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The potential for evolution of resistance to *Gyrodactylus salaris* in Norwegian Atlantic salmon

Sten Karlsson, Geir H. Bolstad, Haakon Hansen, Peder Jansen, Thomas Moen, Leslie Robert Noble

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The potential for evolution of resistance to *Gyrodactylus salaris* in Norwegian Atlantic salmon

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COVER PICTURE
Gyrodactylus salaris on an Atlantic salmon with lesion © Jannicke Wiik-Nielsen, Norwegian Veterinary Institute

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Abstract

Karlsson, S., Bolstad, G.H., Hansen, H., Jansen, P.A., Moen, T. and Noble, L.R. 2020. The potential for evolution of resistance to *Gyrodactylus salaris* in Norwegian Atlantic salmon. NINA Report 1812. Norwegian Institute for Nature Research.

The ectoparasite, *Gyrodactylus salaris*, was introduced to Norway in the early 70's, and has since then been found in 51 salmon rivers. Wherever the parasite has been introduced in Norway, the Atlantic salmon populations have been reduced to very low levels. The policy of management authorities is to eradicate the parasite. *Gyrodactylus salaris* has so far been confirmed eradicated from 38 rivers. In addition 5 rivers have been treated but have not yet been confirmed free from the parasite, and eight rivers have not yet been treated.

In this report we review relevant knowledge to evaluate the possibility for Atlantic salmon in Norway to naturally develop resistance or to develop resistance from selective breeding, and the possible consequences for the Atlantic salmon populations. Our main focus has been to give a summary of knowledge about the genetic basis for developing resistance, the most plausible time frame for such resistance to develop, the effects of migration, the probability of further spreading of the parasite and how this can affect genetic variation, and the genetic integrity and productivity of the salmon populations. We have also evaluated different strategies for breeding for resistance. As a basis for the evaluation of a possible resistance against *G. salaris* a large part of the report is devoted to a review of the general biology of the parasite (*G. salaris*) and the host (Atlantic salmon), and the evolutionary mechanisms behind host-parasite interactions.

We conclude that there is a genetic basis for developing resistance against *G. salaris* in Norwegian Atlantic salmon, but the timeframe to obtain a resistance to maintain viable populations would probably be on the order of hundreds of years or longer. A selective breeding program would probably speed up the process, but would require specific considerations for maintaining genetic integrity and genetic variation. Without supplementary stocking, *G. salaris* infected populations are not expected to reach a productivity at the level of the spawning target, and yield a harvestable surplus, until they have developed resistance against *G. salaris*. A strategy of developing resistance against *G. salaris* as opposed to eradicating the parasite will increase the risk of further spread of the parasite to additional rivers containing salmon that are susceptible to *G. salaris.* This would again lead to low natural productivity in consecutive infected stocks in the unforeseeable future.

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Sammendrag

Karlsson, S., Bolstad, G., Hansen, H., Jansen, P.A., Moen, T. and Noble, L.R. 2020. Potensial for resistensutvikling mot *Gyrodactylus salaris* i norske villaksbestander. 2020. NINA Rapport 1812. Norsk institutt for naturforskning.

Gyrodactylus salaris ble introdusert tidlig på 70-tallet og har siden blitt påvist i totalt 51 villaksbestander. I samtlige bestander som parasitten blitt introdusert til har bestanden av villaks kollapset. Strategien til forvaltningsmyndighetene er å utrydde parasitten. Til nå har 38 elver blitt friskmeldt etter behandling, mens 5 elver er behandlet men ennå ikke friskmeldt. Åtte elver er ennå ikke behandlet.

Denne rapporten oppsummerer relevant kunnskap for å evaluere muligheten for norsk villaks å utvikle resistens mot *G. salaris*, enten naturlig eller ved avl, og hvilke mulige konsekvenser dette vil kunne ha for villaksbestandene. Hovedfokus har vært å gi en oppsummering av kunnskapen om det genetiske grunnlaget for resistensutvikling, det sannsynlige tidsperspektivet for resistensutvikling, effekten av migrasjon, sannsynligheten for videre spredning og hvordan dette kan påvirke genetisk variasjon, genetisk integritet og produktiviteten i laksebestandene, sett i forhold til ulike avlsstrategier for utvikling av resistens. Som grunnlag for evaluering av muligheten for resistensutvikling mot *G. salaris* består en stor del av rapporten av en kunnskapsoppsummering om den generelle biologien til *G. salaris* og verten (laksen), og de evolusjonære mekanismene bak en slik interaksjon mellom vert og parasitt.

Vi konkluderer med at det finnes en genetisk bakgrunn for utvikling av resistens mot *G. salaris* i norske villaksbestander, men at en utvikling av resistens på et nivå der bestandene er levedyktige vil sannsynligvis ta i størrelsesorden noen hundretalls år eller mer. Et seleksjonsprogram vil sannsynligvis kunne gi en raskere utvikling av resistens men vil være en utfordring for å ivareta genetisk variasjon og genetisk integritet for de forskjellige populasjonene. Så lenge infiserte bestander ikke er resistente mot parasitten forventes de ikke å kunne oppnå gytebestandsmålene og høstbare overskudd uten supplerende utsetting av fisk. En strategi ved å utvikle resistens mot *G. salaris* i stedet for å utrydde parasitten vil øke risikoen for videre spredning til andre vassdrag og dermed gi lav naturlig produksjon av laks i påfølgende infiserte vassdrag i uoverskuelig fremtid.

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Contents

Foreword

The Norwegian Environmental Agency appointed an expert group late 2017 to evaluate a possible development of resistance against *Gyrodactylus salaris* in Norwegian Atlantic salmon, and possible consequences of developing such resistance. The members appointed include experts in the genetic structure of wild Atlantic salmon, parasitology, epidemiology, host-parasite co-evolution, genetics, natural selection and selective breeding. The expert group had their first start-up meeting 15 – 16 March 2018, a workshop June 6, a Skype meeting October 26, and a final meeting November 12. The expert group has utilised peer reviewed publications, reports, as well as unpublished data with relevant information. In addition, members of the expert group have been encouraged to discuss with external experts. Our contact at The Norwegian Environmental Agency at that time, Anne Kristin Jøranlid, attended the meetings as an observer.

Mandate

The Norwegian Environment Agency is seeking to commission a summary of relevant knowledge about possible resistance against *Gyrodactylus salaris* in Atlantic salmon, as well as the consequences of developing such resistance. In the event that specific knowledge about this issue does not exist, more general knowledge about the development of resistance should be drawn upon.

The Norwegian Environment Agency wants the report to discuss immunity and resistance against *G. salaris* in Atlantic salmon. We want moreover for the report to discuss what resistance/immunity in individual stocks entails for the risk of *G. salaris* being spread to other stocks. The agency also wants the report to assess how water chemistry and other environmental factors in various rivers affect survival rates in regard to being infected by *G. salaris*.

In the Norwegian Environment Agency's view, there are two ways of developing resistance: natural development and breeding for resistance. We want to shed light on the following questions:

Natural development: Are there resistant Atlantic salmon that have developed naturally? What is the resistance status in Russia, Sweden, and other relevant countries? Is it possible to develop resistance naturally in Norway by letting nature take its course? What is the consequence of natural development? What are the prerequisites (if any) for developing resistance? How will natural straying and escaped farmed salmon affect the potential for developing resistance naturally? What is a realistic time frame? What happens when different stocks vary in their selection? Are there examples in Norway of increased resistance? Why have stocks that have suffered from *G. salaris* for a long while in Norway, sometimes even for decades, failed to develop resistance?

Breeding for resistance: Is it possible to breed a stock of Atlantic salmon that is resistant against *G. salaris*? If a stock is bred for resistance, what consequences will this entail for the stock's genetic integrity? How will breeding for a single trait change a stock? Will *G. salaris* disappear from the river, or will fish be developed that may spread the parasite to other waterways? If a stock is bred that is resistant in the laboratory, how will the fish manage in the wild when they are introduced to rivers? Is it possible to maintain such resistance over time, given that there is immigration of individual fish from other stocks as well as ongoing introgression from escaped farmed salmon?

Such questions on natural development and breeding for resistance must be considered in the context of climate change, which the Atlantic salmon must adapt to. What effect will this have? If we breed for resistance, what will the impact be when the Atlantic salmon must adapt to new conditions? How will this affect natural selection?

Trondheim, May 2020, Sten Karlsson, Geir H. Bolstad, Haakon Hansen, Peder A. Jansen, Thomas Moen, Leslie Robert Noble

Definitions - English

Resistance (resistens) is when a host actively limits parasite population growth on an individual through, for example, behavioural, morphological, physiological and/or immunological responses, but does not necessarily lead to extinction/death of all parasites on a host.

Tolerance (motstandsdyktighet) unlike resistance, does not limit population growth of compatible parasites, instead the host copes with the disease by ameliorating or compensating for parasite-induced damage through reduced immunopathology, increased wound repair mechanisms and a greater resilience to tissue damage, reducing the negative impact of infection on host fitness without directly affecting the parasite.

Immunity (immunitet) is the natural ability of an organism to mobilize a protective response against a disease-causing agent (the interaction triggers a protective response). In this report we use immunity as a state where the population of *G. salaris* fails to grow, and eventually goes extinct.

Pathogenicity (patogenitet) is the potential ability the infectious organism has to cause disease.

Virulence (virulens) is the degree of pathogenicity.

Susceptibility (mottagelighet) is whether the host can be infected by a particular species or variant of a pathogen (parasite) or not. There are varying degrees of susceptibility.

Fitness is affected by individuals' abilities to survive and reproduce. However, it is not a property of an individual, but of a type of individual (genotype of phenotype). Fitness can formally be defined as the expected proportional change in the abundance of a type over a period of time.

Definisjoner - Norsk

Resistens (resistance) er når verten aktivt begrenser populasjonsveksten til parasitten, for eksempel ved endret adferd, morfologi, fysiologiske og/eller immunologiske responser. Responsen leder ikke nødvendigvis til utryddelse/død av alle parasitter på verten.

Motstandsdyktighet (tolerance), til forskjell fra resistens, begrenser ikke populasjonsveksten til parasitten. Verten håndterer sykdom og skader påført av parasitten ved redusert immunopatologi, økt reparasjonsaktivitet av skader og økt motstandskraft mot skader, og dermed reduseres negative effekter fra infeksjonen på verten, uten at parasitten blir direkte påvirket.

Immunitet (immunity) er vertens mulighet for å utvikle en beskyttende respons mot parasitten som forårsaker sykdommen. I denne rapporten bruker vi immunitet som en tilstand der *G. salaris* ikke klarer å leve på verten og til slutt blir utryddet.

Patogenitet (pathogenicity) er en organismes potensiale til å forårsake sykdom.

Virulens (virulence) er graden av patogenitet.

Mottagelighet (susceptibility) er hvorvidt verten kan bli infisert av en spesifikk art eller variant av en patogen (parasitt) eller ikke. Det finnes ulike grader av mottagelighet.

Fitness påvirkes av individers evne til å overleve og reprodusere, men er ikke en egenskap definert for et individ. I stedet er fitness definert for en type individer (genotype eller fenotype). Formelt kan fitness bli definert som den forventede proporsjonale endringen i antall individer av en bestemt type over et tids-steg.

1 Introduction

To understand the possibilities of, and consequences for, evolution of increased resistance to the ectoparasite *Gyrodactylus salaris* in Norwegian Atlantic salmon it is helpful to begin by reviewing some general background knowledge. It is relevant to include the biology of the parasite and the host, general knowledge about host parasite interaction and coevolution in addition to knowledge about management laws, regulations, guidelines, practises, and the current status and threats for Norwegian Atlantic salmon. We have structured our evaluation in three main parts: (1) The evolution of resistance to *G. salaris* by natural selection, where we evaluate the possibility, timescale and consequences of this process. (2) Selective breeding for resistance, where we evaluate the feasibility of different types of breeding programs and their consequences. (3) General considerations that apply to both natural and artificial selection for increased resistance, including implications of evolutionary response in the parasite, the impact of farmed escaped salmon, environmental change, and other pathogens/parasites.

1.1 *Gyrodactylus* and *Gyrodactylus salaris*

Gyrodactylus salaris is the most well-known and studied member of the viviparous genus *Gyrodactylus* (Gyrodactylidae, Monogenea) (Bakke et al. 2007). More than 400 species are described from the genus so far (Bakke et al. 2007, Harris et al. 2004) which is a very small fraction of the estimated 20000 species (Bakke et al. 2007). This estimate is based on a) that *Gyrodactylus* species are relatively host specific, and b) that most described fish species in the world, which at the time of the estimate was 20000, harbor at least one *Gyrodactylus* species. Today, FishBase (www.fishbase.org) lists 34000 fish species and thus the estimated number of *Gyrodactylus* species should be updated accordingly. Some fish, like cod, *Gadus morhua*, and minnows, *Phoxinus phoxinus*, host several species (Harris et al. 2004).

Parasites in this genus are very small in size, normally less than 1 mm in length, and are ectoparasites of teleosts both in marine, brackish, and freshwater environments (Harris et al. 2004). The characters defining these parasites is that they give birth to live offspring (viviparous reproduction), they are hermaphroditic, and they possess an attachment organ (opisthaptor) with two median anchors and 16 marginal hooks (see Bakke et al. 2007). The reproductive mode, where the mother contains a fully-grown daughter inside, which in turn contains a developing embryo, brings to mind the “Russian dolls” and indeed they have been called the “Russian doll-killers” (Bakke et al. 2007, Cable and Harris 2002). Reproduction may be asexual, parthenogenetic, or sexual but the extent of each mode is unknown and assumed to vary between the different species (Harris 1993). The asexual and parthenogenetic mode of reproduction allow for a short generation time and rapid population growth; important features for these parasites when colonizing new water systems and/or switching hosts. *Gyrodactylus* parasites have a direct life cycle (no intermediate hosts), and the most common way of transmission is assumed to be via direct contact between the host fish (including transfer from dead hosts), but they may also transfer indirectly via drifting in the water column and/or via attachment to the substrate (Bakke et al. 1992, Olstad et al. 2006, Soleng et al. 1999). Whenever transmission and attachment on a host is successful, the reproductive mode results in the immediate establishment of a new viable subpopulation on a single susceptible host (infrapopulation).

1.1.1 Systematics and genetic variation in *G. salaris*.

Gyrodactylus salaris was first described by Malmberg in 1957 from Atlantic salmon in the Baltic the River Indalsälven, Sweden, and was later considered a parasite mostly specific to Atlantic salmon and rainbow trout (but see below on host specificity). However, there has been a long debate in the taxonomic community whether the species *G. thymalli*, which infects grayling, *Thymallus thymallus*, is actually a junior synonym of *G. salaris.* This would have implications outside the taxonomic community as e.g. the distribution of *G. salaris* would then need to include the distribution area of grayling where *G. thymalli* has been found present, thus also including countries and watersheds which today are considered free from *G. salaris*. The most recent studies based on analyses of molecular data, both mitochondrial DNA and microRNA (Fromm et al.

2014, Hansen et al. 2003, Hansen et al. 2007a, Hansen et al. 2007b) are in favour of synonymizing the two species and recently all records of *G. thymalli* in the database of The National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) have been synonymized (and are now listed as *G. salaris*). Whether these are two species or one, the parasites infecting grayling are assumed non-pathogenic to Atlantic salmon and are thus not discussed specifically in this report.

There is considerable genetic variation between populations of *G. salaris* as judged by mitochondrial DNA analyses and there are several strains/variants with differing pathogenicity to Atlantic salmon (see Hansen et al. 2007a). The different mitochondrial clades or groups of haplotypes (haplogroups) of *G. salaris* correspond to geography (i.e. parasites from each watershed are genetically different from those from other watersheds) (Hansen et al. 2003). In addition, they are often linked to host specificity where haplotypes from salmon are not found on grayling and vice versa, which has been used as an argument for separate species status of the two. Most importantly, however, potential virulence cannot be inferred from haplotypes directly, something which is also evident from observations that some strains carrying the same haplotype can differ in virulence. This is especially noteworthy for one strain (haplotype F, see Hansen et al. 2003) which is common on rainbow trout from many places in Europe. Parasites with this haplotype have caused epidemics in some Norwegian rivers (Lærdalselva and Drammenselva), but it is also found as a non-virulent strain on e.g. Arctic char in two lakes in Norway (Olstad et al. 2007). Markers that can unambiguously identify pathogenic strains have not yet been developed but are much needed.

1.1.2 Environmental factors influencing the occurrence and intensity of *G. salaris*

Being an ectoparasite of Atlantic salmon, *G. salaris* is exposed to the surrounding environment, the water, throughout its lifetime. Thus, these environmental conditions can influence the occurrence, intensity (growth rate) and mortality rates of the parasite. Several of these environmental factors, such as temperature, salinity, and water chemistry have been shown to have an impact in experiments (for a complete review see Bakke et al. 2007 and references therein).

Gyrodactylus salaris is adapted to cold water and is known to survive temperatures between 0 and 25 °C. Population growth (the number of offspring produced) is positively correlated with increasing temperatures between 6 and 13°C. Several studies have shown that parasite intensity varies with temperature throughout the year (Mo 1992, Winger et al. 2008).

Gyrodactylus salaris is a fresh-water parasite and the salinity is a main factor defining the distribution of *G. salaris* world-wide. The salinity tolerance of *G. salaris* was studied by Soleng and Bakke (1997) who found that the parasite population declined and became extinct in 7,5 ‰ salinity after a maximum of 56 days. At this salinity temperatures between 6,0 ºC and 12,0 ºC had no significant influence on the outcome of the infection. At higher salinities than 7,5 ‰, the survival time decreased.

Both aqueous aluminium (AL) and zinc (ZN) have been experimentally shown to negatively impact the survival of *G. salaris* (Poléo et al. 2004, Soleng et al. 2000). Aqueous aluminium leaches from soil and rocks in acidified watercourses and is a common contaminant in these systems. In fact, *G. salaris* is more sensitive to aqueous aluminium than its host and therefore the use of aqueous aluminium, in combination with acidification of the water, was developed as treatment to exterminate the parasite in rivers (Hagen et al. 2008, 2010) and has been used successfully to treat the River Lærdalselva in Norway (Pettersen et al. 2007). Recently, it has also been shown that *G. salaris* is sensitive to low doses of chlorine (Hagen et al. 2014).

1.1.3 Geographic distribution of *G. salaris*

The natural distribution of *G. salaris* is assumed to lie within the eastern parts of the Baltic area including the drainages of the Russian lakes Onega and Ladoga (Ergens 1983, Malmberg and Malmberg 1993). The parasite also seems to occur naturally in some Swedish and Finnish rivers draining into the Baltic Sea, where it is mostly reported in low intensities (Malmberg and Malmberg 1993) (but see Anttila et al. 2008).

Gyrodactylus salaris has spread from its natural home range and has been reported from several countries in Europe. However, not all of these reports of *G. salaris* have been confirmed, e.g. the

ones from Spain and Portugal are questionable (see Bakke et al. 2007 for an overview). The parasite continues to spread and lately it was also detected in Italy (Paladini et al. 2009a) and Romania (Hansen et al. 2016), and most recently a report of infection in an Atlantic salmon river near Murmansk in the north of Russia (https://www.vetinst.no/nyheter/veterinaerinstituttet-har-diagnostisert-lakseparasitten-gyrodactylus-salaris-i-det-nordlige-russland).

1.1.4 Host specificity of *G. salaris*

Gyrodactylus salaris shows a wider host specificity than most other *Gyrodactylus* species (see Bakke et al. 2002), however, this might also be an artefact related to the high number of studies performed on this species. It is common on Atlantic salmon in northern Europe, and the natural host of *G. salaris* is probably the Baltic group of salmon (Bakke et al. 2007). There are significant differences with respect to the observed intensity of infection on salmon populations: *G. salaris* generally causes heavy infections on the east Atlantic group of Atlantic salmon resulting in catastrophic consequences for the juvenile population in a river. This contrasts with Baltic salmon populations, where generally only mild infections are observed (Bakke et al. 2002, Dalgaard et al. 2003). The difference between east Atlantic and Baltic salmon populations is not straightforward, however, as individuals of salmon from the River Indalsälven, Sweden, had a susceptibility to *G. salaris* corresponding to that of east Atlantic salmon populations (Bakke et al. 2004). In addition to Atlantic salmon, *G. salaris* has also been recorded on other host species such as Arctic char (*Salvelinus alpinus*) (see table 2 Bakke et al. 1992, Robertsen et al. 2007) and rainbow trout (Bakke et al. 1992, Mo 1988). Different populations of Arctic char also show very different susceptibilities to *G. salaris* in infection experiments (Sigurd Hytterød, pers. com.); populations have been observed to maintain infections of *G. salaris* through approximately 20 years without the presence of other suitable hosts (Hytterød et al. 2011). The parasite is also common on farmed rainbow trout across Europe (see Hansen et al. 2016 and references therein). Brown trout (*Salmo trutta*) is considered to have a limited susceptibility to *G. salaris* (Jansen and Bakke 1995, Mo 1988), but recent research on a UK strain of brown trout, showed that susceptibility can vary also between populations of this species (Paladini et al. 2014). As mentioned before, *Gyrodactylus* parasites isolated from grayling are assumed host specific for grayling, but only a few strains from grayling have been experimentally tested on Atlantic salmon.

Very few Baltic strains of *G. salaris* have been tested in infection-experiments, and some might be non-pathogenic. However, it is reasonable to assume that many, if not most, strains from Baltic salmon are pathogenic to Atlantic populations of Atlantic salmon, as exemplified by the epidemics caused by all three different strains that were introduced to Norway (Hansen et al. 2003) and the strains introduced to the River Keret in Russia.

1.2 The history of *Gyrodactylus salaris* in Norway

The (known) history of *Gyrodactylus salaris* in Norway started in 1975 when the parasite was detected at the Research Station for Fish, Sunndalsøra, Western Norway (Johnsen 1978). Later in the same year it was also detected on wild salmon in the River Lakselva, Northern Norway (Johnsen and Jensen 1986, Johnsen and Jensen 1991, Johnsen et al. 1999) and from 1975 to present, pathogenic strains of *G. salaris* have been detected on Atlantic salmon (*Salmo salar*) fingerlings/parr in 51 rivers, 13 hatcheries/farms with Atlantic salmon parr/smolts and 26 hatcheries/farms with rainbow trout (*Oncorhynchus mykiss*) (Hytterød et al. 2020). In addition to this, pathogenic (Hytterød et al. 2011) and non-pathogenic (Olstad et al. 2007) strains of *G. salaris* have been detected on lake-dwelling Arctic char (*Salvelinus alpinus*). Since its first introduction, *G. salaris* has affected many Norwegian populations of Atlantic salmon in a very negative way, both through direct losses of fish (see below on fish population development in infected rivers), but also through lost income for the local communities (fishing licenses, hotels, etc). Not least, the eradication programme for *G. salaris* has been very expensive for the Norwegian government, but a recent report found that the treatments have been successful in economic terms (Andersen et al. 2019).

Four hypotheses for the introduction of *G. salaris* into Norway exist (Johnsen and Jensen 1991, Johnsen et al. 1999) and three of these were later supported by molecular analyses (Hansen et al. 2003). The parasites that were introduced to Norway most likely originated from fish imported

from hatcheries around the Baltic Sea, but the particular parasite sources for all of these have not been identified. The most important spread within Norway mainly occurred through stocking of infected fish to many rivers from the research station at Sunndalsøra and from these rivers *G. salaris* was spread to nearby river systems within the fjord systems via brackish water migration of the host (Jansen et al. 2007, Johnsen and Jensen 1991, Johnsen et al. 1999). This pattern of spreading was evident from the high congruence between the stocking localities and the subsequent observations of infected rivers nearby, and later parasite isolates from all these rivers were found to carry the same mitochondrial haplotype (haplotype A following Hansen et al. 2003). The River Skibotnelva and subsequently the River Signaldalselva and the River Kitdalselva, were infected when a fish transport lorry from the Hölle laboratory in Sweden in 1975 dumped infected fish in the River Skibotnelva, Troms County, Northern Norway. The particular variant of *G. salaris* in these rivers (haplotype B), was found to differ from the one introduced to Sunndalsøra, but was identical to haplotypes in the Swedish Baltic rivers (Vindelälven and Torneälven) (Hansen et al. 2003).

The third route of infection was via infected rainbow trout from Sweden that was introduced to fish farms in the eastern parts of Norway. *Gyrodactylus salaris* was detected on farmed rainbow trout and salmon in Lake Tyrifjorden, Buskerud County, Southern Norway, from where it spread further to the River Drammenselva and the River Lierelva (Johnsen et al. 1999, Mo 1991). Parasite isolates from the River Drammenselva and the River Lierelva, as well as several other isolates from rainbow trout in Europe, carry identical haplotypes (haplotype F according to Hansen et al. 2003), implying that rainbow trout has been important in spreading the parasite, both to Norway and to other countries and locations (Hansen et al. 2003, Hansen et al. 2007b, Hansen et al. 2016, Paladini et al. 2009b). This particular strain has also been found in the River Lærdalselva in Vestland county (former Sogn og Fjordane) on the west coast of Norway (Hansen et al. 2003) that was first found to be infected in 1996 (Johnsen et al. 1999). No official hypothesis for the introduction to this river exists, but the presence of haplotype F indicates that rainbow trout might have been involved.

The fourth introduction route was to a fish farm near the River Langsteinselva in Trøndelag (former Nord-Trøndelag) county, central-Norway (Johnsen et al. 1999). Infected fish were taken into this farm on several occasions in the 1980's. The River Langsteinselva was found infected in 1988 and the same year *G. salaris* was also found nearby in the River Vulluelva. No parasite isolates from this river have been available for later molecular genetic analyses and thus no details of the origin of this introduction have been confirmed.

The policy of the Norwegian Authorities is to eradicate *G. salaris* from infected watersheds and farms (Anon. 2014a). In farms, this is carried out by eliminating the hosts (salmon and rainbow trout). This ensures elimination of the parasite since it lacks specialised free-living stages and does not use intermediate hosts in its life cycle. In rivers, two methods have been used; rotenone treatment and treatment with aluminium sulphate; the latter is used in combination with rotenone (see Hindar et al. 2018 for a review of treatment procedures). In some instances, fish migration barriers are or have been used to shorten the stretches of river for Atlantic salmon and hence limit the stretch which need to be treated. As mentioned previously, research is ongoing on the use of chlorine for eradicating the parasite. In the same way as aluminium sulphate, this chemical kills the parasite without killing the host (Hagen et al. 2014, 2019a). However, more studies are needed before this method is used routinely as an alternative to more established methods.

At the end of 2019, *G. salaris* was confirmed present in only 8 Norwegian rivers. Eradication measures had removed the parasite from 38 rivers and from all hatcheries/fish farms. In an additional 5 rivers, eradication measures have been completed, but not yet declared successful.

1.3 Evolution of resistance, tolerance and virulence

Animal host defence mechanisms are traditionally thought of as an extension of the immune system, the aim of which is to identify and eliminate, or alternatively control, invading pathogens/parasites. This is a rather narrow perspective from which to consider the complex evolutionary dynamics which occur between a host and its parasite/pathogen. By decomposing host

responses into resistance and tolerance, a better understanding of the critical mechanisms involved in host survival (Råberg et al. 2009), and the epidemiology and evolutionary ecology of infectious disease (Medzhitov et al. 2012) can be achieved.

The two defensive responses of resistance and tolerance effectively limit the fitness costs imposed by parasites upon the host (Hedrick 2017). Yet, it is important to recognise their markedly different evolutionary and practical consequences for host-parasite/pathogen interactions (Schneider and Ayres 2008).

Resistance prevents infection, limits parasite growth or clears an infection, through behavioural, morphological and/or immunological responses. The host 'fights' the parasite directly, with both the innate and adaptive immune systems contributing to resistance, but although crucial for host protection there can be substantial costs to host fitness. Resistance is balanced by an acceptable trade-off between disease clearance and immunopathology. However, insufficient resistance can often result in high host mortality, so the level of immunopathology may be high, constituting common symptoms of infectious disease (Little et al. 2010). Tolerance mechanisms can ameliorate the trade-off between protective immunity and immunopathology by limiting tissue damage, permitting longer and more intensive immune responses.

Tolerance, unlike resistance, does not limit infection or reduce parasite burden, instead the host fights the pathogen by ameliorating or compensating for parasite-induced damage through reduced immunopathology, increased wound repair mechanisms and a greater resilience to tissue damage, reducing the negative impact of infection on host fitness without directly affecting the parasite. (*Tolerance in this context is distinct from immunological tolerance, which is defined as unresponsiveness to self-antigens*.) The degree of damage endured depends on tissue type; vascular disruption can be fatal, whereas skin damage is often well tolerated. The concept of tolerance as a defence strategy is well accepted in plant immunity, but has only been more recently considered in animal systems – it is most conveniently defined as the slope of host health (or a fitness trait, like growth rate) against infection intensity (see **Figure 1**).

The relative contributions of resistance and tolerance can be distinguished by plotting parasite burden against a measure of health (see **Figure 1**). Measuring either one of these parameters in isolation makes it impossible to discern the cause of morbidity or mortality. Considering only resistance as the mechanism involved in host survival leads to the, often incorrect, assumption that host mortality is a consequence of a failure of the immune system (see B in **Figure 1**).

Deficiencies in tolerance: Morbidity or mortality can result from failure of tolerance mechanisms, even where resistance is effective (Råberg et al. 2007). This might be signalled by a comparable pathogen burden in hosts with different mortality or morbidity profiles (see **Figure 1**). The distinction between failed resistance and failed tolerance is important because it can dictate the appropriate therapeutic approach (Hedrick 2017); failed tolerance is unlikely to be resolved by strategies boosting immunity or reducing parasite burden.

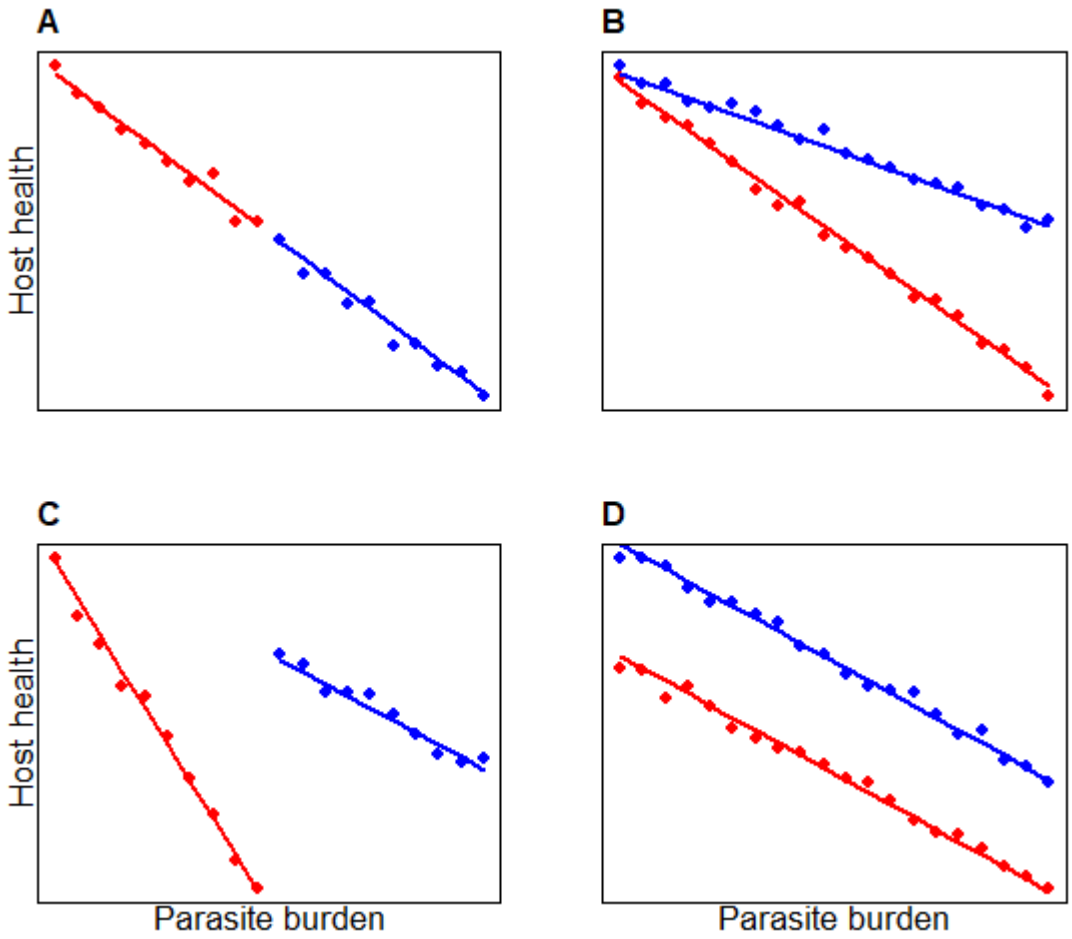

***Figure 1**. Based on Råberg et al. (2007). Schematic of reaction norms of two host genotypes (red or blue line), ignoring any virulence differences in infective agent, for disease severity across a range of infection intensities in individual hosts (dots). (A) Two equally tolerant genotypes differing in resistance; the red genotype has lower parasite burdens (is more resistant) and thereby maintains higher health status when infected. (B) Two equally resistant genotypes (same parasite burden) but the red genotype is less tolerant (health declines faster with increasing parasite burden). (C) Genotypes differ in both tolerance and resistance; the more tolerant genotype (blue) is less resistant, so both have on average the same health status when infected; but the steeper slope and greater health range of the more resistant genotype can be attributed to health declines from immunopathic effects (marked ´relative resistance deficit´) and health benefits of resistance (marked ´relative resistance premium´). (D) Host genotypes differ in neither resistance (same average parasite burden) nor tolerance (same slopes). Instead, the genetic difference in health status is due to a difference in intercept, so that the difference exists even when animals are uninfected; it is indicative of genetic differences in 'general vigor', and does not reflect defence against infectious agents.*

Virulence can be defined as the parasite's ability to cause disease and so mortality, or degree of pathology, in a given host, or can reflect the degree of immunopathology it elicits. The host component is defined by its tolerance to the damage caused by the pathogen (Råberg et al. 2007). Therefore, the evolution of virulence can reflect changes in either pathogen or host. A pathogen/parasite that is more virulent in a new host species is most likely a reflection of the new host's lack of tolerance, as the pathogen/parasite-intrinsic characteristics are unlikely to change immediately (Råberg et al. 2009). A clear example of this is the high virulence in *G. salaris* infected Norwegian salmon. Conversely, a pathogen/parasite can become less virulent in a host because of an increase in host tolerance to damage caused by the infection. This may

evolve to the point where the pathogen colonizes the host constitutively without causing disease (Medzhitov et al. 2012).

1.3.1 Ecological feedbacks and evolution

The expression of each immunological strategy (resistance vs. tolerance) is a consequence of a three-way interaction between the host and parasite genomes and the environment, and the expected evolutionary outcome for virulence in the host-parasite system can be quite different depending upon which strategy is under selection.

There is a naïve expectation that, because parasite survival relies on continuance of the host population, parasites generally evolve to become benign (avirulent). This ignores the many factors affecting parasite fitness which can influence a relationship with the host (Anderson and May 1982, Frank 1996). Clearly, genetic differences controlling parasite traits are the basis of host x parasite interactions. As parasites evolve more rapidly than their hosts much of the infection dynamics is necessarily parasite mediated, through selection of genotypes which affect the level of virulence in response to the parasite´s ecological circumstances. There are three considerations of relevance to infections with *G. salaris*; genetic diversity of parasite demes, parasite responses to host resistance, and the degree of horizontal transmission.

Each host individual may be viewed as containing a temporary population (deme) of parasites. Demes that kill the host before transmission are selected against, as they contribute less to the parasite population than more benign demes, so interdemic selection favours lower virulence. A corollary of this has specific relevance to host infections by the parthenogenic *G. salaris*, which rapidly produces genetically identical clonal daughters on infection. If a number of hosts are infected by a single parasite, or a group of closely related individuals, then the demes are effectively kin groups, so interdemic selection is then equivalent to kin selection, and lower virulence may evolve in all demes. However, if a host infection is composed of multiple unrelated parasite genotypes, selection within demes favours those genotypes with higher reproductive rates, as these will be transmitted in greatest numbers. So increased virulence is expected to evolve in parasites where infection by multiple genotypes is frequent, and conversely lower virulence in cases where there is a single predominant parasite genotype (Frank 1996).

Fitness of parasites transmitted horizontally between unrelated hosts are not dependent upon the long-term survival or reproduction of their host. But if parasites are vertically transmitted (effectively inherited) the fitness of the parasite infecting the host depends directly upon host fitness. Such parasites may be expected to evolve towards a lower virulence (Bull et al. 1991). Conversely, virulence may increase where individuals of *G. salaris* are exchanged frequently between unrelated hosts. Similarly, short residence times, such as on hosts which rapidly become immune to the parasite, and individuals showing an overt immunopathic response could be classed among these, favouring rapid reproduction of the parasite to effectively outrun the host immune system. The parthenogenic *G. salaris* is uniquely adapted in this respect, its enhanced reproductive capacity a mechanism increasing virulence – meaning an effective host immune system may induce a rapid increase in reproduction and so increase virulence.

Because resistance reduces parasite fitness, its evolution is subject to negative frequency-dependent selection. At high parasite prevalence resistance is expected to sweep through a host population (see ´relative resistance premium´ Fig. 1C), but as parasites are eliminated from the population the cost(s) associated with resistance mean it becomes less favoured (see ´relative resistance deficit´ Fig. 1C), and genes promoting resistance are reduced in frequency in the host population. Tolerance, however, does not reduce parasite fitness, so its evolution is expected to produce positive feedback whereby parasite infection selects for tolerant hosts, so increasing parasite prevalence, suggesting virulence must remain high for populations to maintain strong resistance responses. As a consequence of these different dynamics, some theoretical models predict the maintenance of polymorphisms for resistance, but the fixation of tolerance during host-parasite coevolution (Best et al. 2008). By contrast models partitioning tolerance into components of mortality and sterility suggest that tolerance to mortality increases parasite fitness, leading to positive frequency dependence, whereas tolerance to sterility effects can lead to negative frequency dependence and disruptive selection.

1.3.2 Host-parasite coevolution – an ecological perspective

Generally, a simple perception is that absence of pathological effects from infections is evidence of effective host immunity, whereas mortality is evidence of host primary immunodeficiency. However, this perspective lacks the depth of understanding which comes from an appreciation of host-parasite coevolution, and an acknowledgement of the relationship between virulence and replication, and subsequent transmission of the infective agent. An important concept is that the host immune system does not, cannot, act as a universally impenetrable shield, resisting all manner of infections. Instead the intimate and continual contact between rapidly evolving parasites/pathogens and their more slowly evolving hosts leads to an uneasy détente, balanced between parasite virulence and host survival. In this relationship virulence is a property of the parasite/pathogen, but it is manifest only in specific hosts. Similarly, the immune system is a host property, but its efficacy depends on the infective agent.

Virulence is the deleterious consequences to the host of an infection, and is usually closely linked with the parasite/pathogen´s attempts to maximize its replication rate and transmission at cost to the host. However, the demands of replication and transmission are governed by factors which, during coevolution, become balanced with a level of host survival compatible with goals of replication and transmission; consequently, the parasite´s virulence is highly dynamic, varying from life threatening to relatively benign. Where there is no prior history of host-parasite coevolution this balance is lost, and the ability of the immune system to resist or clear the parasite becomes unpredictable, as does the ability of the parasite to obtain the necessary resources from the host. The subtleties of such situations are reminiscent of, and perhaps best illustrated by, zoonotic outbreaks and their subsequent transmission dynamics, such as occurred in HIV, Ebola, and various emerging flu and corona viruses – eventually these may reach an attenuated level of virulence, but for any prediction it is imperative to consider the ecological context.

1.3.3 Host response to parasitism

Until recently the entire focus of immunology was the study of resistance to parasitism/infection, consequently a focus on mechanisms of resistance, clearance of primary infection, acquisition of immunity has encouraged a reductionist approach. Nonetheless, despite the evolutionary advantages of avoiding infection, there are parasites which cannot be avoided and to which there is no effective resistance. Some parasites can infect a host for its entire lifetime in the face of an ineffective immune response. In such cases, where there is a cost to resistance, there can be a self-imposed attenuation of the immune system to avoid fruitless immunopathology; an evolutionary acknowledgement that resistance is futile, and there is instead selection for tolerance (Best et al. 2009).

Most recently, and highly relevant to salmonid parasite defence mechanisms, Klemme et al. (2020) demonstrated a negative association between defence traits involved in the interaction between the eye fluke *Diplostomum pseudospathaceum* with Atlantic salmon and brown trout. Variation of these traits involved a significant genetic component, essential for the evolution of host-parasite interactions.

1.3.4 Trade-off and the evolution of parasite virulence

Based on the work of Anderson and May (1982) and Best et al. (2008) this approach acknowledges that virulence, and its effects on the host population, are intimately linked with transmission between hosts, length of infection, and parasite spread between rivers. Virulence, the host cost of infection, is associated with rapidity and extent of on-host parasite replication. Transmission can occur during infection, so is associated with host lifespan; its rate is a measure of successful host to host infections, minus the host death rate due to infection and rate of parasite clearance. These factors define the parasite fitness – the number of new infections caused by each infected host. The trade-off increases of on-host reproduction are paralleled by increases in transmission and virulence. But as virulence increases beyond a certain point the length of infection begins to decline, and with it the rate of transmission (see **Figure 2**). Parasite fitness is thus a trade-off of the opposing forces of virulence and transmission rate versus length of infection. Although undoubtedly a simplification of the real world, trade-off theory nevertheless constitutes an effective means of describing theoretical and practical aspects of parasite propagation.

Perhaps the best known and most widely appreciated demonstration of the trade-off theory is provided by biological control experiments involving the myxoma virus from the South American tapeti (*Sylvilagus brasiliensis*) released into populations of the European rabbit (*Oryctolagus cuniculus*) (Keer 2012). In its natural host the virus causes a benign cutaneous fibroma, but standard laboratory strains produced a fatality rate of >99% in rabbits. Released in Australia in 1950 over the following 30 years the virus evolved an attenuated virulence, with a 70-95% fatality rate in laboratory rabbits and an extended time of infection. The pattern was repeated in France upon release of a separate viral strain. Of relevance to the topic of this report, a corresponding coevolutionary increase in resistance in the wild rabbit population was followed by a corresponding ´Red Queen` rebound of virulence in the myxoma virus. In contrast, and in line with the trade-off theory, re-isolated strains from Europe and Australia contained geographically unique mutations which appeared to diminish the immune inhibition properties of the virulence factors of the natural virus, so attenuating its virulence and increasing its length of time of infection.

1.3.5 The role of host population density and size in determining virulence

The success of the trade-off theory has proven useful in predicting real world parameters that might affect the dynamics of disease spread and virulence. Parasite spread and virulence arises from host population density, size and spatial structure. The denser the population of hosts the more rapidly the parasite can spread, and the less selection there is for an extended time of infection. Consequently, virulence evolves to higher levels, until the host population declines to the level where the length of infection required to maintain the parasite approaches the life time of the host, that is, persistence.

Population size can influence virulence, with larger populations able to maintain more virulent parasites/pathogens. Acutely infectious diseases can eliminate all potential hosts, causing them to become extinguished from small populations, unless they reduce their virulence before this happens. Black (1975) showed people of the Amazon Basin living in small communities, such as must have predated the rise of cities, showed serological evidence for persistent viruses, such as herpes and varicella, but little acute illness. Thus, evidence supports epidemiological theory that the evolution of virulence is strongly influenced by host population size and density.

1.3.6 Host population structure determines infectivity

Another aspect of the host population which can influence virulence is spatial structure, or more correctly the interactions a structured population promotes. Network connectivity, the clustering and number of interactions, is a property of the host population capable of increasing or decreasing virulence. The greater the level of clustering and connectivity between host individuals the greater the level of virulence the parasite can sustain.

So, by considering the density, structure and parasite transmission, the characteristics of the coevolved host-pathogen/parasite interactions can be deduced. Parasites endemic within a low dispersal, low density population evolve persistence and elicit tolerance. Those that exhibit acute infectivity and rapid transmission elicit stronger resistance responses. Tolerance and resistance correlate with the extremes of host immunopathology and affect the virulence of the parasite.

Therefore, an ecological perspective of the coevolution of the *G. salaris*-salmon system predicts that virulence is likely to remain high where salmon population structure consists of large, mobile groups of predominantly unrelated individuals, at high stocking densities which promote parasite transmission. Stocks possessing an effective immune response to the parasite, often marked by pathogenic skin lesions, may be expected to promote increased virulence, in response to the shortened residence time for *G. salaris* on individuals. Indeed, observation of *G. salaris*-salmon immunopathogenic interactions (Conon vs Neva stocks) are consistent with theoretical expectations, and evidence from other fish-parasite/pathogen systems, that host resistance inevitably increases parasite virulence (see Anon. 2004, Gilbey et al. 2006, and literature therein). Alternatively, development of tolerance in salmon stocks (often manifested as attenuated immune responses in other fish-parasite/pathogen systems), together with reduced population connectivity and lower stocking densities, are more likely to lead to reduced virulence and increased parasite prevalence, eliciting further host tolerance.

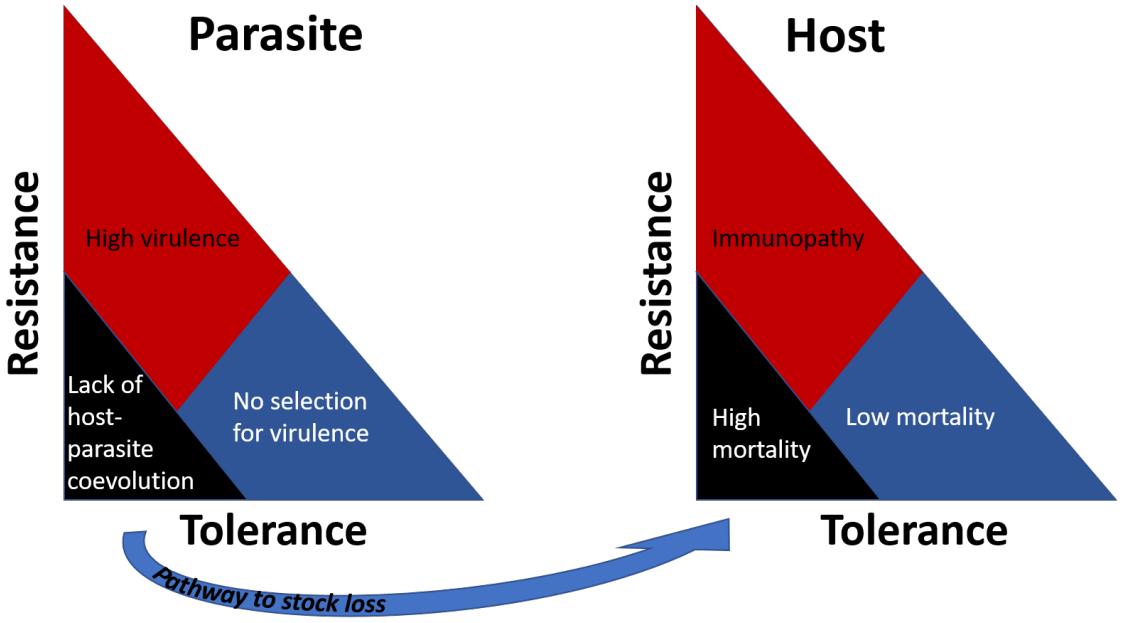

Figure 2. *Phase space diagrams showing resistance vs tolerance for hosts and their parasites/pathogens. Host resistance or tolerance will place parasites respectively under more or less pressure to evolve virulence; a mechanism to overcome resistance. Hence, strong resistance of a host is associated with immunopathy and increased selective pressure for virulent parasites. Alternatively, tolerance results in widespread but low level infection within host populations. Where there is absence of effective resistance (though not necessarily absence of immunopathy) and tolerance to a newly encountered parasite there is potential for high host mortality.*

1.4 The genetic basis of resistance to *Gyrodactylus salaris*

The variation in a particular trait within a population can be due to genetic variation, variation in the environment experienced by the animals within the population, or to random factors such as developmental noise. For evolution to occur, there must be genetic variation in the trait in question.

The genetic component of the variation in a trait is often measured as the similarity between relatives, known as the heritability. The heritability is a main determinant of how easy it will be to breed for changes in a trait. If the heritability is very high, most of the observed merits of an animal is due to genetics. Hence, if one breeds further on a well-performing animal, the offspring of that animal are highly likely to be well-performing as well. On the other hand, if heritability is low, a well-performing animal may very well give poor-performing offspring. However, a low heritability does not imply that there is not substantial scope for genetic improvement; to achieve it will just take longer and require more effort. For example, the heritability of sea lice resistance in farmed Atlantic salmon is only moderate, ranging from 0.12 to 0.33 in reported experiments (Kolstad et al. 2005, Gjerde et al. 2011, Ødegård et al. 2014, Tsai et al. 2016a). Due to the importance of the trait to the salmon farming industry, that moderate heritability does not stop the breeding companies from putting emphasis on sea lice; instead they compensate by investing in large-scale, routine testing of sea lice resistance among their breeding candidates.

Only one study has presented estimates of heritability which are relevant for *G. salaris* resistance and/or tolerance. Salte et al. (2010) challenge-tested 984 fish from 25 paternal half-sib groups (where all fish within each group shared the same father), originating from the River Drammenselva in Norway. In the challenge test, the fish were infected at a size of about 8 g, and survival was measured over a period of approximately two months. The heritability (± standard

error) in survival (on the liability scale) was found to be 0.32±0.10 (which corresponds to 0.17 on the binary scale), while the heritability for days of survival was 0.29±0.07. This strongly suggest that the level of resistance and/or tolerance is selectable, though its ability to evolve may vary across populations. There is therefore scope both for natural selection and artificial breeding in increasing the resistance and/or tolerance against *G. salaris*.

A different approach to study the genetic component of trait variation is to take advantage of genetic markers and to statistically associate these with the trait in question, this approach identifies so-called quantitative trait loci (QTL). Gilbey et al. (2006) employed a QTL screening approach in order to identify markers linked to QTL influencing *G. salaris* resistance in first generation backcrosses of Baltic (resistant) and Scottish (susceptible) salmon. The fish were screened for 39 microsatellite markers. Marker-trait combinations showing a statistically significant change in the amount of variance explained when the influence of marker alleles was incorporated as suggestive of marker-QTL linkages. Markers showing significant associations in individual marker-trait analyses were combined in a generalized linear model (GLM) that allowed the total amount of variance in parasite numbers associated with the genetic markers identified to be determined. A total of seven traits based on parasite counts were examined. Ten genomic regions associated with heterogeneity in resistance explained up to 27.3% of the total variation in parasite loads. This study shows that resistance to infection by *G. salaris* is heritable and suggests it is controlled by many genes (i.e. polygenic).

The QTL from Gilbey et al. (2006) showed an exponential distribution in their effects, however, as large effects are identified before smaller ones this distribution may be artefactual. Also, estimates of QTL effect can be upwardly biased, especially for loci with large effect, making the true effect of the QTL smaller than the experimental estimate. Additionally, estimates of numbers and effects of QTL in this study are necessarily estimates pertaining to chromosomal regions, each of which may contain hundreds or thousands of genes. This means the estimated single locus large effects might actually be due to additive small effects of many loci. This is especially relevant in this study as the markers represent entire linkage groups which may even be complete chromosomes, requiring further fine scale mapping to determine the true numbers and strengths of factors involved.

An approach similar to that followed by Gilbey et al. (2006) was used to investigate the challenge-tested salmon from the River Drammenselva (Salte et al. 2010) in more detail: Sixteen hundred challenge-tested individuals were genotyped on a SNP-chip developed by the breeding company AquaGen and the Norwegian University of Life Science, containing 50,000 SNPs distributed across the salmon genome. Genotypes were correlated to survival (a binary trait) and days-of-survival in a genome-wide association study (GWAS). Preliminary results from this study support the hypothesis that resistance to *G. salaris* is a polygenic trait: although the heritabilities reported by Salte et al. (2010) were confirmed using genotypes (rather than pedigree), no experiment-wide significant QTL was detected for the survival or days-of-survival. The results indicate that, at least in the River Drammenselva, resistance to *G. salaris* is determined by a large number of genes with small individual effects.

1.5 Management laws, regulations and guidelines for Atlantic salmon

Insight into the general management laws, regulations, and guidelines is a prerequisite for evaluating the possibility of developing resistance and/or tolerance against *G. salaris* in Norwegian Atlantic salmon populations. A possible strategy for developing resistance against *G. salaris* needs to consider that Atlantic salmon populations are managed separately, making sure that genetic integrity and genetic variation in each population is preserved, and that each population according to law reaches its spawning target, allowing for a sustainable harvest. Consequently, if we were to aim for development of resistance in Norwegian salmon populations, either from establishing selective breeding programmes or by allowing a natural development of resistance, genetic integrity and genetic variation needs to be preserved at the same time, and each population should have a productivity allowing for sustainable harvest.

The Nature Diversity Act (Naturmangfoldloven) on species management states that the species and their genetic diversity is to be maintained in viable populations in their native range for the

foreseeable future, while the purpose of the salmon and inland fish act (Lakse- og innlandsfisk-loven) is to ensure that native populations of anadromous salmonids, freshwater fish and other aquatic organisms as well as their habitat, are managed such that biodiversity and productivity is maintained in accordance with the nature diversity act. Regulations and guidelines for management of Atlantic salmon follow the purpose of these acts, and include management and regulations of physical constructions in the rivers, stocking activities, fishing regulations, etc. One fundamental principle for management of Atlantic salmon is that each population is managed separately. This management strategy follows from the fact that Atlantic salmon are subdivided into separate populations or stocks that are more or less genetically separated from each other and therefore more or less maintain productivity and genetic integrity independent of each other. Populations, or stocks, are generally allocated to rivers, because of the nature of natal homing of Atlantic salmon.

Annual stocking of Atlantic salmon is conducted in about 60 rivers (Karlsson et al. 2018). Recently the motivation for stocking has gradually shifted from increasing the number of fish for recreational and commercial fishing towards conservation of natural populations. The guidelines from the Norwegian environmental agency state that release of hatchery produced fish should be a last resort, after efforts have been made to remove factors preventing sufficient natural production and sustainable harvest. Furthermore, it states that where there is a need for stocking it should be done in a way to maintain the population´s genetic integrity and genetic variation (Anon. 2014b). Specific guidelines for the latter have been developed by Karlsson et al. (2016a). Genetic introgression of escaped farmed Atlantic salmon in wild salmon populations is one of the largest threats to Norwegian wild salmon populations (Forseth et al. 2017) and is widespread in Norwegian wild salmon populations (Karlsson et al. 2016b, Diserud et al. 2019). Consequently, since 2014 genetic analyses of all potential broodfish has been mandatory, to exclude broodfish of likely farmed escape ancestry (Karlsson et al. 2015).

1.6 Population structure and local genetic adaptation in Atlantic salmon

Atlantic salmon is subdivided into populations at many hierarchical levels across the distribution range, with the largest differences being between salmon from Eastern- and Western Atlantic, followed by several genetically distinct groups of populations within the Eastern- and Western Atlantic (Nielsen et al. 1996, King et al. 2001, Verspoor et al. 2005, Bourret et al. 2013, Gilbey et al. 2017), and genetic differences between populations within regions at large and at small geographical scales (Ståhl 1987, Säisä et al. 2005, Karlsson et al. 2011, Gilbey et al. 2017, Vähä et al. 2017, Ozerov et al. 2017). Background information for the purpose of understanding and evaluating the possibility for development of resistance against *G. salaris* in Norway is that the Baltic salmon makes a distinct phylogenetic cluster different from the Norwegian Atlantic salmon (Bourret et al. 2013). This is important to consider because *G. salaris* was evidently imported from the Baltic and Baltic strains of Atlantic salmon appear to show much higher resistance. Norway has more than 400 Atlantic salmon rivers with populations varying greatly in size and magnitude of human impacts (Forseth et al. 2017). Norwegian salmon constitutes two phylogenetic groups: Barents-White Sea and Atlantic (Bourret et al. 2013), with a geographical separation around the border between Troms and Finnmark counties but with a hybrid zone between the phylogenetic groups (Karlsson et al. 2016b). The Atlantic phylogenetic group in Norway is further subdivided into three genetic groups (Mid-Norway, South-West Norway, and South Norway), as revealed by an extensive study of genetic variation in microsatellite loci in Europe (Gilbey et al. 2017). In Norway, the Barents-White Sea phylogenetic group is also divided into several distinct genetic groups (Ozerov et al. 2017). Finally, in general, each river holds populations genetically different from other neighbouring rivers, also within identified genetic groups of populations (Gilbey et al. 2017, Ozerov et al. 2017). In addition, some large water courses with several tributaries, may contain genetically distinct populations within the river system (e.g. the River Teno, Vähä et al. 2017).

The large body of research on genetic structuring of Atlantic salmon suggest that populations are mainly defined by rivers (but see Cauwelier et al. 2018). Because of restricted gene flow

between populations, and because there are large environmental differences between rivers, there is great potential for the evolution of local genetic adaptations. Experimental trials and comparative studies of fitness related traits constitute a large body of circumstantial evidence for local genetic adaptations (reviewed in Garcia de Leaniz et al. 2007). Development of molecular genetic methods enabling studies of the whole genome, or a large coverage of genetic variants across the genome in Atlantic salmon (Davidson et al. 2010, Lien et al. 2016, Tsai et al. 2016b) has made it possible to identify single genes, or complexes of genes, for fitness related traits (Gutierrez et al. 2012, 2015, Bourret et al. 2013, Johnston et al. 2014, Tsai et al. 2015, Ayllon et al. 2015, Barson et al. 2015). A recent study of genetic variation in 208 704 SNP-markers revealed strong signatures of natural selection in a gene with a large effect on age at maturity. This gene was polymorphic in all but one out of 54 Norwegian populations analysed, and it was shown that this variation is maintained by balancing selection with varying patterns of dominance between sexes (Barson et al. 2015). These observations suggest strong contemporary natural selection for optimal age at maturity and that the large differences in sea-age composition, also between neighbouring rivers, has a genetic component, representing local genetic adaptation. Additional evidence for local genetic adaptation was shown by Mobley et al. (2018), with strong selection against strayers, where local salmon had higher reproductive success compared to dispersers.

1.7 Stocking of Atlantic salmon

Stocking of salmon has a long tradition in Norway going back to 1855. There is a growing recognition of the potential negative genetic consequences of stocking (Laikre et al. 2010), including loss of genetic variation and genetic integrity (Christie et al. 2012a, Baillie et al. 2014), loss of genetic adaptation from unintentional selection during domestication (Araki et al. 2007, Christie et al. 2012b), reduction in effective population size (Ryman and Laikre 1991, Christie et al. 2012a) and epigenetic changes (Christie et al. 2016, Le Luyer et al. 2017). Consequently, motivation for releasing hatchery produced fish in Norway has shifted from enhancing fisheries towards conserving genetic variation and integrity (Skår et al. 2011). Since 1986, 33 populations of Atlantic salmon are now in a live gene bank in Norway, motivated by one or several of these reasons for the populations being at risk: *G. salaris*, hydro power regulation, acidification, salmon lice from aquaculture, and genetic introgression of escaped farmed salmon (http://tema.miljodirektoratet.no/no/Tema/Arter-og-naturtyper/Villaksportalen/Bevaringstiltak/Genbank-for-villaks/, Arne Sivertsen, Norwegian Environmental Agency, pers. com.). About 60 salmon populations are stocked annually (Karlsson et al. 2018). Since 2014 genetic analyses to exclude brood fish likely of not being of pure wild origin, as opposed to farmed origin, have been mandatory (Karlsson et al. 2015). In practise, brood-salmon caught in the river are tagged and scale samples sent to the Norwegian veterinary institute. Results from scale analyses are then used to exclude potential first generation escaped farmed salmon, and genetic analyses used to exclude brood-salmon that are offspring of escaped farmed salmon (**Figure 3**).

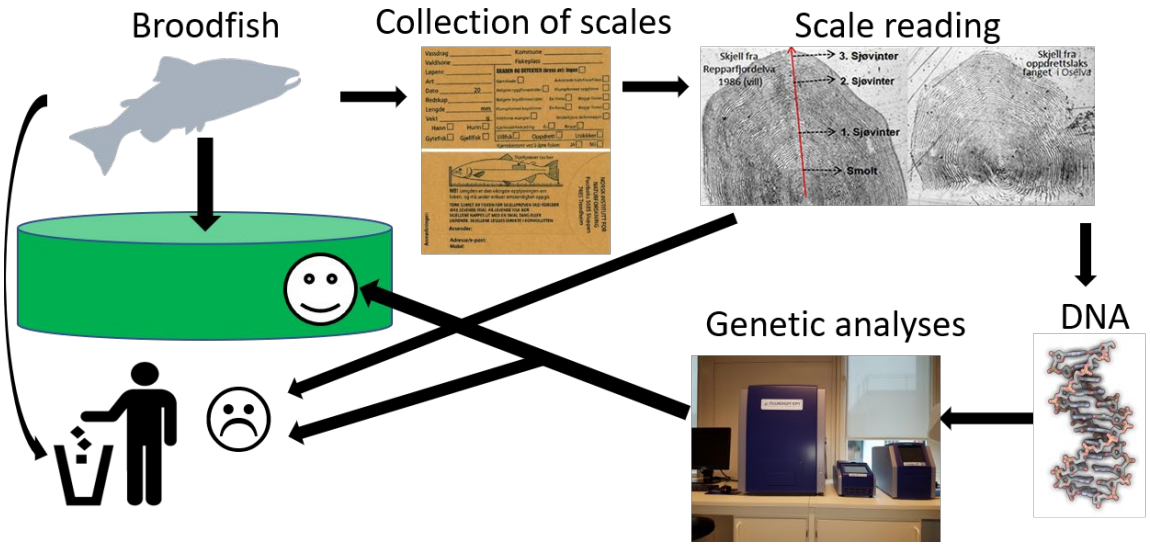

Figure 3. *Work flow of broodfish control. Scale samples from brood-salmon are being sent for scale analyses to exclude potential escaped farmed salmon. Broodfish not first generation escaped farmed salmon are further analysed genetically to exclude brood-salmon which are unlikely to be of pure wild origin. Photo of scales, scale envelope, Fluidigm instrument and cartoon of salmon ©NINA, Illustration of DNA ©Bjørnar Sporsheim.*

Good stocking practice includes not only using local brood-salmon, but also avoiding inbreeding, reduction of effective population size, loss of genetic variation, and unintentional domestic selection and epigenetic effects. Currently there are no regulations which instruct how stocking should be conducted to meet these challenges. Nevertheless, there are general guidelines to follow (Skår et al. 2011, Anon. 2014b, Karlsson et al. 2016a).

1.8 Genetic introgression of escaped farmed in wild salmon

Norwegian farmed salmon have their origin in many wild salmon populations (Gjedrem 1991, Gjøen and Bentsen 1997). Selective breeding programs started in the early seventies and have been very successful in changing the genetics of farmed salmon from its wild origin, selecting for commercially important traits (Thodesen et al. 1999, Gjedrem and Baranski 2009, Solberg et al. 2013). The traits of farmed salmon have been shown to be inferior to the traits of wild salmon in nature (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012, Reed et al. 2015, Glover et al. 2017), and genetic introgression of escaped farmed salmon with wild salmon is leading to a change in important life-history traits in wild salmon populations (Bolstad et al. 2017). Furthermore, Norwegian farmed salmon has less genetic variation than wild salmon (Mjølnerød et al. 1997, Skaala et al. 2004, 2005, Karlsson et al. 2010). Hence, farmed genetic introgression in wild salmon populations is of great concern and is currently identified as the largest current expanding threat to wild salmon populations (Forseth et al. 2017). Unfortunately, genetic introgression from farmed salmon in wild salmon populations is widespread (Karlsson et al. 2016b). As the first animal in Norway, a quality norm under the Nature diversity act was established in 2013 (Anon. 2016). One of the quality elements in the norm is “genetic integrity”, with focus on genetic introgression of escaped farmed salmon in wild salmon populations. Among 225 analysed populations, only in one-third of the populations (75) was no genetic introgression observed, while the remaining populations showed indications (67), moderate genetic changes (16) or large genetic changes from farmed genetic introgression (67) (Diserud et al. 2019).

2 Evolution of *Gyrodactylus salaris* resistance by natural selection

In this section we discuss the possibility and timescale for evolution of *G. salaris* resistance by natural selection. We evaluate the evidence for evolution of resistance against *G. salaris* in natural populations and discuss the implications of straying (migration) between infected and non-infected populations, both when it comes to evolution of resistance and spreading of the parasite.

2.1 Tolerance, resistance and immunity to *G. salaris*.

In experiments comparing population growth, survival and reproduction of *G. salaris* on salmon from the River Neva in the Baltic (where *G. salaris* is native) with Norwegian salmon stocks it is clear that the Norwegian salmon is much less resistant towards the parasite than the Neva salmon. For example, Bakke et al. (1990) reports that the number of *G. salaris* on Lone and Alta salmon increased steadily during the experiment, while the number of *G. salaris* on Neva salmon declined after three weeks, approaching zero after 7-10 weeks. In an experiment where 10 Lone and 10 Neva fish were infected with one gravid female worm, there was a steady increase of *G. salaris* on all Lone fish, while on Neva fish the parasite population was unable to persist during the five weeks of the experiment. On some Neva fish the parasite was unable to successfully reproduce after the initial infection or soon disappeared. Cable et al. (2000) reports from an experiment that 45% of the parasites survived to give birth on Neva fish, compared to 60% on Alta and Lierelva fish. The mean survival was 3.5 days on Neva fish and 7.9 and 5.2 days on Alta and Lierelva fish, respectively. The time until first birth was longer on Neva fish, and the fecundity was lower on Neva fish, where only two births occurred compared to three to four births occurring on Alta and Lierelva fish.

The reduction in number of parasites after a few weeks on the Neva fish is good evidence for higher resistance compared to Norwegian salmon. For tolerance, we do not have the same level of evidence for a difference between Neva and Norwegian salmon. However, resistance and tolerance are not mutually exclusive host characteristics, and both may play important roles in the host's evolution of a strategy to cope with *G. salaris* infections; as might the parasite's attenuation of virulence. The parasite's lowered fecundity on Neva fish can be explained by an effective host immune system producing resistance, and its continued persistence by a more compatible host-parasite interaction (i.e. host tolerance). As the parasite is still present in Neva and other Baltic stocks, though often at low prevalence (Kuusela et al., 2005), there is no evidence for immunity (full resistance), but here the parasite causes little acute illness amongst hosts. What roles attenuation of parasite virulence, greater host tolerance, and an effective immune response play in maintaining this balance is unknown. However, because we have good evidence for evolution of increased resistance in Baltic stocks, we have chosen to focus on this useful aspect of the host reaction in the rest of the text, despite the fact that the evolution of tolerance cannot be ruled out as a mechanism contributing to host fitness in the interaction between *G. salaris* and Atlantic salmon. In this context, it is worth mentioning that populations of Atlantic salmon vary in their level of resistance and tolerance to eye fluke *Diplostomum pseudospathaceum*, with highly tolerant populations having low resistance and *vice versa* (Klemme et al. 2020). The significant genetic component of these traits suggests a mosaic of genetic responses may, therefore, also be expected from the selection pressure caused by *G. salaris.*

2.2 Conditions for *G. salaris* resistance to evolve

Because mortality is very high in rivers where East Atlantic and Barents/White Sea populations of Atlantic salmon are infected with *G. salaris*, it seems clear that there will be strong selection for increased resistance in these natural populations.

For evolution to occur, the traits need to be heritable. This means that the traits (e.g. resistance) have to have a genetic component inherited from parents to offspring, and that there is variation in the population for this heritable component (see 1.4 "The genetic basis of resistance to *G. salaris* "). A standard measure of genetic variation is the additive genetic variance (*G*). If only

one trait is under selection, the expected genetic response (evolution) of this trait is directly proportional to the additive genetic variance in the population. The expected change in the average trait value, or response to selection (R), can be computed from the equation:

$R = G\beta$,

where β is the selection gradient, a measure of the strength of selection on the trait (Lande 1979).

If more than one trait is under selection and the traits are inherited together (i.e. they are influenced partly by the same genes), predicting the evolutionary response to natural selection would need to take into account selection on all relevant traits. A consequence of this is that selection on other traits (e.g. life history traits or survival due to other factors or in other parts of the life cycle) can capture part of the additive genetic variation in resistance. In other words, the additive genetic variation in resistance is not necessarily freely available, and in a natural system where we would expect natural selection to keep the other relevant traits at their current (optimal) values, only the free component of the additive genetic variation will contribute to evolutionary change for increased resistance (for theory and a general discussion on this topic, see Hansen and Houle 2008, Walsh and Blows 2009). This means that the expected evolutionary response in resistance could be substantially reduced compared to what we would expect from measures of its additive genetic variance (see further discussion 2.5 “The potential for development of resistance by natural selection in Norwegian salmon populations”).

A premise for evolution of increased resistance or tolerance is that the infected population does not go extinct. An infected population will have dramatically reduced size and therefore an increased risk of extinction by stochastic processes (Lande et al. 2003), even if part of the population survives the infection. A population with few individuals may also be in trouble because of a phenomenon called Allee effects or depensation (Allee et al. 1949, Dennis 2002). This phenomenon arises when small populations experience lower survival or reproduction per capita than larger populations, and therefore faces an increased risk of extinction. This may be due to, for example, problems of finding mates or increased risk of predation. Indeed, there is evidence of high levels of hybridization between Atlantic salmon and brown trout in *G. salaris* infected rivers (Johnsen 2007, Arnekleiv et al. 2010, Solem et al. 2017), something that can be due to scarcity of mates. Harvesting on an infected population, well below its spawning target, will further reduce the number of spawners and hamper evolution of resistance and increase risk of extinction.

An infected population at risk of going extinct can be demographically rescued by natural immigration from other populations, so called strayers, or by stocking the river with laboratory/hatchery reared fish. However, immigration or stocking may also hamper the evolution of increased resistance through a phenomenon called *maladapted gene flow* if the immigrants have genetically low resistance. Immigrants with genetically high resistance will have the opposite effect and can speed up the evolution of resistance in the recipient population. (See further discussion of the effects of migration under 2.6 “Implications of migration”.)

2.3 The possible evolution of resistance to *G. salaris* in Norway.

Trends in density of parr in the years following the first introduction *G. salaris* to a river can give an indication of the ability of salmon populations to adaptively respond to the presence of the parasite. Data on such trends are collected by Johnsen et al. (1999), who reviewed the status for infections with *G. salaris* in Norwegian rivers up until the end of 1999. The review presents detailed data from infected salmon stocks, among others, density estimates of salmon parr older than young of year (age 0+) along with suggested years for the first introduction of the parasite in the various rivers. Data on density of parr (older than 0+) for several rivers are presented in **Figure 4**. Data from the River Driva and the River Vefsna, where we also have information on parr density after 1999, are presented in **Figure 5**. Most rivers show very low parr densities during the infection period, and Johnsen et al. (1999) estimated the mean decrease in density at 86%. In their review, Johnsen et al. (1999) also present data on the prevalence of *G. salaris* in

each population. The general pattern emerging from these data is a continuously high prevalence, in many cases all the captured fish were infected, and a continuous low abundance of parr with no sign of recovery during the infection period.

The River Batnfjordelva and the River Lierelva stand out with relatively high densities of juvenile abundance even several years after infection. Johnsen et al. (1999) in their review of the River Batnfjordelva comment that relatively high estimates of the densities of juvenile salmon many years post *G. salaris* introduction in this river must be considered highly deviant from other infected rivers. The high densities were maintained despite a high prevalence (96 - 100%) of infection among the parr (Johnsen et al. 1999). Appleby et al. (1997) studied the skin morphology of Atlantic salmon parr and found that the parr in the River Batnfjordelva had significant more cell layers and a thicker skin compared to the parr in the River Oselva that was uninfected. Arguably, a thicker skin can contribute to a higher tolerance and partly explain an apparent lower mortality from G. salaris in the River Batnfjordelva. Jansen and Bakke (1993) studied the population dynamics of *G. salaris* in the River Lierelva which drains into the Drammensfjord on the South-East coast of Norway. They found a significant decrease in the abundance of *G. salaris* with increasing size and age of the juvenile salmon. Since all fish became infected already at age 0+ and small sizes, it was argued that larger and older fish with relatively low parasite intensities apparently have some capability in controlling parasite numbers. This capability in controlling parasite intensities may in turn explain the rather high densities of salmon parr observed in this river (Jansen and Bakke, 1993). However, stocking practices in the years following infection and the water chemistry might also influence the outcome of the infections. Indeed, Hagen et al. (2019a) concluded that the water chemistry of the River Batnfjordelva is naturally restricting the infection of *G. salaris* on the salmon in this river.

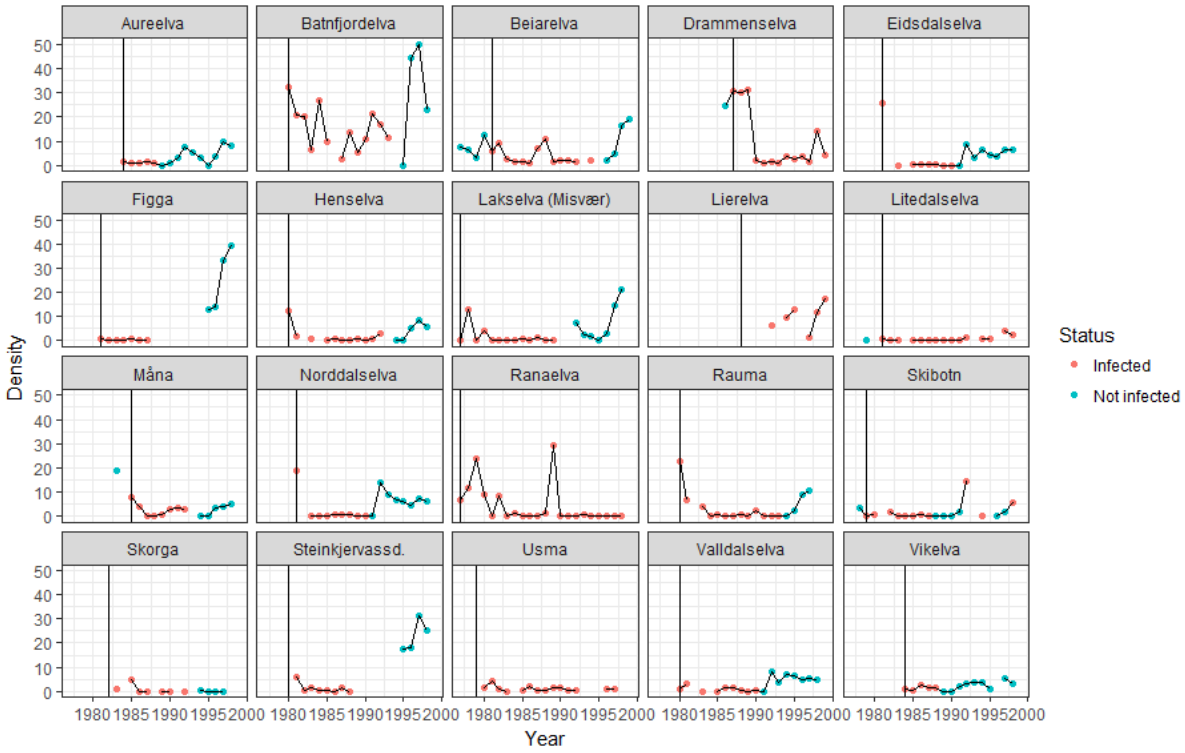

***Figure 4**. Density (N/100 m^2) for parr older than young of year in Norwegian rivers with a history of G. salaris infection. Colours indicate years with and without evidence of G. salaris infection, years without infection includes years before infection and years after treatment (rotenone). Some rivers have had several outbreaks of the parasite. Vertical line shows first year with evidence of infection. The data is reported in Johnsen et al. (1999).*

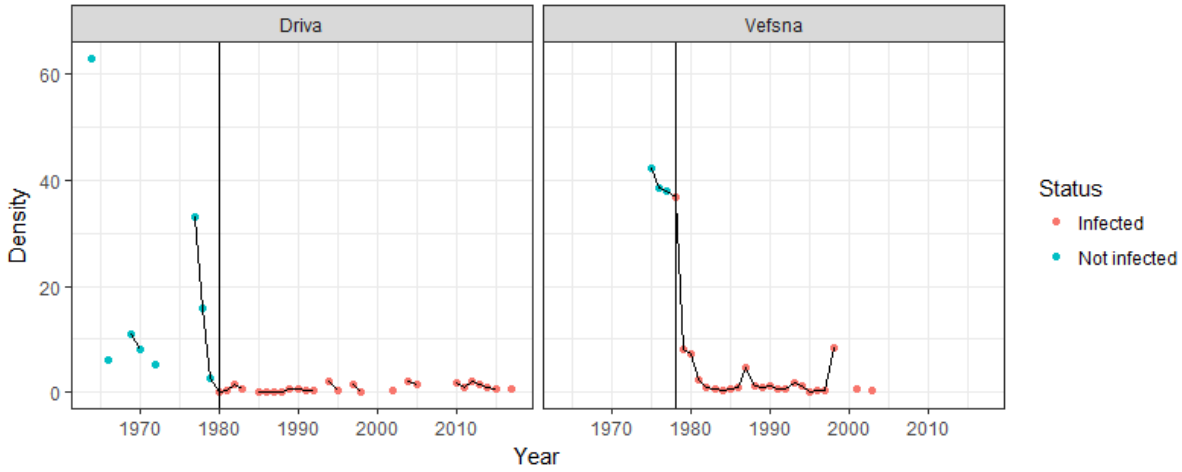

Figure 5. *Density (N/100 m^2) for parr older than young of year in the River Driva and the River Vefsna. Colours indicate years with and without evidence of G. salaris infection, years without infection includes years before infection and years after treatment (rotenone). Vertical line shows first year with evidence of infection. The data is reported in Johnsen et al. (1999, 2005), Holthe et al. (2015) and Solem et al. (2017, 2018). In the river Vefsna the density was still very low in the period 2005-2010 (Arne J. Jensen pers. com.).*

The non-recovery of juvenile abundance during the infection period can have several explanations. First, the population crash following the *G. salaris* outbreak have decreased the number of individuals well below the carrying capacity in each river. The generation time of salmon in Norway is usually between 5 and 7 years, implying that the natural recovery time of a population is quite a few years even in the absence of *G. salaris*. Second, the non-recovery suggests that there is only marginal evolutionary response in resistance or tolerance to *G. salaris* within the time frame of these infections (30-40 years). The lack of evolutionary response may be due to lack of available genetic variation, or it may be due to maladapted gene flow through straying or farm escapees. In addition, several rivers have been stocked during the considered infection period, and are therefore potentially strongly influenced by maladaptive gene flow (See Appendix 1, Table S1 for an overview of stocking).

In a recent publication, Gjerde et al. (2018) present an experiment where the survival of juveniles infected by *G. salaris* is compared between fish from three Norwegian rivers (Skibotnelva, Driva and Drammenselva) with a history of *G. salaris* infection and to rivers (Altaelva, Numedalslågen) and one region (Eira/Surna/Toåa) without such a history. In addition, fish from the Baltic River Neva in Russia (with a long history of *G. salaris* infection) was included in the experiment. Survival was measured over 84 days of infection, starting when the fish were on average 17 g. The Neva salmon had the highest survival, estimated at 69.7%, compared to both the *G. salaris* free rivers/regions in Norway (average survival of 25.3%) and the *G. salaris* infected rivers in Norway (average survival of 31.6%). In their statistical pairwise comparison between the most adjacent rivers/regions with and without *G. salaris*, the average difference in survival was estimated at 5.7±1.6%. The authors interpret this as an increase in resistance due to evolution by natural selection in Norwegian rivers. The magnitude of this evolutionary response is in concordance with the continuous low abundance of parr in infected rivers (**Figure 4** and **5**).

2.4 Resistance to *G. salaris* outside Norway

Gyrodactylus salaris is endemic in the watersheds of Sweden, Finland and Russia that drain into the Baltic sea and in many of these rivers and lakes *G. salaris* is rare on salmon and is found in low prevalence and intensity on natural populations (see e.g. Bakke et al. 2007). It is assumed that most of these Baltic strains are resistant against *G. salaris*. This assumption is based on the fact that few parasites are found on wild populations and on experimental infection experiments carried out on a few Baltic salmon populations (See Bakke et al. 2007). However, relatively few

populations have been tested and in at least one river, the River Indalsälven, several individuals showed limited resistance to the parasite (Bakke et al. 2004).

In the Russian River Keret, a river that drains into the White sea and has a population of Atlantic salmon, *G. salaris* was introduced in 1992 and had a devastating effect on the salmon population, a population which has remained low ever since (Ieshko et al. 2008., Evgeny Ieshko, pers. com.).

Atlantic salmon from Scotland has been tested and found very susceptible to *G. salaris* (Bakke and MacKenzie 1993, Dalgaard et al. 2003, Gilbey et al. 2006).

According to Nilsson et al. (2001) the populations of Atlantic salmon on the Swedish west coast carry both Baltic and Atlantic haplotypes and might thus be expected to show some resistance to *G. salaris*. In most major rivers on the Swedish west coast with a population of Atlantic salmon *G. salaris* is now confirmed present, the last being the River Kungsbackaån that was found infected in 2017 (ref www.vetinst.no). The infection levels seem to vary both within and between the different rivers and the impact on the salmon populations are less clear than in Norway, but *G. salaris* is found in high intensities on fish in at least some rivers (Alenäs 1998). The explanation for the observed differences in infection levels between the Swedish populations with Atlantic salmon and the Norwegian ones, is probably a combination of causes where resistance might be one factor, but there is reason to believe that environmental factors are more important than resistance. Alenäs (1998) speculates that temperature during summer might be suboptimal (too high) for *G. salaris* and therefore contributes to decreasing intensities in this period. From studies in the River Batnfjordelva, water quality parameters, like aluminium content, periodically reduces the infection levels (Hagen et al. 2019a).

Several experimental studies have looked at differences in resistance between populations, and at differences within population which are generally not ascribed to any particular factor (such as genetics) (Bakke et al. 1990, 2002, 2004, Bakke and MacKenzie 1993, Dalgaard et al. 2003, Lindenstrøm et al. 2006, Kania et al. 2007, 2010, Ramirez et al. 2015). These studies are based on controlled challenge tests, and in most of them both parasite counts and mortality rates have been recorded. It is important to note, however, that not many Baltic populations of salmon have been tested and that only a few strains of *G. salaris* have been used in the experiments.

Jointly, the studies show that:

- Baltic salmon populations are generally speaking more resistant than East Atlantic (Norwegian, Danish, or Scottish) populations of salmon. However, there is a substantial overlap in levels of resistance between Baltic and East Atlantic populations, and one should not regard one group simply as resistant and the other as susceptible without further evidence. Most evidence regarding the resistance in Baltic populations comes from studies on offspring from the Neva stock held at Ims research station, NINA. This stock was imported as eggs from the Laukaa central hatchery station in Finland in 1983, originally imported as eggs from Neva hatchery station in Russia, hatched in 1972, 1974, and 1976 from wild salmon caught in the River Neva (Bakke et al. 1990). This population is not representative for all Baltic populations and the strain held at Ims might not represent the genetic variation in the natural Neva population. Nevertheless, similar to the low infection levels observed on most natural populations of salmon in the Baltic, experimental work shows that intensities of *G. salaris* on the Neva strain from Ims never reach epidemic levels and are reduced to low intensities after a few weeks.
- Although experimental trials have been carried out on only a few Baltic populations, there seems to be large variation in resistance within several populations, with some animals appearing innately resistant (parasite levels remaining low throughout the experiment), some appearing responsive (parasite population grows at first, then decreases), some appearing susceptible (parasite population grows until the host eventually dies).
- In general, the growth rate of the parasite population declines from the outset of experiments. and there is little support in the data for exponential population growth.

These studies do not present formal proof that differences between Baltic and Atlantic populations are (partly) due to adaptive evolution. However, the observed differences between populations, coinciding partly with the historical range of the parasite, suggesting that adaptive evolution is a contributing factor. The recent study by Gjerde et al. (2018), described above, supports this hypothesis when comparing one Baltic population (Neva) with a selection of Norwegian populations.

2.5 The potential for development of resistance by natural selection in Norwegian salmon populations

It is likely that increased levels of resistance will evolve by natural selection, as has happened in Baltic populations. There is both additive genetic variance in survival under *G. salaris* infection (Salte et al. 2010) and Gjerde et al. (2018) report a 5.7% difference in survival in a pairwise comparison between three infected and three non-infected Norwegian rivers/regions. Based on the presence of *G. salaris* in Baltic rivers, it seems unlikely that the parasite will disappear from the rivers and it is unknown if the rivers will reach the same productivity as without the parasite. Denholm et al. (2016) predicts that salmon populations are likely to recover to high densities (90%) of that observed before the infection. However, their prediction comes from a mathematical model with several underlying assumptions (e.g. parasite attach rate to hosts, rate that the host develops the immune response and the decay rate in the immune response, listed in Table 1 of their paper).

When evaluating the potential evolutionary possibility for the development of resistance in Norwegian salmon by comparing them with Baltic salmon it is important to bear in mind that the Norwegian and the Baltic salmon represent different phylogenetic groups with a long evolutionary history in isolation, and the genetic conditions for evolving resistance in Norwegian salmon might be different from that of the Baltic salmon.

While it is likely that increased levels of resistance will eventually evolve in Norwegian salmon populations it is uncertain how long this will take. We have two lines of evidence that can inform our understanding of the time required for a salmon population to reach a certain degree of resistance/tolerance to *G. salaris*: (1) the degree of resistance/tolerance reached in Norwegian salmon stocks, some that have been subjected to the parasite for more than 40 years (however with the impact of stocking), and (2) quantitative genetic theory used in combination with available estimates of genetic variation in resistance/tolerance.

The fact that none of the infected Norwegian salmon stocks have shown recovery of juvenile abundance through the infection period, suggests that the time it will take for a natural system that is influenced by external sources, such as maladapted gene flow, is much longer than a few decades. This is supported by the relatively small difference (5.7%) in survival to *G. salaris* between infected and non-infected systems reported by Gjerde et al. (2018). It should be noted that the situation would not necessarily be better without immigration of maladapted individuals, as the severe population crash following *G. salaris* infection could have driven several populations to extinction, or potential Allee effects in addition to the cost of being in an environment with *G. salaris* could have kept the population size at a low level for a long time.

Quantitative genetic theory (see e.g. Lynch and Walsh 1998) can give us an indication of the time it takes for different levels of resistance and/or tolerance to evolve. This theory is, to a large degree, built around the breeder's equation (and its various extensions). In this equation the response to selection is given by

$R = h^2 S$,

where h^2 is the heritability and S is the selection differential.

Salte et al. (2010) estimated a heritability of survival. Their sample size was 984 fish of 25 half-sib groups (where all fish within each group shared the same father), and measured survival over a period of approximately two months, on fish with initial body weight of about 8 g. The heritability of survival on the liability scale was estimated at 0.32 ±0.10 (which corresponds to

0.17 on the binary scale). They observed an average survival of 11.3%. The response to selection can be estimated using the relation $S = i\sigma_P$, where the selection intensity i is a direct function of the survival (see Appendix 2) and, because the environmental variance is standardized to unit on the liability scale, the phenotypic standard deviation (σ_P) equals the square root of $1\text{-}h^2$. If these values are taken at face value and we assume constant heritability the expected evolutionary response in survival will be as shown in **Figure 6**.

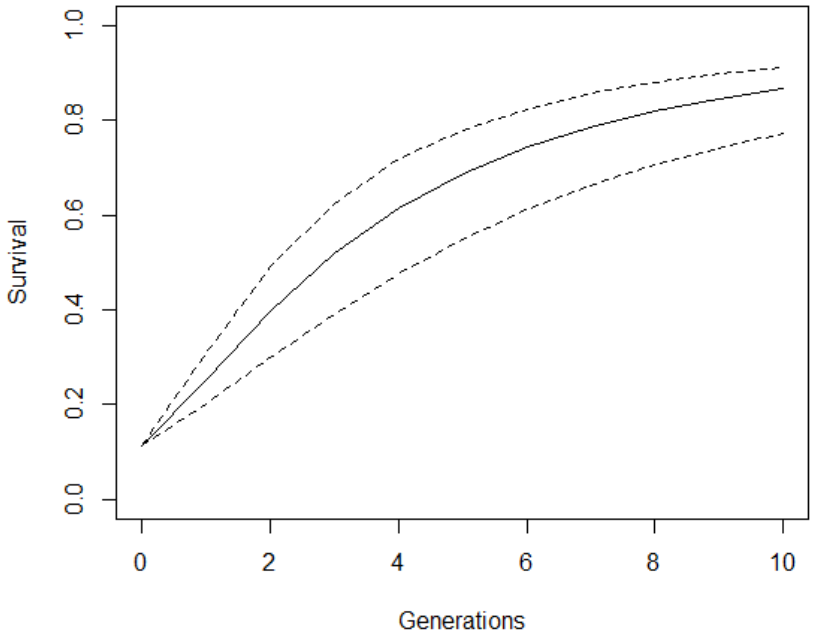

Figure 6. *Expected change in survival using the estimates from Salte et al. 2010, the solid line shows the expected change and the dotted lines show the uncertainty (± one standard error).*

The predicted response using the estimates from Salte et. al (2010) (**Figure 6**) is strong and not in concordance with the observed continuous low abundance observed in infected rivers, and the small increase in survival reported in Gjerde et al. (2018). According to the predictions, the survival is expected to more than double after the first generation. For comparison with **Figures 4** and **5**, a salmon generation in Norway is usually between 5 and 7 years, meaning that the infection period of the River Driva, 38 years, corresponds to between 5 and 8 salmon generations.

The predicted response shown in **Figure 6** assumes a constant heritability, this is a simplification of the truth. Strong selection will reduce additive genetic variance by building up genetic disequilibrium (Bulmer 1971). In addition, the additive genetic variance will be reduced due to genetic drift. To get a grip on the effect of these processes on the evolutionary response, we can use a standard theoretical model of evolution (that is developed to simplify the mathematics). This model is called the infinitesimal model as it assumes an infinite number of alleles with infinitesimal effect. Under a version of the infinitesimal model that includes the effect of linkage and drift, the evolutionary dynamics will follow the dynamics of the equation given in Appendix 2 (this equation is modified from equation 10 in Le Rouzic et al. 2011). The expected change in survival is shown in **Figure 7A.**

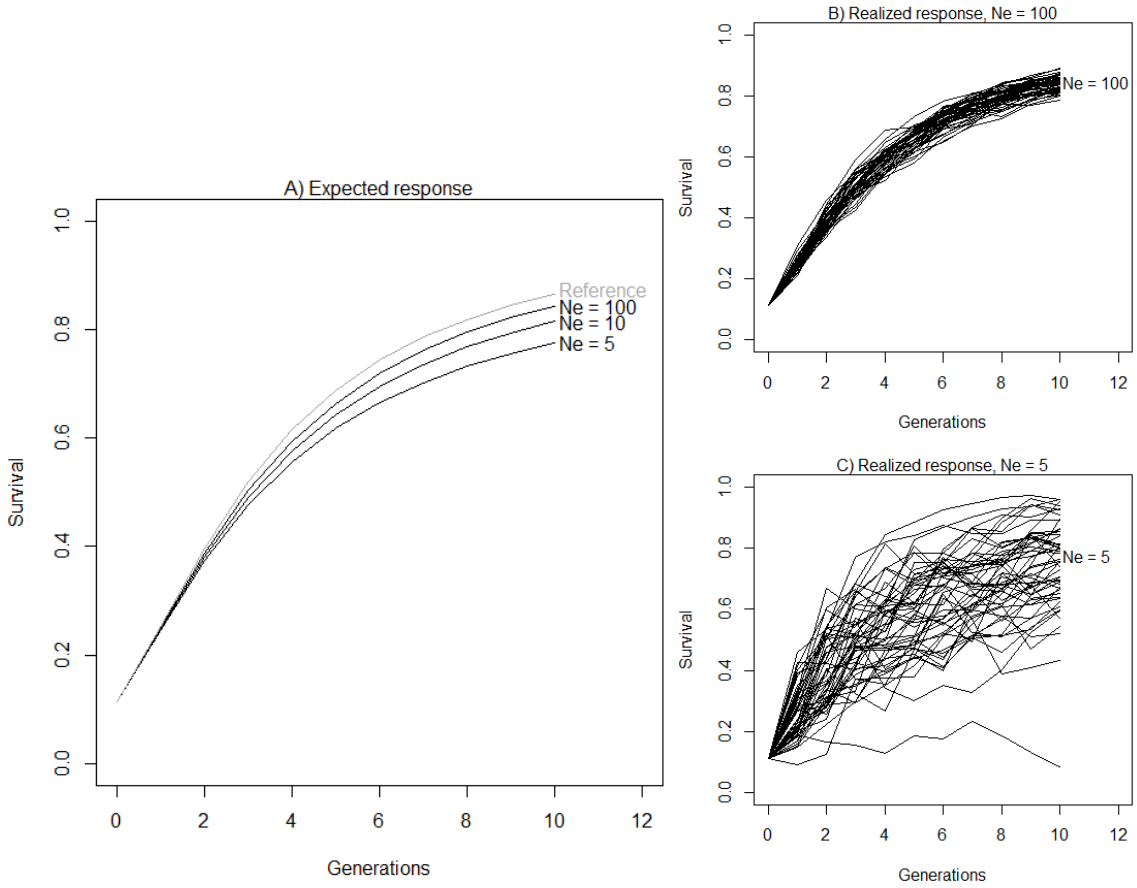

***Figure 7**. **A**) Expected change in survival with truncated selection at different effective population sizes (Ne). The naïve expectation from Figure 6 is shown in grey for comparison. See Appendix 2 for equation of expected selection response. **B**) and **C**) shows the realized response in 100 simulations with Ne = 100 and Ne = 5, respectively.*

The effects of truncated selection and drift on the expected evolutionary response of survival is relatively minor (**Figure 7A**) and rapid evolution of increased resistance is still expected even with effective population sizes as low as 5. Note that, what is shown in **Figure 7A** is the expected response, the realized response in a river with small effective population size will be highly erratic (**Figure 7C** compared to **7B**), as genetic drift will dominate over the response to natural selection in small populations. The response shown in **Figure 7** assumes that there are many alleles with small effect and is not representative if the genetic architecture of *G. salaris* resistance is dominated by a few alleles with large effects. In the case of few alleles with large effect, we expect loss of beneficial alleles of low frequency by genetic drift and the evolutionary response would slow down. Consequently, a high effective population size is important for evolution of resistance.

It is important to realize that the survival measured over approximately two months in the lab is not the same as the survival up until smoltification or maturity in the wild. Infection by *G. salaris* may lead to a reduction in survival probability throughout the life of an individual salmon. In other words, the trait that is measured in the laboratory is probably genetically correlated with the relevant trait in the wild, but it is not the same trait. It is not easy to infer the evolutionary potential of the relevant trait in question, but there are many reasons to believe that evolutionary responses shown in **Figures 6** and **7** are overly optimistic. The heritability of survival during the first couple of months of *G. salaris* infection is probably lower in the wild than in the laboratory. Survival in the laboratory for non-infected fish is generally high, and much of the variation in survival in the Salte et al. (2010) experiment was therefore likely due to *G. salaris* infection. In contrast, the survival of non-infected fish in the wild is generally low (approximately 1.5% survives from egg to smolt, Hutchings and Jones 1998), and a larger part of the variation in early survival in infected rivers is expected to be due to non-genetic sources. This will increase the

environmental variance in survival and reduce the heritability, and therefore the expected evolutionary response for a given selection differential.

Natural selection on resistance to *G. salaris* is probably not as strong as what is used for deriving the theoretical expectations above. Selection arising from mortality due to *G. salaris* is just part of the total selection. Early survival in the wild probably reflects several selective challenges, and not just *G. salaris*. In addition, the selection differentials of the various life stages must be multiplied with the Fisherian stable age distribution to calculate the contribution of selection at a particular life stage to the total selection differential of the trait (Engen et al. 2012). As the reproductive value, one of the component of the Fisherian stable age distribution, is typically low at young ages, the selection differential in the above quantitative genetic predictions are overestimated.

Conflicting selection on genetically correlated traits, for example due to trade-offs between surviving infection by *G. salaris* and surviving other environmental challenges or trade-offs between early survival and other life-history traits, can constrain the evolution of resistance to *G. salaris* (Walsh and Blows 2009, Hansen and Houle 2008). In the same way, selection for resistance can constrain adaptation to other environmental challenges and even disrupt local adaptation in traits important for fitness by indirect selection.

Taken together the different factors reducing both the heritability of and selection on resistance to *G. salaris*, and the evolutionary response shown in the figures above is surely highly overestimated. If the heritability is reduced by a factor of 1/5 to 1/10 and the selection by a factor of 1/3 to 1/6, the evolutionary response over 5 to 8 generations is small (**Figure 8**) and not in conflict with the observed lack of recovery of infected populations (**Figure 4** and **5**). These reduction factors are purely guesses, but they show that there need not be a conflict with the quantitative genetic predictions and the lack of recovery over 40 years of infection. Under these scenarios, after 100 salmon generations (corresponding to 500 – 700 years) the survival from an infection with *G. salaris* would have increased to between 32% and 75%, depending on scenario, and after 200 salmon generations (1000 – 1400 years) the corresponding numbers would be 51% and 90% (using the naïve assumptions of constant heritability and non-overlapping generations) (**Figure 8**).

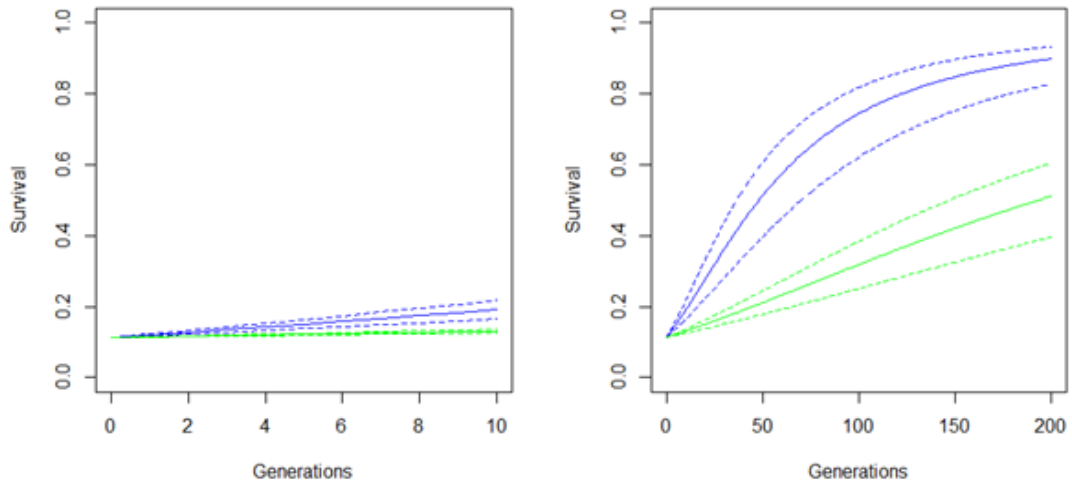

***Figure 8**. Expected change in survival using the estimates from Salte et al. (2010) with heritability reduced by a factor of 1/10 (green) and 1/5 (blue) and a corresponding reduction of selection differential by a factor of 1/6 and 1/3. The dotted lines give the predicted response for the h2± one standard error. In the left panel, the change is plotted over 10 generations of selection, and in the right panel, over 200 generations of selection.*

An additional factor that may affect the response to selection is systematic interaction among genes (epistasis) (Hansen 2006). If the genes systematically increase the effect of each other

this can speed up evolution, while the opposite is true for genes that systematically decrease the effect of each other. This, and the above discussion serves to illustrate that predicting evolution in the wild is hard. Our quantitative predictions based on quantitative genetic theory are only in the best of cases uncertain and not wrong. However, the empirical evidence (see sections 2.3 and 2.4) and the above considerations suggest that the time it will take until a substantial degree of resistance has evolved is probably on the order of hundreds of years (or longer) rather than decades. Here it must be noted that large evolutionary responses over hundreds of years is considered very rapid evolution, although there are many examples of rapid evolution in the literature (see e.g. Uyeda et al. 2011).

2.6 Implications of migration

The natural rivers are not closed systems, and thus, so called strayers will be part of the spawning population and reproduce in a river where it is not born. This leads to gene flow, exchange of genetic material, between rivers. There is gene flow between neighbouring rivers in Norway (Ozerov et al. 2017), though not to the extent that it hampers local adaption (Jonsson et al. 1991, Barson et al. 2015). Degree of gene flow can be measured using the fixation index (F_{ST}). This index measures the amount of molecular genetic variation that is not shared among populations relative to the total molecular genetic variation. A typical F_{ST} for rivers in a fjord system is on the order of 1-5% (Ozerov et al. 2017, Wacker et al. 2019).

Using theoretical models of idealized population structure, we can transform estimate of F_{ST} into effective numbers of immigrants. Effective numbers of immigrants is defined by the effective population size (N_e) multiplied with the per generation rate of immigration (m). The effective population size is a theoretical concept introduced by Ronald Fisher and Sewall Wright to simplify evolutionary theory. In natural populations it is almost always lower than the census population size, but to what degree depends on the biology of the population. Under Wright's classical island model (Wright 1931, 1943), that assumes infinite number of populations each with the same effective population size and the same number of immigrants the effective number of immigrants can be approximated by

$$N_e m \approx \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right).$$

For F_{ST} ranging from 5% to 1%, this gives approximately 5 to 25 effective number of immigrants per generation. For finite number of populations, the approximation becomes (Takahata and Nei 1984):

$$N_e m \approx \frac{N_{pop}-1}{4N_{pop}}\left(\frac{1}{F_{ST}} - 1\right),$$

where N_{pop} is the number of populations. Hence for two populations with symmetric gene flow effective number of immigrants is reduced to approximately 2 to 12 per. generation.

To understand the impact of immigration on the evolution of resistance to *G. salaris* it is useful to consult theoretical models on how a phenotypic trait changes with immigration. The mean trait value after immigration (z') can be approximated by the following equation (Tufto 2000):

$$z' \approx (1-m)z + mz_{imm},$$

where z is the average trait value in the recipient population and z_{imm} is the average trait value of the immigrants. In addition to a direct change in the mean trait value, the genetic variance in the trait will increase because of the genetic differentiation between the populations. The genetic variance after immigration G' can be approximated by the equation

$$G' \approx (1-m)G + mG_{imm} + m(1-m)(z - z_{imm})^2,$$

where G is the additive genetic variance of the recipient population and G_{imm} is the additive genetic variance of the immigrants. If the additive genetic variance of the population and the immigrants is approximately the same, the equation simplifies to

$$G' \approx G + m(1-m)(z - z_{imm})^2,$$

where only one term in the equation will influence the change in additive genetic variance. This term will be large when the two populations have a large genetic difference in average trait value and the migration rate is close to 0.5.

For a small population, say with an effective population size of 30, the immigration rate *m* may be between 0.07 and 0.40, and the effect of the immigrants on the average trait value in the population, in our case the resistance to *G. salaris*, can be substantial. This means that such populations will be hampered from evolving resistance when facing gene flow from neighbouring populations without *G. salaris*. On the other hand, neighbouring rivers that have evolved a higher degree of resistance will in such systems be of great benefit. In larger rivers with an effective population size of about 100 and an immigration rate m between 0.02 and 0.12, the effect will be smaller. The increase in additive genetic variance following gene flow will in all cases speed up the evolution towards higher resistance, but more so in small rivers with high immigration rates than in large rivers where the immigration rates are expected to be smaller.

Immigration of maladapted individuals may not only come from strayers. Several populations with *G. salaris* infection have been stocked (see Appendix 1 Table S1). The effect of stocking on the evolution of resistance depends on the stocking practise. If the broodstock is chosen among fish that have survived *G. salaris* infection, the stocking may potentially speed up evolution (also see discussion under selective breeding for resistance), while if the broodstock is chosen among fish not selected for increased survival towards *G. salaris* infection, the stocking may strongly hamper the evolution, depending on the magnitude of the stocking. Similarly, we would expect gene flow from escaped farm salmon (Karlsson et al. 2016b) to hamper evolution of resistance.

In non-infected rivers, gene flow from rivers that have developed high resistance to *G. salaris* infection will lead to evolution of higher resistance. This effect will be larger in small populations than in large. If the traits underlying resistance to *G. salaris* are costly, however, there will be selection for reduced resistance in these rivers, and the evolutionary response following this selection will be increased because of the increase in additive genetic variance following gene flow. The degree of resistance evolved will depend on the migration rate, population size, degree of resistance in the immigrants and the cost associated with resistance.

2.7 Evolution of resistance and the risk of spreading *G. salaris*

The spread of *G. salaris* from resistant populations will depend on the possibility of *G. salaris* to survive on a fish migrating between rivers in a fjord system, but also to what degree host-resistance has an impact on the probability of spreading the parasite by altering host - parasite population dynamics.

With respect to inter-river spread of *G. salaris*, it is worthwhile to be aware of the mechanisms by which the parasite spreads to new rivers. In a risk assessment considering various potential pathways for the inter-river spread of *G. salaris*, Høgåsen et al. (2009) concludes that such spread is unlikely by any other means than the spread of infected hosts. Here we adhere to this conclusion, implying that inter-river transmission by movement of free-living parasites is not considered. Spread of infected hosts can occur naturally in brackish water between neighbouring rivers, or by humans translocation of infected fish.

The historic spread of *G. salaris* to Norwegian rivers is relatively well accounted for. Johnsen and Jensen (1986, 1991) and Johnsen et al. (1999) traced the sources for spreading the parasite to the initially infected rivers in Norway (see the introduction section for more details). Based on this work, Jansen et al. (2007) identified 18 primary infected rivers in Norway, of which only the River Lærdalselva and the River Beiarelva were infected by an unidentified source. The rest of the primary infected rivers were either infected directly through stocking of infected fish from a known source farm (9 rivers), through associations with farms that had received infected fish from the same source farm (6 rivers) or in association with a transport of infected fish (1 river). From these primary infected rivers, the spread to all other known *G. salaris* infected rivers can be explained by secondary infections arising from migrating infected fish. The introduction history

and further spreading within Norway was later supported by molecular genetic analyses (Hansen et al. 2003). Jansen et al. (2007) found an intimate association between postulated inter-river transmission of *G. salaris* and fjord-wise inter-river distance, along with freshwater inflow into the area through which potentially infected fish must migrate to spread the parasite. In short, this suggests that decreasing inter-river distance between river outlets increases the probability of inter-river transmission. Furthermore, decreasing salinity through increasing freshwater inflow into fjords implies that *G. salaris* transmission can be expected over larger fjord-wise distances. It is important, however, to emphasize that inter-river transmission of *G. salaris* by migrating infected fish through brackish fjord areas has not been observed directly. Nevertheless, local distributions of infected rivers (Johnsen and Jensen 1986, 1991, Johnsen et al., 1999) and studies of the salinity tolerance of this parasite (Soleng and Bakke 1997, Soleng et al. 1998) supported the hypothesis suggesting brackish water migration of infected fish as a pathway for the spread of *G. salaris*. This principle has also been the subject of quantitative risk-analyses. Høgåsen and Brun (2003) quantified the annual risk of *G. salaris* spreading from the River Drammenselva and the River Lierelva to nearby rivers. They calculated an annual probability of 31% for the parasite to spread to the River Sandeelva, but also showed that the probability is strongly dependent on the number of smolt leaving the rivers. This river was shortly after diagnosed as *G. salaris* infected. In sum, a large body of circumstantial evidence has built up to support the hypothesis of inter-river dispersal of *G. salaris* through migrating infected fish in fjord-systems (Jansen et al. 2007).

The probability of spreading the parasite from a resistant host population may be affected by the development of resistance in itself. At one end of the scale, a host-population may develop innate immunity to the parasite, in which case the parasite may go extinct in the host-population and further spread would be prevented. To our knowledge, however, there are no salmon populations in Norway that are innately immune to pathogenic strains of *G. salaris* in Norway.

At the other end of the scale, a host population may develop higher tolerance to parasite infection, in which case the host-population could sustain a higher host population density without reducing parasite prevalence or abundance (Appleby et al. 1997, Appleby and Mo 1997, Mo 1992). In this case, development of tolerance may increase the probability of spreading the parasite to neighbouring rivers since an increasing number of infected fish would migrate out from the tolerant population. Subtle development towards tolerance to *G. salaris* infection in Norwegian populations of salmon has been discussed, for example with regard to The populations in the River Lierelva and the River Batnfjordselva (Jansen and Bakke 1993, Johnsen et al. 1999). These two rivers have been reported to sustain relatively high densities of juvenile salmon, but nevertheless with an abundance of *G. salaris* on juvenile salmon (Jansen and Bakke 1993, Johnsen et al. 1999). From this, it is consensus in the present project group that development of tolerance to *G. salaris* infection in Norwegian river-populations of salmon most likely would contribute to increase the rate of spread of the parasite to neighboring rivers within an infection region.

There are now only two regions in Norway where the salmon remain infected by *G. salaris*; the Driva region and the Drammens region. Development of tolerance and consequences for further spread of infection is therefore of interest to evaluate specifically for these regions.

Consider for example a hypothetical situation where *G. salaris* was cleared from all rivers in the Drammen region except for the River Drammenselva, in which a degree of tolerance to the parasite developed. The spread of *G. salaris* to the River Lierelva would then be expected to occur very soon since these rivers drain into the fjord practically at the same location. Further spread to the River Sandeelva would also be expected to be inevitable since this river drains into the fjord 39 km from the outlet of the River Drammenselva, and there are large volumes of freshwater that drain into the area that separates the outlets of these rivers (Jansen et al. 2007). *G. salaris* has not spread further through the fjordsystem from the Drammens region during more than 30 years of *G. salaris* infection in the River Drammenselva and the River Lierelva. The Drammens region is described in detail regarding fish communities and hydro-morphology and further risks of spread of *G. salaris* to other regions by Hindar et al. (2018). The probability of further fjordwise

spread to the large salmon river, the River Numedalslågen, or to the River Åroselva were estimated to a yearly probability of less than 1% in the original risk analyses of Høgåsen and Brun (2003). An update of this analysis, however, takes account of new information about extreme freshwater inflow to coastal areas. In this analysis, the yearly probability estimate for the River Åroselva increases to 8% for new observed measurements of salinity. A higher freshwater inflow would not increase the risk of spreading the parasite to the River Numedalslågen in itself, but there would be an increased risk (2%) from a larger number of smolts that would migrate out from the infected rivers in the Drammensregion (Høgåsen 2016).

3 Selective breeding for resistance

In this section we discuss the potential and implications for establishing a breeding program for increased resistance to mitigate infected rivers.

3.1 Breeding programmes for Atlantic salmon

Norwegian farmers of Atlantic salmon use eggs from one or more out of a handful of domesticated strains. These strains originate from Norwegian rivers (Gjedrem et al. 1991), and they have been selectively bred for up to 13 generations. Farmers of Atlantic salmon require a fish which grows fast in the farm environment, does not sexually mature until the production cycle has ended, and resistant to the diseases and parasites found in aquaculture. Up until the end of the 1980's, growth rate and (absence of) premature maturation were the only direct selection criteria. Since then, resistance to bacterial, viral, and amoebic pathogens, and to parasites (*Lepeophtheirus salmonis*; sea lice), has become a major part of the breeding goal of most or all domesticated Atlantic salmon strains in Norway. The breeding companies regard these as isolated, though sometimes correlated, problems (i.e. they select for resistance to specific diseases). The means to do selective breeding on resistance to diseases/pathogens is to subject fish to challenge tests. In challenge tests for resistance against bacteria or viruses, the fish are exposed to the pathogen through intra-peritoneal injection of pathogen (i.p. challenge), through pathogen added to the water (bath challenge) or through contact with pre-infected cohabitants (cohabitant challenge). The recorded phenotype (trait) is typically the survival/mortality status, or days of survival, of each fish. In some instances, the challenge test does not induce mortalities, or the Food Safety Authorities do not permit mortalities. Challenge tests for some viral diseases, for example, use viral concentrations, estimated through quantitative real-time PCR (qPCR), as phenotype. Resistance to sea lice is quantified through counting of number of lice per fish, following standardised exposures to lice. Lice are counted manually or semi-manually, using digital imaging.

Only very rarely can challenge tests for disease resistance, sea lice etc., used in aquaculture breeding programmes, be applied directly on the breeding candidates. As a rule, pathogens and parasites must be kept away from the breeding candidates, in order not to spread the pathogens and/or parasites further. In such cases, the breeding companies resort to so-called family selection. In a family-based breeding programme, the breeding population consists of a number of more or less equally-sized families (sibling groups), and the family-ID of each animal is known and retained through the life cycle using physical tagging or DNA-based family assignment. Family-based selection facilitates breeding for traits that cannot be registered in breeding candidates, such as disease- and lice resistance. In a family-based breeding programme, siblings of the breeding candidates are tested for resistance to diseases and/or parasites. The parents of the next generation are then selected from the families that displayed the highest survival or lowest lice count. Importantly, family selection also facilitates more precise inbreeding control.

Up until 2009, family-based selection facilitated selection of fish from the best families, but selection was random within family. That is, successful breeding candidates were randomly picked animals from the families that had, for example, highest survival in challenge tests. In 2009, a new era began, with the introduction of DNA markers in breeding, so-called marker-assisted selection (MAS). Helped by development in molecular genetics, the breeding companies and their research partners were able to locate genes with large effect on traits of importance, or more precisely, DNA markers linked to such genes (reviewed e.g. in Yañez 2015). The first trait targeted by this methodology in salmon was resistance to IPN. Research revealed that the genetic component of resistance to IPN was determined almost exclusively by one gene (Houston et al. 2008, Moen et al. 2009). By employing DNA-markers located within or close to this gene, the breeding companies could identify the animals carrying 2 or 1 copy of the resistance allele at the gene. Thus, selection could target not only the best families, but also the best individuals within the best families. Following the successful use of MAS for IPN resistance (the number of IPN outbreaks was reduced by 75 % from 2009 to 2016) (Moen et al. 2015), MAS was introduced for various other disease and quality traits. In 2013, another milestone was reached, with the

implementation of so-called genomic selection (GS), a more mature form of MAS (Meuwissen et al. 2001). In GS, breeding candidates are genotyped using a very large number of DNA markers scattered throughout the genome. Siblings of the breeding candidates are also genotyped, but these are also phenotyped, e.g. tested for resistance to particular diseases or sea lice. Using the (very many) DNA markers, very precise genetic relationships between animals are calculated, and the successful breeding candidates are those who, genetically speaking, most resemble the most disease resistant/sea lice resistant/fastest growing animals. In a typical scheme for selective breeding in salmon aquaculture today, the breeding candidates would be genotyped for ~50,000 DNA markers.

The response to selection for increased sea lice resistance is of interest in this context. No scientific papers have yet been published on the matter, but some benchmarking results have been presented by the breeding companies. One company compared the lice resistance of 1) a high-resistance (HR) sub-population having been selected for high lice resistance for two generations using genomic selection, 2) a low-resistance (LR) sub-population having been similarly selected for low lice resistance for two generations, and 3) a sub-population which had not been selected for sea lice resistance (see https://aquagen.no/2017/12/08/avl-luseresistens-virker-2/). Smolts from these three groups were challenged with relatively high doses of lice in a common garden experiment, and number of lice per fish were counted after a few days. Following de-lousing treatment, the fish were challenged again, and a second lice count was performed. At the first round, the mean lice count was 40 % lower in the HR group compared to the unselected control. At the second round, the mean lice count was 45 % lower in the HR group compared to the control. The corresponding differences between the HR group and the LR group was 53 % and 55 % in the two rounds, indicating that selection for high resistance had been more effective than selection for low resistance. In a similar study, another breeding company found that the HR group had on average 29 % fewer lice than the LR group, after genomic selection for lice resistance had been carried out for one generation (Matt Baranski, Mowi, pers. comm.). These studies were performed in laboratory settings, where high lice pressures can be applied and noise factors can be controlled. In aquaculture production settings, good estimates of gain in lice resistance due to selective breeding are harder to obtain, because lice pressures are affected by many variables and because the number of lice is kept very low at any site due to legislations.

3.2 Factors to consider when setting up a breeding programme

The purpose of a selective breeding programme is to facilitate genetic gain for one or more traits. Selection should be long-term, i.e. the possibilities for continuous improvement on a long term must not be exhausted. The trait which is selected for must be relevant for the biological improvements one wishes to bring about.

The breeding value is a central concept in breeding. The breeding value is the genetic merit of an animal for the trait in question; if the breeding value is above average, then the animal has “better than average genes” for the trait in question. The true breeding value cannot be observed, but an estimated breeding value can be calculated on the basis of available data.

The rate of genetic gain is determined by four factors (e.g. Falconer and MacKay 1996):

- It is proportional to the accuracy of selection. The accuracy of selection is the strength of the relationship between the true and the estimated breeding value. If selection is done only on the basis of direct observations in the breeding candidates, the accuracy of selection is equal to the heritability. If additional data can be made available, e.g. by inclusion of data from relatives, the accuracy of selection can be made higher than the heritability. In other words, if many sources of data are used, the accuracy can be high even when heritability is low.
- It is proportional to the intensity of selection. The intensity of selection is the (standardised) difference between the mean trait value of the selected animals and the overall population mean. Thus, the higher the intensity of selection, the more superior the selected animals are.

- It is proportional to the phenotypic variation within the population. In other words, the larger the variation among animals in the population are, the more potential there is for genetic improvement.
- It is inversely proportional to the generation time. If the generation time is three years, the rate of genetic change will be twice as fast compared to if the generation time is six years.

The accuracy of selection can be improved in several ways. In modern breeding programmes, the accuracy is commonly improved by incorporating registrations from relatives on top of registrations from the breeding candidates themselves. The observed phenotypic value of an animal is influenced by environment, uncertainty in registration, and other random factors. Similar data from relatives will bring the estimate closer to the true breeding value, and the closer the relatives are to the candidate, the better.

The accuracy of selection can also be improved by incorporating other information about the candidates' genes, on top of the observed phenotype. For example, an animal which has 'good genes' may by chance end up with poor observed trait value. Other observations which could shed light on the selection candidate's genes would be helpful. During the past two decades, methodology (marker-assisted- and genomic selection) has matured which makes it possible to directly read the genes of animals, predicting whether the animal carries 'good genes' or not.

A breeding programme is long-term, aimed at continuous improvements without an end-point. However, in order not to exhaust the potential for long-term improvements, inbreeding must be kept in place. Thus, a breeding programme must be formed on a base population with sufficient genetic variation, and care must be taken not to mate close relatives. In a modern breeding programme, the rate of increase in inbreeding is estimated, and is not allowed to rise about a set threshold value (e.g. Gjerde et al. 1996).

Finally, the observed trait must be relevant for the trait that one wishes to improve. Very often one can measure only traits which are correlated to the trait of interest. In aquaculture, for example, resistance to diseases is a key breeding goal. But since one usually cannot do selective breeding on animals that have been exposed to disease outbreaks, one must resort to controlled challenge tests performed in the laboratory. These tests are often done using only one particular strain of the pathogen, delivered in doses which may be different from the ones experienced by fish in the sea cages, in an environment which is very different from the sea cage environment. Sometimes, challenge tests lead to infection but not mortality in the tested animals, and one must resort to alternative phenotypes correlated to survival, such as virus concentration in tissue (measured using e.g. quantitative real-time PCR). In such cases, the response to selection hinges on the measured trait being sufficiently correlated to the trait one wishes to improve.

In regard to a practical feasibility of setting up a potential breeding program for wild salmon, there is much experience, knowledge, and several facilities for setting up hatcheries that can include a selective breeding regime for developing resistance for *G. salaris.* There are 49 different stocks of Atlantic salmon, brown trout or Arctic char kept in the live gene bank of Norway. These are kept in five different hatchery facilities (Bjerka, Haukvik, Herje, Hamre and Ims under development), in addition to three other supporting facilities (Kårvika, Leirfjord, and Eidfjord). In addition to the live gene bank there are cryopreserved sperm (milt) from about 230 different stocks (see https://www.miljodirektoratet.no/). The live gene bank was established in 1989 and cryopreservation of sperm started in 1986. The main objective of the salmonid gene bank is to secure genetic integrity and genetic variation in threatened populations until the environmental conditions are good enough for the stocks to uphold a natural strong production and viability. In addition, about 60 populations of Atlantic salmon are being stocked by locally hatchery produced fish.

3.3 How to breed for resistance to *Gyrodactylus salaris*?

If left alone, a river population of Atlantic salmon might develop resistance to *G. salaris* through natural selection. According to experience from infected rivers, however, chances are high that

the mortality inflicted by *G. salaris* would reduce the size of the population severely before a sufficient degree of resistance can be obtained. The reduced number of broodstock could lead to a population bottleneck, leading to inbreeding and potentially reduced fitness.

As an alternative, one might envision a breeding programme for resistance to *G. salaris*, modelled on the breeding programmes for disease- or parasite-resistance found in aquaculture. Broodstock could be taken from rivers to form a base population. The population could be propagated in the hatchery for multiple generations in order to build up resistance. Controlled challenge tests could be used in order to assess the *G. salaris* resistance of individuals. Survivors from these tests could be used as parents for the next generation, or some other criteria such as *G. salaris* count could be used.

The two strategies laid out above (no intervention vs a closed breeding programme) are extremes from a continuum of options. If the first strategy is followed, natural selection for *G. salaris* resistance in the population's home environment will act, but the population may be severely harmed because the selection intensity cannot be controlled. If the second strategy is followed, resistance can be built up across multiple generations in an isolated environment, but domestication selection and genetic drift may diminish the populations' fitness in its own home environment. In between the two options, however, there are several 'hybrid' strategies:

1. **Reinforcement** of the population through stocking: Stocking of juveniles could be used in order to keep up a population which is threatened by *G. salaris*. In some *G. salaris*-infected rivers like e.g. the River Drammenselva, juveniles have been stocked in *G. salaris*-free sections of the river. In this way, the population is reinforced in number, but the stocking does not contribute to increase the population's resistance to *G. salaris*. On the contrary, stocking is likely to counteract the build-up of resistance, because the stocked fish are not subject to natural selection in the same way as naturally produced juveniles are. If juveniles were stocked in *G. salaris*-infected parts of the river, however, the strategy would perhaps serve to keep the population's numbers up while resistance is slowly built up through natural resistance.
2. **Capture of surviving smolts**: Out-migrating smolts, hatched in *G. salaris*-infected sections of the river, could be captured and grown to maturity in a shielded environment (tanks on land or net-pens in the sea), and be used as broodfish for stocking. Light- and temperature manipulation could be used in order to shorten their time to maturity, i.e. to increase the rate of genetic gain across generations.
3. **A semi-closed breeding programme for resistance to *G. salaris***: Controlled challenge-tests for resistance to *G. salaris* could be carried out in the local hatchery. Resistant or responsive individuals from these tests could be raised to maturity in the hatchery, with light- and temperature control in order to bring down the generation interval. Resistant/responding individuals from challenge test would be used as parents of the next generation, but also supplemented by naturally born individuals that have survived a whole life cycle in the infected river. Excess eggs, from production of the next generation, could be stocked in the river. Likewise, excess resistant individuals from challenge test could be released as parr or smolts. The fraction of broodstock taken from the river (and not from the hatchery) could be varied according to the status of the population, as could the number of juveniles released. Thus, for the first generations, one might need to use mostly broodstock coming from the hatchery. As resistance builds up in the river due to the concerted actions of artificial and natural selection, one would gradually increase the fraction of broodstock born in the river. Subsequently, one would start to reduce the number of juveniles stocked. The genetic progress could be monitored by comparing survival of hatchery-derived offspring with that of river-derived offspring.

Controlled challenge tests can be run until mortality only with permission from the Food Safety Authorities. If such a permission is not given, challenge tests could utilise parasite counts on individuals. Counting *G. salaris* on hundreds or thousands of animals would be labour-demanding, but feasible. Technology for automated, imaging-based counting of sea lice has been devel-

oped for aquaculture (see e.g. http://en.stingray.no/). Possibly, similar technology could be developed for automated counting of *G. salaris*. However, there is no guarantee that the technology can be developed for use on live fish, as a *G. salaris* is much smaller than a sea lice and nearly invisible on live fish. To our knowledge, no study has estimated genetic correlations between *G. salaris* counts and survival during a *G. salaris* infection, and more research is needed. However, as noted above the research that has been done indicates that the ability to "turn" an infection is found in many individuals; the important matter is the time it takes to achieve this change (e.g. Bakke et al. 2002, Ramirez et al. 2015). Therefore, although more research is needed, it seems likely that the most resistant individuals will harbour few parasites rather than many, so that one might expect a (numerically) negative correlation between survival and parasite intensity.

As described above, marker-assisted and genomic selection can be used in order to increase the accuracy of selection. Marker-assisted selection (MAS) can be used if a large fraction of the genetic variance is explained by a single locus. Genomic selection is a more generic, though also more expensive method, which acts on the entire genome. Selection for a trait like resistance to *G. salaris* could be carried out without either methodology, and with sufficient funding, use of these methods would increase the rate of genetic gain and provide better inbreeding control.

In a typical MAS scheme, DNA-markers 'tagging' the locus which has an effect on the trait (called a quantitative trait locus, QTL) have already been identified through research experiment, and the identity of the good and bad alleles at these markers are known. The DNA-markers in question are interrogated using some inexpensive genotyping assay, where the readout is the alleles at the DNA-markers in question and little more. Costs per animal would be approximately 100 NOK per animal, for DNA-extraction and genotyping. The results could be used to narrow down the list of good breeding candidates. For example, if the number of survivors from a *G. salaris* challenge exceeded the number of broodstock needed, one could select only those animals carrying the good allele at the QTL.

In a genomic selection (GS) scheme, a very large number of DNA markers scattered across the genome are needed. Thus, the cost per animal for DNA extraction plus genotyping would be something in the range of 200-400 NOK per animal. Furthermore, with GS, a reference data set is needed, consisting of relatives of the breeding candidates, having both genotypes and phenotypes. Genomic selection will increase the rate at which resistance is built up, by increase our ability to pinpoint the very best animals. GS will also allow a reduction in inbreeding, because very precise estimates of the relatedness between animals are obtained, which can be used when matings are performed.

3.4 Impact of genetic architecture

The term 'genetic architecture' refers to how genetics influence a trait. Some traits may be genetically determined mostly by only one gene, the case of IPN in Atlantic salmon being one rare example. More commonly, traits are polygenic, i.e. they are controlled by tens, hundreds or even thousands of genes. Usually, very little is known about how many genes a trait is influenced by, or what the identities of these genes are. Obtaining good answers to these question is very demanding, because 1) many genes may have real, but very small effects, 2) genes may interact with each other in complex ways, 3) the effects of any particular gene may vary from population to population, and according to which phenotype is measured or how it is measured, 4) the genomes of higher organisms contain millions or tens of millions of variable positions (DNA polymorphisms), very many of which have not yet been uncovered, and 5) in any one study, only a fraction of the DNA polymorphisms can be assayed. Note that the use of the work 'gene' is a simplification: in reality, phenotypic variation is created by DNA polymorphisms in the genome, but since each of these DNA polymorphisms affects some gene or another, these are often referred to as 'genes'.

One thing can, however, be assessed with relative ease and certainty: whether or not a trait is influenced strongly by only one or a handful of genes. If a trait is found to be controlled by only a few genes, marker-assisted selection can be used to select for the trait. MAS is a relatively inexpensive and simple method, which may yield significant genetic improvements within a short

time frame, if but only if the trait is controlled by genes of large effect. Once the genes of large effect have become fixed or almost fixed in the population, MAS is no longer relevant.

MAS may be relevant for some traits. Many viral resistance traits, in particular, have been found to be controlled by single genes to a large extent, in Atlantic salmon. Most traits are, however, not controlled by single genes to any large extent, i.e. most traits are polygenic. Polygenic traits should be handled by family selection or genomic selection, as described above.

The available data indicate that resistance to *G. salaris* is a typical polygenic trait, influenced by a large number of unknown genes. This finding is in line with findings regarding resistance to sea lice, another ectoparasite. Data coming from the breeding companies have indicated this trait to be highly polymorphic.

Genetic architecture will, reasonably, have an impact also on what genetic changes are facilitated through selection. If a trait is controlled partly by single genes with large effect, selection will act strongly on the loci in question, eliciting substantial changes in allele frequencies, and a local reduction in heterozygosity (genetic variation), at the loci in question. If selection is carried out in multiple rivers, unidirectional selection will occur in multiple populations, leading to reduced variation at that locus on the across-population level. Additionally, if resistance is to a large extent determined by only one or a few loci, resistance built up through selective breeding might be more easily reversed through adaptation in the parasite: fewer biological pathways might be underlying the built-up resistance, so that the parasite would have fewer barriers to break in order to regain virulence.

If a trait is not controlled by major genes, selection will work on a large number of loci with small individual effects. According to theories of quantitative genetics, selection will then lead to only small allele frequency changes at individual loci. Furthermore, slightly different sets of genes are likely to be targeted in different populations, due to differences in genetic background and environment. Indeed, Karlsson et al. (2011) searched for loci subject to unidirectional selection across all Norwegian breeding populations of Atlantic salmon, using a ‘metapopulation’ of wild salmon from 13 different rivers as control. Although a set of DNA markers was found which could, jointly, be used to distinguish wild salmon from farmed salmon, no single DNA markers were found displaying a very large overall contrast in allele frequency between wild- and farmed salmon (among the ~6,000 which were interrogated). No loci were found to be fixed in opposite directions in the wild- and farmed metapopulations.

The available data indicate that resistance to *G. salaris* is a polygenic trait, not affected by major genes. Selection for resistance to *G. salaris* in itself is therefore not likely to elicit dramatic genetic changes at individual loci. Domestication selection and inbreeding are other processes which may come into play. These are discussed below.

Genes can affect more than one trait, a phenomenon known as pleiotropy. This means that selection on a trait affect other traits through shared genes. We know little about the genetic architecture of resistance to *G. salaris* and the genes it may share with other traits. In aquaculture breeding, resistance to specific disease (in particular, resistance to viruses) has generally been found to be uncorrelated to other measured traits, and negative correlations between disease traits have not been documented. It would be naïve, however, to assume that increased resistance would have no effect on other fitness related traits. Life-history theory predicts trade-offs between fitness related traits (Stearns 1989), and it seems reasonable to assume trade-offs between surviving infection by *G. salaris* and surviving other environmental challenges or trade-offs between early survival and other life-history traits (see also discussion under 2.2 “Conditions for *G. salaris* resistance to evolve” and 2.5 “The potential for development of resistance by natural selection in Norwegian salmon populations”).

3.5 Consequences of selective breeding for resistance to *Gyrodactylus salaris* for the genetics of wild stocks

As noted above, selection for increased resistance is not likely to elicit substantial allele frequency changes, or locally reduced heterozygosity, as long as the trait is polygenic in nature. A

closed breeding programme, taking place in the hatchery for several generations without gene flow to and from the river, could lead to domestication selection. Such a programme could also potentially lead to inbreeding. This is one reason why we recommend an open programme with gene flow to and from the river. However, measures could be taken to reduce increase in inbreeding to a sufficiently low level. General important guidelines for hatchery produced and released fish are: 1) use broodfish of local origin 2) a good balance between number of broodfish and number of stocked offspring in relation to the size of the natural population 3) avoid crossing between close relatives 4) stock as young stages of fish as possible to minimize time in captivity 5) minimize mortality (or in this particular case unintentional mortality) to minimize domestication selection 6) documentation and evaluation of the stocking practise to enable correct adjustments if necessary.

In addition to potential negative effects from domestication selection by keeping fish in an artificial environment, epigenetic effects are also important to consider (Christie et al. 2016). Epigenetic effects are heritable traits from parents to offspring that are adjusted depending on the environment of the parents, i.e. traits not adjusted by changes in the genetic code but from changes in the way genes are being expresses (Goldberg et al. 2007). Offspring from parents of hatchery origin often have lower survival than fish from wild parents, and this is partly explained by epigenetic effects (Christie et al. 2016, Hagen et al. 2019b).

The risk of spreading the parasite between rivers is expected to increase with increasing levels of resistance as there will be more out-migrating smolts carrying the parasite, and hence a higher probability of spreading to a neighbouring river.

3.6 Artificial selection - how much faster will resistance build up?

As noted above, a natural population of salmon would probably require hundreds of years rather than tens of years to become resistant to *G. salaris*. How much faster could resistance be built up within the context of a closed, artificial breeding programme? The most tangible factor in this equation is the generation interval, which the rate of genetic gain is inversely proportional to. As noted above, a typical generation interval in wild salmon is 6-8 years. In captivity, the generation interval can easily be brought down to 3-4 years, doubling the rate of genetic gain. Other factors come into play, but are harder to quantify: in a controlled challenge test for resistance to *G. salaris*, survival and/or *G. salaris* counts are measured, and they are both directly linked to *G. salaris* resistance. In the wild, fish die or lose fitness for many different reasons, of which *G. salaris* is only one. Therefore, the heritability of *G. salaris* resistance will be higher in a controlled challenge test than in the wild, given that survival/fitness are the only traits which can be "observed" in the wild. Another term in the breeders equation (see above) also speaks in the favour of selective breeding: the selection accuracy can be made higher in selective breeding relative to natural selection. Nature "culls" breeding candidates partly on the basis of the animal's genes. In selective breeding, information from relatives and information from DNA markers can be taken into account when breeders are selected, on top of the animals' own genes. The last factor in the breeders' equation, the selection intensity, may favour natural selection over artificial selection, depending on circumstances: a large river may be able to "test" more candidates than a hatchery can, but the hatchery may outcompete a small river. In addition, natural selection will act directly on resistance while artificial selection can only use challenge tests as a proxy for resistance.

In conclusion, even if all other factors were disregarded, the reduced generation interval alone would make sure that artificial selection acted at least twice as fast as natural selection. However, because most or all other factors in the breeding equation would favour artificial selection over natural selection, the difference between the two is expected to be significantly larger.

3.7 Would a selective breeding program be more effective compared to natural selection? Why/why not?

Natural selection would, by definition, be the most appropriate selection regime, because natural selection would select jointly for resistance to *G. salaris* and fitness in the local environment. For

that reason, we do not recommend a closed breeding programme based on challenge testing for *G. salaris* where natural selection would be highly relaxed. A solution could be an open breeding programme, in which artificial selection is used to speed up the genetic progress, where broodstock are taken partly from the river, partly from the hatchery, and where excess juveniles are used to stock the river, as described above. Alternatively, the approach using captured surviving smolts as broodstock for the next generation (see 3.3 “How to breed for resistance to *Gyrodactylus salaris*”) could be a viable solution. An important advantage of the different breeding programs is that they could substantially increase the number of individuals under selection, making selection more effective and reducing genetic drift (i.e. random genetic changes).

4 General considerations

In this section we discuss factors that are relevant for both the natural and artificial selection for *G. salaris* resistance and tolerance.

4.1 Genetic response in the parasite

In the above discussion virulence of *G. salaris* is treated as static. This is of course an over simplification of the truth. The generation time of the parasite is a few days (Jansen and Bakke 1991). Compared with the generation time of Norwegian salmon populations that are typically 5-7 years, this gives the parasite an enormous evolutionary advantage. Rapid evolution is, for example, demonstrated in sea louse *Lepeophtheirus salmonis* (an ectoparasite on salmon, with a generation time of a few months) following the increase in the salmon farming industry (Messmer et al. 2018), and there is no reason to believe that *G. salaris* has a lower evolutionary capacity. This being said, the sea louse example differs from the *G. salaris* situation as the Norwegian salmon populations are historically naïve to *G. salaris*, while the introduced parasite has a long history of coevolution with Baltic salmon. The selection pressure to increase virulence is therefore probably low or non-existent. Hence, it may be a good approximation to treat *G. salaris* as a static parameter in the first generation of evolution towards increased *G. salaris* resistance. Nevertheless, there are several different strains of *G. salaris* with different degrees of virulence, a highly plastic and dynamic trait, which may add to the complexity of evolving resistance to *G. salaris*; e.g. will evolution of resistance to one *G. salaris* strain also confer resistance to a different strain of *G. salaris*?

A consequence of pursuing a strategy of evolving resistance against *G. salaris* is the continued spread of *G. salaris* to naïve Atlantic salmon stocks, which (as identified by Gilbey et al. 2006) are likely to possess genes promoting a highly immunopathic resistance response. Yet, in a case of frequent invasions of contiguous catchments by *G. salaris*, it might be necessary to abandon costly and destructive strategies focused on eradication, and biodiversity and stock integrity may be better protected in the long run by evolving resistance. Instead, accepting the parasite as a permanent component of the ecosystem allows exploration of more novel strategies which seek to eliminate damaging virulent interactions by exploiting factors promoting parasite tolerance.

Nonetheless, virulence is recognised as a highly dynamic trait in evolutionary theory. The number of secondary infections resulting from a single infection in a naïve host population, will be increased by parasite transmission, but decreased by virulence *per se*, where it reduces the infectious period because it limits the host's life span. In the absence of any constraints, theory predicts that parasites evolve to maximise their ability to infect new hosts (infectivity) and may reduce their virulence to do this if increased virulence does not result in more infections (Ebert 1998, Bull and Lauring 2014). In *G. salaris*, infectivity requires virulence as the parasite feeds off the host in order to grow and produce new parasites that can infect new hosts.

However, spatial structure acts to constrain the evolution of infectivity. Predominantly local interactions mean highly infectious parasite strains tend to "self-shade", so local susceptible hosts are rapidly exploited, infectious individuals becoming surrounded with a halo of infected ones (Boots and Mealor 2007). In contrast, strains with lower infectivity produce a structure within the host population as they spread, leaving a higher proportion of susceptible individuals next to infected ones. Hence, less infective strains gain an advantage by not surrounding themselves with infected individuals likely to die. Consequently, there is an optimal transmission rate balancing maximized infectivity with maximized available susceptible hosts in the vicinity of infected ones.

Evolutionary theory (Anderson and May 1982, Best et al. 2008, Hedrick 2017) predicts that where neighbouring hosts are occupied by highly infective parasites of the same strain or close kin, this will lead to reduced virulence and lower transmission rate; a scenario relevant to *G. salaris* infections. In systems where parasite productivity is an important component of transmission, reduced movement of hosts and the consequent increase in local interactions in the population would be predicted to lower transmission rate, probably through a reduction in virulence. This is

one of the few predictions on parasite evolution in spatially structured environments to have been tested empirically in a mesocosm (Boots and Mealor 2007). By changing the dispersal ability of hosts in a homogeneous environment - making the environment more viscous to dispersal - to increase local interactions of parasites, infectivity was lowered. By contrast increasing globalization and population connectivity - making the environment more fluid to dispersal - can increase the extent of disease outbreaks and emergence of infective strains of parasites. The influence of spatial structure is increasingly recognized in theoretical models to be crucial to evolutionary outcomes (Lion and Boots 2010), justifying consideration of host and parasite population structure and dispersal.

4.2 Environmental change

Any population of strongly reduced size (e.g. because of *G. salaris* infection) is generally considered vulnerable towards other stressors. An extra fitness load from a new environmental challenge could drive the population to extinction. In addition, small populations loose genetic variation through the process of genetic drift, and hence have less potential to adapt to a new environment, caused for example by climate change (Allendorf and Lukiart 2009).

Denholm et al. (2013) evaluated the effect of temperature on population growth of *G. salaris*. They argued that as warmer temperature leads to faster maturation there presumably will be a higher population growth of *G. salaris*. Their simulation did not support this, however, because of trade-offs between the total number of offspring the parasite gives birth to and the first birth timing. Hence, the effect of climate change on the virulence of the parasite may not be as substantial as previously thought. However, in a field observation study the abundance of *G. salaris* in the River Glitra (a tributary to the River Lierelva), was positively related to water temperature; it was highest in the summer and lowest in the end of winter (Jansen and Bakke 1993).

Increasing precipitation following climate change can lead to lower salinity levels in estuaries and therefore increased risk of spreading the parasite between rivers.

4.3 Gene flow from farmed salmon

A special case of maladapted gene flow is widespread the genetic introgression from farmed escaped salmon (Karlson et al. 2016). A large body of empirical studies documents a negative effect of farmed salmon introgression on wild salmon populations (Glover et al. 2017). Escaped farmed salmon is likely to be more successful in small than large wild populations (Heino et al. 2015). Hence, *G. salaris* infected rivers are highly susceptible to be negatively affected by gene flow from escaped farmed salmon. In addition, gene flow from farmed salmon will counteract the evolution of resistance towards *G. salaris* in wild populations (see 2.6 “Implications of migration”), because we expect the farmed salmon to have low resistance.

Geneflow from farmed salmon has implications for supplementary stocking and a semi closed program in *G. salaris* infected rivers. Hagen et al. (2019b) documented that broodfish of farmed origin produced four times more returning adult salmon compared to broodfish of pure wild origin in a stocking program. This shows that a breeding program can unintentionally select for domesticated genotypes and therefore compromise the fitness of the wild population that is supplemented.

4.4 Hydropower regulation

Hydropower regulation has a large negative effect on natural salmon populations, by changes in the water discharge (amount and timing), water temperature, and by removing a large part of the habitat (Forseth et al. 2017). This environmental change leads to a reduced population size and to changes in natural selection, making the salmon more vulnerable and less capable of evolving resistance.

4.5 Other parasites

The presence of *G. salaris* itself and evolutionary changes towards resistance against *G. salaris* may have consequences for interaction with other ectoparasites.

Interacting parasites can affect each other's survival and virulence (Lion and Boots 2010). The strength of interactions depends on a variety of factors, such as host behaviour, the infection dose to which the host is exposed, or the order in which multiple infections occurred. Besides these intrinsic host properties, parasites can be largely affected by environmental factors to which their hosts are exposed (Ebert 1998). The effect of host resources, mediated by diet quality on the interspecific association between a parasite and a virus of *Daphnia magna* (an iridovirus – Daphnia Iridescent Virus 1 (DIV-1) actually now known to be the cause of White Bacterial Disease (WBD) (Toenshoff et al. 2018), and the Unicellular Gut Parasite (UGP), a microsporidian known as Micro1 (Decaestecker et al. 2003) infecting this freshwater crustacean reflect interspecific parasite-pathogen interactions. This study showed a low-virulent microsporidian (UGP) protected *D. magna* from co-infection with a highly-virulent parasite (WBD) when food quality was high, but this protective effect was not apparent when food quality was low (Lange et al. 2014). This study indicates that exploitation competition in multispecies infections is environmentally dependent, with diet quality influencing interspecies competition within a single host.

Similarly, and of direct relevance to *G. salaris* infections of naïve salmon stocks, earlier studies on within-host competition showed fitness disadvantages for less virulent parasites. Such fitness declines are explained by competition favouring increased host exploitation and thus increased virulence (Choisy and de Roode 2010), but could also be attributed to increased host defences, where co-infecting high-virulence parasites strongly activate the host immune system. Although these within-generation comparisons have limited relevance to existing evolutionary theory, they demonstrate that within-host parasite interactions are not restricted to competition for resources, as envisaged by theory. Instead, mixed infections may result in phenotypic changes in parasite growth rate or impaired immune clearance.

5 Knowledge gaps

Evaluating the potential for Norwegian salmon populations to evolve high levels of resistance or tolerance for *G. salaris* infection is a difficult task. Even arriving at a useful operational definition of tolerance (or resistance) is challenging, in that it must not be confounded with other host and parasite factors affecting virulence. Nevertheless, despite the lack of studies simultaneously assessing selection on tolerance and resistance, evolutionary theory suggests selection of these traits should be correlated, with specific combinations favoured (such as high tolerance and low resistance, or low tolerance and high resistance, or intermediate values of both) (Fornoni et al. 2004). These combinations derive from their mutually redundant relationship; the fitness of completely resistant individuals cannot be increased by increased tolerance, nor would the fitness of tolerant individuals benefit from increased resistance. However, although demonstrated in plants (Fornoni et al. 2004) such correlational selection has not been widely investigated in animals.

Measures of tolerance require estimation of the relationship between fitness, or health, and parasite load for a given host genotype (the reaction norm). Tolerance of a given genotype may be assessed by estimating the relationship between disease progression and parasite load across individuals of that genotype. Approaches can use families, or longitudinal data from individuals; the latter is an especially promising alternative facilitating genome-wide association studies (GWAS) to investigate the genetic basis of tolerance. However, studies of this kind between *G. salaris* and specific salmon host genotypes have not yet been made.

In this section we present a list of knowledge gaps that if filled would have helped us to better answer the questions posed in the mandate.

- Genetic variation in tolerance and resistance measured in wild Atlantic salmon.
- Empirical estimates of the per generation genetic response in resistance and tolerance in the natural environment (with gene flow and farm escapees).
- Influence of environment (e.g. water chemistry) on *G. salaris* virulence and the evolutionary response in salmon.
- The level of productivity in rivers with a long history *G. salaris*.
- Estimates of genetic correlations between resistance and tolerance and other traits important for fitness.
- Information about evolvability in virulence and optimal virulence of *G. salaris* in different environments.
- Disentangling the heritable and non-heritable components of defence strategies is vital for assessing the co-evolutionary outcome of the host-parasite interactions. The few available examples of wild populations (Mazé-Guilmo et al. 2014) suggest transmitted variance for tolerance is as high as for resistance, and show it to be subject to strong and significant environmental effects.

Several of these gaps could have been filled with better data from infected rivers. The best would be to have data from a long-term monitoring program of several infected rivers, with surveillance of demography and genetics of the salmon, environment, prevalence and infection intensity of the parasite. The genetics would have allowed us to build a pedigree for the salmon population, and in combination with data on prevalence and infection intensity it would be possible to get estimates of genetic variation in resistance, tolerance, and survival and genetic response in these traits over time. Data on strayers and their reproductive success would have allowed us to investigate the effect of maladapted gene flow on the evolution of resistance or tolerance in *G. salaris*.

6 Conclusions

- Higher levels of resistance seem possible to obtain through both natural and artificial selection in Norwegian populations of Atlantic salmon.
- Natural evolution of high resistance levels will probably take a long time (on the order of hundreds of years or longer rather than tens of years), with populations remaining below the spawning target and no harvestable surplus.
- A selective breeding programme will most likely speed up the evolution of *G. salaris* resistance. A closed breeding programme, kept isolated from the parental stock for many generations, will reduce the fitness of the population towards other aspects of its home environment. An open breeding programme, having gene flow both to and from the river, will mitigate, but not completely remove, this shortcoming. An open breeding programme is likely to build up resistance faster than natural selection would, but slower than a closed breeding programme would.
- It seems unlikely that *G. salaris* will disappear from Norwegian rivers as a result of evolution of resistance by natural selection or artificial breeding. The current knowledge suggest that the salmon populations will eventually reach a high productivity level with low prevalence of *G. salaris,* as is the case in Baltic stocks.
- Selection for and evolution of *G. salaris* resistance can cause maladaptation of genetically correlated traits closely related to fitness and reduce the adaptive potential of the infected populations.
- A strategy of developing resistance as opposed to eradication will increase the risk of spreading *G. salaris* to additional rivers. Hence, we expect further spread and an increased rate of spreading the parasite as more rivers are being infected.

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Appendix 1: Supplementary tables

***Table S1**. Overview of stocking in rivers infected with G. salaris. The information is gathered from Johnsen et al. (1999) and Håvard Lo and Espen Holte at the Norwegian Veterinary Institute (pers. com.). Considered infection period gives the period with G. salaris as shown in Figure 4 and 5 (not necessarily the complete infection period). Data that is not available are abbreviated by NA.*

River	Stocking
Aureelva	Considered infection period: 1984 - 1987 Stocking of fry: 1983 (n = NA), 1984 (n = NA)
Batnfjordelva	No stocking during the considered infection period (1980-1993)
Beiarelva	Considered infection period: 1981 - 1994 Stocking of parr: 1982 (n = 1000), 1986 (n = 1000), Stocking of fry: 1987 (n = 3000), 1988 (n = 1000), 1990 (n = 3000), 1991 (n = 20 000), 1995 (n = 19 000) Stocking of smolt: 1994 (n = 14 000), 1995 (n = 17 000).
Drammenselva	Considered infection period: 1987-1999 Stocking of fry: 200 000-300 000 yearly. Stocking of smolt: about 50 000 yearly.
Driva	Considered infection period: 1980-2017 Stocking of egg 1994-2000 (n = 596 960). Stocking of fry: 1960s-1979 (n = NA), 1980-1985 (n = 1 604 000). Stocking of smolt: 1960s-1979 (n = NA), 1980-1985 (n = 210 300), 1993 (n = 55 000), 1994 (n = 110 000), 1995 (n = NA), 1996 (n = NA), 1997 (n = NA), 1998 (n = NA), 1999 (n = NA), 2000 (n = NA), 2001 (n = 80 000).
Eidsdalselva	Considered infection period: 1981-1990 (no stocking 1982-1991) Stocking of alevins: 1994 (n = 15 000 - 18 000), 1995 (n = 80 000 - 85 000) Stocking of fry: 1980 (n = 30 000), 1981 (n = 30 000), 1992 (n = 25 000), 1993 (18 000).
Figga	Considered infection period: 1980-1993 Stocking of fry: 1995 (n = 1500) Stocking of smolt: 1988 (n = 1350), 1993 (n = 100).
Henselva	No stocking during the considered infection period (1980-1993)
Lakselva (Misvær)	No stocking during the considered infection period (1975-1989)
Lierelva	Considered infection period: 1987 – 1999 Stocking of fry: 1987 (n = 90 000) Stocking of smolt: 1987 (n = 3600), 1988 (n = 1000)

	No reports on stocking available from 1989 – 1999
Litedalselva	No stocking during the considered infection period (1981-1998)
Måna	Considered infection period: 1980 - 1993 Stocking of fry: 1980 (n = 49 000), 1981 (n = 86 000), 1982 (n = 75 000) Stocking of smolt: 1988 (n = 15 000), 1993 (n = 18 300), 1994 (n = 31 700)
Norddalselva	No stocking during the considered infection period (1981-1990)
Ranaelva	Considered infection period: 1977-1998 Stocking of fry: 1983-87 (n = 40 000) Stocking of parr: 1983-87 (a small number of 1- and 2-year olds) Stocking of smolt: 1975-91 (n = 596 400)
Rauma	Considered infection period: 1980-1993 Stocking of alevins: 1995 (n = 180 000), 1996 (n = 350 000) Stocking of fry: 1983 (n = 63 000), 1984 (n = 290 000) Stocking of parr: 1987-1990 (yearly n = 30 000), 1995 (n = 50 000). Stocking of smolt 1987-1991 (yearly n = 20 000), 1992 (n = 40 000), 1993 (n = 57 300), 1994 (n = 31 000), 1995 (n = 42 500), 1996 (n = 4 550)
Skibotn	Considered infection period: 1979-1998 Stocking of fry: 1980-1984 (n = 110 700) Stocking of smolt: 1984 (n = 6 000), 1993 (n = 9 000), 1994 (n = 7 500)
Skorga	No stocking during the considered infection period (1982-1993)
Steinkjervassd.	Considered infection period: 1980-1992 Stocking of smolt: 1988 (n = 1000), 1989 (n = 4000), 1990 (n = 3000), 1993 (n = 10 000)
Usma	Considered infection period: 1980-1997 Stocking of smolt: 1997 (n = 23 000), 1998 (n = 22 000).
Valldalselva	Considered infection period: 1980-1990 (no stocking 1981-1991) Stocking of alevins: 1994 (n = 20 000), 1995 (n = 100 000 - 110 000) Stocking of fry: 1988 (n = 10 000), 1992 (n = 60 000), 1993 (n = 14 000).

Vefsna	Considered infection period: 1978-2003 Stocking of eggs: 1997-2001 (n = 61 500) Stocking of fry: 1971-1978 (n = 906 000), 1979-1983 (n = NA) 1984-1991 (n = 3 155 500) Stocking of one-year-old parr: 1970-1977 (n = 23 000) Stocking of smolt 1966-1991 (n = 382 700)
Vikelva	No stocking during the considered infection period (1984-1988)

Appendix 2: Theory on selection response in survival

Liability of survival is defined as

$$z = \Phi_{0,P}^{-1}(\mathrm{P[survival]}),$$

where $\mathrm{P[survival]}$ is the probability of survival and Φ_{μ,σ^2}^{-1} is the quantile function (inverse of the cumulative distribution function) of a Normal distribution with mean μ and variance σ^2. In our case $\mu = 0$ and $\sigma^2 = P = \frac{1}{1-h^2}$, where h^2 is the heritability.
For truncated selection, the selection intensity (the variance standardized selection differential) on liability of survival is given by

$$i = \int_{\Phi^{-1}(1-\overline{\mathrm{P[survival]}})}^{\infty} x \frac{\varphi(x)}{\overline{\mathrm{P[survival]}}}\,\mathrm{d}x,$$

where φ is the probability density function of a standard Normal distribution ($\mu = 0$, $\sigma^2 = 1$), Φ^{-1} is the quantile function of a standard Normal distribution, and $\overline{\mathrm{P[survival]}}$ is the average probability of survival.
Assuming truncated selection and the infinitesimal model, the evolution of liability of survival z (which is assumed to be normally distributed after reproduction, with mean = $\bar{z}$ and variance = P) from generation t to $t+1$ can be approximated by (modified after equation 10 in Le Rouzic et al. 2011):

$$\begin{aligned}
\bar{z}_{t+1} &= \bar{z}_t + h_t^2 i_t \sqrt{P_t},\\
h_{t+1}^2 &= \frac{G_{t+1}}{P_{t+1}},\\
P_{t+1} &= G_{t+1} + 1\\
G_{t+1} &= g_{t+1} + d_{t+1}\\
g_{t+1} &= g_t\left(1 - \frac{1}{2N_e}\right),\\
d_{t+1} &= \frac{1}{2}\left(1 - \frac{1}{N_e}\right)(d_t + h_t^4 \Delta P_t),\\
\Delta P_{t+1} &= P_{t+1}\left[1 + \alpha_{t+1}\frac{\varphi(\alpha_{t+1})}{1-\Phi(\alpha_{t+1})} - \left(\frac{\varphi(\alpha_{t+1})}{1-\Phi(\alpha_{t+1})}\right)^2\right] - P_{t+1}\\
&= P_{t+1}\left[\alpha_{t+1}\frac{\varphi(\alpha_{t+1})}{1-\Phi(\alpha_{t+1})} - \left(\frac{\varphi(\alpha_{t+1})}{1-\Phi(\alpha_{t+1})}\right)^2\right],\\
\alpha_{t+1} &= \bar{z}_{t+1}/\sqrt{P_{t+1}},
\end{aligned}$$

where G is the additive genetic variance that consists of the additive genetic variance at gametic phase equilibrium g and the change due to linkage disequilibrium d, N_e is the effective population size, ΔP is the change in variance due to selection (given by standard formulas for the truncated Normal distribution), and α is the standardized truncation point between survivors and non-survivors (i.e. $\Phi^{-1}\left(1 - \overline{\mathrm{P[survival]}}\right)$)

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