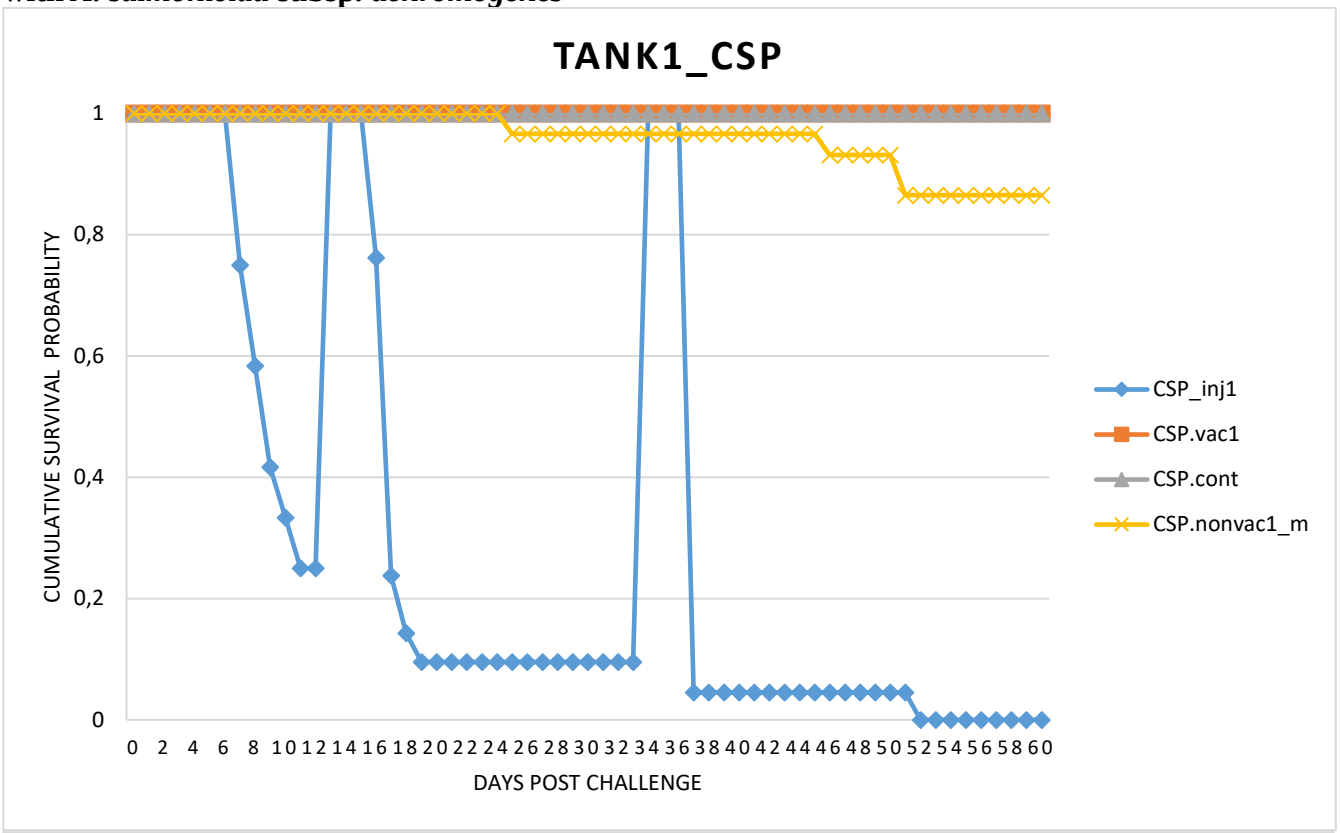
Appendix 4: Analysis of Kaplan-Meier survival estimates between injections

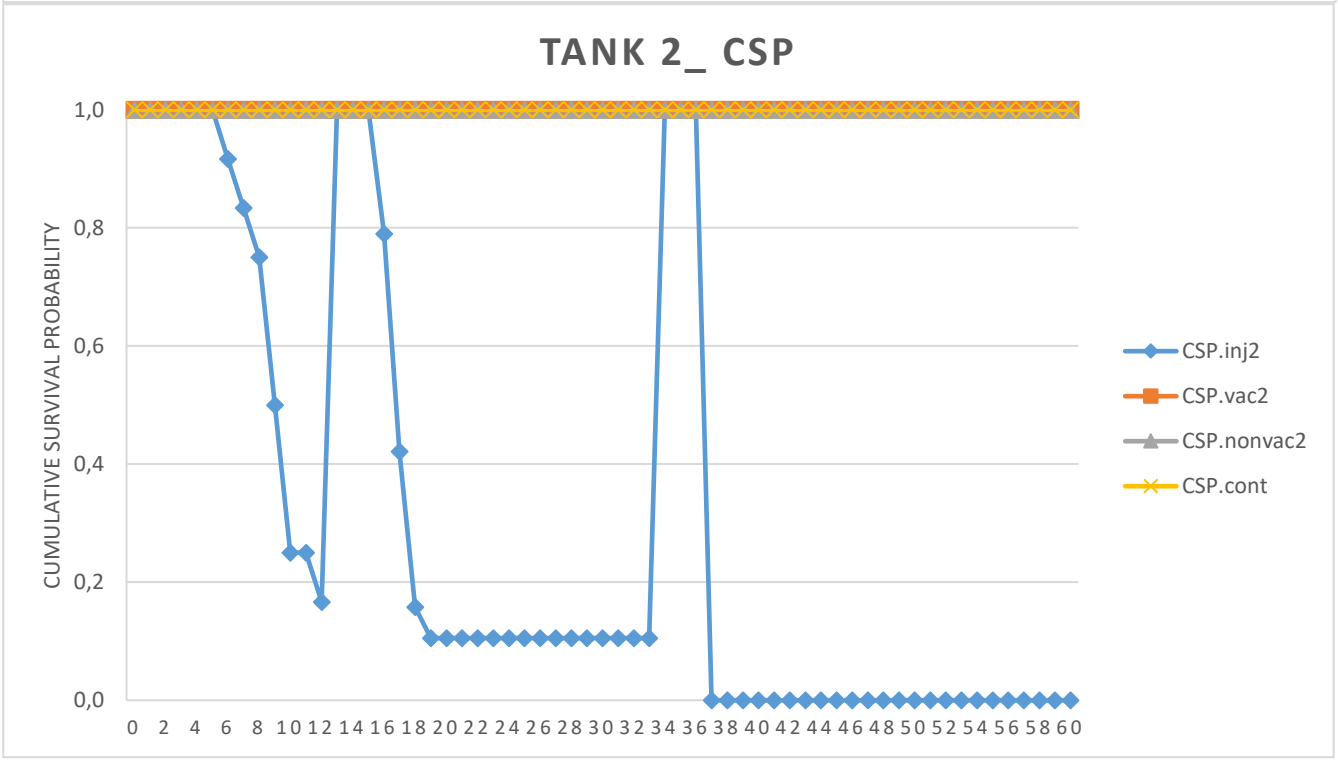


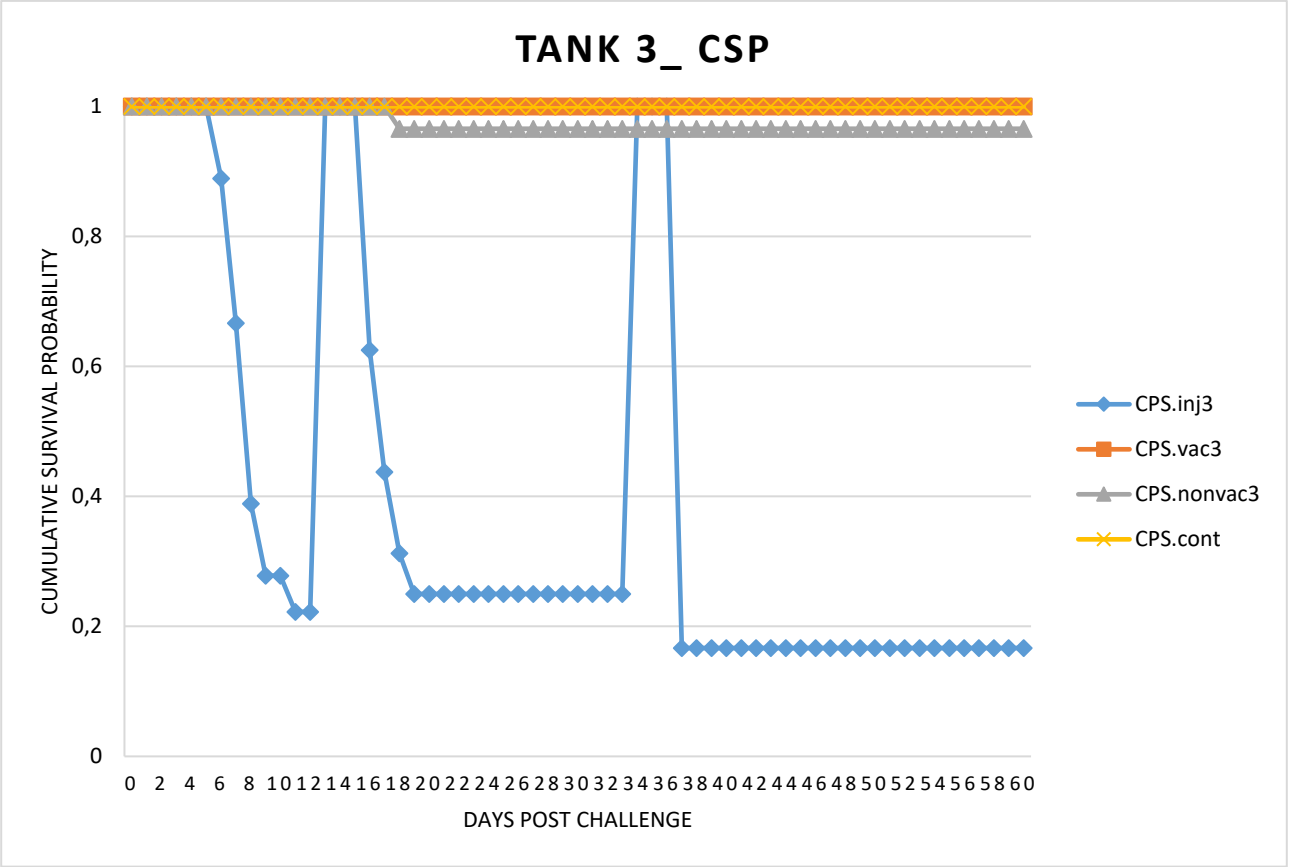

Injection
Cumulative Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
Challenge
Injection 1
Injection 3
Days post challenge
Chisq= 17.6 on 1 degrees of freedom, p= 2.67e-05

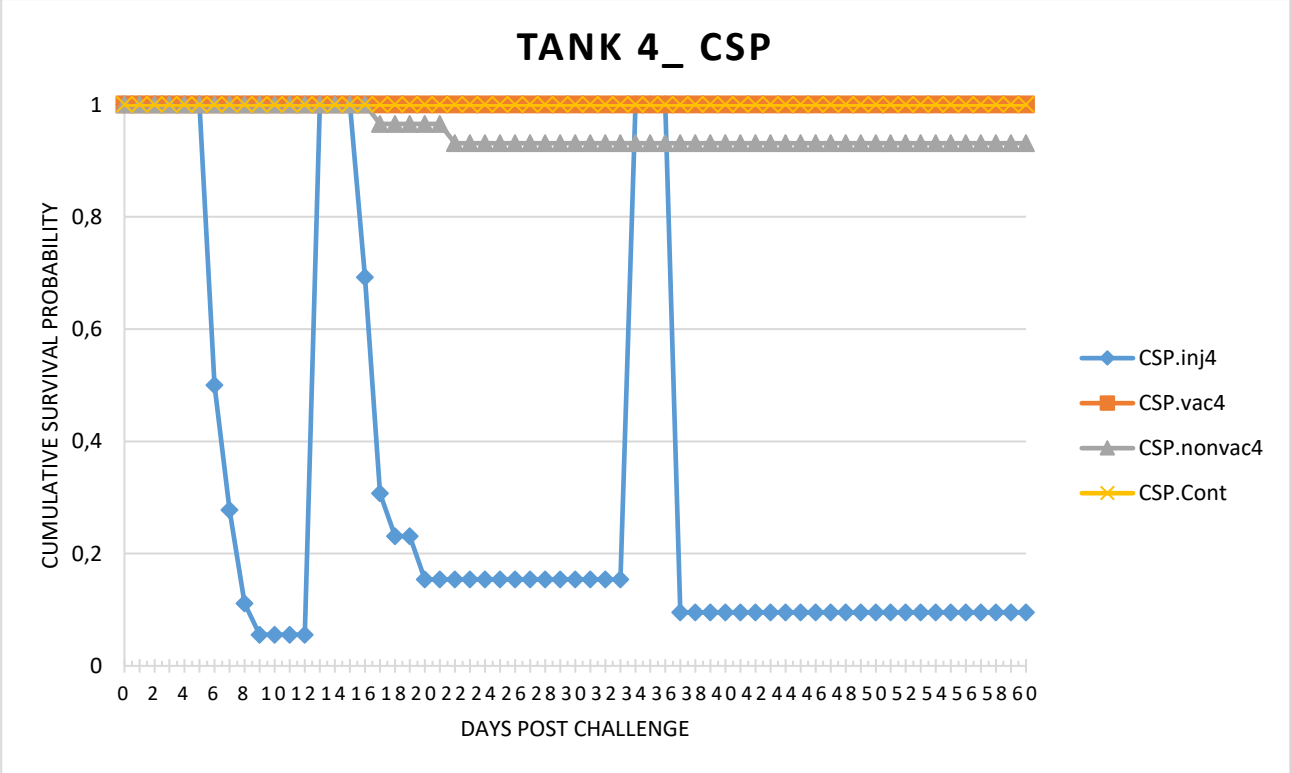

Injection
Cumulative Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
Challenge
Injection 3
Injection 2
Days post challenge
Chisq= 10.9 on 1 degrees of freedom, p= 0.000937

Appendix 5. Cumulative survival probability following cohabitation challenge of Arctic char in with *A. salmonicida* subsp. *achromogenes*






TANK 3_ CSP
CUMULATIVE SURVIVAL PROBABILITY
1
0,8
0,6
0,4
0,2
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
DAYS POST CHALLENGE
CPS.inj3
CPS.vac3
CPS.nonvac3
CPS.cont


TANK 4_ CSP
CUMULATIVE SURVIVAL PROBABILITY
1
0,8
0,6
0,4
0,2
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
DAYS POST CHALLENGE
CSP.inj4
CSP.vac4
CSP.nonvac4
CSP.Cont