Source, and effect of Bacterial Kidney Disease (BKD) in Icelandic aquaculture

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Ágrip

Sjúkdómsvaldandi örverur finnast í villtum fiskum í náttúrunni, en virðast að jafnaði ekki valda þar sjúkdómsfaröldrum eða merkjanlegum skaða. Í eldisstöðvum, þar sem fiskar eru aldir við mikinn þéttleika, eru smitleiðir greiðar milli fiska og geta sjúkdómar magnast þar upp og valdið stórvægilegum vandræðum í eldinu.

Ein af grunnforsendum farsæls og arðvænlegs fiskeldis á laxfiskum er staðgóð þekking á eðli sjúkdóma og áreiðanlegum aðferðum til að greina smitsjúkdómavalda. Auk þessa, er afar mikilvægt að það sé í eins góðri sátt við náttúrulegt umhverfi þess og frekast er unnt. Áform um stóraukið laxfiskaeldi hafa valdið umtalsverðum áhyggjum er varða hugsanleg neikvæð áhrif þeirra á umhverfið. Möguleg áhrif sjúkdóma, sem jafnan fylgja stríðeldi, á villta laxfiska í nágrenni eldissvæða er einn þáttur sem ástæða er til að fylgjast með. Nýrnaveiki (BKD - **B**acterial **K**idney **D**isease) er einn af þessum sjúkdómum. Nýrnaveiki er sjúkdómur af völdum bakteríunnar *Renibacterium salmoninarum* (Rs) (Sanders & Fryer, 1980), og er sá sjúkdómur sem valdið hefur hvað mestu tjóni í laxfiskaeldi á Íslandi síðustu áratugina.

Meginmarkmið þessa verkefnis var að: (1) Rekja uppruna nýrnaveikissmits í þremur landeldisstöðvum/seiðastöðvum í Tálknafirði. (2) Athuga stöðu nýrnaveikismits í villtum laxfiskum í: (i) nágrenni eldiskvía sem í voru nýrnaveikismitaðir fiskar og (ii) í villtum laxfiskum fjarri eldissvæðum til samanburðar. (3) Kanna hvort erfðabreytileiki sé til staðar í Rs-stofnum frá mismunandi svæðum á Íslandi. (4) Bera saman aðferðir við einangrun á erfðaefni Rs í mismunandi tegundum sýna.

Þrjár landeldisstöðvar (Stöðvar A, B og C), staðsettar í Tálknafirði, þar sem nýrnaveiki hefur komið upp síðastliðin ár, tóku þátt í þessari rannsókn. Allar þessar stöðvar hafa notað, að mismiklu marki, inntaksvatn úr nærliggjandi lækjum og beindist grunur að því að uppruna Rs-smits væri að finna í þeim og að smit gæti borist inn í eldisstöðvarnar með inntaksvatni. Takmarkaðar upplýsingar lágu fyrir um tilvist laxfiska í sumum þessara lækja og engar um það hvort þessir laxfiskar bæru Rs-smit.

Niðurstöður eru byggðar á tölfræði- og sameindalíffræðilegum rannsóknaraðferðum. Viðurkenndar greiningaraðferðir voru notaðar til greiningar á Rs-mótefnavökum (pELISA) og erfðaefni (qPCR) bakteríunnar. Þar sem þessi tvö próf greina mismunandi sameindir, ætti notkun þeirra að gefa góða mynd af stöðu nýrnaveikissmits.

Alls 95% laxfiska sem teknir voru úr lækjum í nágrenni eldisstöðvanna greindust Rs-jákvæðir í pELISA en aðeins 14% þeirra í qPCR. Öll laxaseiði frá Stöð A greindust Rs-jákvæð í báðum greiningarprófum. Allar bleikjur frá Stöð B greindust neikvæðar í pELISA, en ein jákvæð í qPCR. Þegar sýnataka fór fram voru báðar eldisstöðvarnar með vatnstöku úr þessum eða nærliggjandi lækjum. Niðurstöðurnar benda sterklega til þess að uppruni smits í báðum þessum eldisstöðvum sé í fyrrnefndum lækjum. Allir laxar frá Stöð C greindust neikvæðir í báðum prófum, líklega vegna þess að sú stöð er nýlega byggð, tæknivædd og notar nú eingöngu borholu vatn.

Aðeins 4% villtra laxfiska úr stöðuvötnunum, þar sem ekki gætir fiskeldisáhrifa, greindust Rs-jákvæðir í qPCR en 40% í pELISA. Til samanburðar þá voru allir laxfiskar veiddir í tveimur fjörðum, þar sem fiskeldisáhrifa gætir, neikvæðir í í qPCR og aðeins einn jákvæður í pELISA. Þessar niðurstöður

gefa vísbendingar um að nýrnaveikissmitaður fiskur í sjókvíum hafi ekki neikvæð áhrif á villta laxfiska í nágrenni eldisins, með tilliti til tíðni nýrnaveikissmits. Niðurstöðurnar eru þó takmarkaðar og frekari rannsókna er þörf, með fleiri sýnatökum yfir lengra tímabil, svo hægt sé að fullyrða að svo sé.

Enginn breytileiki greindist í erfðaefni Rs-stofna sem einangraðir voru úr fiskum frá mismunandi svæðum á Íslandi. Það samræmist niðurstöðum erlendra rannsókna.

Niðurstöður úr fylgniprófi (Pearson r) sýndu ekki fram á marktækann mun á einangrun erfðaefnis úr sýnum (floti) sem meðhöndluð höfðu verið fyrir pELISA og hefðbundinni meðhöndlun sýna fyrir qPCR úr nýrnabitum. Handvirk einangrun erfðaefnis og sjálfvirk einangrun í þjarka (e. Robot), reyndust ekki marktækt frábrugðnar. Hinsvegar, var hærri fylgni milli flots og nýrnabita þegar sjálfvirk einangrun átti sér stað, samanborið við sýni þar sem erfðaefni var einangrað handvirkt.

Niðurstöður benda sterklega til þess að uppruni nýrnaveikissmits í öllum þremur eldisstöðvunum, sem hafa komið upp síðastliðin ár, megi rekja til inntaksvatns úr nlærliggjandi lækjum. Þar sem hvorki virk meðhöndlun né bóluefni eru tiltæk gegn sjúkdómnum, er áhrifaríkasta leiðin til að stemma stigu við því að nýrnaveiki berist í eldisfisk, að koma í veg fyrir að smitefnið berist inn í eldisstöðvar.

Abstract

Certain disease-causing agents in fish seem to live in some sort of balance in wild salmonids without causing a disease outbreak. In fish farming, where salmonids are cultured in high biomass and proximity, infectious agents can proliferate extensively, effectively transmit between fish and cause a disease outbreak in the farms.

One of the crucial premises for healthy and prosperous salmonid farming, is proper knowledge on both preventive measures and disease causing agents. Plans for increased salmonid farming around the world has caused big debates about the possible negative effects it could have on the surrounding environment. One of the greatest concerns are infectious diseases in aquaculture and their presumable impact on wild salmonids in the vicinity of farming areas. BKD (**B**acterial **K**idney **D**isease) is one of those diseases. The disease is caused by the bacterium *Renibacterium salmoninarum* (Rs) (Sanders & Fryer, 1980), and is the disease that has caused the most damage in Icelandic salmonid aquaculture the last decades.

The main objectives of this study were to: (1) Trace the source of Rs-infections in land-based salmonid farms located in Tálknafjörður fjord in Iceland. (2) To investigate Rs-status of wild salmonids in: (i) fjords with sea cages containing Rs infected fish and (ii) in wild salmonids from areas far from farming areas and not affected by them, for comparison. (3) Examine whether genetic variation exists in Rs isolates originating from different areas in Iceland. (4) Examine whether different DNA isolation methods in diagnostic qPCR tests using two different sample types, would give comparable results.

Three land-based salmonid farms took part in this project (Farms A, B and C), all of which have experienced BKD outbreaks in recent years. All these farms have used, to a varying degree, water from nearby mountain brooks as a water supply. Consequently, suspicions arose whether this brook water might be the source of Rs-infection in the farms. Available data on the presence of salmonids in these brooks were wage or hardly existing, and no data existed on the Rs-status of the salmonids inhabiting them.

Results are based on statistical-, and molecular scientific research methods. Reliable diagnostic methods were used to detect Rs-antigens using polyclonal ELISA (pELISA) and the bacterial DNA with the use of PCR (qPCR). Whereas these two methods detect two different elements, their combined results should give a good representation of infection status.

A total of 95% of all salmonids caught in mountain brooks in vicinity of the fish farms, tested positive in pELISA while only 14% in qPCR. All salmon from Farm A tested positive in both diagnostic tests, while all Arctic charr from Farm B tested negative in pELISA but one fish positive in qPCR. At the time these samples were collected, both Farms A and B used these brooks as source water to some extent. All salmon from Farm C tested negative in both diagnostic tests. That is most likely because this farm is a newly built, hi-tech facility and recently started utilizing solely UV treated borehole water. These results strongly indicate the bacterium had entered all three fish farms from surrounding natural brooks and caused previous or ongoing outbreaks experienced in these farms.

Only 4% of salmonids caught in lakes with no fish farms nearby, tested positive in qPCR but 40% in pELISA. For comparison, all salmonids caught in the two fjords with fish farms nearby, tested negative in qPCR and only one positive in pELISA. These results suggest Rs-infected salmonids in sea-cages do not have a negative effect on nearby migrating salmonids in terms of Rs infection. However, these data from one-time sampling are limited and further sampling, over a longer period of time, is required for confirmation.

Analysis on the 16S DNA of Rs-strains, originating from different areas in Iceland, revealed no genetic variation, which is in agreement with comparable studies abroad.

A correlation test (Pearson r) did not reveal a statistical difference in qPCR results when DNA was isolated from samples that had been processed for pELISA supernatant and those where DNA was isolated by conventional methods, i.e. directly from kidney tissue. Similarly, comparison of results from samples were DNA isolation was performed either manually or automatically (in a robot) were not statistically different. However, there was higher correlation between sample types (i.e. pELISA supernatant and kidney tissue) when DNA isolation was performed automatically, compared to those were DNA was isolated manually.

The results strongly suggest the BKD outbreaks, experienced recently in all three farms, can be traced to Rs-infected water from nearby brooks the farms used as water source. Since there are neither active vaccines nor antibiotics available against the disease, the most effective way to minimize the occurrence and effect of BKD is to prevent the bacterium from entering land-based fish farms, by using solely UV-treated borehole water.

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ATL	Tissue lysis buffer
BKD	Bacterial Kidney Disease
°C	Degree celsius
Cm	Centimeter
CT	Cycle threshold
DNA	Deoxyribonucleic acid
kg	Kilograms
km^2	Square kilometer
m	Meters
ma.s.l.	Meters above sea level
min	Minutes
mL	Milliliter
msa	Genomic DNA for p57
MSA	Major soluble antigen (p57)
MV	Multiplication value
n	Number of sample
ng	Nanogram
OD	Optical density
PCR	Polymerase chain reaction
pELISA	Polyclonal enzyme-linked immunosorbent assay
qPCR	Quantitative polymerase chain reaction
rpm	Revolutions per minute
Rs	*Renibacterium salmoninarum*
RT	Room Temperature
SKDM	Selective Kidney Disease Medium
µl	Microliter
µm	Micrometer
UV	Ultraviolet
V	Volt
-	Undocumented

1 Introduction

1.1 Aquaculture

1.1.1 Introduction to salmonid aquaculture

Culturing of salmonid fish date back to the 19th century, when salmon hatcheries were established in both North America and Scotland. Initially, production was aimed on enhancement of declining wild salmon stocks to feed people living on this earth or to simply augment declining natural stocks. It was not until the mid-20th century that a commercial production of salmonids as foodfish started in the world, initially consisting mainly of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.). Therefore, the first century or so, salmonids were mostly farmed in freshwater (Barnabé, 1990; Stickney & Treece, 2012)

In Iceland, the enhancement of natural salmonid stocks has been employed since the late 19th century. That includes stripping of broodfish, fertilization and hatching of eggs and culture of the offspring for subsequent release into their home river. Initially, mostly yolk sack larvae were released into rivers. Later, and up to date, mostly smolts are cultured and released for that purpose. One could say that the starting of a “conventional” aquaculture, where fish are cultured from hatching to slaughter size, started in the 1940s, when local farmers in NE-Iceland started a small scale culturing Atlantic salmon and other salmonids. However, the initial import of rainbow trout eggs, and subsequent culture of the offspring, could be considered as the first “real” aquaculture in Iceland. In addition, the foundation of “Fiskeldisstöð Ríkisins” in 1961, was a breaking point in the development of aquaculture in Iceland (Hannesson, 1977; Jónasson, 1991).

Aquaculture in Iceland has had its ups and downs the last few decades. Sea ranching started in 1963 but first attempt of culturing salmon in sea cages was in 1972 in Hvammsvík in Hvalfjörður by Fiskifélag Íslands. In the 1980s, small fish farming companies set up six sea cages at different locations with accidental escapes being fairly common (Gunnarsson, 2008). Slow growth, early sexual maturity, cold water, lack of research and knowledge, made it hard for aquaculture companies to culture salmon in Iceland. Furthermore, during the 1980s and early 1990s, outbreaks of bacterial kidney disease (BKD) severely affected the aquaculture sector. This resulted in many companies going out of business (Halldórsson, 1992; Guðmundsdóttir *et al*., 2000). At the start of a new century, an interest in aquaculture increased again with large seafood companies increasing research and developing better methods in fish farming. In 2006, Iceland reached an all-time high in production with over 10.000 tons of salmonids cultured of which 7.000 tons were Atlantic salmon. However, in the following years the aquaculture sector experienced various problems, the production in salmon farming decreased to 1.100 tons and total production dropped to around 5.000 tons in 2008, of which the majority came from Arctic charr (*Salvelinus alpinus* L.) (Landssamband Fiskeldisstöðva, 2009). Among factors, which negatively affected the farming industry was BKD, which affected many farms in Iceland, culturing Atlantic salmon and Arctic charr, during the years 2003-2008. Without much doubt, BKD played a significant part in the collapse of salmon farming experienced in the late 2000s (data from the Fish disease Laboratory at Keldur). With increasing demand for salmon, sea cage rearing of

salmon started again, and the production reached 8.420 tons in 2017 and 27.000 tons in 2019 and is still increasing. The production of Arctic Charr has been relatively stable through the last decades. It has, however, been slowly increasing from 1987 with around 4000-6000 tons cultured in recent years. This makes Iceland the largest Arctic charr farmers in the world. Production of rainbow trout was 2.138 ton in 2017 but since then has dropped significantly. The present farming of other species, such as Senegalese sole (*Solea senegalensis*) and Atlantic cod (*Gadus morhua),* is merely a fraction of the total production (Jónsson, 2020). Data on the production in Icelandic aquaculture, from 1987-2019 is shown in Figure 1.

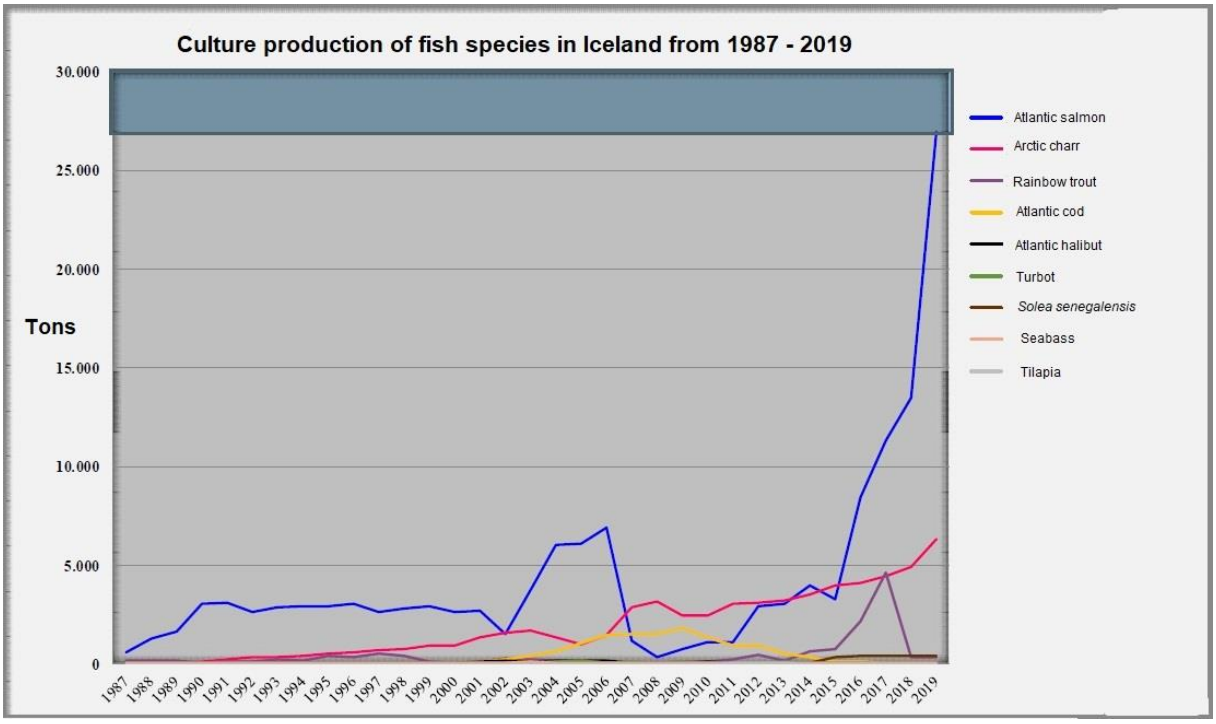


Figure 1 : Total production of farmed fish species in Iceland from 1987 – 2019 (Jónsson, 2020)

Aquaculture is one of the most important long-term growth areas for high protein food production and continues to grow faster than any other major food sector. Global aquaculture production in 2016 was 110.2 million tons. As fisheries are a limited resource, aquaculture has increasingly been contributing to the global production of fish protein, reaching 46.8% of the world's total production in 2016 compared to 25.7% in 2000. In 2016, aquaculture accounted for 17-18% of total fish production in Africa, the Americas and Europe. (FAO, 2018). Aquaculture is one way of providing food for the increasing population of the world. As population has reached 7 billion, it has become crucial to have an eco-friendly way to meet the increased consumption. The aquaculture sector has recently been criticized for their activities, i.e. being harmful to the environment and it has been pointed out that these factors could have a large impact on nearby wildlife (Tidwell & Allan, 2001), directly through the output of fish cages or indirectly through the biological environment or community. The culture of aquatic animals has great potential for efficient food production with the ever-growing human population. However, it can have a negative effect if not done properly in consideration to the surrounding environment. The potential threat of fish farming to its surroundings can vary greatly

among farms, depending on numerous aspects, such as their location, both in terms of their vicinity to valuable wild salmon rivers, as well as exclusively land-based farms or in sea cages (Iwama, 1991).

1.1.2 Offshore and land-based aquaculture

As mentioned above, there is an increasing demand for fish protein in the world. In relation to that, offshore aquaculture, in open sea cages, has been under a lot of pressure to meet these consumption demands, that cannot be met by fisheries. Offshore salmonid aquaculture uses net pens which are relatively inexpensive, can contain hundreds of tons of fish and do not take long to construct. These sea cages protect the salmonids from predators and provide constant flow of seawater which provides the salmonids with oxygen and nutrients while at the same time dispose feces and left-over feed into their surroundings (Mánason, 2017).

Although favorable, in terms of productions, fish farms utilizing sea cages can have negative impact on the marine environment in several different ways e.g. the use of toxic chemicals, the escape of cultured salmonids from cages by accidental release, which can compete with wild salmonids over food and habitat as well as a potential breeding of the farmed salmon with wild ones which can result in nonnative DNA entering the wild gene pool. Furthermore, there is a risk of transmission of diseases from the farmed fish to wild populations of fish (WWF, 2006). However, it seems that sea cage rearing, in itself, is not actually causing the biggest concern but rather the inadequate management or development and irresponsible practices in use (Mánason, 2017).

Land-based farms are considered more environmentally friendly and could presumably solve most of the potential problems related to farming in sea cages. However, building and maintaining aquaculture on land can be difficult because of many factors. Unlike the offshore aquaculture, the land-based one has to take into consideration factors like water flow-rate and quality of available water, topography of the building site, area available for use, existence of other farms upstream, i.e. when rivers are used as water source, difference in level across the site and soil permeability (Barnabé, 1990). However, the land-based fish farms have many advantages over the offshore ones, e.g. less risk of the farmed fish acquiring infections from wild fish from the surroundings, and vice versa and almost no risk of fish escaping into nearby waters, with the potential breeding with wild fish. Most of the land-based farms in Iceland use borehole water, which, if done properly, greatly minimizes infectious agents entering the farming facility. Furthermore, some preventive measures can be applied, such as exposing the water supply to UV, which should eradicate most of the possible infectious agents in the water. However, land-based fish farms cannot keep up the same amount of food production as offshore companies regarding magnitude of water supply, land/sea volume being used for culturing as well as being more expensive to build and manage (Mánason, 2017).

1.1.3 Common fish diseases in Icelandic aquaculture- brief introduction

Overall, the health of farmed aquatic animals in Iceland is good, compared to other neighboring countries. Since aquaculture began in Iceland, the fish farms have mostly had to deal with bacterial

and parasitic infections. The most problematic disease affecting farmed fish, through the decades is BKD, and still is. The experience in other neighboring countries is somewhat different where the biggest threat to fish farms are viral diseases (Hjeltnes *et al.*, 2019). At present, they are not of major concern in Icelandic aquaculture (Data from Fish disease Laboratory at Keldur; Annual report from Keldur, 2019).

Bacteria causing fin- or tail rot in fish is common in both fish farmed in fresh- and saltwater in Iceland and is caused by bacteria of the genera *Tenacibaculum* and *Flacobacterium. Tenacibaculum* spp. mostly causes ulcers in fish in saltwater and *Flavobacterium* spp. in fresh water (Woo & Bruno, 1999). There were 8 cases reported in 2019 (Jónsson, 2020). Winter ulcers (*Moritella viscosa*) have appeared in fish cultured in saltwater which mostly appear in period of low temperature during the winter (Annual report from Keldur, 2019; Jónsson, 2020). Among other fish diseases caused by bacteria is atypical furunculosis, which is caused by *Aeromonas salmonicida* subspecies *achromogenes* and has caused extensive losses in salmonid aquaculture in Iceland, particularly in fish cultivated in brackish water. Main clinical signs from this disease is haemorrhaging and boil-like lesions (Woo & Bruno, 1999). It was first diagnosed in 1980, in the early days of the salmonid fish farming industry (Guðmundsdóttir, 1998) but is managed with vaccination and was reported in two cases in Iceland in 2019 (Jónsson, 2020). The bacterium is also known to infect wild salmon in Iceland (Data from Fish disease laboratory at Keldur). Enteric red mouth disease caused by (*Yersinia ruckeri*), is fairly common in fish farms which causes septicaemial infections and reddening of the mouth (Woo & Bruno, 1999). Due to effective vaccine, it can be managed and only three cases were reported in the year 2019 (Jónsson, 2020). Vibriosis, is bacterial disease caused by *Vibrio anguillarum* causing red spots on the ventral and lateral areas of the fish and swollen and dark skin lesions that ulcerate (Woo & Bruno, 1999). It was first diagnosed in 2001 in cultured cod and has occasionally been identified in salmonid aquaculture the last few years. It is kept down with vaccination but was diagnosed in 2019 in Arctic charr and lumpfish (Jónsson, 2020). Most of the bacterial diseases mentioned above are seldom seen because of proper vaccination in the fish farms (Jónsson, 2020).

Although not considered a major problem, some viruses have been detected in Icelandic aquaculture. Viral hemorrhagic septicemia virus (VHSV), is a serious virus which affects many fish species. It causes a widespread haemorrhaging seen internally, in the liver, in adipose tissue and within the muscle (Woo & Bruno, 1999). The first and only detection of this virus in Iceland, was in wild lumpfish, caught in Breiðafjörður fjord in 2015, for use as broodfish in production of lumpfish juveniles intended as cleanerfish for salmon lice found on salmon in sea cages. The VHS episode in Iceland was restricted to one farm, where wild broodfish and farmed juveniles were cultured in the same facility (Guðmundsdóttir *et al.*, 2019). In 2019, The Institute of Experimental Pathology at Keldur detected, for the first time in Iceland, infectious pancreatic necrosis virus (IPNV), which can severely affect the pancreas as well as causing various other pathological changes (Woo & Bruno, 1999), in ten salmon, located in sea-cages in a fjord in East Iceland. Furthermore, an apathogenic form of Infectious salmon anemia virus (ISAV), i.e. ISAV-HPR0 in salmon, have regularly been detected the last decade (Annual report from Keldur, 2019). The disease caused by the virulent form of ISAV, which has not

been detected in Iceland, is a serious disease causing, among other symptoms, anemia, necrosis and haemorrhage in the liver (Woo & Bruno, 1999). Piscine orthoreovirus (PRV) and salmon gill pox virus (SGPV), seems to be quite common in Icelandic aquaculture, causing mortality in some cases, which often seems to be related to suboptimal environmental conditions (Annual report from Keldur, 2019). Despite this, the status in Iceland, with regard to viral diseases is good, and has ESA approval for being free from all of the most serious fish viruses, such as VHSV, IHNV (Infectious Hematopoietic Necrosis Virus), ISAV-del (the non-virulent form of Infectious Salmon Anaemia Virus), Pancreas disease (PD), caused by SAV (Salmonid Alphavirus) and Cardiomyopathy syndrome (CMS), caused by PMCV (Piscine Myocarditis Virus) (Data from Fish disease laboratory at Keldur; Jónsson, 2020). An extensive and regular screening for viral infections, in accordance with the requirements of OIE (World Organization of Animal Health), has been performed in Iceland for decades.

The main parasites affecting fish in sea cages are sea lice, in particular the salmon louse (*Lepeophtheirus salmonis)* and the fish louse (*Caligus elongatus*). Both these species are common on wild fish, the salmon louse only infects salmonids while the fish louse is nonspecific with regard to fish host, infecting numerous species of marine fish. The salmon louse can cause large open wounds on the fish skin which can create pathways for other pathogens. The fish louse is smaller and causes some physical damage to the fish's skin, using suction disks to hold on to the fish (Woo & Bruno, 1999). Until recently, problems related to sea lice were minimal. However, the last few years, the sea lice problem has increased significantly, particularly in the Westfjords, most likely due to increasing biomass of farmed fish and rising sea temperature (Annual report from Keldur 2019; Jónsson, 2020).

1.2 The salmonid species in Iceland

The Family Salmonidae includes species such as salmon, trouts, charrs, freshwater whitefishes and graylings, which are collectively known as salmonids (Jobling *et al*., 2010). Three different native species of salmonids are present in Iceland, i.e. Atlantic salmon, brown trout (*Salmo trutta*) and Arctic charr. Furthermore, there is one introduced, i.e. rainbow trout, which was imported to Iceland in 1950 for farming purposes. It can be found in the wild, to some extent, due to escape from fish farms. However, it does not seem to be able to breed in the Icelandic ecosystem. Below is a brief description of the three native Icelandic salmonids (Guðbergsson & Antonsson, 1996).

The Arctic charr has a circumpolar distribution and mainly resides in cold waters (Jónsson & Pálsson, 2013). No freshwater fish species has as northern distribution as Arctic charr, being found at 80°N in Lake Hazen on Ellesmere Island in Canada (Helfman *et al*., 2009). Arctic charr can either be resident and spend its whole life in fresh water or anadromous, spending parts of their life in seawater. However, it always breeds in freshwater. A common length of a mature adult Arctic charr is approximately 40 cm. The largest one reported in Iceland, caught in Lake Skorradalsvatn, measured 87.5 cm in length and weighted 10 kg (Guðbergsson & Antonsson, 1996).

Anadromous Arctic charr favour coastal areas and estuaries, during their marine period. The sexually mature fish migrate back to its home river or lake in August and September. It resides in freshwater during the winter, where it spawns in December (Jónsson & Pálsson, 2013). The spawning

female Arctic charr lay their eggs in rocky gravel bottoms so they can receive shelter. Males release their sperm close to the eggs for fertilization to occur (Guðbergsson & Antonsson, 1996). After hatching of eggs, the juveniles live in their home river for the next 2-5 years before migrating down to sea for feeding in May-August (Jónsson & Pálsson, 2013).

Resident Arctic charr are more likely to be found in the southern part of Iceland where they live in their home lake or river their entire life. These fish can live at high altitudes with easy access to the sea but for some reason they do not migrate to sea. This is thought to be by, as of yet, unexplained environmental conditions (Guðbergsson & Antonsson, 1996). Resident Arctic charr prefer colder lakes, both deep and shallow, and live in the majority of lakes in Iceland. They spawn in the shallow parts of their habitat and have been found at altitudes as high as 700 ma.s.l. in Iceland (Jónsson & Pálsson, 2013). The size of resident Arctic charr differs between each population in Iceland. They tend to grow larger in warmer rivers, up to 50-60 cm, but to only around 7-12 cm in colder lakes (Guðbergsson & Antonsson, 1996).

Arctic charr tolerates many diseases better and adapts well to different environments compared to other salmonid species as well as surviving colder and harsher conditions. It can utilize more food sources, based on availability and competition, than the other two species present in Iceland while also being able to bottom feed in darkness, which is a very good trait for fish farming (Björnsson, 2001; Jónsson & Pálsson, 2013; Thorarensen, 2011).

Atlantic salmon has a wide distribution in temperate and subarctic regions of the North Atlantic Ocean. In the Northwest Atlantic it is found from Ungava Bay in Canada and south to New England in the United States. In the Northeast, its distribution extends from the White Sea in Russia in the north and to Spain in the south (Thorstad *et al.*, 2011). The Atlantic salmon is an anadromous fish species (Guðbergsson & Antonsson, 1996).

Adult pre-spawning Icelandic salmon migrate to their home rivers in late May to September with majority of them migrating in July (Jónsson, 1983). Spawning takes place from September to December and hatching of eggs takes about 6-8 months, depending on water temperature. The juveniles spend their first 3-5 years in freshwater before they smoltify and migrate to the sea in spring and early summer, then about 10-12 cm in length. The smoltified salmon spend 1-2 years in the sea until reaching sexual maturity when they return to their native river (Guðjónsson, 1978; Mills, 1989; Guðbergsson & Antonsson, 1996; Guðbergsson & Guðjónsson 2005). The average size of mature wild Icelandic salmon is around 50-100 cm in length and 2-12 kg in weight, but it can reach 150 cm and 50 kg (Jónsson, 1983).

The brown trout's distribution extends much further south than the Arctic charr and is much more widely distributed around Europe. It is found all over Iceland and is very common. Generally, the brown trout is darker than the Arctic charr with black spots spread all over its body which is what distinguishes it from the other two salmonid species in terms of color patterns. Furthermore, the ventral side is usually brighter. However, color variations can be very different depending on their environment but also their age or stage of maturity. The average length of an adult trout is around 40-45 cm but they can grow up to 100 cm (Jónsson & Pálsson, 2013).

Like the Arctic charr, the brown trout can be either resident or anadromous. The anadromous trout spawns in the autumn and lays the eggs in gravel reeds in oxygen rich water. Eggs hatch in springtime and juveniles stay in their home river for around 2-3 years. Subsequently, they migrate to the sea for feeding. Mature and immature trout return to their home river later in the summer or autumn and stay there until next spring, when they migrate back to the sea. Resident brown trout normally travel to the nearest lake where they stay until mature (around 4-6 years old) until they return to their home river to breed (Guðbergsson & Antonsson, 1996; Jónsson & Pálsson, 2013).

1.3 Bacterial Kidney Disease and its causative agent

1.3.1 Introduction of BKD

Bacterial Kidney Disease (BKD) is a systematic infection which progresses slowly, is seldom visible until the fish are at least 6-12 months old and can often be fatal to cultured salmonids. BKD was first described in wild Atlantic salmon, caught in two Scottish rivers in the early 1930s (Smith, 1964). Similar lesions were described in rainbow trout in a North American hatchery in 1935, however the causative bacterium was not cultured until 1956. BKD is recognized as a very costly problem in the farming industry. Although Rs has been found in various non-salmonids, BKD outbreaks have, to this date, only been reported in salmonids (Elliott, 2017).

1.3.2 *Renibacterium salmoninarum*

Renibacterium salmoninarum is the causative agent of BKD. Rs is a Gram-positive bacterium which often stays in pairs or sometimes in short chains. It is a small (0.3-1.5µm x 0.1-1.0µm) non-spore forming, non-motile, non-acid fast and not encapsulated (Sanders & Fryer, 1980). However, scientists have been able to culture from sub-clinically infected fish, coccoid cells in small aggregates and more slender rod-shaped bacteria from atypical colonies (Hirvelä-Koski *et al.*, 2006). Rs is a facultative intracellular bacterium which infects the cytoplasm of epi/endothelial cells and neutrophils (Bruno, 1986; Ferguson, 1989; Elliott, 2017), as well as in sinusoidal cells, reticular cells or fibroblastic barrier cells of spleen and kidney haematopoietic tissue (Flaño *et al.*, 1996; Elliott, 2017). However, the bacterium can survive, and most likely replicate, in mononuclear phagocytic cells which apparently protects the bacterium from extracellular host defence mechanisms, such as compliment fixation and antibody binding. The highest quantity of the bacterium in salmonids is often inside macrophages (Hardie *et al*., 1996; Pascho *et al.*, 2002; Munang'andu, 2018).

For years, the aetiological agent of BKD was thought to belong to the genus *Corynebacteria* and was referred to as "kidney disease *Corynebacteria*" because of its morphological appearance as well as its Gram-positive nature, catalase positive characteristics, proteolysis and lack of endospores (Ordal & Earp, 1956; Smith, 1964) and later *Corynebacterium salmoninus* (Sanders & Fryer, 1978; Bullock *et al*., 1980). Because the bacterium's wall composition was significantly different from *Corynebacteria* species, its generic identity was assigned to the novel genus *Renibacterium*. However,

it was still thought to be most closely related to the coryneform group (Sanders & Fryer, 1980) but later it was revealed to be most closely related to *Arthrobacter* species (Wiens *et al.*, 2008).

A 57 kDa extracellular major soluble antigen MSA, has been identified as a key mediator in immunosuppression in the fish host and has been shown to be the most dominant virulence factor of Rs. The MSA antigen causes reduction in phagocytic activity as well as antibody production (Pascho *et al*., 2002; Wiens, 2011). Alongside it, a 22kDa surface protein of the bacterium (p22), is also thought to cause a suppression of antibody production (Fredriksen *et al*., 1997).

1.3.3 Isolation and growth properties

Renibacterium salmoninarum is a slow-growing bacterium compared to most other species of fish bacteria and does not grow on conventional media such as general blood agar. After 12 weeks incubation period on three different media, Kidney Disease Medium 2 (KMD2) and "kidney disease medium charcoal" (KDMC) it was observed that the bacterium grows best on selective medium (SKDM) (Guðmundsdóttir *et al*., 1991). All these media contain L-cysteine, which is essential for growth of Rs (Ordal & Earp, 1956). Rs was first cultured in the 1950s, i.e. about twenty years after it was first reported (Smith, 1964). Rs produces white or creamy, shiny, smooth, round, raised, entire colonies that vary from pinpoint to 2 mm in size. On average, visible colonies in culture will form after 2-3 weeks from a BKD diseased salmonid. However, up to 8 weeks have been reported for initial growth on KDM2, and 19 weeks on SKDM (Benediktsdóttir *et al*., 1991). The bacterium is aerobic and grows at all temperatures between 5-22°C. However, optimum growth is at 15-18°C and not at all at temperatures 25°C and above (Smith, 1964).

The biggest reason behind the slow pace of information gathered on Rs, is mainly because of how slowly the bacterium grows as well as the chronic nature of the disease that it usually causes. Other properties of the pathogen, especially its ability to enter and survive inside eggs and phagocytic cells, has made it difficult for scientists to develop methods for controlling infections. It has been shown *in vitro*, that Rs, taken from infected primary cultures of rainbow trout survives inside their mononuclear phagocytes (Bruno, 1986; Gutenberger *et al*., 1997; Elliott, 2017). Furthermore, it has been shown using transmission electron microscopy, that the bacterium partially depends on its ability to move from the phagosome into the cytoplasm to survive. Formalin-killed Rs also were able to enter the cytoplasm, but at a slower rate and after sustaining greater cell wall damage, suggesting that the extracellular protein of the bacteria plays a role in intracellular survival (Gutenberger *et al.,* 1997; Elliott, 2017).

1.3.4 Modes of transmission

Different from most other fish-infecting bacteria, Rs can be transmitted vertically, from parent to progeny with infected eggs. Egg disinfection procedures using organic iodine disinfectants does not prevent transmission of the disease via eggs (Bullock *et al*., 1978). For this reason alone, it is highly complicated to stop the disease from spreading further (Warren, 1983).

The bacterium can also spread horizontally, from individual fish to another e.g. via the fecal-oral route or sharing the same water supply with Rs-infected fish This has been reported both in hatcheries and in the wild. Frantsi *et al.* (1975) reported cultured Atlantic salmon fingerlings to be exposed to BKD from “naturally” infected wild salmonids habituating the farm’s water supply. Guðmundsdóttir *et al*. (2017b) performed a cohabitation trial where naïve Arctic charr received water from intraperitoneal infected fish and infection started to take hold after 3 weeks. Mitchum *et al*. (1979) also reported a stocked hatchery trout contracting BKD from wild trout. Rs has been shown to be able to survive for weeks outside the host (Balfry *et al*., 1996; Hirvelä-Koski, 2004).

Infected fish products have been shown to transmit BKD to cultured fish. Until the mid-1960s, the main diet used for culturing salmon fingerlings was a compilation of ground carcasses of spent broodfish, raw ground salmon viscera and other fresh fish products mixed with beef spleen, liver and other animal organs. These types of wet diets often carried fish disease agents like Rs and played a big role in spreading BKD and other serious diseases in many Pacific Northwest salmon hatchery stocks (Wood, 1974).

1.3.5 Susceptibility

The primary hosts of the bacterium are considered fish of the genera *Oncorhynchus*, *Salmo*, *Salvelinus* and *Coregonus* even though it has occasionally been detected in non-salmonids (Wiens & Kaattari 1999; Faisal *et al*., 2010). Susceptibility to the disease varies quite considerably in terms of fish species. Pacific salmon species (genus *Oncorhyncus*) are considered the most susceptible species while the Atlantic salmon is considered to be more susceptible than rainbow/steelhead trout, brown trout and charr species (Elliott *et al.*, 2014). Many environmental and physiological factors are considered to play a major role in susceptibility to BKD (Bruno, 1986).

Stress is generally known to increase the susceptibility of fish towards diseases. Physical stress can be monitored by measuring cortisol levels in fish which increases during smoltification and causes a reduction in the number of circulating leucocytes (Maule *et al*., 1989). A good example of stress inducing conditions is high biomass of fish in sea cages. The proximity in such conditions enhances transmission of the bacterium between individual fishes, via the fecal-oral route (Warren, 1983).

Change in salinity is known to increase susceptibility in salmon, although BKD does not seem to obstruct key indicators of smoltification, such as Na+- and K+-ATPase in the gills. It seems to rather impair acclimatization to seawater. This causes a subsequent reduction in ocean survival of the salmonids (Mesa *et al*., 1999). It is not certain whether high salinity has diminishing effect on BKD infections in fish since results from experiments have been contradictory, but research suggest it plays a role in infection load (Meyers *et al*., 1999)**.**

Smoltification can trigger an outbreak of the disease as the fish undergo a number of morphological, physiological and behavioural changes in preparation for ocean residence (Folmar & Dickhoff, 1980).

Temperature seems to play a big part in BKD outbreaks in cultured trout populations. It has been reported that BKD mortality increases during spring, that it is at its highest in May and then slows

down in July. Rising temperatures are considered to be a key factor in the increase of BKD in March to May but might also enhance the production of immunological factors that suppress the disease in the fish, which might explain why the disease declines by July. Another reason for the decline in the disease might be stocking of yearling trout which removes many of the infected fish from the hatchery. However, many researchers have shown that BKD infections can occur over a wide range of water temperature (Belding & Merrill, 1935; Earp *et al*., 1953; Fryer & Sanders, 1981; Bullock & Herman, 1988).

Water quality is very important to keep the fish stress-free and healthy. Increased BKD-resistance in fish has been linked to increased water hardness (containing high concentrations of dissolved calcium and magnesium ions). The reason most likely being the pathogen´s ability to survive in the water or the salmonid's ability to resist infections (Warren, 1963).

Older studies have shown that fish diet may affect BKD infection load. The feed ration as well as the composition of the diet may influence susceptibility of salmonids by altering innate and specific immune responses (Fryer & Sanders, 1981; Waagbø, 1994; Alcorn *et al*., 2003). Paterson *et al.* (1981) showed that, at one hatchery, yearling Atlantic salmon which had been fed a diet with increased levels of trace minerals (Fe, Cu, Mn, Co, I and F) and low levels of calcium, resulted in decreased incidence of BKD. On the other hand, a high incidence of BKD occurred in similar fish fed a standard commercial feed (Warren, 1983; Paterson, 1981).

1.3.6 Disease symptoms

It can take several months, or even years, for clinical symptoms to appear in the fish. Factors such as, temperature, host-species and possibly also bacterial strain, have a big impact on the duration of the incubation period. Consequently, all fish in a particular aquaculture facility might be infected once the disease has been discovered (Fryer & Lannan, 1993). In contrast to other bacterial diseases in fish, fish that are severely infected with BKD may show no obvious external clinical/pathological signs, or may show one or the following: pale gills indicative of anaemia, exophthalmia, abdominal distension (due to the accumulation of ascitic fluid), skin blisters filled with clear or turbid fluid, shallow ulcers (the results of broken skin blisters), haemorrhages (particularly around the vent) and, infrequently intramuscular cavities filled with blood-tinged caseous or necrotic material. Turbid fluid may gather in the abdominal and pericardial cavities, haemorrhages are seen on the walls of the abdominal cavity and on the viscera, fibrinous layer may cover one or more of the visceral organs, and most characteristically, creamy-white granulomatous lesions develop in the kidney and, less frequently, in the spleen and the liver (Elliott, 2017).

These lesions can be encapsulated and may even be resolved in species such as Atlantic salmon. However, lesions are rarely encapsulated in Pacific salmon (Genus *Onchorynchus*), which is, as noted above, considered more susceptible to BKD than the Atlantic salmon. Histologically, the lesions are mainly in haemopoietic tissue in chronic granuloma form but in the final stages of the disease they may have extended to any organ e.g. liver, or cardiac and skeletal muscles (Elliott, 2017).

1.3.7 BKD in Iceland

BKD outbreaks have never been seen in wild salmonids in Iceland (Kristmundsson *et al.*, 2016). Jónsdóttir *et al.* (1998) performed a study in 1993 to examine the prevalence of infection and intensities of Rs antigens in 961 resident Arctic charr and brown trout from 23 lakes around Iceland, there amongst Lake Elliðavatn. Results revealed all 22 populations of Arctic charr and nine of brown trout to test positive for Rs-antigens. Furthermore, Kristmundsson *et al.* (2016) and Guðmundsdóttir *et al.* (2017a) reported wild salmonids from Lake Elliðavatn positive for Rs-antigens, without showing any symptoms of BKD. This suggests the bacterium has been endemic for a long time in Iceland and is probably a normal, low density resident in wild fish (Jónsdóttir *et al.,* 1998). A disturbance in the host-pathogen homeostasis could possibly lead to an BKD outbreak (Kristmundsson *et al.*, 2016) as often is seen in fish farms. For more than three decades, wild female salmonids, meant for restocking purposes have been screened for Rs, according to Icelandic regulations, at the Institute of Keldur. Eggs from Rs-positive broodfish are discarded.

In 1968, BKD was first diagnosed in Iceland in farmed Atlantic salmon (Helgason, 1985). Since then, Rs has regularly been detected in farmed, ranched and wild populations of salmonids fish (Sigurjónsdóttir *et al.*, 1995; Kristmundsson *et al.,* 2009; Kristmundsson *et al.*, 2016).

The fish farming industry in Iceland grew rapidly in the 1980s and in 1985 four fish farms had BKD outbreaks (Guðmundsdóttir *et al*., 2000). This was the start of Iceland's first BKD epidemic. A year later, systematic screening for the causative bacterium began in Iceland. The infection load increased dramatically in fish farms and the overall percentage of infected fish in affected farms was around 35%, until a brood stock culling program was initiated. The epidemic ended in 1992 but sporadic incidents of BKD outbreaks occurred in the following years (Guðmundsdóttir *et al*., 2000).

The second epidemic started in 2003 and lasted until 2008. This affected cultured Atlantic salmon, Arctic charr and rainbow trout and Rs was detected in 18 fish farms (around half of all fish farms in Iceland at the time). According to the records of the Fish disease Laboratory at Keldur, the outbreak can be traced back to two separate initial incidents. Iceland gained control over the disease by practicing culling. Millions of fish were slaughtered and the damage to the fish farming industry amounted to millions of Euros (€) (Kristmundsson *et al*., 2008; Kristmundsson *et al*., 2009).

1.3.8 Prevention and vaccination

The first and strongest line of defense against BKD is preventive measures, i.e. to practice whatever means that are necessary to prevent the bacterium from entering the system of the fish farm. Thorough monitoring programs, sanitary policies, careful planning and a good understanding of the etiology of BKD is necessary for a successful program of prevention and control. If these efforts fail then the next step is to try and contain the spread and minimize the overall effect of BKD (Warren, 1983).

At present, there are no effective drugs available that will cure fish of BKD. Some antibiotics have been used, e.g. erythromycin and sulfamethazine, but have been shown to merely suppress the progress of the disease to some extent, as long as the drugs are being administered. Once these

drugs are withdrawn, mortality due to BKD commonly resumes within few weeks (Warren, 1983). The only currently available and commercially licensed vaccine against Rs is Renogen®1 (Elanco Animal Health). It is a lyophilized preparation containing live cells of an apathogenic environmental bacterium, *Arthrobacter davidanieli*. It is not available in Europe nor Japan but is applied in North America and Chile (Elliott *et al*., 2014). According to laboratory and field trials, the vaccine provides limited protection against the bacterium in experimentally infected Chinook salmon and some protection in Atlantic salmon juveniles. Renogen®-vaccinated Atlantic salmon were reported to have a significantly higher survival rate. However, it is unknown how long the duration of protection lasts (Rhodes *et al*., 2004; Alcorn *et al*., 2005; Salonius *et al*., 2005; Elliott *et al*., 2014).

Once BKD has been confirmed in an aquaculture facility, the immediate reaction is to remove the bacterium by use of short-term cleanup procedures, i.e. slaughtering BKD infected fish and disinfecting all rearing units. Only if these cleanup procedures have been done properly, eggs or fish can re-enter the farming facilities (Warren, 1983). Population densities, stress, water characteristics, and the severity of the BKD infection seem to play the biggest role with regard to long term effects. Erythromycin and sulfamethazine can be used as a part of a strategy of “dilution”, i.e. to lower the intensity of infection to result in an increasing number of healthy fish (Warren, 1983).

Even though *Renibacterium salmoninarum* can transmit horizontally in the wild, BKD does not appear to be a significant or widespread problem in most wild salmonid populations. As mentioned before, the bacterium seems to live in some sort of balance with wild salmonids but certain environmental factors can stir that balance and lead to an outbreak. All things considered, the “dilution” strategy remains the only practical approach available to reduce the disease in wild populations (Warren, 1983).

1.4 Diagnostic methods

1.4.1 Culture

The only way to be sure that samples from a given infected fish harbor viable Rs is by culturing the bacterium (Pascho *et al*., 2002; Wiens, 2011). However, that is not enough evidence to prove that you have the right bacterium. For that purpose, specific tests are needed based on biochemical-, serological- and/or molecular characteristics of the bacterium. Currently, the most used media for isolation of the bacterium is selective medium (SKDM) which is KDM agar supplemented with four antibiotics to prevent unwanted organisms from growing on the medium. Rs optimum temperature while on SKDM is around 15-18°C. Usually it takes several weeks for viable colonies to emerge. This may occur within 5-7 days or as late as 19 weeks (Benediktsdóttir *et al*., 1991).

1.4.2 Enzyme-linked immunosorbent assay (pELISA) for Rs detection

The enzyme-linked immunosorbent assay (ELISA) has been the preferred diagnostic-, as well as screening method, for BKD in hatcheries and captive brood stock programs for decades because of

several desirable features. It allows for processing large sample numbers with its 96 well format and it is easy to procure reagents. The assay gives reliable results and has a sensitivity of approximately 20 to 50 ng ml^{-1} of stable target antigens in the homogenate. It is relatively inexpensive and is considered more sensitive than FAT (fluorescent antibody test) techniques. Furthermore, sample preparation is relatively simple (Meyers, 1993).

The ELISA test detects epitopes on the “major soluble antigen” (MSA) (Pascho & Mulcahy, 1987), which Rs secretes abundantly and have been known to persist for months (Pascho *et al.*, 1997; Guðmundsdóttir, 2020). ELISA protocols for detection of the bacterium are either based on polyclonal or monoclonal antibodies, which detect epitopes on the MSA protein. Monoclonal antibodies detect a single epitope of the MSA protein, while the polyclonal antibodies detect multiple epitopes as well as other antigens produced and secreted by the bacterium (Guðmundsdóttir *et al***.,** 1993; Árnason *et al*., 2013). Polyclonal ELISA (pELISA) is reported to be more sensitive than monoclonal ELISA (mELISA) (Jansson *et al.*, 1996; Árnason *et al*., 2013; Guðmundsdóttir *et al*., 1993; Elliott, 2017).

Although ELISA is a good diagnostic method and well suited for screening, other methods are more suitable as a confirmatory assay (Elliott, 2017). The prediction can be inaccurate when target antigens in tissue are not correlated with the number of bacteria present, since these antigens can persist without the presence of live bacteria (Guðmundsdóttir *et al.,* 1993; Wiens, 2011). Guðmundsdóttir *et al.* (2020) performed a study where Rs antigen was injected intra-peritoneally into Arctic charr to study the fate of Rs-antigens in the absence of the bacterium. Results showed that OD values declined over time, but the antigen was still detectable in a majority of the fish after 43 weeks (Guðmundsdóttir *et al.,* 2020).

1.4.3 Polymerase chain reaction (PCR) for Rs detection

Presently, there are many different types of PCR methods for detection of Rs and some of them are recommended as confirmatory tests, e.g. by the OIE and the Fish Health Section (FHS) of the American Fisheries Society (AFS) (Chase & Pascho, 1998; Chase *et al.*, 2006; Elliott, 2017).

Most standard PCR methods for the detection of Rs detect the *msa* genomic DNA indicating whether nucleic acid of the bacterium is present or not. Although such detection of genomic DNA with conventional PCR is more sensitive than ELISA for Rs detection, it does not necessarily indicate that the bacterium is viable, nor can it quantify infection levels. However, such PCR assays are none the less important due to their high sensitivity and specificity, i.e. they are able to amplify DNA from low amounts of bacterial DNA present in samples. Because ELISA detects soluble antigens, which circulate throughout the body, it can detect Rs-antigens in tissues other than the one sampled from (Chase *et al.,* 2006). Therefore, PCR is used along with ELISA for confirmative diagnosis (Chase *et al*., 2006; Jansson *et al*., 2008).

The qPCR method for detection of Rs, developed by Chase *et al.* (2006), allows an accurate determination of the initial template concentration of Rs nucleic acid by monitoring the real-time progress of the PCR assay. Advantages of qPCR over conventional PCR are (1) increased analytical sensitivity by the use of a probe-based detection method, (2) elimination of post-PCR manipulations of

samples and other PCR product-related contamination, but most importantly (3) gives a quantitative assessment of the target nucleic acid in the initial sample, contrary to conventional PCR, thereby giving an estimate of infection levels in fish (Chase *et al.,* 2006*).*

16S sequencing has proven to be useful for differentiating between isolates, strains and species of bacteria (Grayson *et al*., 1999).

2 Objectives

The main objectives of this study were to: (1) Trace the source of infections of the etiological agent of bacterial kidney disease, *Renibacterium salmoninarum* (Rs), in land-based salmonid farms in the Westfjords of Iceland. (2) To investigate whether the presence of Rs infected fish in sea cages could affect the prevalence and intensity of Rs in wild salmonids in nearby aquatic systems. (3) Investigate whether different approaches in diagnostic qPCR tests, could give comparable results. (4) Examine whether genetic difference exists in Rs strains in Iceland.

To reach these goals, the following aspects were addressed:

- The presence of Rs bacterium, or its antigen in:
    - Wild salmonids inhabiting brooks, which serve, or served, as water source (to some extent) for these farms.
    - Brook water used by these fish farms.
    - Tank water in the farms, that has been UV treated.
    - In the juvenile salmon in these farms.
    - Wastewater from the farms released into the sea.
- The Rs status in wild salmonids:
    - Caught in fjords nearby sea cages which contain BKD infected fish.
    - Caught in areas which are not affected by fish farming.
- The possibility of genetic variation of Rs isolates originating from:
    - Different location in Iceland.
    - From other countries in Europe and N-America.
- Testing of multiple organs for Rs, to determine whether kidneys are the best organ to screen for Rs.
- Examine whether the use of samples prepared for ELISA (supernatant) analysis can give reliable results when used in PCR analysis.
- Compare results from PCR using DNA that was extracted manually or automatically by robot.

3 Materials and methods

3.1 Study sites

The following sites were studied in this thesis: (1) Tálknafjörður; (i) three different land-based farms, (ii) Five mountain brooks in the vicinity of these farms, which the farms used as a supply water, (iii) wild salmonids caught close to sea cages in the fjord. (2) Patreksfjörður; wild salmonids caught close to sea cages in the fjord. (3) Four different lakes in Iceland, far from farming areas, i.e. Lake Elliðavatn, Lake Mývatn, Lake Mjóavatn and Lake Steinsmýrarvötn. (4) Various rivers in Iceland (supplementary material), far from farming activities. The location of the study sites are shown in Figure 2. Below is a brief description of each study site.

3.1.1 Tálknafjörður and Patreksfjörður

The three fish farms named A, B and C, who took part in this study, are all land-based in Tálknafjörður. Two of them have their sea cages in Tálknafjörður and in a nearby fjord, Patreksfjörður. All three farms have experienced BKD outbreaks in recent years. Furthermore, active BKD episodes were ongoing in sea cages in both Tálknafjörður and Patreksfjörður during the study. Farms A and C, culture Atlantic salmon for smolt production that are subsequently transferred to the sea cages for onrearing. In Farm B, Arctic charr is cultured until reaching slaughter size.

Until 2018, Farms A and C used water from nearby mountain brooks, as a complimentary supply to borehole water, without UV treatment. At present, both Farms A and C, exclusively use UV treated water. Farm A uses a flow-through system while Farm C utilizes recirculating aquaculture system (RAS), where 95% of the water is filtered and reused. Farm B has, and still does, take most of its water from mountain brooks without any UV treatment (Gísli Jónsson, veterinary officer of Fish diseases, personal communication).

All fish cultured in Farms A, B and C, originally came from Rs-free eggs which previously had been screened at the Fish disease laboratory at Keldur Institute (Gísli Jónsson, veterinary officer of Fish diseases, personal communication; Kristmundsson *et al*., 2009).

Numerous small rivers and brooks are found close to the land-based farms located in Tálknafjörður, some of which have been used as water supply for the farms studied, as noted above. Five of these brooks, here named Brooks 1-5, were studied. Prior to this research project, data on the presence of salmonid fish species in some of these brooks were scarce or non-existing in records. All these rivers/brooks seem to have stocks of brown trout and Arctic charr.

3.1.2 Lakes and rivers studied

Lake Elliðavatn is a shallow lake located within Reykjavík, the capital city of Iceland. Atlantic salmon, brown trout, Arctic charr, European eel (*Anguilla Anguilla*) and three-spined stickleback (*Gasterosteus aculeatus*) are the only fish species residing in the lake (Antonsson *et al*., 2007). Resident Arctic charr

and brown trout are the dominant fish species of the lake. (Antonsson & Guðbergsson, 2000; Harðardóttir et al., 2002; Malmquist et al., 2009).

Lake Mjóavatn is located in Northwest Iceland, more precisely in the highlands of Auðkúluheiði in the interior northern Iceland at 430 - 450 ma.s.l. It is a shallow lake with Arctic charr the only salmonid species inhabiting it (Gudbergsson & Antonsson, 1997).

Lake Mývatn, with its outflowing River Laxá, is located in Northeast Iceland. The dominant fish species in the lake are Arctic charr and the three-spined stickleback but brown trout is also common (Garðarsson & Einarsson, 1991).

Steinsmýrarvötn is a shallow (1-2 m deep) lake, located in Southern Iceland, i.e. in Meðalland in West Skaftafellssýsla. A small brook flows from the lake, which connects the lake to the River Eldvatn, where sea trout is common. Brown trout, both resident and sea migrating, is the dominant species in the lake. Furthermore, Arctic charr, three-spined stickleback and European eel are common (Jóhannsson, 1993; Steingrímsson, 2002).

Fish from eight other rivers were included in this study. These were samples from sea trout from routine Rs-screenings of the Fish Disease Laboratory at Keldur (location shown in Figure 2, labelled 7-14).

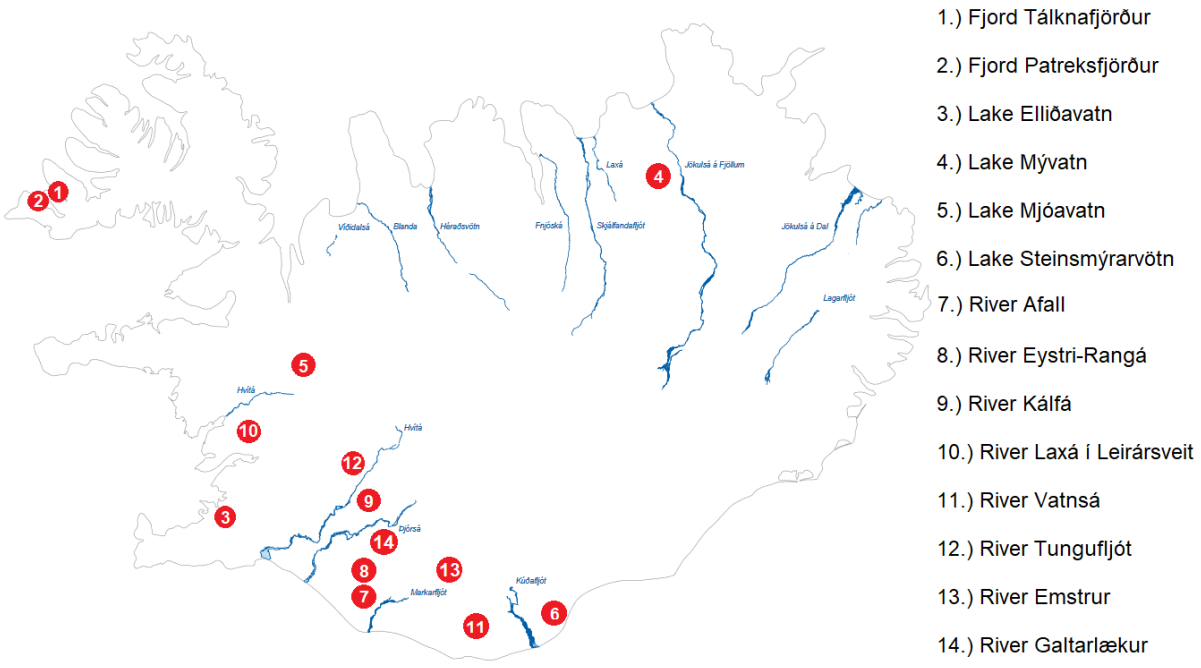


Figure 2. Shows the location of all fish- and water sampling sites in lakes and rivers

3.2 Research material and sampling

3.2.1 Fish

The fish collected for this study consisted of: (1) Salmonids (Atlantic salmon, Arctic charr and brown trout), caught by electrofishing in Brooks 1, 2 and 3, which the three farms studied used as water supply, to some extent; (2) Atlantic salmon juveniles from two land-based farms (Farms A and C) and

Arctic charr from one land-based farm (Farm B); (3) Wild sea trout and sea run Arctic charr caught in gill nets in Patreksfjörður and Tálknafjörður, i.e. areas where sea cage rearing of Atlantic salmon is ongoing; (4) Wild control fish (sea trout and sea run charr) from areas which are not in the vicinity of fish farms: (i) Sea trout and Arctic charr caught on rods in Lake Steinsmýrarvötn, (ii) resident Arctic charr and/or brown trout from Lakes Elliðavatn, Mývatn and Mjóavatn, (iii) samples available at the Fish disease laboratory at Keldur Institute, to accommodate for the lack of wild fish for examination, i.e. samples processed for pELISA assay (supernatant derived from kidney tissue) from breeding sea trout, caught for enhancement programs in various rivers using nets. Basic information about all salmonids collected and examined from each study site is shown in Appendix I. An overview of the number of samples collected, is shown in tables 1, 2 and 3.

All fish were kept fresh until they were examined. Initially, the length and the weight of each fish was determined. Subsequently, the fish were dissected and examined for macroscopic signs, suggestive of bacterial kidney disease. Samples from anterior- and posterior kidney were excised and put in Stomacher bags, for examination by pELISA and frontier kidney samples were taken for PCR and stored in 1,5 mL Eppendorf tubes. The samples were stored at -20°C until further processing.

Table 1. **Wild salmonids caught near farming areas**

Information about date of sampling, number of fish collected and number of PCR and ELISA screenings, in fish caught in two fjords in the Westfjords and three brooks located in Tálknafjörður (which Farms A, B and C used as water supply, to some extent).

Fjord/Brook	Sampling date	No of salmonids	PCR	ELISA
Fjord Tálknafjörður	Aug-19	20	20	20
Fjord Patreksfjörður	Jul-19	23	23	23
Brook 1	Oct-19	30	30	6
Brook 2	Aug-18	37	37	8
Brook 3	Aug-18	18	18	8
Total		**128**	**128**	**65**

Table 2. Wild salmonids far away from farming areas

Information about date of sampling, number of fish collected and number of PCR and ELISA screenings, in fish collected from Icelandic lakes and rivers.

Lake/River	Sampling date	No of salmonids	PCR	ELISA	Culture
Lake Mývatn	Sep-18	41	41	41	42
Lake Mjóavatn	Sep-18	30	30	30	30
Lake Elliðavatn	Sep-18	30	30	29	30
Lake Elliðavatn	Sep-19	34	170	34	0
Lake Steinsmýrarvötn	Sep-19	7	7	7	0
River Affall*	Oct 05-07	13	4	9	0
River Emstrur*	Oct-06	1	1	1	0
River Eystri Rangá*	Oct 09-17	9	1	9	0
River Galtarlækur*	Oct-06	3	1	3	0
River Kálfá*	Oct 06-10	5	1	3	0
River Laxá í Leirársveit*	Oct-06	8	1	3	0
River Tungufljót*	Oct 06-16	6	1	6	0
River Vatnsá*	Oct 06-08	15	3	12	0
Total		**202**	**291**	**187**	**102**

(*older supplementary samples from the Fish Disease laboratory)

Table 3. Cultured salmonid juveniles from farms in Tálknafjörður

Information about date of sampling, number of fish collected and number of PCR and ELISA screenings, in fish collected from three farms located in Tálknafjörður in the Westfjords of Iceland.

Fish farm	Sampling date	No of salmonids	PCR	ELISA
Farm A	Jun-18	32	31	32
Farm B	Oct-18	37	30	37
Farm C	Oct-19	30	30	6
Total		**99**	**91**	**75**

3.2.2 Water samples

Water samples were collected from: (1) Brooks 1-5 in Tálknafjörður, which the fish farms used as water supply (intake water); (2) inside the fish farms (after UV treatment); (3) drainage water released from the fish farms into the sea. Each sample contained 10 L. The samples were stored at -20°C until they were processed for examination, i.e. filtration, followed by pELISA and PCR analyses.

3.3 Methods

3.3.1 Water filtration

The 10 liter tanks were defrosted at room temperature for 24 h and then stored at 4°C, until the ice had melted completely. Filters with pore size of 100 µm, 5 µm, 3 µm, 1 µm, 0.8 µm and finally 0.45 µm were used to filter the water. All filters used per sample were stored in a sample tube of 30-60 mL (dependent on number of filters used) of PBS (phosphate buffered saline) for 24 h at 4°C. After 24 h, the liquid was divided evenly amongst 30 1,5 mL Eppendorf tubes. The tubes were centrifuged at

10.000 revolutions per minute (rpm) for 20 minutes at 4°C. The top layer of the liquid, ca. 50%, was discarded leaving the pellet and left-over liquid in the sample tube. Then the samples were evenly distributed between eight 1.5 mL Eppendorf tubes which were centrifuged and processed as before. The remaining samples were then distributed into four sample tubes, processed as before and merged into one sample. The sample remaining in the final tube was then divided into two samples which were stored at -20°C until they were used in pELISA and qPCR diagnostic methods.

3.3.2 Rs cultivation

Salmonids caught in Icelandic lakes

Homogenized kidney tissue from salmonids caught in Lake Elliðavatn, Lake Mývatn and Lake Mjóavatn were spread on SKDM agar plates. Kidney tissue samples were diluted 1/10 with autoclaved 0.1% peptone in saline and homogenized in a grinder for 1 min. After homogenization, the samples were centrifuged at 3000 rpm for 20 min at 4°C. The supernatant was discarded and pellets were resuspended in peptone saline in the ratio 1:1.

Isolates and kidney tissue from Keldur's sample bank

Nine Rs isolates from Keldur's Institute's sample bank, from past BKD outbreaks, stored in bacterial freezing medium at -80°C, as well as nine kidney tissue samples from BKD positive fish, that had been in long term -20°C storage, were inoculated onto SKDM agar (Table 4).

Kidney tissue (-20°C) was homogenized with same method as samples from Icelandic lakes. Then, a loopful of each sample was spread on SKDM agar plates and incubated at 16°C for at least 16 weeks. A loopful from Rs isolates (-80°C) was spread directly on SKDM agar. Further, a loopful from the growth on the plates was transferred to 800 µl of PBS and several 1/5 dilutions were made and tested in pELISA.

Table 4. Information about all Rs isolates and kidney tissue cultured on SKDM from Keldur's Institute's sample bank

Isolate	Storage (Keldur)	Origin	Species
F-130-87	Bacterial Freezing Medium (-80°C)	Laxalón (in Reykjavík)	Rainbow trout
F-11-12	Bacterial Freezing Medium (-80°C)	Rifós in Kelduhverfi	Atlantic salmon
F-358-87	Bacterial Freezing Medium (-80°C)	Kiðafellsá in Hvalfjörður fjord	Atlantic salmon (w)
2-S-2	Bacterial Freezing Medium (-80°C)	Lækur in Ölfus (Íslenska Fiskeldisfélagið)	Atlantic salmon
F-138-87	Bacterial Freezing Medium (-80°C)	Hvammsvík	Rainbow trout
F-273-87	Bacterial Freezing Medium (-80°C)	Straumsvík (Hafeldi)	Atlantic salmon
S-182-90	Bacterial Freezing Medium (-80°C)	Lækur in Ölfus (Íslenska Fiskeldisfélagið)	Atlantic salmon
F-283-87	Bacterial Freezing Medium (-80°C)	Kollafjörður	Atlantic salmon
F-384-87	Bacterial Freezing Medium (-80°C)	Elliðárstöðin	Atlantic salmon
F5906	Kidney tissue sample (-20°C)	Sæsilfur Mjóafirði	Atlantic salmon
F-107-05	Kidney tissue sample (-20°C)	Silfurstjarnan Núpsmýri í Öxarfirði	Atlantic salmon
F1506	Kidney tissue sample (-20°C)	Húsatóftir í Grindavík	Atlantic salmon
F4807	Kidney tissue sample (-20°C)	Silfurstjarnan Núpsmýri í Öxarfirði	Arctic charr
F4804	Kidney tissue sample (-20°C)	Laugar	Atlantic salmon
M3297	Kidney tissue sample (-20°C)	Eyrareldi í Tálknafirði	Atlantic salmon
F6205	Kidney tissue sample (-20°C)	Íslandslax	Arctic charr
F204	Kidney tissue sample (-20°C)	Fellsmúli	Atlantic salmon
F4903	Kidney tissue sample (-20°C)	Eyrar fiskeldi	Arctic charr

3.3.3 ELISA

Sample preparation

Sample processing of ELISA followed Guðmundsdóttir *et al.* (1993). Kidney samples were placed in sterile Stomacher 80-micro Biomaster (Seward) bags and weighed. If they did not reach 0.3 grams in weight, other kidney samples were added into the Stomacher bag (pooled samples). Samples were diluted in 1:3 (weight/vol) in Dulbecco's PBS (phosphate buffered saline) and 25 µl tween®20 was added for each mL of homogenate. The samples were homogenized in a micro-BIOMASTER grinder (Seward) for 1 min, boiled for 15 minutes and centrifuged at 4.432 rpm for 30 min at 4°C. The supernatant was harvested into a new sample tube and stored at 4°C until tested in pELISA the following day.

ELISA test using polyclonal antibodies (pELISA)

The pELISA assay used was a double sandwich ELISA using polyclonal antibodies as described by Guðmundsdóttir *et al.* (1993). The wells of the pELISA test plate (Maxisorp immunoplate, Nunc), were coated at least 2 days before the assay at 4°C with 100 µl of catching antibodies diluted 10µg ml^{-1} in 0.05M carbonate-bicarbonate buffer, pH 9.6. The antibodies used were both normal and specific rabbit IgG (B-6-6), produced at Keldur Institute (Guðmundsdóttir *et al*., 1993). Plates were washed between all steps with 3 changes of the washing solution (Dulbecco's PBS, pH 7.2 with 0.7M NaCl and 0.1% Tween®20) every 5 minutes.

After plates were coated and washed. Briefly, 100 µl of samples, including negative and positive controls, were added into the wells and left to incubate for two hours at room temperature (RT). Then, the plate was washed as before but adding an extra washing step and 100 µl of the conjugate added into each well. The conjugate (detecting antibodies) was goat polyclonal, affinity purified, horse radish-peroxidase conjugated IgG against heat-treated whole cells of Rs (Kirkegaard & Perry Laboratories Inc.). The antibodies were diluted in a buffer containing PBS, 0.05M NaCl and 0.05% Tween®20 to a concentration of 2ug ml^{-1}. Conjugate was left to incubate with samples and after one hour the plate was washed. Next, 100 µl of substrate buffer was added for conjugate staining (8mg of the substrate o-phenylenediamine dihydrochloride (OPD, DAKO),12 mL of water with 5 µl of 30% hydrogen peroxide (H2O2)) and left to incubate for 15 min at RT. The final step was then to stop the enzymatic reaction by adding 50 µl of 3N hydrochloric acid to each well after 10 min. The OD values were read at 492 nm in Multiskan FC Microplate reader 357 from Thermo Scientific.

3.3.4 DNA isolation methods

GeneJET

The GeneJET Genomic DNA Purification Kit from Thermo Scientific was used for manual DNA isolation from kidney tissue samples. Kidney tissue (ca. 10-20 mg) were lysed in ATL buffer with proteinase K (20 µl of QIAgen proteinase K stock solution and 180 µl of ATL buffer). The procedure was as described by the manufacturer. Purified DNA was eluted in 50 µl of elution buffer and stored at -20°C.

This method was also applied when manually isolating DNA from pELISA supernatant samples, except 80 µl of supernatant was incubated with proteinase K/ATL buffer mix as described.

DNA from all Icelandic Rs isolates (from the sample library at Keldur Institute) was isolated using the GeneJET method before being used in PCR amplification of the Rs 16S fragment. Briefly, growth on culture plates was collected with a sterile inoculation loop and resuspended in 800 µl of PBS. DNA isolation was as described by the GeneJET manual, except 35 µl achromopeptidase (Sigma-Aldrich) was added into the proteinase K/ATL lysis buffer. This was done because this enzyme is known to function well for lysis of Rs (Magnússon *et al*., 1994).

QIAcube

The second DNA isolation method used in this study was with an automated QIAcube HT robot from QIAGEN and QIAamp 96 DNA kit from (#51331) as described by manufacturer.

This isolation method was applied to both lysed kidney tissue samples (180 µl ATL + 20 µl proteinase K) and the filtered water samples (pellet). However, ATL + proteinase K was not added to the pellets straight after filtration. Instead the pellets were stored at -20°C and proteinase K was added 24h at RT before isolated via the QIAcube.

The QIAcube DNA isolation was used for all the Icelandic isolates (Keldur Institute's sample bank) before assayed in qPCR.

3.3.5 DNA quantity and purity assessment

The quantity and quality of isolated DNA was analyzed using NanoDrop®ND-1000 spectrophotometer (NanoDrop technologies, Inc.) following the manufacturer's user manual. The analysis gives a ratio of absorbances. Ratio at 260 and 280 nm (260nm/280nm) gives an indication of how pure the sample is from contaminating proteins and the 260nm/230nm ratio reflects how pure the sample is from salts and other contaminants. A 260nm/280nm ratio of 1.8 is was accepted as "pure" for DNA.

3.3.6 Diagnostic 16S sequencing and qPCR

16S sequencing

The 16s region of Rs was amplified and isolated for sequence analysis. A PCR reaction was performed using the primers in Table 7 and the PCR conditions outlined in Tables 5 and 6. The 16s amplicons were isolated after agarose electrophoresis (Appendix II) and purified using the GeneJET Gel Extraction kit from Thermo, as described by manufacturer. The amplicons were shipped for Sanger sequencing at the company GeneWiz®, Leipzig (Germany). The sequencing primers used were the same primers as used for the PCR amplification (Table 7).

Table 5. The PCR reaction mix for 16s amplification

The PCR master mix was from New England, Biolabs. Note: DNA volume per reaction was increased for samples M3297 and F-197-95 because of low DNA quantity (Table 13)**.**

PCR Reagents	Final Conc.	Vol. per Reaction
Taq 2X Master Mix	1x	12.5 µL
10 µM Forward primer	0.2 µM	0.5 µL
10 µM Reverse primer	0.2 µM	0.5 µL
Water	-	8-10 µL
DNA	<1000 ng	1-3 µL
Total		**25 µL**

Table 6. Thermocycling conditions for the routine 16S PCR amplification

The thermal cycling was done with a 2720 Thermal Cycler from Applied Biosystems.

Step	Temp	Time
Initial Denaturation	95°C	30 seconds
	95°C	15-30 seconds
30 cycles	45-68°C	15-60 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

Table 7. Information of primers and probes used for 16S PCR in this study

16S PCR	Name	Sequence
Forward primer	8F	5'-AGAGTTTGATCCTGGCTCAG-3'
Reverse primer	1544R	5'-AGAAAGGAGGTGATCCAGCC-3'
Hydrolysis probe	805R	5'-GGATTAGATACCCTGGTAGTC-3'

qPCR for quantification of Rs *msa* DNA

DNA isolated from kidney and water samples was subject to qPCR. The primers and probe used are shown in Table 10. The PCR conditions are shown in Table 8 and 9.

In the bulk reaction, Exo IPC has been replaced with Elf1- α. Bland *et al*. (2012) describes Elf1- α as elongation factor 1- α which is gene specific, highly expressed in all fish tissues in many different fish species e.g. Atlantic salmon, rainbow trout, brown trout, cod, Haddock (*Melanogrammus aeglefinus*) and carp (family *Cyprinidae*). The study developed Elf1- α specific assays to be used alongside diagnostic screening assays. The elongation factor targets a number of significant pathogens in a variety of significant host species and cell lines found within Europe and confirms appropriate nucleic acid quantity, quality and amplifying ability within any individual sample as well as providing early warning system of potential contamination during processing. The study then claims that control such as Elf1- α provide further credence in the results gathered from molecular laboratories.

Table 8. Reagent mix for the qPCR reaction

PCR Reagents	Final Conc.	Vol. per Reaction
GenEX Master Mix	1X	12
Forward primer	0.9 µM	1.125 µL
Reverse primer	0.9 µM	1.125 µL
Taq Man probe	0.25 µM	0.3 µL
EF1-α forward primer	0.3 µM	0.375 µL
EF1-α reverse primer	0.3 µM	0.375 µL
EF1-α probe	0.075 µM	0.094 µL
Water	-	4.606 µL
DNA	10-100 ng	5 µL
Total		**25 µL**

Table 9. Thermocycling conditions for qPCR

Thermal cycling was performed on a StepOnePlus Real-time PCR system from Applied Biosystem. The cut off was at Ct 45 and samples had to include a sigma curve to be considered positive.

Step	Temp	Time
Holding stage	50°C	2 minutes
	95°C	10 minutes
45 cycles	95°C	15 seconds
	60°C	45 seconds

Table 10. Information of primers and probes used for qPCR in this study

qPCR	Name	Sequence
Forward primer	RS 1238 F	5'-GTGACCAACACCCAGATATCCA-3'
Reverse primer	RS 1307 R	5'-TCGCCAGACCACCATTTACC-3'
Hydrolysis probe	RS 1262 MGB	FAM-5'-CACCAGATGGAGCAAC-3'-NFQ-MGB

3.3.7 Comparison of 16S sequences

All 16S sequencing results were analyzed in the program Sequencer 5.4.1. and were blasted on NCBI (National Center for Biotechnology Information) to make sure the right sequence was obtained.

The Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to align the 16s sequence from these Rs isolates (F-11-12, F-130-87 and F-358-87) to 16 sequences from the NCBI database using the default settings for DNA sequence alignments in the software. The sequences used from the NCBI database were: AY764166.1 (Canada), AY764165.1 (Canada), AY764163.1 (UK), AY764162.1 (Sweden), AY764161.1 (ISLF358/87), AY764160.1 (Sweden), AY764159.1 (Sweden), MT023376.1 (Russia), MN121120.1 (Kamchatka), AY764164.1 (USA), NR_074198.1 (Seattle), NR_041773.1 (Oregon) and AF180950.1 (Oregon).

3.3.8 Correlation analyses of qPCR and pELISA data

To compare the qPCR data for different sample types, i.e. DNA isolated directly from tissue and DNA isolated from pELISA supernatants (Section 4.5.) the cycle threshold (CT) values from the qPCR analysis, based on a standard curve from each run, were converted to linear values with the ΔCt method. Converted values were then used for correlation coefficient (Pearson r) analyses to measure how strong a relationship there was between qPCR results obtained by the two-purification method/sample types. The analyses were performed in Graphpad Prism v8.3.0.

3.3.9 OD- and CT conversion and classification

In pELISA analysis, sample results are given as OD (optical density) values. The OD value of a sample is compared to a standard curve, which typically is a serial dilution of a known-concentration solution of the target molecule. In qPCR assay, the results are shown in CT units which stands for “Cycle threshold”. This represents the number of circles in a qPCR test it takes to get a positive fluor signal. All results from the pELISA diagnostic method have been converted into multiplication value (MV), as described by Kristmundsson *et al*. (2016). The cut off value for determination of positive individual samples was 2.3 times the mean OD value of three negative controls for individual samples, but 1.8 times the mean OD of three negative controls for pooled samples, because individual low positive salmonids are more likely to get diminished in pooled samples. MV values and CT units were then classified into four categories (Table 11.) created for this study, (1) Negative, (2) Positive+, (3) Positive++ and (4) Positive +++.

Table 11. Classification of pELISA and qPCR results

The classification of pELISA and qPCR results as Negative, Positive+, Positive++ and Positive +++, based on MV and CT values for pELISA and qPCR, respectively.

Categories	pELISA (MV)	qPCR (CT)
Rs negative	<2.3	UD
Rs positive+	2.3 - 4.5	>30
Rs positive++	4.5 - 6.7	25 - 30
Rs positive+++	>6.7	<25

4 Results

4.1 Macroscopic observation during dissection

4.1.1 Farmed salmonids

Clinical signs, typical for BKD, were observed in six of 32 salmon juveniles from Farm A. These were characterized by swollen kidney, which furthermore had focal or diffuse white nodules (Figure 3). No macroscopic abnormalities were observed in the 37 Arctic charr and 30 salmon from Farms B and C (Appendix I).

Figure 3. Macroscopic signs, characteristics for BKD, observed in Atlantic salmon juvenile from Farm A.
The kidney is swollen and numerous white nodules are present.

4.1.2 Wild salmonids

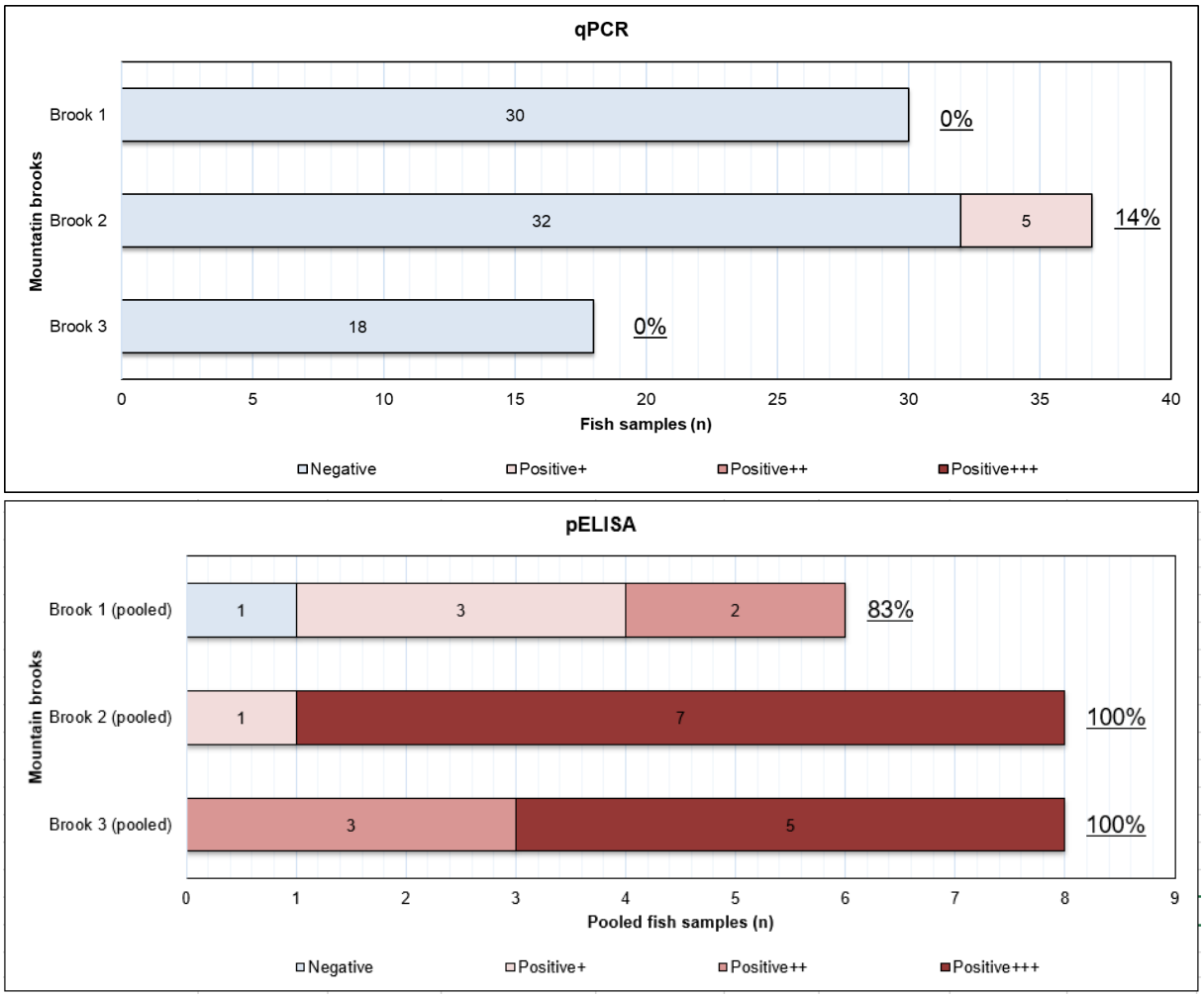
No clinical signs, suggesting BKD, were observed in any of the 142 wild fish, caught in four freshwater lakes, i.e. Lake Elliðavatn (n = 64), Lake Mjóavatn (n = 30), Lake Mývatn (n = 41) and Lake Steinsmýrarvötn (n = 7). Similarly, all 43 fish caught in Tálknafjörður (n = 20) and Patreksfjörður (n = 23), as well as the 85 fish from Brooks 1-3, appeared normal during dissection (Appendix I).

4.2 Rs in salmonid- and water samples from brooks and fish farms

4.2.1 qPCR and pELISA results from salmonids caught in brooks

Both Arctic charr and brown trout were caught in Brooks 1-3 located in Tálknafjörður (Appendix I), which the farms used as source water, to some extent. Results of Rs-screening in pELISA showed that Rs-antigens were highly common in the salmonids inhabiting the brooks as 21 of 22 samples examined (each sample a pool of 5-7 fish) tested positive. Total of 55% (12/22 samples) of the samples yielded high positive values (Pos+++), 23% (5/22 samples) were moderately positive (Pos++), 18% (4/22 samples) were low positive (Pos+) and one sample was negative in pELISA.

Six% (5/85 salmonids) of the salmonids tested low positive in qPCR while the remaining 80 fish gave negative results. All the positive fish originated from Brook 2, which accounts for 14% of samples in this brook. An overview of these results are shown in Figure 4.



Samples for pELISA: Brook 1 (n=30): Each sample is a pool of five fish; Brook 2 (n=37): Three individual samples and five samples are a pool of 5-7 fish; Brook 3 (n=18): Three individual samples while five were a pool of 5 fish.

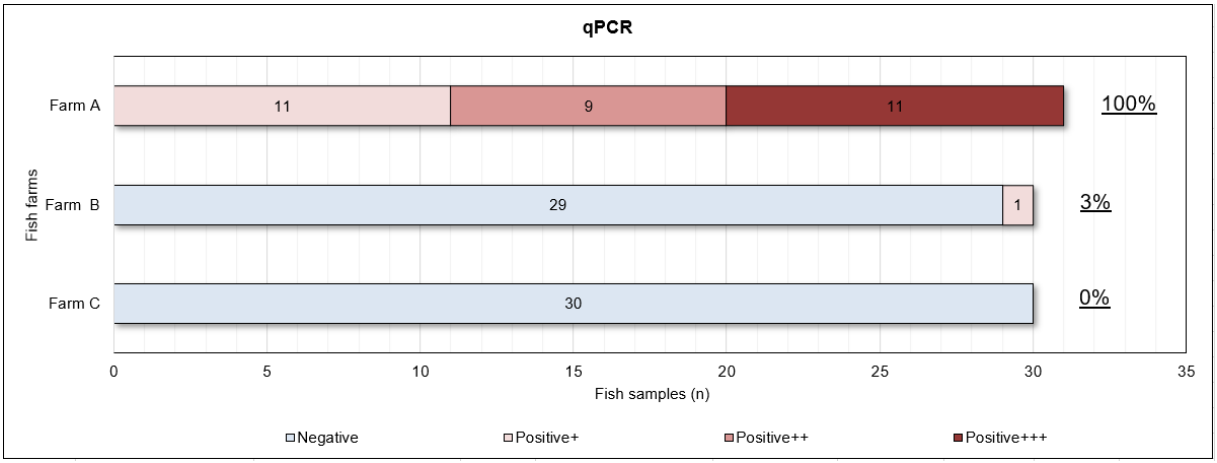
Figure 4. qPCR and pELISA results from salmonids caught in brooks in fjord Tálknafjörður

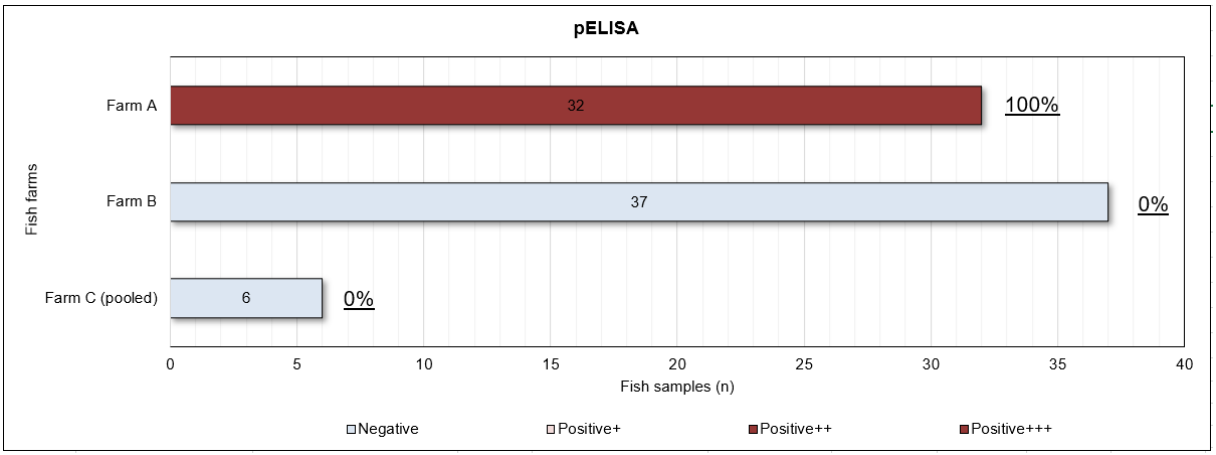
The bar graphs in the figure categorizes qPCR and pELISA results from fish caught in three brooks in Tálknafjörður according to color code as described in Table 11. Percentage (%) at the end of each bar shows the prevalence of Rs positive samples from each brook.

4.2.2 qPCR and pELISA results from salmonids collected from fish farms

The result from screening of Atlantic salmon juveniles cultured at Farm A (Appendix I), revealed that all 32 fish were positive in both pELISA and qPCR. All the salmon tested highly positive in pELISA while 35.5% (11/31 fish) tested highly positive, 29% (9/31) moderately positive and 35.5% (11/31) low positive in qPCR.

All salmonids from Farm B (Arctic charr) and C (Atlantic salmon) (Appendix I) tested negative in both qPCR and pELISA, with an exception of one Arctic charr in Farm B that tested low positive in qPCR. An overview of these results are shown in Figure 5.





Samples for pELISA from Farm C (n=30) were a pool of five fish. From Farms A (n=32) and B (n=37), all samples were from one individual.

Figure 5. qPCR and pELISA results from salmonids collected in fish farms located in fjord Tálknafjörður

The bar graphs in the figure categorizes qPCR and pELISA results from fish collected in three Farms (A, B and C) in Tálknafjörður according to color code described in Table 11. Percentage (%) at the end of each bar shows the prevalence of Rs positive samples from each farm.

4.2.3 qPCR and pELISA results from water collected from brooks and fish farms

Water samples collected from Brooks 1-5, which were either directly or indirectly (interconnected brooks) used as intake water for the farms, all tested highly positive in pELISA but negative in qPCR. Water samples from inside the farms, i.e. after UV treatment, were similarly all positive in pELISA but negative in qPCR. All drainage water tested positive in pELISA. However, these water samples were negative in qPCR, apart from the one from Farm A, which showed low positive reaction in qPCR. An overview of the results are shown in Table 12.

Table 12. qPCR and pELISA results from water collected from brooks and Farms

Results from pELISA and qPCR from intake water, water supply inside the farms (after UV treatment) and drainage water, from the farms studied.

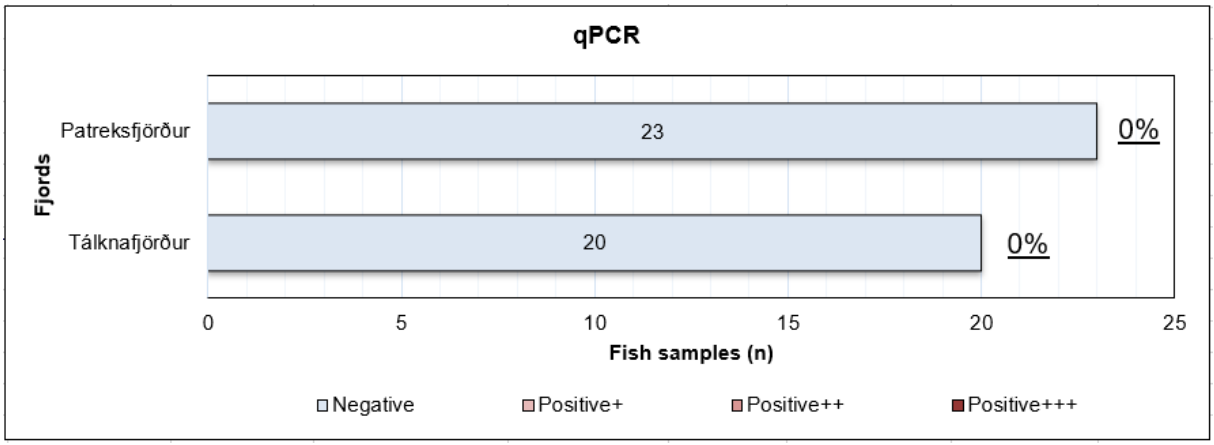
Source	qPCR	pELISA
Brook 4	Neg	Pos+++
Brook 2 (Intake water for Farm B)	Neg	Pos+++
Brook 3 (Intake water for Farm B)	Neg	Pos+++
Farm A (Intake water / Brook 5)	Neg	Pos+++
Farm A (After UV treatment)	Neg	Pos+++
Farm A (Drainage water)	Pos+	Pos+++
Farm C (Intake water / Brook 1)	Neg	Pos+++
Farm C (After UV treatment)	Neg	Pos+++
Farm C (Drainage water)	Neg	Pos+++

Brook 1-5 are all mountain brooks located in fjord Tálknafjörður, some of which were used as water source for Farms A, B and C. "*Intake water*" is water collected from brooks that the farms used as water source (before it reached the tanks). "*After UV treatment*" is water collected after it had been exposed to UV light inside the farms. "*Drainage Water*" is used water the fish farms were exposing into their surroundings.

4.3 Rs in wild salmonids

4.3.1 In vicinity of sea cages - qPCR and pELISA results

Both sea trout and sea charr were caught in Patreksfjörður and Tálknafjörður (Appendix I). In these fjords sea cage farming is ongoing and was during the study. Furthermore, active BKD episodes were in some of sea cages in both fjords. Rs-screening of the migrating salmonids revealed that all of the 43 fish from both fjords, tested negative in qPCR, while one fish tested moderately positive in pELISA (2.3%). An overview of results are shown in Figure 6.



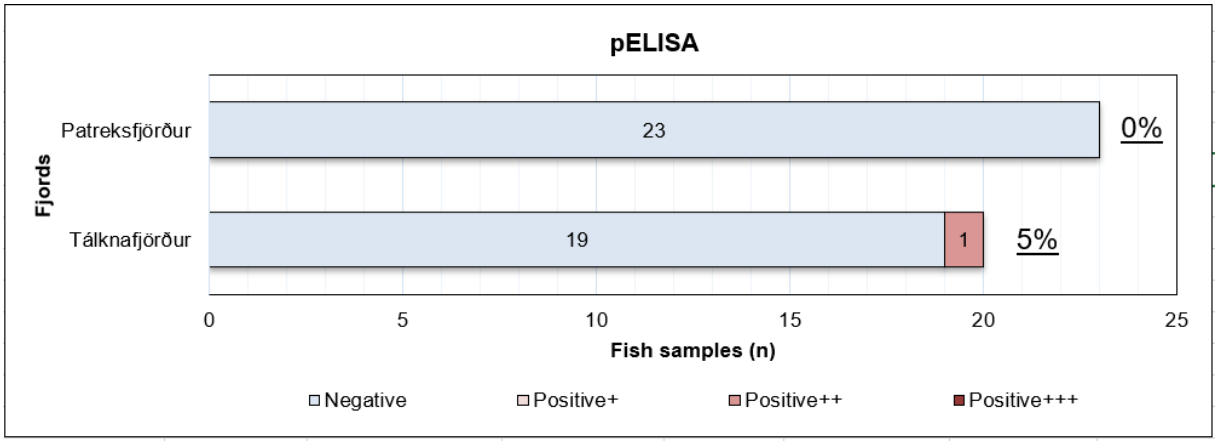
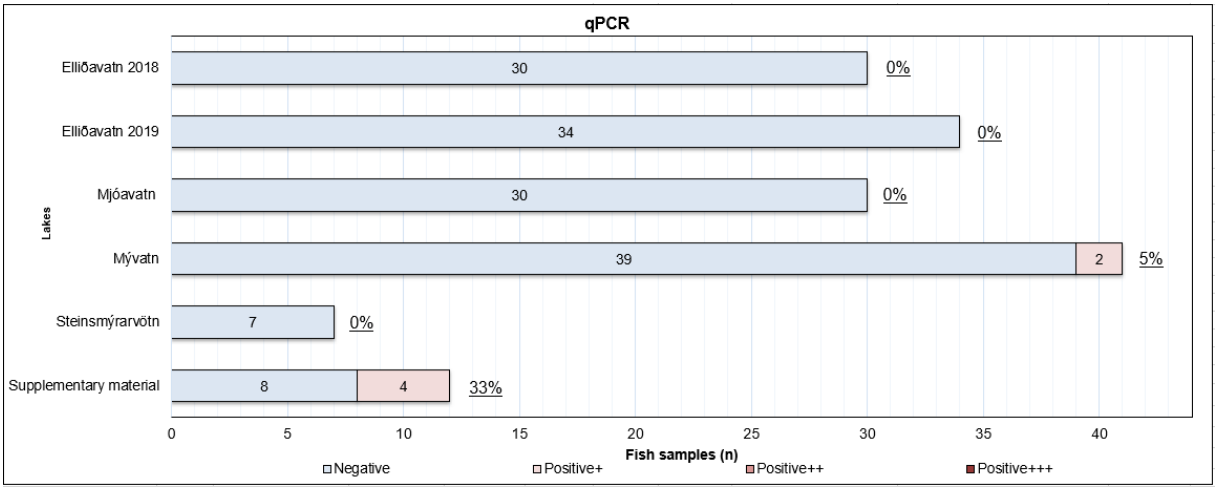


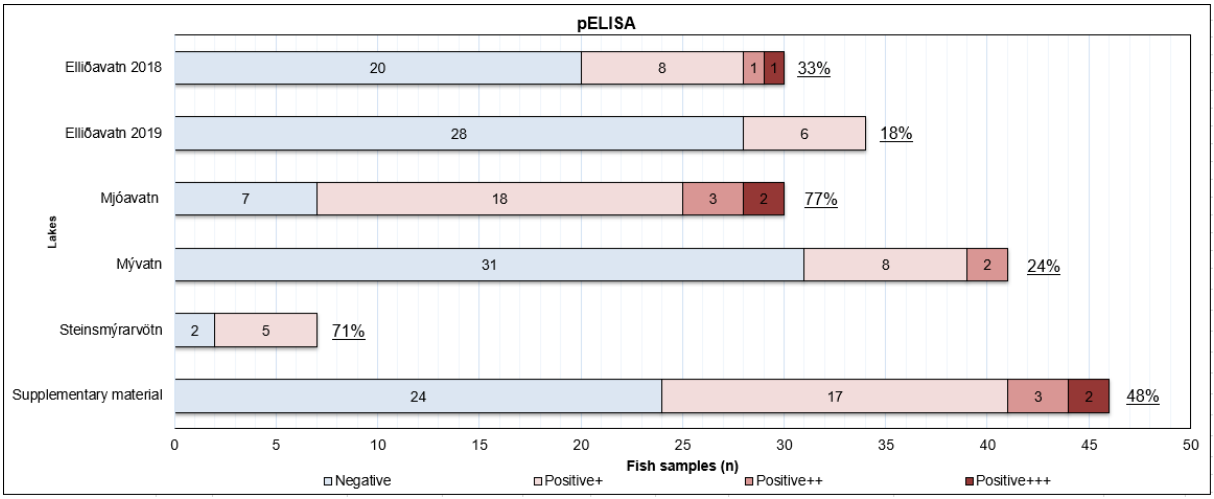
Figure 6. **qPCR and pELISA results from salmonids caught in Patreks- and Tálknafjörður fjord**
The bar graphs in the figure categorizes qPCR and pELISA results from wild salmonids caught in the two fjords studied. Results were categorized according to color code described in Table 11. Percentage (%) at the end of each bar shows the prevalence of Rs positive samples in fish from each fjord.

4.3.2 Distant from fish farming activities - qPCR and pELISA results

In order to determine the natural Rs-status of wild salmonids from areas not affected by fish farming, a total of 142 salmonids from four different lakes in Iceland, both resident salmonids (Lake Elliðavatn, Lake Mjóavatn and Lake Mývatn) and anadromous ones (Lake Steinsmýrarvötn), were screened for Rs-infections. In addition, to further demonstrate the natural Rs-status of wild salmonids, pELISA data available at the Fish disease Laboratory at Keldur Institute (supplementary material) from sea trout caught in the years 2005-2016 (total of 46 samples), from eight different rivers (Figure 2), were used in this study. Twelve of 46 samples were still available at Keldur Institute for qPCR analysis (Appendix I).

Of 154 wild salmonids, caught in the four lakes and 12 samples from supplementary material (not exposed to fish farming activities) showed that 4% (6/154 fish) tested low positive in qPCR. The prevalence of Rs-positive samples was much higher in pELISA, where 3% (5/188) of fish tested as high positive, 5% (9/188) moderately positive, 33% (62/188) low positive and 59% (112/188) as negative. Detailed results from each sampling site are shown in Figure 7.





Supplementary material: Older existing pELISA results, from routine screening at the Fish disease Laboratory. qPCR was performed on 12 samples (supernatant-processed for ELISA) which were still available, while 34 of these samples had been discarded.

Figure 7. **qPCR and pELISA results from salmonids caught in various lakes around Iceland**

The bar graphs in the figure categorizes qPCR and pELISA results from salmonids caught in various lakes and rivers around Iceland, far from any fish farming activities. Results were categorized according to color code described in Table 11. Percentage (%) at the end of each bar shows the prevalence of Rs positive samples in fish from each study site.

4.4 Cultivation and genetic variation of Icelandic Rs isolates

4.4.1 Rs cultivation and results from pELISA and qPCR

Salmonids caught in Icelandic lakes

No colonies, with the characteristics of Rs, grew on the SKDM plates after inoculation for 19 weeks.

Isolates and kidney tissue from Keldur Institute's sample bank

Isolate F-384-87 was the only isolate of nine, from -80°C storage, unable to form colonies on SKDM. Additionally, only two (M3297 and F-107-05) of 9 kidney tissue samples, from -20°C storage, formed colonies.

Samples from bacterial growth from all agar plates, where Rs-like colonies formed, were assayed by pELISA, qPCR and 16s PCR. All eight isolates from -80°C tested highly positive in pELISA and qPCR but the two bacterial isolates from kidney tissue samples, from -20°C storage, tested negative in both assays and therefore were established to be different bacterial species.

4.4.2 DNA quantity and quality

Nanodrop result revealed all eight -80°C isolates to have more concentration of DNA (33,9 ng/µL – 135,4 ng/µL) than the two non-Rs kidney tissue samples from -20°C (7,8 ng/µL – 18 ng/µL). Overview of the results can be seen in Table 13.

Table 13: Quantity and quality of Rs DNA from all cultured isolates and kidney tissues from Keldur Institute's sample bank

Concentration of DNA from nanodrop on eight isolates (-80°C) and two kidney tissue samples (-20°C) from Keldur Institute. Ratio at 260 and 280 nm (260nm/280nm) gives an indication of how pure the sample is from contaminating proteins and the 260nm/230nm ratio reflects how pure the sample is from salts and other contaminants.

Name	Concentration of DNA (ng/ul)	260/280	260/230
F-130-87	42.8	1.62	0.96
F-107-05 (-20°C)	18	1.65	1.33
F-11-12	33.9	1.39	0.57
F-358-87	41.1	1.62	0.86
2-S-2	135.4	1.43	0.65
M3297 (20°C)	7.8	1.58	0.89
F-138-87	86.4	1.58	0.88
F-273-87	111.6	1.52	0.85
S-182-90	124.1	1.51	0.76
F-283-87	130.7	1.41	0.65

4.4.3 16S PCR

A full sequence coverage of the 16S amplicon was only obtained from three of eight Rs isolates (F-11-12, F-130-87 and F-358-87) from -80°C. The 16s sequences from the three isolates were aligned and shown to be identical. Clustal-Omega alignment was performed with their consensus sequence (Figure 8) and sequences from 13 foreign Rs 16s sequences from around the world obtained from the NCBI data base (Appendix II).

TATAGTTGGGGGGGCTAACATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTAATACGTGAGTAAC
CTGCCCTTGACTTCGGGATAAGCCTGGGAAACTGGGTCTAATACTGGATACGACCTATCACCGCATGGTGTGTAGGTGGAAAGTTTTTTGCGG
TTTTGGATGGACTCGCGGCCTATCAGCTTGTTGGTGAGGTAATAGCTAACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCG
CGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAACAAGACATCATTTTTGTGGTGTTGAGGGTACTTGCAGAAGAAGCAC
CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCG
CGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGT
AGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGA
GCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGACATTCCACGTTCTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTA
ATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGGATTAGAAAAGTGCAGAAATGTACTCCCCCTTTTGGGTTGGTTCACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTTATGGTGG
GGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCT
ACAATGGCCGGTACAAAGGGTTGCGATACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCC
CATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAA
GTTGGTAACACCCGAAGCCGGTGGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGGACTGGCGATTGGGACTAAGTCGTAACAAGAGC
CCC

Figure 8. 16S nucleotide sequence isolated from three Icelandic isolates

The 16s nucleotide sequence amplified in strains F-11-12, F-130-87 and F-358-87 as determined by reverse transcriptase sequencing. The sequence (1470 bp) amplified from all three strains were identical to one another.

4.5 Testing of multiple organs

Six of 34 (18%) salmonids (Arctic charr and brown trout) caught in Lake Elliðavatn 2019 (Appendix I), tested low positive in pELISA while all other tested negative. Only one gill- and spleen sample (taken from two different salmonids) of 170 total organ samples tested positive in qPCR (1%). As only few of the salmonids tested positive in both diagnostic tests, we were unable to get a clear picture of how Rs was distributed in tissues.

4.6 Comparison of two different DNA isolation methods and sample types

In this part of the study, we compared qPCR results from samples from same individual fish that were processed differently (see Section 3.2.3. and 3.2.4.). The following comparison of qPCR results was done: (1) DNA extraction done manually (GeneJET) and in a robot (QIAcube). (2) DNA extracted from pELISA samples, i.e. supernatant and (ii) conventional processing for qPCR, i.e. extracting DNA directly from a kidney tissue. All CT values (logarithmic) were converted to linear numbers for better estimation of difference. Correlation between two variables was used to reveal the strength of linear relationship (correlation) in and p-value was calculated for each figure to help significate the results (Section 3.2.8.). An overview of results are shown in figures 9-12 where both isolation methods and sample types have been color coded. Kidney tissue is colored in red, pELISA supernatant in orange, GeneJET in blue and QIAcube in green.

4.6.1 Comparison of GeneJET and QIAcube methods

4.6.1.1 Kidney tissue

There was a significant correlation between the results obtained with the isolation methods (Pearson r = 0.951, p<0.0001). An overview of the results is represented in Figure 9.

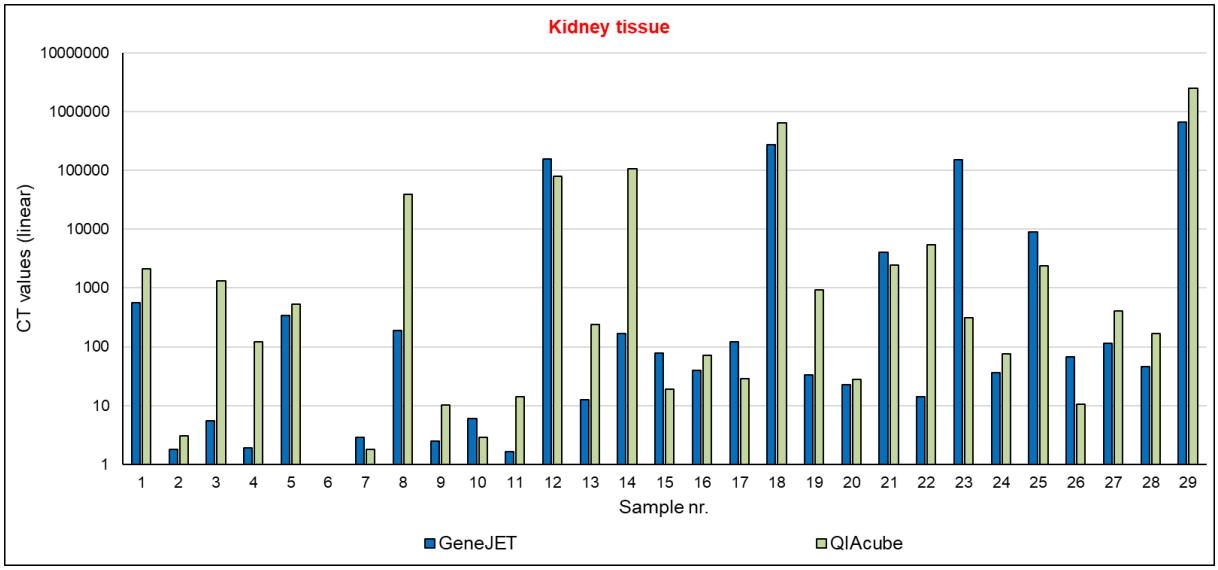


Figure 9. Comparison of GeneJET (manual) and QIAcube (automatic) isolation methods using kidney tissue

The columns in the figure show CT value results from qPCR assay. CT values have been converted into linear form. Therefore, the higher the CT value, the higher the Rs-infection. Sample numbers represent all salmons examined from Farm A (Appendix I), where kidney tissue from 29 salmon was isolated using two different isolation methods (GeneJET and QIAcube).

4.6.1.2 pELISA supernatant

There was a significant correlation between the results obtained with the isolation methods (Pearson r = 0.455, p=0.020) An overview of the results is shown in Figure 10.

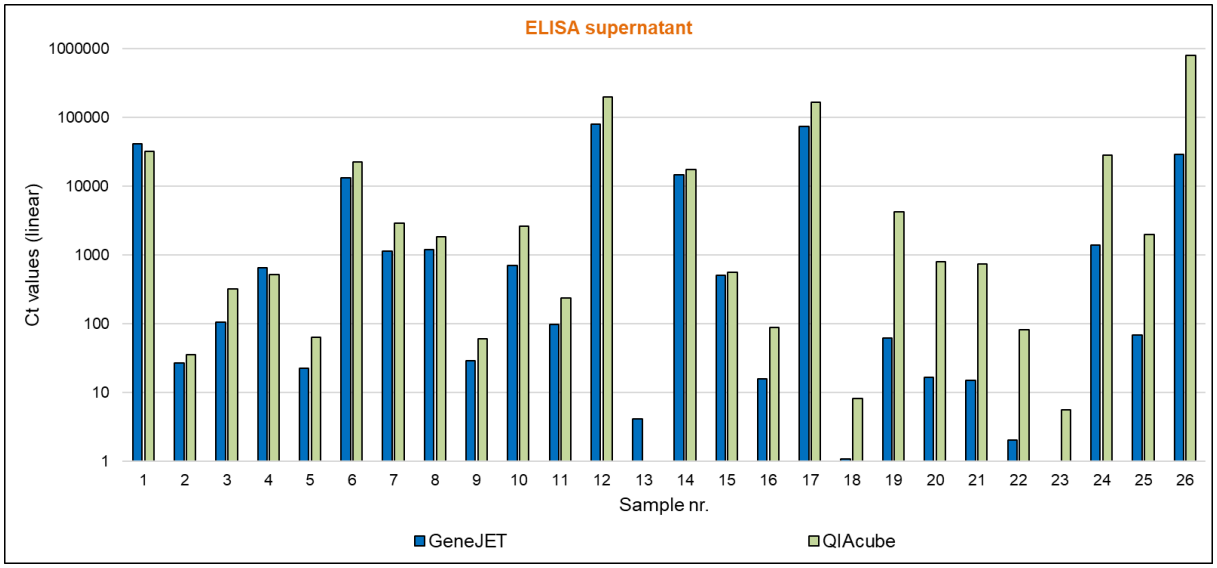


Figure 10. Comparison of GeneJET (manual) and QIAcube (automatic) isolation methods using supernatant

The columns in the figure show CT value results from qPCR assay. CT values have been converted into linear form. Therefore, the higher the CT value is, the higher the Rs-infection. Sample numbers represent all salmons examined from Farm A (Appendix I), where pELISA supernatant from 26 salmon was isolated using two different isolation methods (GeneJET and QIAcube).

4.6.2 Comparison of kidney tissue and supernatant samples

4.6.2.1 GeneJET

There was a significant correlation between the results obtained with the isolation methods (Pearson r = 0.500, p=0.011) An overview of the results is shown in Figure 11.

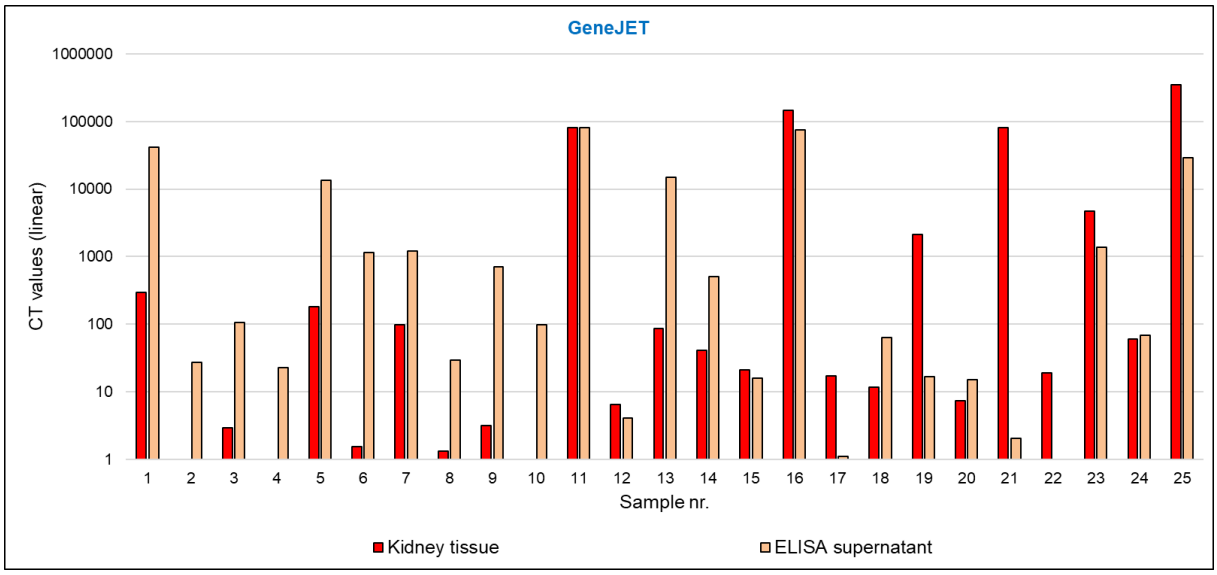


Figure 11. Comparison of kidney tissue and supernatant isolated using the GeneJET method

The columns in the figure show CT value results from qPCR assay. CT values have been converted into linear form. Therefore, the higher the CT value, the higher the Rs-infection. Sample numbers represent all salmons examined from Farm A (Appendix I), where DNA was isolated from 25 salmons using the GeneJET method in both kidney tissue and pELISA supernatant.

4.6.2.2 QIAcube

There was a significant correlation between the results obtained with the isolation methods (Pearson r = 0.975, p<0.0001) An overview of the results is shown in Figure 12.

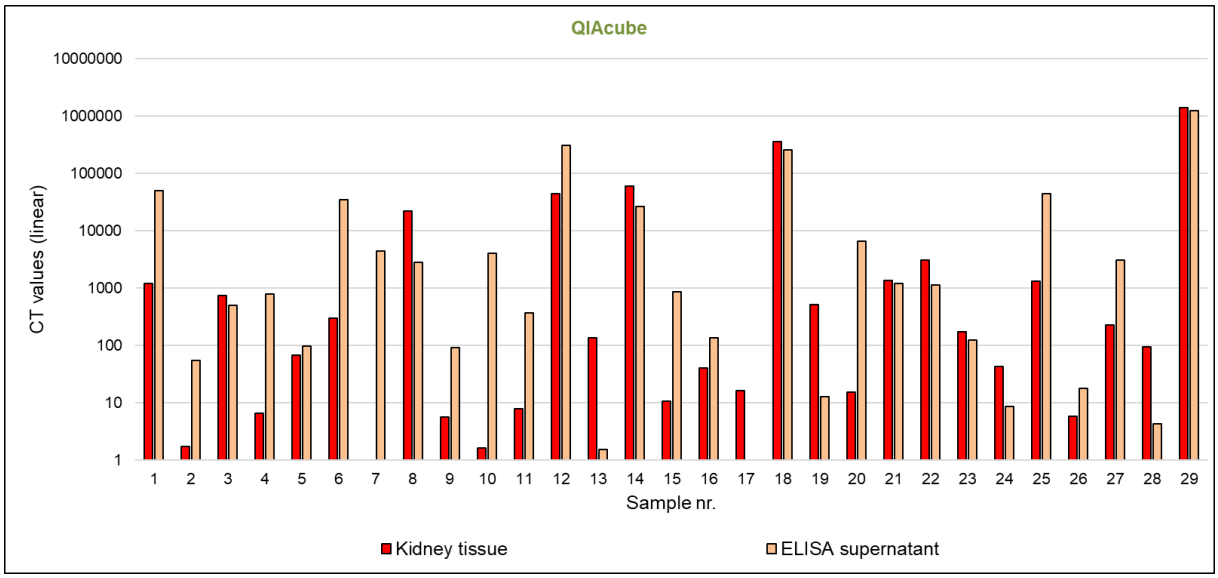


Figure 12. Comparison of kidney tissue and supernatant isolated using the QIAcube method

The columns in the figure show CT value results from qPCR assay. CT values have been converted into linear form. Therefore, the higher the CT value, the higher the Rs-infection. Sample numbers represent all salmons examined from Farm A (Appendix I), where DNA was isolated from 29 salmons using the QIAcube method in both kidney tissue and pELISA supernatant.

5 Discussion

5.1 Tracing the source of Rs in fish farms in the Westfjords of Iceland

5.1.1 Outside the farms; fish and water supply (brooks)

In this study, an attempt was made to determine the source of the Rs-infections experienced in three different land-based farms. The main emphasis was on the water supply of the farms; in particular, the mountain brooks which served, or still serve, as such to a varying degree. Prior to this study, information on whether salmonid fish inhabited these brooks, were wage or even non-existing. Both brown trout and Arctic charr were caught in these brooks, confirming the presence of wild fish in these brooks.

Results showed that 95% of the samples (21 of 22 pooled samples) from the fish caught in three brooks tested positive in pELISA but merely 6% (5/85 salmonids) in qPCR. Discrepancies in results between pELISA and qPCR, such as these are common when dealing with wild fish, where there is a natural balance between the bacteria and wild salmonids (Árnason *et al*., 2013; Kristmundsson *et al.*, 2016; Guðmundsdóttir *et al*., 2017a). Presumable reasons for such dissimilar results in pELISA and qPCR are many: (1) the antigen/bacteria ratio is very high because the bacterium secretes the antigen in such high quantities into its surroundings (salmonid organs, rivers, lakes or ocean) and therefore its far more likely to detect the antigen compared to DNA (Nance *et al.,* 2010), (2) in subclinical infections the bacteria, which is probably in low numbers, can be unevenly distributed in tissues and/or inhabit many different organs other than the kidneys, as shown by Guðmundsdóttir *et al*. (2017a), (3) the fish may have eradicated the bacterium and hence the PCR might be negative. Soluble antigens, however, can persist for months in kidney tissues in the absence of the bacterium (Guðmundsdóttir *et al*., 2020).

All water samples collected from brooks in Tálknafjörður tested highly positive in pELISA but negative in qPCR. The pELISA results reflect the Rs infected salmonids present in these brooks, as noted above. However, the number of bacteria in the water is most likely too low to detect in a sample size of 10 L, to get a positive qPCR. Despite discrepancies in the results from the two tests applied on the samples, it should be clear that Rs is present in salmonids in the brooks, as well as the brook water.

5.1.2 Inside the farms; fish, water supply (brooks) and drainage

All Atlantic salmon juveniles from Farm A tested positive in both pELISA and qPCR assays. That was not unexpected, since macroscopic signs of BKD, were quite commonly observed in these salmons, and therefore obvious that a BKD outbreak was still ongoing in the farm at the time of sampling. All salmon juveniles from Farm C tested negative in both pELISA and qPCR. All Arctic charr from Farm B tested negative in both qPCR and pELISA, with an exception of one Arctic charr in Farm B that was low positive in qPCR. No salmonids from Farm B (Arctic charr) and C (Atlantic salmon) (Appendix I) showed clinical signs of BKD when sampling took place.

At present, Arctic charr is the only salmonid species cultured at Farm B. However, in the past they have also cultured Atlantic salmon and rainbow trout. Since it became operational, it has not utilized UV treatment on the supplying water from nearby rivers. This farm has experienced multiple BKD outbreaks in the past, the last documented outbreak occurring in 2015, according to the records of the Fish disease Laboratory at Keldur Institute. Similarly, Farms A and C have been fighting BKD outbreaks lately (Data from the Fish disease Laboratory at Keldur Institute). Based on the results of the present study, the most likely source of infection in all these farms is the intake water from the brooks. Regarding Farm B, since the farm seems to be constantly supplied with Rs-contaminated water and does not utilize UV treatment, how come more fish are not testing positive? And why doesn't it have more frequent outbreaks on its hands? The reason the farm did not have BKD at the time the study was performed, might be attributed to their present culturing of Arctic charr, as mentioned before, is known to be less susceptible to Rs than Atlantic salmon (Kristmundsson *et al*., 2008; Elliott *et al.*, 2014). Farm B is a small family company, where the whole culture period is employed at the same site and hence there is no transport of live fish between different facilities within the farm. Hence, they are not obligated to send samples for Rs-screening. Considering this, BKD outbreaks in this farm may not always have been reported.

As mentioned above, all salmon from Farm C were negative in qPCR assays as were the six pooled pELISA samples. This was not unexpected as this fish farm is a newly built, hi-tech farm which presently uses UV light to disinfect their water supply while filtering around 95% of their borehole water with recirculating aquaculture system (RAS). However, at the time of BKD outbreak in this farm, they were still using the mountain brooks as a complimentary water resource and did not treat the water with UV.

The BKD outbreak at Farm A, which was still ongoing during the study, started before they began UV treating the water supply. At present this farm is free from Rs-infection, which suggests that the UV treatment is effective in preventing Rs from entering the aquaculture facility.

Until recently all three farms studied used untreated water supply, to varying extent from the mountain brooks studied. Water samples from all five brooks tested highly positive in pELISA but negative in qPCR. At present, and at the time the study was performed, both Farms A and C, treated the water with UV, while no such treatment is yet employed at Farm B (Gísli Jónsson, veterinary officer of Fish diseases, personal communication). UV treated water from Farms A and C were tested with both pELISA and qPCR. The results showed the presence of antigens in samples from both the farms while no Rs-DNA was detected in qPCR. These results agree with the results from water collected from the brooks prior to UV radiation. Therefore, as only antigens were detected after UV filtration but no DNA, one can safely state that the UV does not affect the detection of antigens. Drainage water from both Farm A and C also tested highly positive in pELISA and negative in qPCR, except for drainage water from Farm A, which tested low positive in qPCR, probably due to heavy bacterial load inside the farm as it was experiencing a BKD outbreak when the samples were collected. However, assuming UV filtering was done properly in Farm A, then the bacterium most likely was introduced into the aquaculture facility before UV treatment was employed.

From these results it can be concluded that, (1) the bacterium resides in wild salmonids in the brooks, that all the farms used, or still use, as source water, (2) the bacterium has entered the farms with untreated water from the brooks, causing the outbreak in Farm A and past outbreaks in Farms B and C.

5.2 Is the Rs status of wild salmonids affected by fish farming activities?

Migrating salmonids caught in fjords Patreksfjörður and Tálknafjörður, where BKD infected Atlantic salmon were reared in sea cages at the time of sampling, all tested negative in both qPCR and pELISA except for one fish in Tálknafjörður which was low positive in pELISA.

Results from wild salmonids caught in Icelandic lakes and rivers (from supplementary data), not exposed to fish farming activities, yielded 40% (76/188 salmonids) of all salmonids to test positive in pELISA but 4% (6/154 salmonids) positive in qPCR. Some variations were observed between the three different lakes, with the prevalence of positive samples in pELISA ranging from 18% in Lake Elliðavatn in 2019 to 77% in Lake Mjóavatn.

Results from these lakes and rivers are in full compliance with published papers on salmonids originating from lakes and rivers in Iceland, where percentage of Rs-positives in pELISA is high, but low in PCR assays (Árnason *et al*., 2013; Guðmundsdóttir *et al*., 2017a). The results on the overall Rs-prevalence from fish in vicinity of fish farming activities (1%) and those without any possible effect from fish farming activities (24%), suggest that sea cage rearing does not seem to affect the prevalence and intensity of Rs in nearby wild salmonids. However, these were only one-time-sampling, from each fjord, and hence further research is needed to fully elucidate the possible effect of culturing Rs infected salmonids in sea cages on nearby wild salmonids.

5.3 Do different strains of Rs exist in Iceland and do they vary genetically from Rs strains isolated in other countries?

Results from multiple alignment of the 16s sequence from three Icelandic Rs isolates (Appendix II) showed no sequence variability. The same result was obtained when compared to sequences in the NCBI databank from several locations around the world, except for one single-base substitution in the Canadian isolate AY764166 in bp 519. This result was not surprising as previous research has shown that the 16s sequence is highly conserved and possess only a limited sequence variation in Rs isolates from diverse sources (Grayson *et al.*, 2000). This proves Rs to be highly conserved genospecies with a remarkable degree of genetic uniformity among isolates (Grayson *et al.*, 2000). Most likely there is only a single copy of the 16s rDNA operon in Rs which is consistent with what has been described for several other slow growing organisms (Grayson *et al.*, 1999).

Because the 16s sequence seems to be highly conserved, a whole-genome, paired-end sequencing approach must be used in order to get a better understanding of molecular variations in globally distributed Rs strains, as well as to study potential differences within Iceland. A study from

2014 applied a whole-genome SNP-based comparison on 68 diverse Rs isolates from Norway, Canada, USA, Scotland, England and Wales. Phylogenetic analyses delineated the isolates into two lineages (lineage 1 and lineage 2) and estimated that they diverged 1239 years ago. The study claimed that it was possible that lineage 1 emerged independently within a geographically or ecologically isolated population of the Atlantic salmonid before being transferred into Pacific salmonid populations. It also suggested that the pathogen co-evolved with Atlantic salmonids (genera Salmo and Salvelinus) which might explain why Pacific species are reportedly more susceptible to the pathogen (Brynildsrud *et al*., 2014). The results of the study suggest that the intercontinental spread of lineage 1 over the last century was associated with the anthropogenic movement of live fish, feed and ova for aquaculture purposes and stocking. However, lineage 2 seems only to have been endemic in wild Eastern Atlantic salmonids before commercial activity. Their study describes two major European clusters, i.e. lineage 1 which came about 70 years ago as a result of transmission of fish from North America to the UK and Norway, which spread between fish farms culturing both rainbow trout and Atlantic salmon. (Brynildsrud *et al*., 2014). Although isolates were recovered from many different species (Atlantic salmon, grayling, brook trout, rainbow trout and other Oncorhynchus species) they are all almost identical genetically, indicating free interspecies transmission and broad virulence properties. The study then concludes by stating that previously observed differences in host susceptibility to BKD are likely to reflect host and/or environmental factors rather than variation within the pathogen itself (Brynildsrud *et al*., 2014).

Originally, we planned to perform whole-genome analysis on strains F-11-12, F-130-87 and F-358-87, as well as additional Icelandic isolates, to be able to compare isolates both within Iceland as well as with isolates from other regions of the world. However, these experiments were postponed due to the outbreak of Covid-19 and await more favorable conditions. Further research on Icelandic isolates is important in order to understand the origins of Rs in Iceland. There are many research questions that remain unanswered, e.g. do Icelandic strains differ genetically from each other and/or foreign strains? Was Rs originally brought here by anadromous Atlantic salmon? Or has it been co-evolving with the Arctic charr since it became endemic in Iceland before the arrival of anadromous Atlantic salmon? If so, then perhaps there is a specific Rs strain only found in Iceland. Finally, in light of Iceland's geological location, it would be interesting to examine whether Icelandic strains are more closely related to strains found in North America or Europe.

5.4 Sampling for Rs-screening from multiple organs

The aim of this work was to examine whether sampling of multiple organs in wild salmonids would increase the possibility of detecting the Rs-bacterium. Guðmundsdóttir *et al*. (2017a), performed a study on wild brown trout with no macroscopic signs of BKD in Lake Elliðavatn. Two different qPCR tests, one nPCR test and pELISA were used as diagnostic methods in that study. Kidney samples were collected for pELISA analysis. Various organ samples (kidney, spleen, gills, mid-gut and oesophagus) were collected for PCR analysis. All kidney samples tested positive for Rs antigens in pELISA while only six kidney samples (21%) tested positive in the three different PCRs. When, all

results from all five organs tested in the PCRs were combined, 26 of 28 fish tested positive (93%). These results showed how focal or non-uniform Rs can be distributed in tissues of infected fish and hence severely affect the overall results from fish for the detection of Rs (Elliott *et al*., 2013; Guðmundsdóttir *et al*., 2017a).

In the present study, 34 wild brown trout and Arctic charr (Appendix I) from Lake Elliðavatn (2019) were caught and 170 samples from kidney, spleen, gills, mid-gut and oesophagus were collected to be assayed in pELISA and qPCR. The results from this study were not consistent with those of Guðmundsdóttir *et al*. (2017a), as only six of 34 (18%) salmonids tested low positive in pELISA and two samples taken from gills and spleen (from two separate brown trout and Arctic charr), tested positive in qPCR.

Jónsdóttir *et al*. (1998), thoroughly studied the prevalence and level of Rs-antigens in 961 resident Arctic charr and brown trout in many different populations in Iceland, there amongst from Lake Elliðavatn. From the study, five of 31 Arctic charr (16%) and four of 32 (13%) brown trout caught in Lake Elliðavatn in 1993, tested positive for the Rs-antigen in pELISA. Total prevalence of positive fish being 14%. Kristmundsson *et al*. (2016), also performed a study to screen for the presence of Rs-antigen in wild salmonids from Lake Elliðavatn in 2008. Results revealed sixteen of 18 (89%) Arctic charr and 59 of 60 (98%) of brown trout to test positive in pELISA with a total prevalence of positive fish 96%.

The dissimilarity in results from these four studies can most likely be attributed to annual variation in the prevalence of Rs, as conventional pELISA test on kidney samples resulted in different prevalence figures from the same lake (Lake Elliðavatn), i.e. 14%, 98%, 100%, 33% and 18%, respectively for the years 1993 (Jónsdóttir *et al*., 1998), 2008 (Kristmundsson *et al*., 2016), 2009 (Guðmundsdóttir *et al*., 2017a), 2018 and 2019 (present study).

Annual variation in Rs prevalence has been reported before, e.g. by Meyers *et al.,* (2003), who studied the Rs-status of several species of salmonids for 12 consecutive years. They found that the prevalence of Rs-positive fish, in ELISA, varied greatly between years for most fish species, e.g. from 0-68% for Chum salmon. Furthermore, the total prevalence of Rs antigen was significantly different between some fish species. They concluded that such cyclical fluctuations of pathogen prevalence within animal populations are influenced by numerous undefined biological end environmental variables (Meyers *et al.*, 2003).

5.5 Comparison of PCR results using different sample processing and DNA isolation methods

An Rs-screening using pELISA has been employed at Keldur Institute for roughly 30 years. Consequently, a large number of supernatant samples are available in freezer storage (-20°C) dating years or decades back, when PCR assays were not available. This opens the possibility of re-analysis of the samples by qPCR to gather further information about Rs status in the past. At present, automated robots are often used to isolate DNA, to lessen the workload of scientists, before using the DNA for PCR assays. Therefore, couple of questions were raised: (1) Is it possible to extract DNA

from frozen pELISA supernatants? (2) is manual DNA extraction as accurate as the automated one? (3) Is there a significant difference between PCR results obtained by using different sample types?

Looking at the results shown in Figures 9-12, the first question can be answered with a simple “Yes”. A very high correlation which was statistically significant indicates that pELISA supernatant is just as good a sample type for qPCR as using kidney tissue for nucleic acid isolation. Other researchers have done this experiment before but not received the same results, perhaps the reason lies in better equipment and reagents that are used for DNA isolation nowadays. These results are useful as they show that it is possible to save time and materials when sampling a fish, since collection of one kidney sample, instead of two, would be sufficient. The answer to the second question (2) there is a significant correlation using either sample type in both manual and automated DNA isolation methods as shown in Figures 11 and 12. However, when the correlation coefficients are compared in both figures, it is clear that the QIAcube had less disparity in CT values from both sample types than the GeneJET method. This is most likely due to “operator error” in the manual isolation process. The answer to question three (3) is that by comparing CT values in both isolation methods using either sample type in Figures 9 and 10, it does not indicate that either sample type is a better choice than the other.

6 Conclusions

Bacterial kidney disease (BKD) was first diagnosed in Iceland in 1968. Since then, BKD has periodically caused massive problems in aquaculture, especially during the years 1985-1992, 2003-2007 and 2012-2019 (Guðmundsdóttir *et al*., 2000; Kristmundsson *et al*., 2008, Data from the Fish disease Laboratory at Keldur Institute). These BKD epidemics have caused immense damage to the Icelandic aquaculture industry as millions of fish were slaughtered. Rs is endemic in wild salmonid populations in Iceland (Jónsdóttir *et al*., 1998; Kristmundsson *et al*., 2016) but has only been reported to cause BKD in cultured salmonids, most likely due to stress caused by environmental conditions in aquaculture facilities.

The results of the present study revealed that salmonids- and water samples, from natural brooks in Tálknafjörður, carried heavy loads of Rs antigens. These water systems were utilized as water source, to some extent, by the fish farms studied. This suggests that BKD outbreaks, experienced in these farms, originated from infected wild fish inhabiting these brooks.

Farming of BKD infected Atlantic salmon in sea cages in the Westfjords raised the question of whether that might increase Rs infection load in nearby wild migrating salmonids. Comparing results of Rs-screening of salmonids caught in two fjords in the Westfjords where BKD infected cultured fish are present, and salmonids caught in Icelandic lakes and rivers located far from farming areas, suggest that farming activities do not affect the prevalence and intensity of Rs in nearby migrating wild salmonids. However, further research over a longer period of time is necessary for affirmation.

Kidney tissue is, and has for long, been the preferred organ sampled for Rs-screening. Guðmundsdóttir *et al*. (2017a), who studied brown trout from Lake Elliðavatn (August 2009) showed that the distribution of Rs can be non-uniform in tissue in infected salmonids. By testing multiple organs instead of only the kidney, the prevalence of PCR positive fish increased from 21% to 93%, as the bacterium was detected in other organs than kidney in many cases. Results from similar approach in the current study did not agree with those observed by Guðmundsdóttir *et al.* (2017a) where all fish were found Rs positive by conventional pELISA test (using kidney) compared to 18% and 33% in the present study. Additionally, levels of Rs-antigen in salmonids caught in Lake Elliðavatn have been reported in two other studies. Total prevalence was 14% in 1993 (Jónsdóttir *et al.,* 1998), 98% in 2008 (Kristmundsson *et al.,* 2016), 100% in 2009 (Guðmundsdóttir *et al*., 2017a), 33% in 2018 and 18% in 2019 (present study). Different prevalence reported from these studies is most likely due to annual variation from varying environmental factors. Similar findings have been reported in a study including eight nonanadromous trout species in Alaskan watersheds where the annual prevalence values obtained in 1988-2000 ranged from 18% to 100% (Meyers *et al.*, 2003).

Numerous pELISA frozen supernatant samples from routine Rs-screenings from 2005-2016, when diagnostic PCR was not routinely used, are stored at Keldur Institute. The question was raised whether these samples could be used for PCR, in order to gain further information about Rs status in the past. The use of ELISA processed samples (supernatant) for PCR proved to give similar results as kidney tissue processed for PCR, irrelevant of the isolation method used. Results from samples where DNA was extracted using either manual or automatic (in DNA extraction equipment), were not

statistically different. These results strongly suggest that pELISA supernatant is as good a sample type as kidney tissue for DNA isolation prior to testing for Rs in qPCR.

Sequencing of the 16s region of three Icelandic Rs isolates revealed no sequence variability, as was the case when their sequences were compared to 16s sequences from foreign isolates. This proves that Rs is a highly conserved genospecies where molecular variation in the sequence of the 16S rDNA spacer region from widely separated environments is extremely limited.

Since there are neither effective vaccines nor antibiotics that work against Rs, the most effective way to prevent the bacterium from entering land-based fish farms and subsequently in to sea cages via infected fish, and from there into wild salmonids in the vicinity of aquaculture companies, is the use of UV treated borehole water. Hopefully, this study will help aquaculture companies to avoid the use of untreated surface water in their farms. Benjamin Franklin has described the situation quite fittingly by stating:

“By failing to prepare you are preparing to fail”

7 References

Alcorn, S. W., Pascho R. J., Murray A. L. & Shearer, K. D. (2003). Effects or ration level on immune functions in Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 217, 529–545.

Alcorn, S. W., Murray A. L., Pascho R.J. & Varney J. (2005). A cohabitation challenge to compare the efficacies of vaccines for bacterial kidney disease (BKD) in Chinook salmon *Oncorhynchus tshawytscha*. Dis Aquat Org, 63, 151-60.

Annual report from Keldur. (2019). The Institute of Experimental Pathology of Fish Disease at Keldur at Keldur. University of Iceland.

Antonsson, T., & Guðbergsson, G. (2000). Silungur í Elliðavatni. Samantekt rannsókna 1987-1999. Veiðimálastofnun.

Antonsson, T., Guðbergsson, G., Jónsson, B., & Malmquist, H. J. (2007). Fiskur í stöðuvötnum á höfuðborgarsvæðinu. Málþing um vötn og vatnasvið á höfuðborgarsvæðinu - ástand og horfur, 71-76. Reykjavík.

Árnason, I. Ö., Sigurðardóttir, S., Kristmundsson, Á., Svansson V., Guðmundsdóttir, S. (2013) Evaluation of a semi-nested PCR for detection of *Renibacterium salmoninarum* in samples from kidney, gill and ovarian fluid of Atlantic salmon broodfish. Icel Agric Sci, 26, 49−67.

Balfry, S. K., Albright, L. J., Evelyn, T. P. T. (1996). Horizontal transfer of Renibacterium salmoninarum among farmed salmonids via the fecal-oral route. Diseases of Aquatic Organisms, 25(1), 63-9.

Barnabé, G. (1990). Aquaculture, Volume 2. England. Ellis Horwood Ltd.

Belding, D. L. & Merrill B. (1935). A preliminary report upon a hatchery disease of the Salmonidae. Transaction of the American Fisheries Society, 65, 76-84.

Benediktsdóttir, E., Helgason, S. & Guðmundsóttir, S. (1991) Incubation time for the cultivation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L., brood fish. Journal of Fish Diseases, 14, 97-102.

Björnsson, B. (2001). The trophic ecology of arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in Elliðavatn, a small lake in Southwest Iceland, 31(3), 199-207.

Bland, F., Mcintosh, R., Bain, N., Snow, M. (2012). Development and validation of a range of endogenous controls to support the implementation of practical TaqMan real-time PCR-based surveillance for fish diseases within aquaculture. Journal of Fish Diseases, 35(6), 447-454.

Bruno, D. W. (1986). Histopathology of bacterial kidney disease in laboratory infected rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., with reference to naturally infected fish. Journal of Fish Diseases, 9, 523–537.

Brynildsrud, O., Edward, J. F., Bohlin, J., Castillo-Ramirez, S., Colquhoun, D., McCarthy, U., Matejusova, I. M., Rhodes, L. D., Wiens, G. D. & Verner-Jeffreys, D. W. (2014). Microevolution of *Renibacterium salmoninarum*: evidence for intercontinental dissemination associated with fish movements. The ISME Journal, 8, 746-756.

Bullock, G. L., Griffin, B. R. & Stuckey, H. M. (1980). Detection of *Corynebacterium salmoninus* by direct fluorescent antibody test. Canadian Journal of Fisheries and Aquatic Sciences, 37, 719-721.

Bullock, G. L. & Herman, R. L. (1988). Bacterial kidney disease of salmonid fishes caused by *Renibacterium salmoninarum*. Fish Disease Leaflet No. 78. U. S. Fish and Wildlife Service, Washington, D.C. pp. 10.

Bullock, G.L., Stuckey, H. M. & D. Mulcahy. 1978. Corynebacterial kidney disease: egg transmission following iodophor disinfection. U.S. Fish Wildlife Service. Keamevsville. WV. Fish Health News, 7, 51-52.

Chase, D. M. & Pascho, R. J. (1998). Development of a nested polymerase chain reaction for amplification of a sequence of the p57 gene of *Renibacterium salmoninarum* that provides a highly sensitive method for detection of the bacterium in salmonid kidney. Diseases of Aquatic Organisms, 34, 223-229.

Chase, D. M., Elliott, D. G. & Pascho, R. J. (2006) Detection and quantification of *Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. J Vet Diagn Invest, 18, 375–380.

Earp, B. J., Ellis, C. H. & Ordal, E. J. (1953). Kidney disease in young salmon. Special Report Series No. 1. Washington Department of Fisheries.

Elliott, D. G., Applegate, L. J., Murray, A. L., Purcell, M. K. & Mckibben, C. L. (2013). Bench-top validation testing of selected immunological and molecular *Renibacterium salmoninarum* diagnostic assays by comparison with quantitative bacteriological culture. Journal of Fish Diseases, 9, 779-809.

Elliott, D. G., Wiens G.D., Hammell K.L. & Rhodes L.D. (2014). Vaccination against bacterial kidney disease. In: Gudding, R., Lillehaug, A. and Evensen, Ø. (eds) *Fish Vaccination.* Wiley Blackwell, Chichester/Oxford, UK, pp. 255–272.

Elliott, D. G. (2017). *Renibacterium salmoninarum*. In: Woo and Cipriano (Eds): Fish viruses and bacteria: Pathobiology and protection. pp. 286-297.

Faisal, M., Eissa, A. E. & Starliper, C. E. (2010) Recovery of *Renibacterium salmoninarum* from naturally infected salmonid stocks in Michigan using a modified culture protocol. *Journal of Advanced Research* 1, 95–102**.**

FAO (Food and Agriculture Organization). (2018). The State of World Fisheries and Aquaculture – Meeting the sustainable development goals. Rome.

Ferguson, H. W. (1989). Systemic Pathology of Fish. Iowa State University Press, Ames, IA, USA, pp. 64–89.

Flaño, E., López-Fierro, P., Razquin, B., Kaattari, S. L. & Villena, A. (1996). Histopathology of the renal and splenic haemopoietic tissues of coho salmon *Oncorhynchus kisutch* experimentally infected with *Renibacterium salmoninarum.* Diseases of Aquatic Organisms, 24, 107–115.

Folmar, L. C. & Dickhoff, W. W. (1980). The parrsmolt transformation (smoltification) and seawater adaptation in salmonids. Aquaculture, 21, 1-37.

Frantsi, C., Flewelling, T. C., & Tidswell, K. G. (1975). Investigations on corynebacterial kidney disease and Diplostomum sp. (eye fluke) at Margaree hatchery, 1972-1973. Fish. Mar. Serv., Res. Dev. Br., Dept. Environ. Can., Marit. Reg. Tech. Rep. Ser. No.

Fredriksen, Å., Endresen, C. & Wergeland, H. I. (1997). Immunosuppressive effect of a low molecular weight surface protein from *Renibacterium salmoninarum* on lymphocytes from Atlantic salmon (*Salmo salar* L.). Fish Shellfish Immunol, 7, 273–282.

Fryer, J. L. & Sanders, J. E. (1981). Bacterial Kidney disease of salmonid fish. Annual Review in Microbiology, 35, 273–298.

Fryer, J. L. & Lannan, C. N. (1993). The history and current status of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in Pacific salmon. Fisheries research, 17, 15-33.

Garðarsson, A. & Einarsson, Á. (editors). (1991). Náttúra Mývatns. Prentsmiðjan Oddi. Hið Íslenska Náttúrufræðifélag. Reykjavík.

Grayson, T. H., Cooper, L. F., Atienzar, F. A., Knowles, M. R. & Gilpin M. L. (1999). Molecular differentiation of *Renibacterium salmoninarum* isolates from worldwide locations. American society for Microbiology, 65(3), 961-968.

Grayson, T. H., Atienzar, F. A., Alexander, S. M., Cooper, L. F. & Gilpin, M. L. (2000). Molecular diversity of *Renibacterium salmoninarum* isolates Determined by Randomly Amplified Polymorphic DNA Analysis. American society for Microbiology, 66(1), 435-438.

Guðbergsson, G. & Antonsson, Þ. (1996). Fiskar í ám og vötnum: Landvernd.

Guðbergsson, G. & Antonsson, T. (1997). Bleikja á Auðkúluheiði. Náttúrufræðingurinn, 67(2), 105-124.

Guðbergsson, G. & Guðjónsson, S. (2005). Fækkun stórlaxa í íslenskum veiðiám. Freyr, 101(8), 18-21.

Guðjónsson, T. (1978). The Atlantic salmon in Iceland. J. Agric. Res. Iceland, 10, 11-39.

Guðmundsdóttir, B. K. (1998). Infections by atypical strains of the bacterium *Aeromonas salmonicida*. Búvísindi, 12, 61-72.

Guðmundsdóttir, S., Helgason, S. & Benediktdóttir, E. (1991) Comparison of the effectiveness of three different growth media for primary isolation of *Renibacterium salmoninarum* from Atlantic salmon, *Salmo salar* L., brood fish. Journal of Fish Diseases, 14, 89-96.

Guðmundsdóttir, S., Benediktsdóttir, E., Helgason, S. (1993). Detection of *Renibacterium salmoninarum* in salmonid kidney samples: A comparison of results using double-sandwich ELISA and isolation on selective medium. Journal of Fish Diseases, 16(3), 185-95.

Guðmundsdóttir, S., Helgason, S., Sigurjónsdottir, H., Matthíasdóttir, S., Jónsdóttir, H., Laxdal, B., et al. (2000). Measures applied to control *Renibacterium salmoninarum* infection in Atlantic salmon: a retrospective study of two sea ranches in Iceland. Aquaculture, 186(3-4), 193-203.

Guðmundsdóttir, S., Applegate, L. J., Árnason, Í. Ö., Kristmundsson, Á., Purcell, M. K. & Elliott, D. G. (2017a). Detecting *Renibacterium salmoninarum* in wild brown trout by use of multiple organ samples and diagnostic methods. Bull Eur Ass Fish Pathol, 37, 31-40.

Guðmundsdóttir, S., Kristmundsson, Á. & Árnason, Í. Ö. (2017b). Experimental challenges with *Renibacterium salmoninarum* in Arctic charr *Salvelinus alpinus*. Diseases of Aquatic Organisms, 124, 21-30.

Guðmundsdóttir, S., Vendramin, N., Cuenca, A., Sigurðardóttir, H., Kristmundsson, Á., Moesgaard, I., Olesen, T. & Niels, J. (2019). Outbreak of viral haemorrhagic septicaemia (VHS) in lumpfish (*Cyclopterus lumpus*) in Iceland caused by VHS virus genotype IV. Wiley, Journal of Fish Diseases, 42(1), 47-62.

Guðmundsdóttir, S., Kristmundsson, Á., Árnason, Í. Ö., Björnsdóttir, T., Sóley, Þ., S. R & Bragason, B. (2020). Persistence of soluble antigens of Renibacterium salmoninarum in kidney samples of Arctic charr, *Salvelinus alpinus*, after intraperitoneal injection. Bulletin of the European Association of Fish Pathologists, 40(1), 28-38.

Gunnarsson, V. I. (2008). Reynsla af sjókvíaeldi á Íslandi. Fjölrit No.136.

Gutenberger, S. K., Duimstra, J. R., Rohovec, J. S. & Fryer, J. L. (1997). Intracellular survival of *Renibacterium salmoninarum* in trout mononuclear phagocytes. Diseases of Aquatic Organisms, 28(2), 93-106.

Halldórsson, H. (1992). Laxaveislan mikla og þjóðin borgar brúsann Reykjavík: Fjölvaútgáfan.

Hannesson, E. (1977). Upphaf fiskeldis á Íslandi. Freyr no 9.

Hardie, L. Ellis, A. & Secombes, C. (1996). *In vitro* activation of rainbow trout macrophages stimulates inhibition of *Renibacterium salmoninarum* growth concomitant with augmented generation of respiratory burst products. Dis. Aquat. Org. 25, 175–183.

Harðardóttir, J., Elefsen, S. Ó., Jónsdóttir, J. F., Finnsdóttir, H. P., & Þorláksdóttir, S. B. (2002). Mælingar á dýpi, straumum, botngerð og gróðurþekju í Elliðavatni. Orkustofnun.

Helfman, S. G., Collette, B. B., Facey, D. E. & Bowen, B. W. (2009). The Diversity of Fishes (Second ed.). Chichester, UK: Wiley-Blackwell.

Helgason, S. (1985). Diseases of salmonids on fish farms in Iceland. Aquaculture - miljöproblem; Bergen, Norway: NORDFORSK Miljövårdsserien.

Hirvelä-Koski, V. (2004). *Renibacterium salmoninarum*: effect of hypochlorite treatment, and survival in water. Dis Aquat Organ, 59(1), 27-33.

Hirvelä-Koski, V., Pohjanvirta, T., Koski, P. & Sukura, A. (2006). Atypical growth of *Renibacterium salmoninarum* in subclinical infections. Journal of Fish Diseases, 29, 21-29.

Hjeltnes, B., Bang-Jensen, B., Bornø, G., Haukaas, A. & Walde, C. S. (Eds). (2019). The Health Situation in Norwegian Aquaculture 2018. Norwegian Veterinary Institute 2019.

Iwama, G. K. (1991). Interactions between aquaculture and the environment. Critical Reviews in Environmental Controls, 21(2), 177-216.

Jansson, E., Høngslo, T., Höglund, J. & Ljungberg, O. (1996). Comparative evaluation of bacterial culture and two ELISA techniques for the detection of *Renibacterium salmoninarum* antigens in salmonid kidney tissues. Diseases of Aquatic Organisms, 27, 197-206.

Jansson, E., Lindberg, L., Saker, E. & Aspan, A. (2008) Diagnosis of bacterial kidney disease by detection of *Renibacterium salmoninarum* by real-time PCR. J Fish Dis, 31, 755–763.

Jobling, M., Arnesen, A. M., Benfey T., Carter, C., Hardy, R., Le Francois, N. R., O'Keefe, R., Koskela, J. & Lamarre, S. G. (2010). The Salmonids (Family: Salmonidae). In Le Francois, N. R., M. Jobling, C. Carter & P. Blier (eds), Finfish Aquaculture Diversification. CABI, Wallingford: 234–289.

Jóhannsson, M. (1993). Rannsóknir á vötnum í V-Skaftafellssýslu árið 1992. Veiðimálastofnun.

Jónasson, J. (1991). Kynbætur laxfiska. Ráðunautafundur, 1, 162-171.

Jónsdóttir, H., Malmquist, H. J., Snorrason. S. S., Guðbergsson, G. & Gudmundsdóttir, S. (1998) Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland. Journal of Fish Biology, 53, 322–339.

Jónsson, G. (1983). Íslenskir fiskar. Reykjavík: Fjölvaútgáfan.

Jónsson, G. & Pálsson, J. (2013). Íslenskir fiskar (2 ed.). Reykjavík: Mál og menning.

Jónsson, G. (2020). Ársskýrsla dýralæknis fisksjúkdóma 2019. Matvælastofnun.

Kristmundsson, Á., Helgason, S., Guðmundsdóttir, S. (2008). Epidemiology of *Renibacterium salmoninarum* in farmed salmonids in Iceland. The 60th anniversary of the Institute for Experimental Pathology, University of Iceland, Keldur International Conference of Fish Diseases and Fish Immunology; Reykjavík, Iceland: Book of abstracts.

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

Kristmundsson, Á., Árnason, F., Guðmundsdóttir, S., Antonsson, T. (2016). Levels of *Renibacterium salmoninarum* antigens in resident and anadromous salmonids in the River Elliðaár system in Iceland. J Fish Dis, 39, 681−692.

Landssamband fiskeldisstöðva. (2009). Staða fiskeldis á Íslandi, framtíðaráform og stefnumótun Landssambands fiskeldisstöðva í rannsókna-og þróunarstarfi 2010-2013. Landssamband fiskeldisstöðva.

Magnússon, H. B., Friðjónsson, O. H., Andrésson, O. S., Benediktsdóttir, E., Guðmundsdóttir, S. & Andrésdóttir, V. (1994). *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmonid fish, detected by nested reverse transcription-PCR of 16S rRNA sequences. Applied and Environmental Microbiology, 60(12), 4580-4583.

Malmquist, H. J., Antonsson, Þ. Ingvason, H. R., Ingimarsson, F. & Árnason, F. (2009). Salmonid fish and warming of shallow Lake Elliðavatn in Southwest Iceland. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 30(7), 1127-1132.

Maule, A. G., Tripp, R. A., Kaattari, S. L. & Schreck, C. B. (1989). Stress alters immune function and disease resistance in Chinook salmon *(Oncorhynchus tshawytscha).* Journal of Endocrinology, 120, 135-142.

Mánason, F. N. (2017). Leyfisferli og opinberir kostnaðarþættir fyrir sjókvíaeldi við Ísland – Samanburður við Skotland. Háskólinn á Akureyri.

Mesa, M. G., Maule, A. G., Poe, T. P. & Schreck, C. B. (1999). Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? Aquaculture, 174, 25–41.

Meyers, T. R., Short, S., Farrington, C., Lipson, K., Geiger, H. J. & Gates, R. (1993) Establishment of a negative positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaskan Pacific salmon. Dis Aquat Org, 16, 191–197.

Meyers, T. R., Thrower, F., Short, S., Lipson, K., Joyce, J., Farrington, C. & Doherty, S. (1999). Different prevalences of *Renibacterium salmoninarum* detected by ELISA in Alaskan Chinook salmon *Oncorhynchus tshawytscha* spawned from freshwater and seawater. Dis Aquat Org, 35, 101-105.

Meyers, T. R., Korn, D., Glass, K., Burton, T., Short, S., Lipson, K. *&* Starkey, N. (2003). Retrospective analysis of antigen prevalences of *Renibacterium salmoninarum* (Rs) detected by enzyme-linked immunosorbent assay in Alaskan Pacific salmon and trout from 1988 to 2000 and management of Rs in hatchery Chinook and coho salmon. J Aquat Anim Health, 15(2), 101-10.

Mills, D. (1989). Ecology and management of Atlantic Salmon. Springer Science and Business Media.

Mitchum, D. L., Sherman, L. E. & Baxter, G. T. (1979). Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontin*alis), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*). J. Fish. Res. Board Can, 36, 1370-1376.

Munang'andu, H. M. (2018). Intracellular Bacterial Infections: A challenge for developing cellular mediated immunity vaccines for farmed fish. Microorganisms, 6(2), 33.

Nance, S. L., Riederer, M., Zubkowski, T., Trudel, M. & Rhodes, L. D. (2010). Interpreting dual ELISA and qPCR data for bacterial kidney disease of salmonids. Diseases of aquatic organisms, 91, 113-119.

Ordal, E. J. & Earp, B. J. (1956). Cultivation and transmission of the etiological agent of bacterial kidney disease. Proc Soc Exp Biol Med, 92, 85–88.

Pascho, R. J. & Mulcahy, D. (1987). Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease. Can J Fish Aquat Sci, 44, 183–191.

Pascho, R. J., Goodrich, T. D. & McKibben, C.L. (1997). Evaluation by enzyme-linked immunosorbent assay (ELISA) of *Renibacterium salmoninarum* bacteras affected by persistence of bacterial antigens. J Aquat Anim Health, 9, 99–107.

Pascho, R. J., Elliott, D. G. & Chase, D. M. (2002). Comparison of traditional and molecular methods for detection of *Renibacterium salmoninarum*. Molecular diagnosis of salmonid diseases. pp .157-209.

Paterson, W. D., Lall, S. P. & Desautels, D. (1981). Studies on bacterial kidney disease in Atlantic salmon (*Salmo salar*) in Canada. Fish Pathol, 15, 283.292.

Rhodes, L. D., Rathbone, C. K., Corbett, S. C., Harrell, L. W. & Strom, M. S. (2004). Efficacy of cellular vaccines and genetic adjuvants against bacterial kidney disease in Chinook salmon (*Oncorhynchus tshawytscha*). Fish & Shellfish Immunology, 16, 431-474.

Salonius, K., Siderakis, C., MacKinnon, A. M. & Griffiths, S. G. (2005). Use of *Arthrobacter davidanieli* as a live vaccine against *Renibacterium salmoninarum* and *Piscirickettsia salmonis* in salmonids. Dev Biol (Basel), 121, 189-197.

Sanders, J. E. & Fryer, J. L. (1978). *Corynebacterium salmoninus* sp. nov. the causative agent of bacterial kidney disease; selected biochemical properties and pathogenesis in salmonid fishes. Proc. of the Third Biennial Fish Health Section of the American fisheries Society and Ninth Annual Midwest Fish Disease Workshops at Kansas City, Mishouri. p. 28-33.

Sanders, J. E. & Fryer, J. L. (1980). *Renibacterium salmoninarum,* the causative agent of bacterial kidney disease in salmonid fishes. Int.J. Syst. Bacterial, 30, 496-502.

Sigurjónsdóttir, H., Guðmundsdóttir, S., Matthíasdóttir, S., Jónsdóttir, H., Benediktsdóttir, E. & Helgason, S. (1995). A routine screening for *Renibacterium salmoninarum* in salmonid broodfish in Iceland during the years 1986–1983. In: Conference Handbook, European Association of Fish Pathologists, Seventh International Conference, Diseases of Fish and Shellfish, Palma De Mallorca 10–15, September 1995. p. 57.

Smith, I. W. (1964). The occurrence and pathology of Dee disease. Freshwater and Salmon Fisheries Research, 34, 1–12.

Steingrímsson, S. O. & Gíslason, G. (2002). Body size, diet and growth of landlocked brown trout, *Salmo trutta*, in the subarctic River Laxá, North-East Iceland. Environ. Biol. Fish, 63(4),417-426.

Stickney, R. R. & Treece, G. D. (2012). History of aquaculture. In: Tidwell JH (ed) Aquaculture production systems. Wiley-Blackwell, Oxford, United Kingdom. pp: 15-50.

Thorarensen, H., Gústavsson, A., Karlsson, H. & Sigurgeirsson, Ó. I. (2011). Vöxtur bleikjueldis á Íslandi. Málstofa F- Vatnalíf. Háskólinn á Hólum.

Thorstad, E. B., Whoriskey, F., Rikardsen, A. H. & Aarestrup, K. (2011). Aquatic Nomads: The Life and Migrations of the Atlantic Salmon. Atlantic Salmon Ecology. pp. 419.

Tidwell, J. H. & Allan, G. L. (2001). Fish as food: aquaculture's contribution. EMBO reports, 2(11). 958-963.

Waagbø, R. (1994). The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar* L.: a review. Aquac Fish Manage, 25, 175–197.

Warren, J. W. (1963). Kidney disease of salmonid fishes and the analysis of hatchery waters. Prog. Fish-Cult, 25, 121-131.

Warren, J. W. (1983). Bacterial Kidney Disease. U.S. Department of the interior. Fish and Wildlife Service, 21, 190-191.

Wiens, G. D. & Kaattari, S. L. (1999). Bacterial kidney disease (*Renibacterium salmoninarum*). In: Fish Diseases and Disorders, Vol. 3, Viral, Bacterial and Fungal Infections. CABI Publishing, New York, USA. pp. 269–301**.**

Wiens, G. D., Rockey, D. D., Wu, Z., Chang, J., Levy, R., Crane, S., Chen, D. S., Capri, G. R., Burnett, J. R., Sudheesh, P. S., Schipma, M. J., Burd, H., Bhattacharyya, A., Rhodes, L. D., Kaul, R. & Strom, M. S. (2008). Genome sequence of the fish pathogen *Renibacterium salmoninarum* suggests reductive evolution away from an environmental *Arthrobacter* ancestor. J Bacterial, 190, 6970–6982**.**

Wiens, G. D. (2011). Bacterial kidney disease (*Renibacterium salmoninarum*), In: Woo, P.T.K. & Bruno D.W. (eds.), Fish diseases and disorders-Viral, bacterial and fungal infections, Second edition Centre for Agricultural Bioscience International, Wallingford, pp. 338–374.

Woo, P. T. K & Bruno, D. W. (1999). Fish diseases and Disorders, Volume 3. Viral, Bacterial and Fungal Infections. CABI publishing.

Wood, J. W. (1974). Diseases of Pacific salmon: their prevention and treatment. 2nd edition. State of Washington, Department of Fisheries, Hatchery Division.

WWF (World Wildlife Fund). (2006). Aquaculture and the Environment. Information report.

Appendix I

Basic information about salmonids caught in Lake Elliðavatn 2018

Elliðavatn 2018					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	16.4	52.0	Female	Arctic charr	27.09.18
2	26.3	202.2	Female	Arctic charr	27.09.18
3	26.7	210.1	Female	Arctic charr	27.09.18
4	26.0	217.5	Female	Arctic charr	27.09.18
5	24.3	166.0	Male	Arctic charr	27.09.18
6	24.0	166.0	Male	Arctic charr	27.09.18
7	31.0	318.0	-	Arctic charr	27.09.18
8	23.0	140.0	Female	Arctic charr	27.09.18
9	26.5	242.0	Female	Arctic charr	27.09.18
10	26.5	212.0	Female	Arctic charr	27.09.18
11	28.9	308.0	Male	Arctic charr	27.09.18
12	16.8	58.0	Male	Arctic charr	27.09.18
13	22.2	136.0	Female	Arctic charr	27.09.18
14	24.5	178.0	Male	Arctic charr	27.09.18
15	24.7	168.0	Female	Arctic charr	27.09.18
16	25.2	182.0	Female	Arctic charr	27.09.18
17	24.3	166.0	Female	Arctic charr	27.09.18
18	28.0	300.0	Female	Arctic charr	27.09.18
19	22.1	130.0	Female	Arctic charr	27.09.18
20	26.5	220.0	Female	Arctic charr	27.09.18
21	29.5	340.0	Male	Arctic charr	27.09.18
22	37.5	774.0	Female	Arctic charr	27.09.18
23	10.5	12.1	Male	Arctic charr	27.09.18
24	13.7	28.0	Male	Arctic charr	27.09.18
25	17.0	52.0	Male	Arctic charr	27.09.18
26	13.5	32.0	Female	Arctic charr	27.09.18
27	14.0	32.0	Male	Arctic charr	27.09.18
28	13.8	32.0	Female	Arctic charr	27.09.18
29	13.9	34.0	Female	Arctic charr	27.09.18
30	13.0	30.0	Female	Arctic charr	27.09.18

Basic information about salmonids caught in Lake Elliðavatn 2019

Elliðavatn 2019					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	31.4	414.0	Female	Arctic charr	19.09.19
2	40.4	828.0	Male	Arctic charr	19.09.19
3	10.6	10.0	Male	Arctic charr	19.09.19
4	8.6	6.0	Female	Arctic charr	19.09.19
5	15.9	36.0	Female	Arctic charr	19.09.19
6	22.5	124.0	Male	Arctic charr	19.09.19
7	40.9	936.0	Female	Arctic charr	19.09.19
8	37.4	624.0	Male	Arctic charr	19.09.19
9	16.3	52.0	Male	brown trout	19.09.19
10	16.6	65.0	Female	brown trout	19.09.19
11	29.0	346.0	Female	brown trout	19.09.19
12	19.4	96.0	Male	brown trout	19.09.19
13	29.5	350.0	Male	brown trout	19.09.19
14	25.0	214.0	Female	brown trout	19.09.19
15	23.3	182.0	Male	brown trout	19.09.19
16	24.7	196.0	Male	brown trout	19.09.19
17	28.6	320.0	Male	brown trout	19.09.19
18	32.1	474.0	Male	brown trout	19.09.19
19	43.6	1202.0	Female	brown trout	19.09.19
20	20.5	114.0	Female	brown trout	19.09.19
21	18.2	80.0	Female	brown trout	19.09.19
22	19.6	100.0	Male	brown trout	19.09.19
23	23.2	172.0	Female	brown trout	19.09.19
24	22.3	140.0	Male	brown trout	19.09.19
25	31.1	390.0	Female	brown trout	19.09.19
26	21.6	130.0	Male	brown trout	19.09.19
27	28.5	276.0	male	brown trout	19.09.19
28	18.3	84.0	Male	brown trout	19.09.19
29	41.4	962.0	Female	brown trout	19.09.19
30	37.6	740.0	Female	brown trout	19.09.19
31	34.8	568.0	Female	brown trout	19.09.19
32	47.0	1442.0	Male	brown trout	19.09.19
33	45.0	1300.0	Male	brown trout	19.09.19
34	43.4	1102.0	Female	brown trout	19.09.19

Basic information about salmonids caught in Lake Mývatn

Mývatn					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	21.0	73.8	Female	Arctic charr	04.09.18
2	24.0	105.7	Female	Arctic charr	04.09.18
3	17.5	42.3	Male	Arctic charr	04.09.18
4	17.5	39.2	Female	Arctic charr	04.09.18
5	17.0	38.5	Female	Arctic charr	04.09.18
6	15.5	30.4	Female	Arctic charr	03.09.18
7	17.5	40.9	Male	Arctic charr	03.09.18
8	27.5	180.2	Female	Arctic charr	03.09.18
9	27.0	159.1	Male	Arctic charr	03.09.18
10	17.0	37.8	Male	Arctic charr	03.09.18
11	17.0	39.6	Male	Arctic charr	04.09.18
12	18.0	41.2	Male	Arctic charr	03.09.18
13	17.5	40.3	Female	Arctic charr	03.09.18
14	16.5	37.3	Male	Arctic charr	03.09.18
15	31.0	324.3	Female	Arctic charr	04.09.18
16	17.0	32.2	Female	Arctic charr	03.09.18
17	16.5	37.2	Male	Arctic charr	03.09.18
18	17.5	41.4	Male	Arctic charr	04.09.18
19	17.5	40.0	Female	Arctic charr	04.09.18
20	19.0	54.1	Female	Arctic charr	03.09.18
21	19.5	51.9	Male	Arctic charr	04.09.18
22	22.0	92.4	Male	Arctic charr	04.09.18
23	15.8	27.6	Male	Arctic charr	03.09.18
24	18.5	60.2	Male	Arctic charr	04.09.18
25	16.5	35.3	Male	Arctic charr	03.09.18
26	17.0	35.4	Male	Arctic charr	04.09.18
27	24.5	113.4	Female	Arctic charr	04.09.18
28	15.0	30.1	Female	Arctic charr	03.09.18
29	22.5	102.0	Female	Arctic charr	03.09.18
30	23.0	106.2	Female	Arctic charr	04.09.18
31	18.5	52.4	Female	Arctic charr	03.09.18
32	16.5	32.9	Female	Arctic charr	04.09.18
33	27.0	178.8	Female	Arctic charr	05.09.18
34	33.0	349.0	Female	Arctic charr	04.09.18
35	22.0	95.3	Female	Arctic charr	04.09.18
36	25.5	144.7	Male	Arctic charr	04.09.18
37	16.0	34.6	Female	Arctic charr	04.09.18
38	25.0	175.6	Female	Arctic charr	04.09.18
39	25.0	191.7	Female	Arctic charr	03.09.18
40	18.5	49.9	Female	Arctic charr	03.09.18
41	18.0	42.5	Male	Arctic charr	04.09.18

Basic information about salmonids caught in Lake Mjóavatn

Mjóavatn					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	27.5	276.0	Male	Arctic charr	07.09.18
2	27.2	262.0	Male	Arctic charr	07.09.18
3	32.8	442.0	Female	Arctic charr	07.09.18
4	29.0	366.0	Male	Arctic charr	07.09.18
5	20.3	88.0	Female	Arctic charr	07.09.18
6	18.8	78.0	Male	Arctic charr	07.09.18
7	17.5	56.0	Female	Arctic charr	07.09.18
8	21.5	114.0	Male	Arctic charr	07.09.18
9	25.5	178.0	Male	Arctic charr	07.09.18
10	21.5	120.0	Female	Arctic charr	07.09.18
11	24.5	142.0	Male	Arctic charr	07.09.18
12	25.0	173.0	Male	Arctic charr	07.09.18
13	25.0	168.0	Female	Arctic charr	07.09.18
14	31.5	364.0	Female	Arctic charr	07.09.18
15	28.8	284.0	Male	Arctic charr	07.09.18
16	36.0	696.0	Male	Arctic charr	07.09.18
17	42.5	994.0	Male	Arctic charr	07.09.18
18	29.2	338.0	Female	Arctic charr	07.09.18
19	29.8	346.0	Female	Arctic charr	07.09.18
20	27.2	232.0	Female	Arctic charr	07.09.18
21	33.4	470.0	Male	Arctic charr	07.09.18
22	32.0	372.0	Female	Arctic charr	07.09.18
23	36.0	584.0	Male	Arctic charr	07.09.18
24	44.0	1012.0	Male	Arctic charr	07.09.18
25	29.7	310.0	Male	Arctic charr	07.09.18
26	34.5	512.0	Male	Arctic charr	07.09.18
27	28.5	306.0	Male	Arctic charr	07.09.18
28	28.5	270.0	Female	Arctic charr	07.09.18
29	37.5	666.0	Female	Arctic charr	07.09.18
30	45.0	1114.0	Female	Arctic charr	07.09.18

Basic information about salmonids caught in Lake Steinsmýrarvötn

Steinsmýrarvötn					
Sample nr.	**ca. length (cm)**	**Ca. weight (kg)**	**Sex**	**Species**	**Date of capture**
1	38-55	1 - 2.5	-	brown trout (sea run)	29.09.19
2	38-55	1 - 2.5	-	Arctic charr (sea run?)	29.09.19
3	38-55	1 - 2.5	-	brown trout (sea run)	29.09.19
4	38-55	1 - 2.5	-	brown trout (sea run)	29.09.19
5	38-55	1 - 2.5	-	brown trout (sea run)	29.09.19
6	38-55	1 - 2.5	-	Arctic charr (sea run?)	29.09.19
7	38-55	1 - 2.5	-	brown trout (sea run)	29.09.19

Most information about these fish were lost. The approximate weight and length was assessed by the fisherman.

Basic information about twelve of 46 samples assayed in qPCR, from supplementary material

Supplementary material					
Sample nr.	**Length (cm)**	**Weight (g)**	**River**	**Species**	**Date of capture (year)**
1	-	-	Afall	brown trout	2007
2	-	-	Afall	brown trout	2007
3	-	-	Afall	brown trout	2007
4	-	-	Afall	brown trout	2007
5	-	-	Eystri Rangá	brown trout	2017
6	-	-	Kálfá	brown trout	2006
7	-	-	Laxá í Leir	brown trout	2006
8	-	-	Laxá í Leir	brown trout	2006
9	-	-	Vatnsá	brown trout	2006
10	-	-	Vatnsá	brown trout	2006
11	-	-	Vatnsá	brown trout	2006
12	-	-	Tungufljót	brown trout	2015

Basic information about salmonids caught in Brook 1

Brook 1					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	10.6	13.4	-	Atlantic salmon	07.10.19
2	8.7	6.3	-	Brown trout	07.10.19
3	7.5	4.4	-	Brown trout	07.10.19
4	7.7	5.0	-	Atlantic salmon	07.10.19
5	9.5	8.6	-	Atlantic salmon	07.10.19
6	7.6	4.5	-	Brown trout	07.10.19
7	6.9	3.5	-	Atlantic salmon	07.10.19
8	11.9	17.2	-	Atlantic salmon	07.10.19
9	11.9	19.2	-	Brown trout	07.10.19
10	7.7	5.5	-	Atlantic salmon	07.10.19
11	7.9	5.7	-	Atlantic salmon	07.10.19
12	10.4	11.6	-	Brown trout	07.10.19
13	11.3	15.5	-	Atlantic salmon	07.10.19
14	8.0	5.8	-	Atlantic salmon	07.10.19
15	8.2	6.1	-	Brown trout	07.10.19
16	6.9	4.1	-	Brown trout	07.10.19
17	8.1	6.0	-	Brown trout	07.10.19
18	7.8	5.0	-	Brown trout	07.10.19
19	7.6	5.3	-	Brown trout	07.10.19
20	10.9	16.0	-	Atlantic salmon	07.10.19
21	11.7	19.9	Male	Atlantic salmon	07.10.19
22	7.8	5.4	-	Atlantic salmon	07.10.19
23	7.7	6.1	-	Atlantic salmon	07.10.19
24	8.2	6.4	-	Atlantic salmon	07.10.19
25	8.2	7.7	-	Atlantic salmon	07.10.19
26	8.7	7.2	-	Atlantic salmon	07.10.19
27	11.6	17.0	-	Atlantic salmon	07.10.19
28	12.2	20.3	-	Atlantic salmon	07.10.19
29	11.6	19.1	-	Atlantic salmon	07.10.19
30	12.9	23.5	-	Atlantic salmon	07.10.19

Basic information about salmonids caught in Brook 2

Brook 2					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	21.5	80.3	-	Brown trout	31.08.18
2	18.0	49.1	-	Brown trout	31.08.18
3	11.5	15.7	-	Brown trout	31.08.18
4	12.8	20.0	-	Brown trout	31.08.18
5	9.5	7.3	-	Brown trout	31.08.18
6	22.6	82.2	Female	Arctic charr	31.08.18
7	10.5	12.5	-	Arctic charr	31.08.18
8	11.3	16.2	Female	Arctic charr	31.08.18
9	8.4	5.5	-	Arctic charr	31.08.18
10	7.0	2.8	-	Arctic charr	31.08.18
11	7.9	4.5	-	Arctic charr	31.08.18
12	9.6	7.7	-	Arctic charr	31.08.18
13	7.2	3.6	-	Arctic charr	31.08.18
14	7.7	4.7	-	Arctic charr	31.08.18
15	7.4	3.9	-	Arctic charr	31.08.18
16	8.0	4.6	-	Arctic charr	31.08.18
17	8.5	5.6	-	Arctic charr	31.08.18
18	7.9	4.1	-	Arctic charr	31.08.18
19	8.0	4.3	-	Arctic charr	31.08.18
20	8.4	4.8	-	Arctic charr	31.08.18
21	9.0	5.3	-	Arctic charr	31.08.18
22	7.6	3.7	-	Arctic charr	31.08.18
23	7.8	4.5	-	Arctic charr	31.08.18
24	8.3	5.2	-	Arctic charr	31.08.18
25	8.7	5.9	-	Arctic charr	31.08.18
26	11.5	13.1	Female	Arctic charr	31.08.18
27	8.7	5.9	-	Arctic charr	31.08.18
28	7.7	3.9	-	Arctic charr	31.08.18
29	7.9	4.4	-	Arctic charr	31.08.18
30	7.3	3.8	-	Arctic charr	31.08.18
31	8.2	4.9	-	Arctic charr	31.08.18
32	8.6	5.4	-	Arctic charr	31.08.18
33	8.6	5.9	-	Arctic charr	31.08.18
34	6.9	3.3	-	Arctic charr	31.08.18
35	7.5	3.7	-	Arctic charr	31.08.18
36	4.5	0.8	-	Arctic charr	31.08.18
37	3.7	0.3	-	Arctic charr	31.08.18

Basic information about salmonids caught in Brook 3

Brook3					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	18.5	65.3	-	Arctic charr	31.08.18
2	17.5	71.5	Female	Arctic charr	31.08.18
3	15.0	37.8	Female	Arctic charr	31.08.18
4	15.0	37.2	Male	Arctic charr	31.08.18
5	16.0	43.5	Female	Arctic charr	31.08.18
6	12.7	22.6	-	Arctic charr	31.08.18
7	12.0	17.9	-	Arctic charr	31.08.18
8	12.0	16.2	-	Arctic charr	31.08.18
9	11.5	16.9	-	Arctic charr	31.08.18
10	10.0	11.2	-	Arctic charr	31.08.18
11	13.0	22.3	-	Arctic charr	31.08.18
12	10.7	11.0	-	Arctic charr	31.08.18
13	11.0	12.9	-	Arctic charr	31.08.18
14	10.7	10.9	-	Arctic charr	31.08.18
15	11.2	13.8	-	Arctic charr	31.08.18
16	11.3	17.0	-	Arctic charr	31.08.18
17	5.4	1.1	-	Arctic charr	31.08.18
18	4.8	1.1	-	Three-spined stickleback	31.08.18

Basic information about salmonids caught in Tálknafjörður fjord

Tálknafjörður					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	-	-	-	Arctic charr	20.08.19
2	-	-	-	brown trout	20.08.19
3	-	-	-	brown trout	20.08.19
4	-	-	-	brown trout	20.08.19
5	-	-	-	brown trout	20.08.19
6	-	-	-	brown trout	20.08.19
7	-	-	-	Arctic charr	20.08.19
8	-	-	-	Arctic charr	20.08.19
9	-	-	-	brown trout	20.08.19
10	-	-	-	brown trout	20.08.19
11	-	-	-	brown trout	20.08.19
12	-	-	-	brown trout	20.08.19
13	-	-	-	brown trout	20.08.19
14	-	-	-	brown trout	20.08.19
15	-	-	-	brown trout	20.08.19
16	-	-	-	brown trout	20.08.19
17	-	-	-	Arctic charr	20.08.19
18	-	-	-	brown trout	20.08.19
19	-	-	-	Atlantic salmon	20.08.19
20	-	-	-	Atlantic salmon	20.08.19

Basic information about salmonids caught in Patreksfjörður fjord

Patreksfjörður					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	38.0	579.0	Male	brown trout	11.07.19
2	24.3	169.0	Male	brown trout	11.07.19
3	27.8	258.0	Male	Arctic charr	11.07.19
4	26.1	212.0	Female	brown trout	11.07.19
5	22.9	136.0	Male	Arctic charr	11.07.19
6	18.8	78.0	Female	Arctic charr	11.07.19
7	30.0	333.0	Male	brown trout	11.07.19
8	28.1	259.0	Female	brown trout	11.07.19
9	16.1	51.0	Female	Arctic charr	11.07.19
10	20.4	98.0	Male	Arctic charr	11.07.19
11	30.0	296.0	Female	brown trout	11.07.19
12	21.5	106.0	Male	Arctic charr	11.07.19
13	24.7	171.0	Male	Arctic charr	11.07.19
14	20.1	98.0	Female	Arctic charr	12.07.19
15	26.0	194.0	Female	brown trout	12.07.19
16	20.8	98.0	Female	brown trout	12.07.19
17	19.0	78.0	Male	brown trout	12.07.19
18	19.0	82.0	Male	brown trout	12.07.19
19	20.5	103.0	Male	Arctic charr	12.07.19
20	22.9	140.0	Male	brown trout	12.07.19
21	23.0	126.0	Male	brown trout	12.07.19
22	20.7	106.0	Female	brown trout	12.07.19
23	19.8	90.0	Male	Arctic charr	12.07.19

Basic information about salmonids collected from Farm A

Fish farm A					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	-	-	-	Atlantic salmon	06.06.18
2	-	-	-	Atlantic salmon	06.06.18
3	-	-	-	Atlantic salmon	06.06.18
4	-	-	-	Atlantic salmon	06.06.18
5	-	-	-	Atlantic salmon	06.06.18
6	-	-	-	Atlantic salmon	06.06.18
7	-	-	-	Atlantic salmon	06.06.18
8	-	-	-	Atlantic salmon	06.06.18
9	-	-	-	Atlantic salmon	06.06.18
10	-	-	-	Atlantic salmon	06.06.18
11	-	-	-	Atlantic salmon	06.06.18
12	-	-	-	Atlantic salmon	06.06.18
13	-	-	-	Atlantic salmon	06.06.18
14	-	-	-	Atlantic salmon	06.06.18
15	-	-	-	Atlantic salmon	06.06.18
16	-	-	-	Atlantic salmon	06.06.18
17	-	-	-	Atlantic salmon	06.06.18
18	-	-	-	Atlantic salmon	06.06.18
19	-	-	-	Atlantic salmon	06.06.18
20	-	-	-	Atlantic salmon	06.06.18
21	-	-	-	Atlantic salmon	06.06.18
22	-	-	-	Atlantic salmon	06.06.18
24	-	-	-	Atlantic salmon	06.06.18
25	-	-	-	Atlantic salmon	06.06.18
26	-	-	-	Atlantic salmon	06.06.18
27	-	-	-	Atlantic salmon	06.06.18
28	-	-	-	Atlantic salmon	06.06.18
29	-	-	-	Atlantic salmon	06.06.18
30	-	-	-	Atlantic salmon	06.06.18
31	-	-	-	Atlantic salmon	06.06.18
32	-	-	-	Atlantic salmon	06.06.18

Basic information about salmonids collected from Farm B

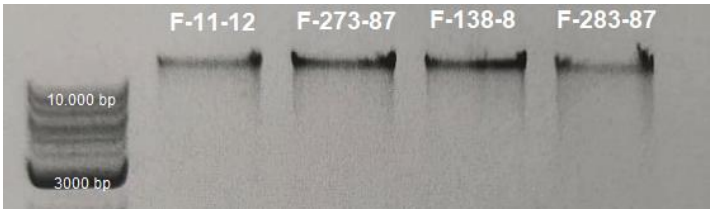
Fish farm B					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	16.0	45.9	-	Arctic charr	24.10.18
2	17.5	64.4	-	Arctic charr	24.10.18
3	16.5	52.8	-	Arctic charr	24.10.18
4	16.0	39.6	-	Arctic charr	24.10.18
5	16.5	49.2	-	Arctic charr	24.10.18
6	15.0	37.7	-	Arctic charr	24.10.18
7	16.5	49.8	-	Arctic charr	24.10.18
8	14.5	36.0	-	Arctic charr	24.10.18
9	15.0	38.6	-	Arctic charr	24.10.18
10	14.5	27.8	-	Arctic charr	24.10.18
11	14.5	29.4	-	Arctic charr	24.10.18
12	14.0	29.1	-	Arctic charr	24.10.18
13	15.0	39.1	-	Arctic charr	24.10.18
14	15.5	40.1	-	Arctic charr	24.10.18
15	13.0	29.1	-	Arctic charr	24.10.18
16	14.5	32.6	-	Arctic charr	24.10.18
17	13.5	26.0	-	Arctic charr	24.10.18
18	14.0	36.6	-	Arctic charr	24.10.18
19	13.0	23.2	-	Arctic charr	24.10.18
20	13.5	28.2	-	Arctic charr	24.10.18
21	14.5	39.8	-	Arctic charr	24.10.18
22	13.0	27.3	-	Arctic charr	24.10.18
23	14.0	26.8	-	Arctic charr	24.10.18
24	-	-	-	Arctic charr	24.10.18
25	13.0	23.6	-	Arctic charr	24.10.18
26	13.0	25.0	-	Arctic charr	24.10.18
27	13.5	23.0	-	Arctic charr	24.10.18
28	12.0	22.8	-	Arctic charr	24.10.18
29	12.0	18.2	-	Arctic charr	24.10.18
30	11.0	16.4	-	Arctic charr	24.10.18
31	11.5	18.3	-	Arctic charr	24.10.18
32	-	-	-	Arctic charr	24.10.18
33	-	-	-	Arctic charr	24.10.18
34	-	-	-	Arctic charr	24.10.18
35	-	-	-	Arctic charr	24.10.18
36	-	-	-	Arctic charr	24.10.18
37	-	-	-	Arctic charr	24.10.18

Basic information about salmonids collected from Farm C

Fish farm c					
Sample nr.	**Length (cm)**	**Weight (g)**	**Sex**	**Species**	**Date of capture**
1	-	-	-	Atlantic salmon	07.10.19
2	-	-	-	Atlantic salmon	07.10.19
3	-	-	-	Atlantic salmon	07.10.19
4	-	-	-	Atlantic salmon	07.10.19
5	-	-	-	Atlantic salmon	07.10.19
6	-	-	-	Atlantic salmon	07.10.19
7	-	-	-	Atlantic salmon	07.10.19
8	-	-	-	Atlantic salmon	07.10.19
9	-	-	-	Atlantic salmon	07.10.19
10	-	-	-	Atlantic salmon	07.10.19
11	-	-	-	Atlantic salmon	07.10.19
12	-	-	-	Atlantic salmon	07.10.19
13	-	-	-	Atlantic salmon	07.10.19
14	-	-	-	Atlantic salmon	07.10.19
15	-	-	-	Atlantic salmon	07.10.19
16	-	-	-	Atlantic salmon	07.10.19
17	-	-	-	Atlantic salmon	07.10.19
18	-	-	-	Atlantic salmon	07.10.19
19	-	-	-	Atlantic salmon	07.10.19
20	-	-	-	Atlantic salmon	07.10.19
21	-	-	-	Atlantic salmon	07.10.19
22	-	-	-	Atlantic salmon	07.10.19
23	-	-	-	Atlantic salmon	07.10.19
24	-	-	-	Atlantic salmon	07.10.19
25	-	-	-	Atlantic salmon	07.10.19
26	-	-	-	Atlantic salmon	07.10.19
27	-	-	-	Atlantic salmon	07.10.19
28	-	-	-	Atlantic salmon	07.10.19
29	-	-	-	Atlantic salmon	07.10.19
30	-	-	-	Atlantic salmon	07.10.19

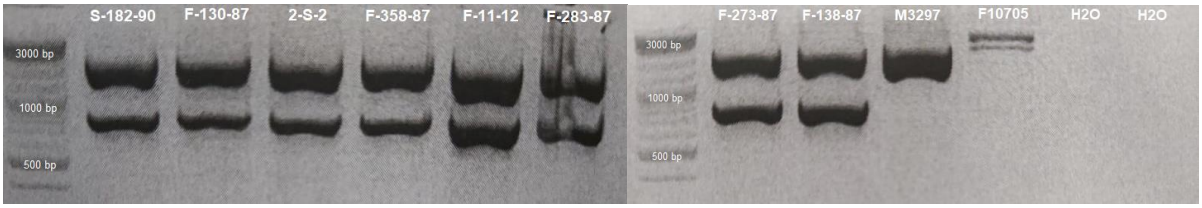
Appendix II

DNA Electrophoresis results from Icelandic isolates (Keldur Institute's sample bank)



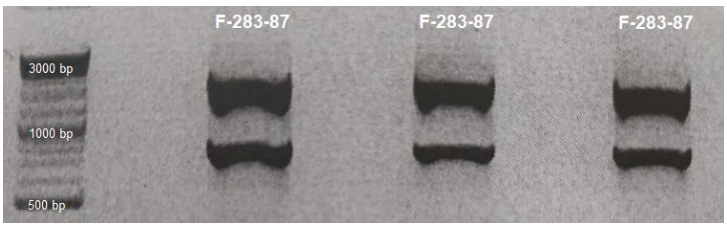
Gel electrophoresis from four -80°C isolates after DNA isolation

After all -80°C isolates had been isolated manually with GeneJET, electrophoresis was performed on samples F-11-12, F-273-87, F-138-87 and F-283-87 to examine the size and state of the DNA before 16S PCR analysis was performed.



Gel electrophoresis analysis from all isolates, after 16S PCR

M3297 and F10705 are kidney tissue samples from -20°C while all other samples are isolates from -80°C freezing medium. H_2O was placed in two wells as negative control.



Repeated gel electrophoresis from isolate F-283-87

The two DNA strands isolated in sample F-283-87 did not separate completely and therefore a gel electrophoresis was repeated.

Multiple sequence alignment results from Icelandic and foreign isolates

Nucleotide comparison on the three Icelandic strains used in this study (F-358-87, F-130-87 and F-11-12) and 13 other foreign R. salmoninarum strains from the NCBI data base

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(Canada)AY764165.1          CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(USA)AY764164.1             CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(UK)AY764163.1              CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(Sweden)AY764162.1          CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(ISLF358/87)AY764161.1      CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(Sweden)AY764160.1          CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(Sweden)AY764159.1          CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
(Russia)MT023376.1          CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
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(Kamchatka)MN121120.1       CATGCAAGTCGAACGATGAAGCGGTGCTTGCGCCGTGGATTAGTGGCGAACGGGTGAGTA       60
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(ISL)F13087                 GGTACTTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGT        480
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(Russia)MT023376.1          GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
(Seattle?)NR_074198.1       GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
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(Oregon)AF180950.1          GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
(Kamchatka)MN121120.1       GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
(ISL)F35887                 GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
(ISL)F13087                 GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540
(ISL)F1112                  GCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC        540

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(UK)AY764163.1              GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
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(Russia)MT023376.1          GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
(Seattle?)NR_074198.1       GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
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(Oregon)AF180950.1           GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
(Kamchatka)MN121120.1        GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
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(ISL)F13087                  GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600
(ISL)F1112                   GTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTA        600

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(UK)AY764163.1               GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
(Sweden)AY764162.1           GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
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(Sweden)AY764160.1           GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
(Sweden)AY764159.1           GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
(Russia)MT023376.1           GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
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(Oregon)AF180950.1           GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
(Kamchatka)MN121120.1        GGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGAT        660
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(Sweden)AY764162.1           GGCGAAGGCAGGTCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAAC        720
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(Oregon)AF180950.1           GGCGAAGGCAGGTCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAAC        720
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(ISL)F13087                  GGCGAAGGCAGGTCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAAC        720
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(Kamchatka)MN121120.1        CCACGTTCTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAA        840
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(Sweden)AY764160.1          GGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATT       900
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(UK)AY764163.1              TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGTTATGGT      1080
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(Oregon)NR_041773.1         TACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACT       1260
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(Kamchatka)MN121120.1       TACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACT       1260
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(Seattle?)NR_074198.1       CGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGT       1320
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(Kamchatka)MN121120.1       CGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGT       1320
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(Sweden)AY764160.1          TCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTTGGTAACACCCGAAGCCGGT       1380
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(USA)AY764164.1             GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
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(UK)AY764163.1              GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
(Sweden)AY764162.1          GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
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(Sweden)AY764160.1          GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
(Sweden)AY764159.1          GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
(Russia)MT023376.1          GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
(Seattle?)NR_074198.1       GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
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(ISL)F1112                  GGCCTAACCCTTTTGGGAGGGAACCGTCGAAGGTGGG  1417
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