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Genome editing for sustainability: Improving host resistance to combat late blight in potato and sea lice in Atlantic salmon

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Master Thesis

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Abstract

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Few species are as central to Norwegian society and culture as the potato and the Atlantic salmon. Yet these industries face considerable threats to production by pests, namely potato late blight (*Phytophthora infestans*) and sea lice (*Lepeophtheirus salmonis*). Current pest-control strategies, such as the use of fungicides and mechanical delousing methods, endanger the sectors' sustainability. Breeding for increased host resistance against these pests offers a strong preventative strategy to ensure future potato and Atlantic salmon production, in a sustainable way. Nevertheless, traditional breeding methods and GMO technology do not offer durable solutions for improved resistance. New breeding technologies like genome editing using CRISPR/Cas9 offer a unique, rapid solution to introduce much-needed resistance in these species. CRISPR technology revolutionises how we can target specific genes to strengthen host resistance. In potato, we explored how CRISPR may improve resistance by introducing race-specific (qualitative) and non-race-specific (quantitative) genes as well as by knocking out susceptibility genes. We further investigated how CRISPR may enable pyramiding of resistance and susceptibility genes to achieve durability against *P. infestans.* Research in Atlantic salmon shows that sea lice resistance can be explained by genetics but that it is a highly polygenic trait, with many genes having minor effects. CRISPR can be deployed as a way to study gene function to identify the causative DNA sequences underlying sea lice resistance. Once discovered, CRISPR can be used to promote certain alleles having the largest effects on resistance (PAGE method), or by harnessing genetic biodiversity from a closely related species (introgression-by-editing), or even by introducing small, novel insertions or mutations in the target genes. We found, however, that if the aim is to release an organism for cultivation and consumption, the type of changes to the DNA determines how that organism will navigate the legal framework. The Gene Technology Act determines that organisms edited using CRISPR are defined as GMO and must undergo the appropriate assessments for deliberate release. Part of that assessment investigates the organism's contribution to sustainability, a criterion maintained in the Norwegian Biotechnology Advisory Board's proposal for a tiered regulatory system. A potato demonstrating strong partial to complete resistance against late blight, with minor changes to its DNA might significantly reduce, possibly even eliminate, fungicide use, thereby providing food that positively impacts environmental health and sustainability. Farming of Atlantic salmon with improved resistance not only improves fish welfare and possibly the necessity for delousing, but it may also reduce the concentrating effect of infestations at farm sites and the resultant impacts on wild salmon populations. This thesis shows that with less risky genome edits, done with a sustainable purpose may pave the way for release approval under the Gene Technology Act, securing sustainable food production in Norway. We cannot, however, disrupt the status quo unless policymakers and regulators can strike a fine balance between regulating the risk and fostering technological innovation.

Sammendrag

Få arter er like sentrale i det norske samfunnets matvaner som potet og laks. Samtidig står produksjonen av disse matvarene overfor betydelige trusler fra sykdommer og skadedyr, henholdsvis potettørråte (*Phytophthora infestans*) og lakselus (*Lepeophtheirus salmonis*). Bekjempelsesstrategier, som bruk av soppdrepende midler og mekaniske avlusingsmetoder, setter søkelys på bærekraften i produksjonen. Avl for økt resistens mot disse skadegjørerne er nødvendig i en forebyggende strategi for å sikre fremtidig bærekraftig potet- og lakseproduksjon. Tradisjonelle avlsmetoder og GMO-teknologi gir ikke nødvendigvis umiddelbare løsninger for forbedret resistens. Nye avlsteknologier slik som genomredigering ved bruk av CRISPR/Cas9 kan tilby raskere løsninger for å introdusere resistens mot skadedyr hos disse artene. CRISPR-teknologi revolusjonerer hvordan vi kan målrette spesifikke gener for å styrke resistens mot skadegjørere. For potet har vi sett på mulighetene for hvordan CRISPR kan øke resistensen mot tørråte ved å introdusere sorts-spesifikke (kvalitative) og ikke-sorts-spesifikke (kvantitative) gener, samt ved å slå ut mottakelighetsgener som bidrar til økt angrep. Videre har vi sett på hvordan CRISPR kan muliggjøre 'pyramidisering' av resistens- og mottakelighetsgener, slik at resistensen kan vare lenge og virke mot flere raser av soppen *P. infestans*. Forskning på atlantisk laks viser at luseresistens kan forklares med genetikk, men at det er en svært polygenisk egenskap, med mange gener som hver har mindre effekt. CRISPR kan brukes for å studere genfunksjon og for å identifisere de underliggende DNA-sekvensene som kan gi resistens mot lakselus. Hvis slike gener oppdages, kan dette brukes til å fremme spesifikke alleler med påvist størst effekt på resistens (PAGE-metoden), eller ved å utnytte genetisk biologisk mangfold fra en nært beslektet art (introgresjon ved redigering), eller til og med ved å introdusere små, nye geninnsettinger eller mutasjoner. Hvis målet er utsetting for produksjon, bestemmer typen endringer i DNA hvordan organismen vil kunne navigere i det juridiske rammeverket. Genteknologiloven av 1993, som er underlagt EØS-avtalen, innebærer at organismer redigert ved bruk av CRISPR er definert som GMO og dermed blir de gjenstand for de samme vurderingene og godkjenning for utsetting for vi har i dag ved konvensjonell GMO. Neste del av vurderingene i denne oppgaven er å diskutere organismenes mulige bidrag til bærekraft, et kriterium som er opprettholdt i Bioteknologirådets forslag til revisjon av Genteknologiloven som innebærer et trinnvis reguleringssystem, med ulik grad av risikovurdering i konsekvensutredningen. En potet som viser sterk til fullstendig resistens mot tørråte, med få endringer i DNA, kan redusere og muligens til og med eliminere, bruken av soppdrepende midler, som i dagens landbruk står for halvparten av all bruk av soppmidler i Norge. Den vil derved bidra til mat med en positiv innvirkning på miljø, helse, og bærekraft. Oppdrett av laks med økt luseresistens forbedrer fiskevelferden og sannsynligvis redusere antall avlusinger. Den vil også kunne redusere konsentrasjonseffekten av luseangrep på oppdrettslokaliteter og dermed redusere luseangrep på villaksbestandene. Denne masteroppgaven diskuterer hvorvidt mindre risikable genom-redigeringer som utføres med formål om bærekraftig matproduksjon i Norge, kan eller bør bli godkjent for utsetting i henhold til genteknologiloven. Diskusjonen spiller inn til den politiske og forvaltningsmessige debatten om balansen mellom å regulere risiko for helse og miljø og å fremme teknologisk innovasjon.

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List of Abbreviations

AVR	Avirulence
AWA	Animal Welfare Act
Cas	CRISPR-associated protein
CMS	Cardiomyopathy syndrome
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribose nucleic acid
DSB	Double stranded break
EEA	European Economic Area
EU	European Union
FAO	Food and Agricultural Organization
GM	Genetically modified
GMO	Genetically Modified Organism
GS	Genomic selection
GTA	Gene Technology Act
GWAS	Genome wide association study
HR	Homologous recombination
LMO	Living Modified Organism
MAS	Marker Assisted Selection
MNC	Multinational corporation
NBAB	Norwegian Biotechnology Advisory Board
NGO	Non-Governmental Organisations
NHEJ	Non-homologous end-joining
NOK	Norwegian kroner
PAGE	Promotion of alleles by genome editing
QTL	Quantitative trail loci
QTN	Quantitative trait nucleotide
RNA	Ribonucleic Acid
RNAi	Ribonucleic Acid interference
SAFA	Sustainability Assessment of Food and Agricultural systems
SAGE	Standard application of gene editing
SDN	Site-directed nucleases
sgRNA	Single guide Ribonucleic Acid
SNP	Single nucleotide polymorphism
UN	United Nations
USD	United States dollar

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Chapter 1: Introduction

1. A full dinner plate

Few species are as iconic and tightly linked to Norwegian cultural identity and dinner plate as the potato and Atlantic salmon. A typical dinner meal in Norway consists of meat or fish with boiled potatoes and vegetables (Bugge and Almås, 2006). Agriculture and aquaculture industry have both played significant historical roles in the development of Norwegian society and culture (Gjedrem, 1993; Holtet, 2020). However, the Norwegian potato and Atlantic salmon industries face significant challenges in remaining profitable and sustainable when confronted with two systemic, widespread, and intractable pests: *P. infestans* causing late blight in potato and sea lice in Atlantic salmon.

The increased success of potato farming during the early 1900s was a major contributor to a decrease in death rates and an increase in the Norwegian population growth. Potato provided a bigger yield for the same amount of space of wheat, the harvest was less affected by bad weather, and it contributed to much better nutrition in the general population (Sejersted, 1973). Today, potato (*Solanum tuberosum* L.) is the third most consumed crop worldwide, making its production central to enhancing future food security (Campos and Ortiz, 2020). *Phytophthora infestans* Mont. de Bary is a major pathogenic threat to potato production in Norway and around the world. Breeding for disease resistance is part of the key research options to ensure future potato production (Devaux *et al.*, 2020).

Since the 1970s, the Norwegian aquaculture industry has grown into a global industry, with fish and fish products being the second largest export from Norway, after oil (FAO, 2020; Workman, 2021). Atlantic salmon (*Salmo salar* Linnaeus, 1758) was the first successfully farmed fish in floating open net pens, laying the foundations for modern aquaculture (Norwegian Seafood Council, 2020). Within a handful of years, the Norwegian parliament enacted the first law on salmon farming, regulating fish welfare and quality[1]. Currently, Atlantic salmon is one of the most successful aquaculture species, with Norway consistently holding the largest production share globally (Iversen *et al.*, 2020). As a result of intensified aquaculture activities along the Norwegian coastline and thus, a high number of salmon hosts available, there is an accompanying prevalence of parasitic salmon lice (Misund, 2019). Salmon lice, or sea lice (*Lepeophtheirus salmonis* Krøyer), has progressed from a naturally occurring parasite to a management issue associated with aquaculture (Misund, 2019). Just as with potato, a key research option to combat sea lice infestations is to breed for increased host resistance.

Humans have applied selective breeding practices in both plants and animals for centuries to obtain desired traits, usually related to growth, yield, pest and disease resistance, and other environmental tolerances (Derry, 2015, p. 13; Pacher and Puchta, 2017). These same breeding goals endure today

[1] Lov 8 juni 1973 nr. 48 om bygging, innredning, etablering og utvidelse av anlegg for klekking av rogn og for oppdrett av fisk.

but with the additional pressures of exponential population growth, climate breakdown, finite resources, and the conflicting interests of stakeholders (farmers, breeders, researchers, consumers etc). Naturally, breeding practices have developed with the growing knowledge of genetics and genome science, with biotechnology being one of the key tools to produce improved crops and animals. Breeding goals can be met in several ways, by applying conventional breeding, mutation breeding, modern biotechnology, or a combination of these. Conventional breeding relies on the inherent genetic variation in individuals in a defined species. Individuals with desired traits are crossed to produce offspring with the desired combination of traits (Bonierbale *et al.*, 2020). Mutation breeding (or mutagenesis) on the other hand introduces novel genetic variation into a species where that variation is not immediately available. Genetic variation can thus be expanded by applying physical mutagens like X-rays and gamma radiation, or strong chemical mutagens to cause random mutations in the organism's DNA (Holme, Gregersen and Brinch-Pedersen, 2019). Modern biotechnology techniques may also introduce genetic information by inserting preferred genes into an organism's DNA. The genes can derive from the same species (cisgenesis) or from an unrelated species (transgenesis). Biotechnology thus represents one of the key tools to produce improved crops and animals. This thesis focuses on one such biotechnological development: genome editing, or precision breeding, using the CRISPR/Cas system to breed for resistance traits (see ***Box 1***).

Box 1

CRISPR/Cas is the acronym for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats. These are specialised regions of DNA with both repeat sequences of DNA (21-40 base pairs) and some spacers (25-40 bp).

The *Cas* refers to the **C**RISPR-**as**sociated enzyme capable of making cuts in the DNA.

1.1. Genome editing using CRISPR/Cas system

Genome editing (or gene editing) employs site-directed nucleases (SDNs) to make controlled changes to predetermined sites in an organism's DNA (Doudna and Charpentier, 2014). There are two parts to the CRISPR/Cas system to induce precise changes to the DNA. The first part is the endonuclease (the Cas enzyme) that cleaves the chemical bonds within a DNA strand (Figure 1). Much research currently uses the Cas9 enzyme to cleave the DNA but there are indeed two class categories (class I and II) which are further subdivided into six types (types I-VI) of Cas enzymes (Makarova *et al.*, 2015; Liu *et al.*, 2020). The second part is the single guide RNA (sgRNA) exhibiting two crucial features: (1) a complementary RNA sequence that will pair with the target DNA sequence and (2) the duplex RNA structure that binds to the endonuclease (Figure 1).

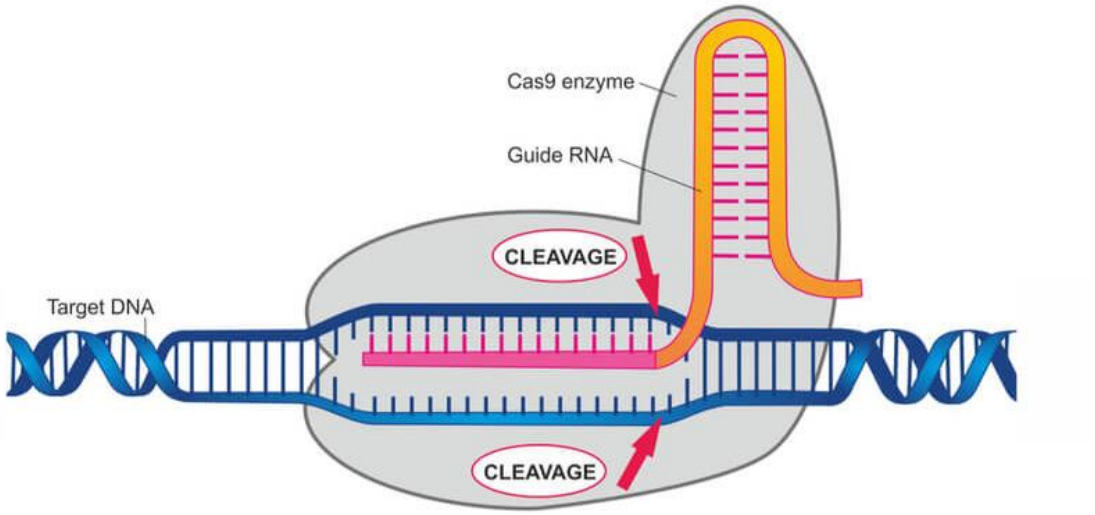

Figure 1: The CRISPR/Cas system, depicting the two parts required to induce precise genomic changes. The first is the endonuclease, the Cas9 enzyme, depicted in shaded blue behind the DNA and RNA strands. The endonuclease is responsible for the cleavage occurring within the DNA strands, noted by the red arrows. The second is the sgRNA (indicated in this figure as 'guide RNA'). The pink nucleotides of the RNA are complementary to the predetermined, target DNA sequence. The orange nucleotides indicate the duplex RNA structure that binds to the endonuclease. Image adapted from Rodríguez Fernández (2020).

Researchers employing CRISPR/Cas rely on prior gene sequence information to synthesize a complementary guide RNA sequence. Hence, cleavage by the Cas endonuclease occurs at precise points in the DNA. Cleavage induces a double stranded break (DSB) in the DNA. All organisms can employ their natural DNA repair mechanisms once a DSB occurs. Repair generally happens either by homologous recombination (HR) or non-homologous end-joining (NHEJ). The latter mechanism is most common in somatic plant and animal cells (Gomez and Hergovich, 2016; Pacher and Puchta, 2017). As an intrinsic repair mechanism, NHEJ repairs the DSB without needing a template to direct such repair, and thus is described as error prone, often inserting or deleting nucleotides at the repair locus (Pacher and Puchta, 2017). Repair by NHEJ can give rise to heterozygous mutations (mutation of one allele), biallelic mutations (a different mutation on each allele), and homozygous mutations (identical mutations on each allele) (Arora and Narula, 2017). These small changes to the sequence can bring about gene knockouts (by causing frame shifts), small deletions and insertions. By comparison, HR repair generally requires homologous sequence overhangs to process the DSB. There are several intricate mechanisms of HR to bring about desired deletions, small insertions and larger gene insertions in the DNA sequence (Guirouilh-Barbat *et al.*, 2014; Pacher and Puchta, 2017).

1.2. Outlining the research problem

Genome editing with the CRISPR/Cas system promises to simplify gene or nucleotide deletion, editing and insertion from a technical point of view. The potential for researchers to use genome sequence information from a wide range of plant and animal species acts as a catalyst for an increase in research and application (LaManna and Barrangou, 2018). Precision breeding using genome editing is a powerful and complementary tool to traditional breeding practices: both strategies can yield the same genetic outcome, but which can be achieved more precisely (and rapidly) using genome editing. The Norwegian Gene Technology Act regulates products derived from gene technology (GMOs), and as a safeguard, release approval for field experiments, farming and food

production will only be granted if there is no risk of adverse effects on health and the environment. There is, however, disagreement on the suitability of current regulations to govern genome editing and its derived products (Lassoued *et al.*, 2020). Naturally, any emerging technology and its products must be risk assessed, but innovation in technology also requires innovation in governance (Turnbull, Lillemo and Hvoslef-Eide, 2021). This thesis thus explores the potential for integrating gene editing into plant and animal breeding programmes with application in Norway – from both a technical perspective as well as a regulatory perspective. Accordingly, the first research question asks what genetic strategies and methods are currently available to mitigate or solve pest problems by improving host resistance. The second research question asks how the current legal framework works and how possible future frameworks might work. The third research question probes if it is possible to move to using new technology like CRISPR to provide food that has a positive impact on sustainability, specifically environmental health, and fish welfare.

There is major interest in developing agriculture and aquaculture in a more sustainable way in Norway – sustainability forms part of law regulating both industries (further explored in Chapter 4: Legislation). Assessing an industry's sustainability necessitates identifying the primary issues that prevent or impede sustainability in that sector. In potato production, the use and potential overuse of fungicides is having major impacts and costs on environmental health (explored in Section 2.1). In the salmon industry, sea lice infections are the single biggest indicator of welfare of the farmed salmon and the sustainability of the industry (explored below in Section 2.2). By using science and all the tools in the breeding arsenal, this thesis aims to address the existing concerns of industry practice (that of using fungicides and issues of sea lice) by proposing genome edited potato and Atlantic salmon. We also explore and delimit the study by investigating how Norwegian law demands that these organisms contribute to sustainable development by focusing on specific concerns raised by stakeholders: environment and welfare. Although the science is in its early stages, and there are knowledge gaps, we explore the genetic strategies that may provide a solution and how the benefits of these organisms contribute so largely to solving two big sustainability concerns that it might tip the scale in favour of approval for release. The importance of regulations supporting innovation and early adoption of technology to industry challenges is a particular focus for export-driven nations like Norway.

This thesis is broadly presented in two parts: it begins by exploring the technical potential of CRISPR technology to tackle challenges when breeding for resistance to pest and pathogens in the Norwegian agri- and aquaculture industries (Chapter 1: Introduction, Chapter 2: CRISPR technology in potato, Chapter 3: CRISPR technology in Atlantic salmon). Chapter 1 investigates the Norwegian potato industry, touching on desirable traits for potato seed supplied by companies like Graminor AS. The investigation includes a focus on the *P. infestans* pathogen causing potato late blight, costing the industry thousands in preventative fungicide applications each year, not to mention the environmental cost by those same fungicides. We then delve into the Atlantic salmon aquaculture industry, looking at the challenges and costs linked to sea lice infestations, with a particular focus on health and welfare of the farmed fish. The second part of this thesis (Chapter 4: Legislation and Chapter 5: Discussion) investigates the regulatory potential of genome edited organisms in Norway, by considering the present scope of the Gene Technology Act (Genteknologiloven) as well as the proposed policy change

by the Biotechnology Advisory Board. Since we consider a genome edited salmon exhibiting increased resistance to sea lice, we must also consider the present Animal Welfare Act (Dyrevelferdloven) and how public perception drives the shaping of what is 'welfare'.

Finally, the discussion considers whether it is possible, in light of the genetic changes in the product and the corresponding contribution it may have on sustainability, that genome-edited potato or Atlantic salmon become a part of Norwegian food production. The hope is that the science together with the views expressed in this thesis may be used by Norwegian policymakers and interest groups when evaluating whether to embrace genome edited plants and animals as a solution to costly industry challenges. Whether that necessarily means lowering the regulatory hurdles for future cultivation and production or not, the hope is for recognition that we can achieve innovative, science-based alternatives to the impact we have on our planet.

2. Two costly industry challenges

2.1. *Phytophthora infestans* and the potato late blight disease

Norwegian farmers produce around 350 000 tons of potato each year, with production occurring even in the most northern regions of the country (Landbruk.no, 2020; Statistics Norway, 2021). One of the costliest and most destructive challenges to their production is potato late blight disease (*potettørråte* in Norwegian). The disease had such devastating effects causing the Great Irish Famine between 1845 and 1849, a tragedy that today is marked by the Famine Memorial in Dublin (Figure 2). It is caused by the fungus-like oomycete *Phytophthora infestans*, manifesting symptoms of necrosis on leaves, stems and potato tubers. Farmers can experience up to 100% yield loss in just a few weeks (Andrivon and Savini, 2019). Various methods and strategies are used to combat infection and spread of the disease, including phytosanitary measures against the primary source of the infection (like infected seed potatoes, tubers destined for cull or waste piles, infected neighbouring plots and volunteer plants), using resistant cultivars and chemical (fungicide) treatments (Adolf *et al.*, 2020). The latter treatments can involve sprays before symptoms appear (prophylactic application) and/or after symptoms appear (curative application) (Adolf *et al.*, 2020).

Figure 2: The Famine Memorial in Dublin, by the sculptor Rowan Gillespie, commemorating more than a million lives lost due to starvation when the potato crops were obliterated by *P. infestans.* Image by Ron Cogswell (2018).

The unique climactic conditions in the different potato production areas of Norway determines the application of the fungicidal treatments. It is well understood that spread and infection of late blight is highly weather-dependent (Hjelkrem *et al.*, 2021). In some areas of Norway, the climate is suboptimal for blight for long periods of time, with infection only starting late into the growing season (Nærstad, Hermansen and Bjor, 2007). Hence, a regular late-blight forecasting system was

established in 1957 enabling controlled and more effective fungicide applications (Førsund, 1983). Such a forecasting system is still used today (Ficke, 2021) and is in keeping with the expectations of governments, supermarkets, consumers and environmental NGOs for less fungicide use (Hjelkrem *et al.*, 2021). For the producer, controlled fungicide input can reduce cost of production, considering that the total annual cost associated with late blight is around 55-65 million NOK (Sæthre, Hermansen and Nærstad, 2006).

Environmental costs are not always as readily measurable. In their report, Sæthre and colleagues (2006) identified two potential sources of environmental impact. The first threat derives from the *P. infestans* pathogen itself affecting other plant species outside of cultivated potato and tomato (*Solanaceae* family). Just over 20 plants distributed across Norway are identified as being capable of infection by *P. infestans*, either by natural or artificial infection. Although capable of infection, the conclusion is of minor damage and a temporary effect on the ecosystem (Sæthre *et al.*, 2006, p. 26). The second cost to the environment is the effect that the two principal fungicides (mancozeb and fluazinam) may have on the natural ecosystem. In December 2020, the European Commission withdrew their approval of mancozeb as an active substance for use in pesticides as it was found to be toxic for reproduction and displayed endocrine-disrupting effects in humans and other organisms[2]. This will result in the removal of three pesticide products used in Norway during 2021 (Regjeringen.no, 2021). Such a development could be an additional trigger for stakeholders to consider more seriously that breeding for host resistance should be prioritised (Sæthre *et al.*, 2006; White and Shaw, 2010; van Hove and Gillund, 2017).

Breeding potato cultivars with increased resistance to the pathogen itself presents a promising strategy to reducing fungicide input. For example, Graminor AS, the plant variety developer in Norway, lists a variety of breeding goals for potato, including agronomical qualities (short growing time, high crop yield, early maturation and good storage capacity), consumption quality (like taste, consistency, size, form, colour etc), industrial quality for chips and fries (such as size, form, starch content and storage capacity) and good resistance traits against disease (Graminor AS, 2021b). A potato variety exhibiting strong resistance to late blight means a marked decrease – possibly even an elimination – of fungicide use, thus less environmental pollution, increased sustainable production and a reflection of consumer demands. Breeders have approached this challenge in two ways: the first is by traditional breeding methods and the second, by employing conventional biotechnology tools to create a cisgenic cultivar with resistance genes from wild potatoes found in the Andes mountains. Both strategies rely on the advances in plant genetics, with the former introducing resistance factors by for example, crossing related wild species with commercial varieties. Naturally, potato breeding in the traditional sense presents a host of different challenges, a topic outside of the focus of this thesis, but best covered by Bonierbale *et al*. (2020). Most cultured potatoes are tetraploids with four sets of 12 chromosomes (2n = 4x12 = 48) presenting the primary challenge in traditional potato breeding (Watanabe, 2015). This tetraploidy makes it considerably challenging to fix resistance genes across all four loci. However,

[2] Commission Implementing Regulation (EU) 2020/2087 of 14 December 2020 concerning the non-renewal of the approval of the active substance mancozeb, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011, C/2020/8805, OJ L 423, 15.12.2020, p. 50–52.

once the desired resistance genes are fixed, it is simple and rapid to vegetatively clone through growing the tubers.

The second approach to generating a late blight resistant cultivar using biotechnology promises to overcome the challenges associated with traditional breeding methods (Gillund and Myhr, 2016). Biotechnology in this sense refers to the methods associated with 'traditional' genetic modification, that is recombinant nucleic acid techniques, or fusion of cells beyond the taxonomic family (see Chapter 4 of this work for a discussion on the legal definitions). Further along, this work discusses the unique challenges associated with breeding for resistance traits in potato and salmon and how genome editing presents a potential alternative, even to challenges experienced using GM technology. Over the years, researchers worldwide have developed various resistant cultivars using GM technology (Haverkort *et al.*, 2009, 2016; Zhu *et al.*, 2012; Ghislain *et al.*, 2019). And yet, there are no GM potatoes cultivated, consumed or imported into Norway. Much of the argument for the lack of using GM potato as a solution is the rigorous regulatory pathway that biotech products face before their release, along with a host of other concerns from various stakeholders (van Hove and Gillund, 2017).

2.2. *Lepeophtheirus salmonis* – the salmon / sea lice

Norway is the second largest exporter of fishery commodities, after China, exporting close to 12 billion USD (FAO, 2020). In such a critical export industry, Atlantic salmon is by far the most important aquaculture species, accounting for over 80% of production (FAO, 2021). Farming of Atlantic salmon also contributes significantly to food, economic and employment security in many countries, like Norway, Canada, Chile, and the United Kingdom (Houston and Macqueen, 2019). Along with intensified farming along the coast of Norway, comes the issue of sea lice infestations (Frazer, Morton and Krkošek, 2012). *L. salmonis* is the most prevalent species of sea lice in Norwegian salmon farms, a common external parasite belonging to the order of Copepoda. There are however increasing reports of infestations of *Caligus elongatus* in northern Norway (Hemmingsen *et al.*, 2020). Although not usually a deadly pest to salmon, the welfare cost to the salmon and the accompanying economic cost to the industry are important factors.

Sea lice are macroparasites, attaching and feeding on the salmon, causing skin lesions which can lead to secondary bacterial or viral infections and osmotic and ionic imbalances in the skin layer (Thorstad and Finstad, 2018). Infestations can lead to increasing degrees of stress, resulting in loss of appetite, a depressed immune system and thus decreased performance (Finstad *et al.*, 2000; Tully and Nolan, 2002; Lhorente *et al.*, 2012). Fish that are stressed by natural events such as smoltification, migration or sexual maturation, or by handling, crowding or feeding are also more susceptible to sea lice infections (MacKinnon, 1998). Lice also act as potential vectors for other infectious diseases (Oelckers *et al.*, 2014). Sea lice thus have direct effects on the individual welfare of salmon, and in turn, welfare of fish is used as an indicator of sustainable production efforts (Brakstad *et al.*, 2019). In an attempt to avert such effects on individual fish, the Norwegian Ministry of Trade, Industry and Fisheries permits a limit of 0.5 adult female sea lice per fish before sea lice treatments are required (Heuch *et al.*, 2005). If this number is exceeded, the facility is forced to slaughter the salmon early – causing an indirect cost to production (Iversen *et al.*, 2020).

In addition to direct welfare of the farmed salmon, harsh criticism is levelled at the aquaculture industry for the environmental consequences related to the industry: particularly for the impact of sea lice on wild populations of salmon (Tiller, Brekken and Bailey, 2012; Osmundsen and Olsen, 2017) and to a lesser degree, environmental costs associated with chemical treatments of sea lice (Burridge *et al.*, 2010; Langford *et al.*, 2014). Like lice infections on farmed salmon, sea lice infections on wild populations of salmon is considered a further indicator for sustainable growth of the aquaculture industry (Misund, 2019).

In economic terms, researchers estimated that in 2011, sea lice parasitism cost the Norwegian salmon industry approximately 2.5 billion NOK in damages via production and quality loss as well as direct costs of control measures (using 0.17 USD = 1 NOK) (Abolofia, Asche and Wilen, 2017). In 2014, damages were estimated to be between 3 and 4 billion NOK (Iversen *et al.*, 2018). Suffice to say that preventing and treating sea lice infections has evolved to become a major cost to industry, after feed costs (Iversen *et al.*, 2020). In Chapter 3, we tackle the methods that industry presently employs to maintain a low number of lice count on the farmed salmon and how emerging technologies may provide a necessary solution for the aquaculture industry.

Chapter 2: CRISPR technology in potato

Resistance traits in both plants and animals occur in three forms: non-host, qualitative and quantitative resistance. *Non-host resistance*, or species resistance, sees the complete exclusion of the pathogen so that a host-pathogen relationship can never be established. It is the most common and durable type of resistance (Thordal-Christensen, 2003). Exclusion occurs either by a constitutive barrier or by some inducible defense mechanism existing prior to contact with the pathogen (Nürnberger and Lipka, 2005). Total exclusion of the late blight pathogen presents an attractive outcome of gene editing goals, potentially eliminating the need for fungicides. Nevertheless, it requires a thorough understanding of the genes and molecular mechanisms bequeathing such complete resistance in plants not affected by *P. infestans*.

If the pathogen overcomes the initial barriers to establish a host-pathogen relationship, resistance is then determined by the organism's capacity to limit the consequences of that relationship (Andrivon and Savini, 2019). The organism's *qualitative* and/or *quantitative resistance* underpins its capacity to limit the host-pathogen relationship. *Qualitative resistance* (***Box 2***) is a form of total resistance, or near-total resistance, whereby the consequences of a host-pathogen relationship is almost completely limited (Nelson *et al.*, 2018). The host resistance limits extension and reproduction of the pathogen, i.e. where a plant's immune system might recognise and restrict the spread of fungal hyphae along plant cells (Jones and Dangl, 2006). Qualitative resistance is usually mono- or oligogenic, those genes referred to as "major resistance" genes and referred to as R-genes. Their action is not general in nature – in other words, the resistance demonstrated is usually wholly dependent on both the plant's R-genes and the pathogen's avirulence (AVR) genes (Nelson *et al.*, 2018). Hence resistance is only present for some plant genotypes and against some pathogen genotypes (Andrivon and Savini, 2019). Due to there being fewer genes involved, if a pathogen undergoes a mutation in their AVR gene, the corresponding relationship with the R-gene can be overcome.

Box 2

Qualitative resistance Other terms for this type of resistance include hypersensitivity resistance, vertical, or race-specific resistance. The genes are denoted as R-genes.

Quantitative resistance Other terms for this type of resistance include broad or field resistance, horizontal, general, or non-race-specific resistance. In potato breeding, the genes are denoted with the prefix Rpi.

On the other hand, *quantitative resistance* (***Box 2***) is generally polygenic – due to a great many genes all with small to moderate effects. Organisms display a partial resistance phenotype, that is, reduced symptom severity in the size of lesions and the rate of spread compared to susceptible organisms (Pilet-Nayel *et al.*, 2017; Andrivon and Savini, 2019). The advantage of polygenic resistance lies in its improved durability, enabling resistance to remain effective when deployed over a large area under substantial disease pressure over a long time (Pilet-Nayel *et al.*, 2017; Nelson *et al.*, 2018). However, the molecular mechanisms underlying quantitative traits are not well described and the polygenic nature presents some difficulty when using traditional breeding methods to introduce resistance from closely-related species (Nelson *et al.*, 2018).

1. Late blight resistance

Box 3

Introgression The incorporation of desirable alleles from a donor species into the gene pool of a divergent recipient species, usually by means of hybridisation or backcrossing.

Investigation for resistance genes against *P. infestans* began soon after the Irish Famine in the 1840s (Fry, 2008). Some wild potato species demonstrated total resistance by means of major R-genes, which were introgressed (see ***Box 3***) into the agronomic varieties (Harrison and Larson, 2014; Muktar *et al.*, 2015). The species *Solanum demissum* has come to be the most exploited source of resistance genes to late blight (Verzaux, 2010). Over the years, several major race-specific genes were identified and denoted R_1, R_2, R_3 etc (Sleper and Poehlman, 2006, p. 372; Rodewald and Trognitz, 2013). The resistance, however, lacked durability as the *P. infestans* avirulence alleles evolved over time and in response to widespread cultivation of the resistant varieties (Fry, 2008). Considering the continued defeat of R-genes by *P. infestans*, researchers and breeders now focus their efforts into breeding for durability through 'field resistance' (***Box 2***) (Fry, 2008). Although it seems there is little consensus across the literature, genes conferring quantitative field resistance can be denoted with the prefix Rpi.

Potato is a strong candidate crop for genome editing for several reasons. First, most potato cultivars are autotetraploid, highly heterozygous and suffer acute inbreeding depression, demonstrating reduced biological fitness due to lower genetic variation (The Potato Genome Sequencing Consortium *et al.*, 2011). These characteristics make using classical breeding rather difficult, especially when attempting to incorporate a large number of agronomic, market quality, and resistance traits into the final product. (Nadakuduti *et al.*, 2018). Second, genome sequence information as well as established transformation and regeneration protocols can facilitate CRISPR editing of favoured cultivars (Nadakuduti *et al.*, 2018). Third, a modified CRISPR/Cas9 system can facilitate alteration of all four loci at the same time in polyploids (Kusano *et al.*, 2018). Fourth, the Nordic *P. infestans* populations demonstrate particularly high genetic diversity, thus possessing high adaptative ability (Brurberg *et al.*, 2011). Although the primary goal is resistance durability, the relative ease of genome editing allows the rapid production of resistant cultivars before the pathogen has a chance to overcome the resistance.

2. What we know about R-genes in the *Solanum* genus

The remarkable evolving nature of *P. infestans* has driven many attempts to generate resistant potato varieties by harnessing R-genes from wild potato relatives. However, when introducing a novel trait that is not already present in the organism, three primary challenges must be considered (Table 1). First, it takes up to 50 years to introgress a single resistance gene using a classical breeding approach. Researchers and private breeding companies in the Netherlands started breeding activities in 1959 to introduce a single resistance gene from *S. bulbocastanum*. In 2005, two resistant cultivars, Bionica and Toluca, were released for organic potato production (Haverkort *et al.*, 2009). Second, a common issue with classical breeding is the simultaneous hitchhiking of linked negative traits (***Box 4***).

Box 4

Hitchhiking Occurs when an allele experiences a change in frequency, not because it was the target of such change but because it is associated with the target allele by linkage disequilibrium.

As a result, farmers tend to rely on known superior quality varieties and chemical control of late blight than on new resistant varieties. Finally, despite widespread interest in introducing single race-specific R-genes from *S. demissum* in the early 1900s, durability issues persisted (Fry, 2008). When the new variety was grown at scale for a few years, the pathogen evolved to defeat the resistance (Fry, 2008).

To overcome the first and second challenges, CRISPR can directly introduce a single gene into the recipient species by inducing a double-stranded break (DSB) in the DNA and thereafter directing homologous recombination (HR). This technique alone negates the consequences of linkage drag. Of course, this requires prior knowledge of the size, sequence, and position of the gene. Yet, the timeframe of three to six years required for knocking in an entirely new gene is exceedingly less than the average 50 years required by classical breeding (Bullock, Wilson and Neadeau, 2021). Indeed, the expected time for development of an edited plant is also significantly shorter than that of conventional GM technology, where R&D can take up to 13 years (Table 2) (Calyxt, 2017; Bullock *et al.*, 2021). The major drawback of generating such a cisgenic variety is that it falls firmly within the definition of a GMO in almost all jurisdictions around the globe, including Norway (Turnbull *et al.*, 2021).

Table 1: The benefits and drawbacks of introducing qualitative or quantitative resistance into *S. tuberosum* using classical breeding approaches.

Resistance	Benefits	Drawbacks	Reference
R-gene	▪ Complete resistance ▪ Marked reduction/elimination of fungicide application ▪ Monogenic ▪ Introgression possible	▪ Complete resistance can be defeated ▪ Low durability ▪ Linkage drag ▪ ~50 years for introgression ▪ Possible difference in foliage and tuber resistance	(Sleper and Poehlman, 2006, chap. 21; Haverkort *et al.*, 2009)
Rpi-gene	▪ Spectrum of resistance ▪ Increased durability ▪ Reduce fungicide application	▪ Strong association of QTL with late foliage maturity ▪ Polygenic resistance ▪ Resistance dependent on size of cultivation area and dynamics of pathogen population ▪ Higher demand on introducing trait	(Rodewald and Trognitz, 2013; Adolf *et al.*, 2020)

Pyramiding or stacking of resistance genes into a single organism could potentially overcome all three challenges associated with conventional breeding (time, linkage drag and durability) (Table 2). Pyramiding can occur in three ways: pyramiding of major R-genes, pyramiding of several broad spectrum Rpi-genes, or a combination of R- and Rpi-genes (see Figure 3). Breeding programs have been designed for all three types of pyramiding in various crops like wheat and rice (Collard and Mackill, 2008). Again, traditional breeding to stack resistance genes is expected to be more complicated and time-consuming than the 50 years for a single trait (Haverkort *et al.*, 2009; Ghislain *et al.*, 2019).

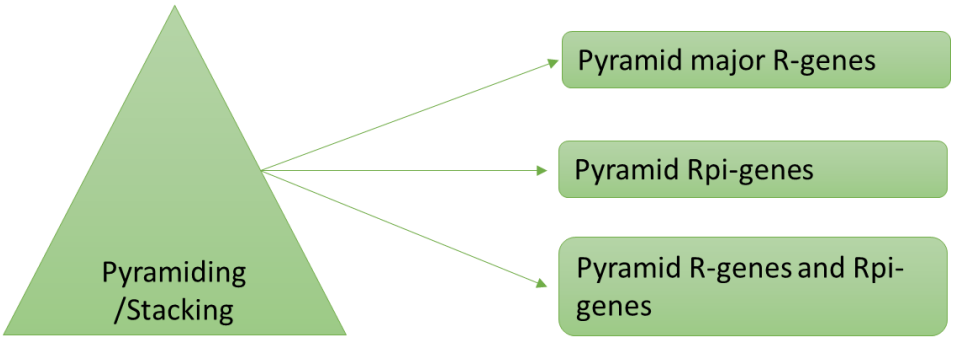

Figure 3: Breeding programs using pyramided or stacked genes to confer resistance to disease or pathogens. Pyramiding can occur by (i) combining several major genes (R-genes), (ii) combining several quantitative minor genes (Rpi-genes) and (iii) combining both major R-genes and some broad spectrum Rpi-genes. Figure constructed based on information from Pilet-Nayel *et al.* (2017).

Prior to the development of the CRISPR system, several resistance genes were stacked using marker-assisted selection (MAS) and recombinant GM technology. Tan *et al.* (2010) demonstrated the additive effect of stacking an R-gene with a strong resistance (*Rpi-ber1*) and one with a weak resistance (*Rpi-mcd1*) to late blight. Zhu *et al.* (2012) simultaneously transformed three broad spectrum R-genes (*Rpi-sto1*, *Rpi-vnt1.1* and *Rpi-blb3*) using one binary vector into the susceptible Desiree cultivar. Stacking of R-genes (in this case, a triple stacked transformant) exhibited increased durability to AVR gene evolution in *P. infestans* (Zhu *et al.*, 2012). In a field trial in Uganda, Ghislain *et al.* (2019) used a triple-stacked combination of R- and Rpi-genes (*RB, Rpi-blb2* and *Rpi-vnt1.1*) to create a resistant GMO potato. The varieties demonstrated strong resistance and continuing durability against late blight over three consecutive seasons, without fungicide application. Although the authors were optimistic, they acknowledged that new stacked events would eventually be required due to the pathogen's adaptability, a concern further explored in the discussion section of this thesis (Ghislain *et al.*, 2019).

3. What we know about S-genes in *S. tuberosum*

Susceptibility genes represent a different side to the same coin. S-genes are those genes that facilitate infection or support compatibility with the pathogen (Zaidi, Mukhtar and Mansoor, 2018). Thus, disrupting susceptibility to late blight by knocking out S-genes could confer much needed resistance, so-called *S-gene-mediated resistance* (Zaidi *et al.*, 2018). The resistance can be either be pathogen-specific, affecting the penetration requirements of a certain pathogen into the plant, or broad-spectrum (pathogen-unspecific), involving a constitutive, barrier defence (Zaidi *et al.*, 2018). In contrast to R-gene resistance, S-gene-mediated resistance is a recessive trait, often associated with a fitness cost to the plant. For example, the recessive mildew resistance locus O (*mlo*) allele in apple, barley, tomato and wheat confers increased resistance against powdery mildew. However, modification to the *mlo* allele may result in enhanced susceptibility to other pathogens (van Esse, Reuber and van der Does, 2020). It seems also that the stronger the resistance by *mlo* alleles, the stronger the pleiotropic effects, like leaf spots and early leaf senescence, whether the recessive trait was induced or occurred by traditional breeding (Kusch and Panstruga, 2017). All of these factors must be considered when editing potatoes for late blight resistance.

It was only in the last two decades that plant genes for susceptibility to certain pathogens were discovered (Eckardt, 2002). More recently, exploiting S-genes to confer resistance was proposed as a novel breeding strategy (Pavan *et al.*, 2009). Disrupting S-genes to confer late blight resistance in potato was first explored using the RNA-interference (RNAi) technique (Sun *et al.*, 2016). By separately silencing five S-genes in the susceptible Desiree cultivar, the transformed varieties displayed complete resistance to late blight. Additionally, a silenced sixth gene of the 11 candidate S-genes, showed partial resistance against late blight. However, the authors noted several instances of phenotypic fitness costs, including dwarf plants, autonecrosis on older leaves and green colour loss – all undesirable for agricultural purposes. Silencing genes by RNAi can be effective, as evidenced by the commercially-available Innate® potato, which is resistant to browning, black spots, late blight and forms less acrylamide when baked or fried (Simplot, 2017). Even so, *where* the gene encoding the RNA is incorporated into the plant's DNA may not be precise, potentially leading to unintended consequences (Table 2). Furthermore, organisms modified using the RNAi technique are classified as GMOs and must undergo more stringent biosafety assessments.

Genome editing opens the possibility of exploiting recessive traits in tetraploid potato with more precision and control (van Esse *et al.*, 2020). Very recently, researchers in Sweden and Denmark introduced mutations to S-genes using the CRISPR system resulting in increased late blight resistance (Kieu *et al.*, 2021). Building on the results using RNAi, the researchers showed that knockout in two of the candidate genes (*StCHL1* and *StDMR6*-1) resulted in resistance to late blight, without any associated phenotypic fitness costs. The authors however, did not test the possible enhanced susceptibility these edited lines may experience to other potato pathogens (van Esse *et al.*, 2020). Using knockout techniques is particularly attractive as no new genetic information is introduced into the organism, and thus the final product may potentially avoid the legislative demands as a GMO. Solutions depend on further work on candidate S-genes as targets for knockout to establish the durability of resistance contributed by mutant S-genes and how that resistance might interact when pyramided with R-genes (Table 2).

Table 2: An abbreviated summary of the various strategies available to researchers or breeders when targeting specific genes for purposes of increased host resistance to late blight. The table identifies the genetic targets and highlights the methods possible to introduce/change the desired genes. The table also explores the amount of resistance demonstrated by the genetic change as well as the longevity of the resistance conferred (where known). The final column provides an estimation of the time for development of a resistant organism and, where known, the time required for market approval. * indicates time for commercial R&D

Strategy	Method	Benefits	Drawbacks	Resistance	Durability	Time	References
Classic breeding	Introgression	See Table 1	Crossing barriers; linkage drag	Partial-complete	Some R-genes defeated, others persist	50+ years	(Fry, 2008; Adolf *et al.*, 2020)
Single R-gene	GM technology	Natural immunity from wild relatives can be achieved; history of genetic information available	Depends on GM technology used	Effective against prevailing *P.infestans* population	Depends on plasticity of pathogen – over 100 years history of defeated resistance	~13 years*	(Vleeshouwers *et al.*, 2011; Du *et al.*, 2015; Calyxt, 2017)

Rpi-gene	GM technology	Resistance is broad spectrum	Depends on GM technology used	Partial	Likely more durable than R-genes alone	~13 years*	(Calyxt, 2017; Ortiz and Mihovilovich, 2020)
Pyramid R-genes	GM technology	Reduce fungicide use by over 80%	Low commercial success or interest	Strong partial-complete	Currently undefeated – resistance management research	~13 years*	(Haverkort *et al.*, 2009, 2016; Bullock *et al.*, 2021)
S gene 1	CRISPR knock out	Possible to induce non-host resistance	Fitness costs possible and linked to strength of resistance conferred	Enhanced	Unclear	3-6 years	(Pavan *et al.*, 2009; Calyxt, 2017; Zaidi *et al.*, 2018; Kieu *et al.*, 2021)
S gene 2	RNAi (GM technology)	Generally high efficiency of silencing; Stable and long-term silencing	Varying fitness costs; Inaccurate specificity; Fluctuation in silencing during plant development	Partial-complete	Unclear but S-gene mutation shown to be undefeated for over 35 years in barley	13 years for market approval	(Lyngkjær *et al.*, 2000; Mansoor *et al.*, 2006; Haverkort *et al.*, 2016; Sun *et al.*, 2016)
Pyramid R-genes and S-genes	CRISPR knock in and knock out	Combine natural immunity from R-genes with standing genetic variation of S-gene	Requires further work in multiplexing protocols	Strong partial to complete	Expected strong durability, possibly remain undefeated	3-6 years	(Pavan *et al.*, 2009; Calyxt, 2017; Wulff-Vester, 2019)

4. Pyramiding as an option

Just as researchers have shown success in stacking R-genes and Rpi-genes, perhaps answers can be found when considering pyramiding of R/Rpi-genes together with knockout of S-genes using CRISPR technology (Wulff-Vester, 2019). Although this is not true pyramiding in the sense that multiple genes are introduced into one species, it is a *multi-edit* of a single species to achieve true durable resistance (Table 2). To avoid rapid breakdown in resistance of a single gene, editing both resistance and susceptibility genes may offer long-term durability against late blight. Yet the goal of genome editing depends on the aim to be achieved: whether genome editing will be used as a proof-of-concept or as a means to introduce a new potato crop product for cultivation and consumption.

As a proof-of-concept, the researcher is not confined by the legislative demands for release. Rather, the experiment is required to be exercised within the experimental and field trial regulations. To this end, the researcher can work relatively unfettered in his focus on which genes to combine: the knock in of race-specific R-genes, broad spectrum Rpi-genes and the knock out of S-genes (Wulff-Vester, 2019). The combination of knock in and knock out depends on the standing resistance of the variety used – for instance, in Norway, 'Nansen' is a popular consumer cultivar which also exhibits its own medium to high resistance to late blight (Graminor AS, 2021a). To achieve a multi-edit will require an efficient multiplex CRISPR system, using several guide RNAs to target more than one gene at a time (Nadakuduti *et al.*, 2018). Nevertheless, it is prudent to consider unintended effects of both knock in and knock out, particularly affecting agricultural traits such as yield, quality indicators and

resistance to other diseases. If the aim is to release the variety for farmers to cultivate and trade, the legislative realm applicable in the country of release is a strong director. To this end, chapter 4 of this thesis explores the Gene Technology Act in Norway and how such multi-edited potato product might be assessed.

Chapter 3: CRISPR technology in Atlantic salmon

The high concentration of salmon lice associated with salmon farming causes a two-fold problem: high lethality in wild salmonids and economic and welfare issues in farmed salmon. It is identified as the most acute sustainability issue facing expanding farming practices, costing in excess of USD 880 million per annum globally (Gratacap *et al.*, 2019; Wargelius, 2019; Iversen *et al.*, 2020). When it comes to combat strategies against sea lice, Barrett et al. (2020) characterises two strategies: a reactive strategy to treat infestations already present and a proactive strategy to prevent successful infestations (Figure 4).

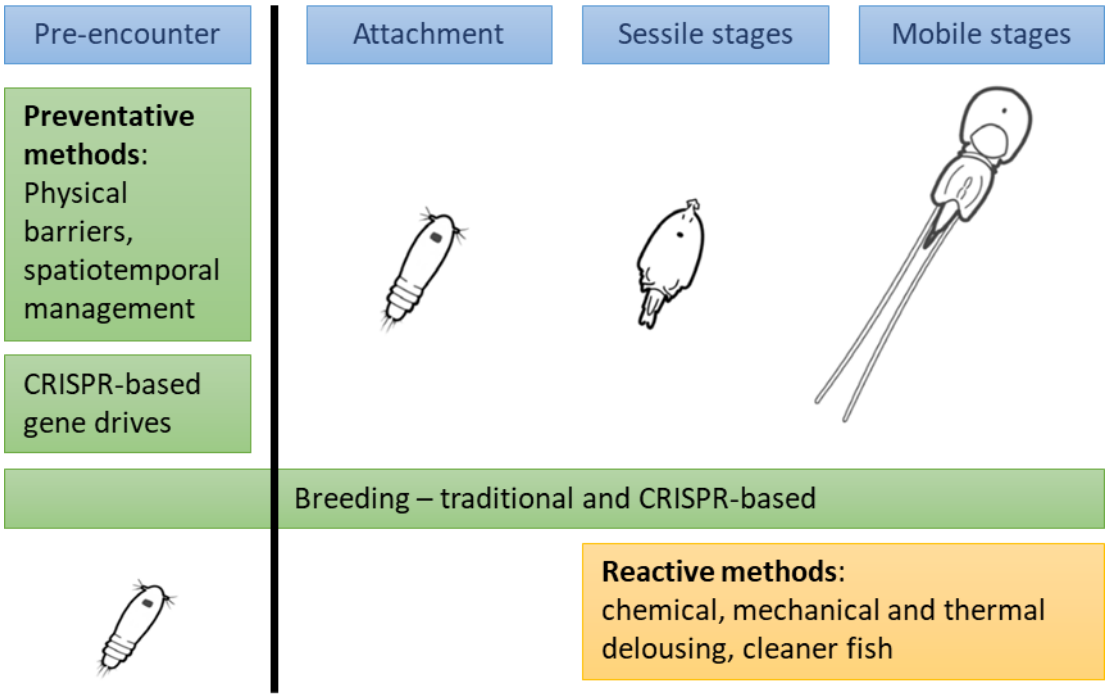

Figure 4: Indication of targeted life-cycle stage for preventative strategies and reactive strategies. Green shading illustrates preventative methods and orange illustrates reactive methods. Image adapted with permission from Barrett *et al*. (2020).

Reactive strategies (orange shaded text box in Figure 4) have historically been employed, for example, by administering medicine or chemicals in bath treatments or in feed. Employing medicinal therapeutants has however, led to drug resistance and negative impacts on the environment and on non-target species (Aaen *et al.*, 2015). As a result the use of other reactive strategies, like mechanical and thermal delousing have become the most prevalent in Norway (Overton *et al.*, 2018; Barrett *et al.*, 2020). Mechanical delousing methods apply low-pressure water jets or brushes to dislodge lice, which are then filtered away – see for instance the Hydrolicer system (Smir AS, 2021) and the SkaMik system (SkaMik AS, 2021). Similarly, the thermal delousing process exposes the salmon to warmed seawater (28ºC to 34ºC) for about 30 seconds, inactivating the lice and causing their detachment (Brunsvik, 1997; Overton *et al.*, 2018). Although highly effective and without negative environmental impacts, there is evidence that both methods cause stress to the fish, resulting in elevated mortality after treatment and indicating a fish welfare issue (Overton *et al.*, 2018; Nilsson *et al.*, 2019).

A biological delousing alternative is to include cleaner fish such as ballan wrasse (*Labrus bergylta*), or lump fish (*Cyclopterus lumpus*) which consume lice directly off the salmon. However, this solution is not without controversy as the welfare and health of cleaner fish is not directly measured and managed (Overton *et al.*, 2020). In addition, cleaner fish are typically harvested from regionally distinct wild populations and their escape or release from salmon pens poses a threat to the genetic diversity of both species (Faust *et al.*, 2021). Lastly, some question the effectiveness of cleaner fish as there has been no documented reductions in lice infestations or in the number of reactive treatments when using cleaner fish (Barrett *et al.*, 2020; Overton *et al.*, 2020).

On the other hand, proactive or preventative strategies offer additional benefits by reducing necessity and costs of delousing, and improving fish welfare, productivity and sustainability (green shaded text boxes in Figure 4). Preventative strategies can be divided into two subcategories: those that (i) reduce encounters between salmon and lice and (ii) those that reduce the success of lice infestation post-encounter (Barrett *et al.*, 2020). Briefly, methods that reduce encounters include barrier technologies that are depth-specific, that filter lice from the water column or that hide salmon host cues, manipulating the swimming depths of salmon, managing when and where farming activities take place (spatiotemporal management) or incapacitation of lice using light or sound (Barrett *et al.*, 2020). It is also proposed that we can reduce pre-encounters by controlling the overall lice population using gene drives engineered by CRISPR (Esvelt *et al.*, 2014). The gene drive would control the wild lice population by employing a 'suppressive drive' type, a strategy that reduces the target species by introducing sterility or lethality (Champer, Buchman and Akbari, 2016). Our interest however, lies in preventative methods utilising breeding and CRISPR technology to control pre- and post-encounter infestations.

1. Traditional breeding for resistance in Atlantic salmon

Breeding for louse resistance presents a strong possibility to reduce both pre-encounters and post-encounter lice infestations (Figure 4). Breeding for resistance currently applies traditional breeding techniques but could possibly include CRISPR-based techniques in future. Several studies have shown that louse resistance in Atlantic salmon is *heritable* (***Box 5***), meaning that genetic variation explains why some individuals in a population are more resistant to lice than others (Glover *et al.*, 2005; Kolstad *et al.*, 2005; Wray and Visscher, 2008; Gjerde, Ødegård and Thorland, 2011; Tsai *et al.*, 2016). Lice resistance is also shown to be highly *polygenic*, meaning many genes underlie the heritability (Gharbi *et al.*, 2015; Holborn *et al.*, 2019).

Box 5

Heritability A term used in genetics and breeding. It summarises how much of the variation in a single trait is caused by the variation in genetics between individuals in a population (Wray and Visscher, 2008).

The *heritability* of a trait assists breeders in using genomic selection (GS) to drive genetic improvement for a target trait (Meuwissen, Hayes and Goddard, 2001). When the trait is highly *polygenic*, breeders routinely use Genome-Wide Association Studies (GWAS) to find and explain the genetic variation of a trait. Both GS and GWAS rely on dense single nucleotide polymorphism (SNP) marker maps. SNP markers are assumed to be in association with the genes causing that trait phenotype by linkage disequilibrium (Jonas *et al.*, 2019). The location of SNP markers in the genome

makes it possible to broadly infer the regions where a causative allele may be located. Importantly, when the exact causative gene sequences are not known, they are referred to as quantitative trait loci (QTL). For GS, you do not need to know the prior effect and location of the QTL to make genetic progress (Ødegård *et al.*, 2011). However, for gene editing, you do need to determine the causative QTL and its size, hence the need for GWAS to determine if a heritable trait is controlled by a few loci with large effects or due to many loci, each with minor effects (Miles and Wayne, 2008; Houston *et al.*, 2020).

Kjetså *et al*. (2020) found that resistance to *L. salmonis* in Norwegian populations of Atlantic salmon is highly polygenic and without major QTL regions. Similarly, in Canadian salmon populations, resistance was not explained by major QTL but that two QTL explained 6% of the genetic variation (Rochus *et al.*, 2018). Comparatively, Chilean salmon populations exposed to a different species of sea lice (*C. rogercresseyi*) exhibited three QTL that explained 7 to 13% of the genetic variation in resistance (Robledo *et al.*, 2019). Finding these QTL provide a good starting point for further work, because QTL information is linked to markers rather than the causative variants. Causative variants are the polymorphisms within the genome that directly affect the trait of interest (Houston *et al.*, 2020). Crucially, in the case of sea lice resistance in Atlantic salmon, causative variant information is still lacking.

2. Indirect CRISPR to identify lice resistance genes

Before applying CRISPR, it is essential to first know the genomic sequence and location of the causative alleles, as well as their relative importance as targets. So, how then can we begin to find and understand the genes involved in sea lice resistance? Mapping and understanding QTL is just one way to discover the genetic causes for variation in lice resistance (Houston *et al.*, 2020). Fine mapping based on more detailed sequencing in QTL regions together with RNA expression studies can assist us to identify the causative sequences (Houston *et al.*, 2020). Functional genomic studies generates vast data on genome and RNA sequencing, which contributes to the identification of candidate causative gene variants, a task undertaken by the Functional Annotation of All Salmonid Genomes (FAASG) initiative (Macqueen *et al.*, 2017). CRISPR offers a second way to identify causative sequences and gene function of sea lice resistance. We describe it in this thesis as *indirect CRISPR application* – to distinguish it from direct CRISPR edits to introduce lice resistance (Figure 5).

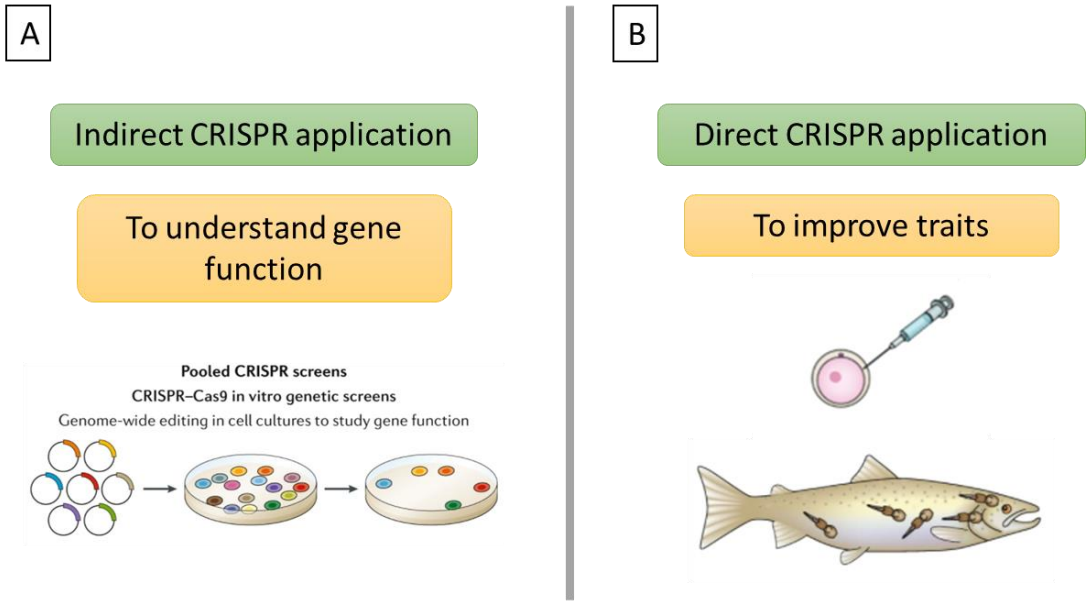

Figure 5: Gene editing can assist in breeding for lice resistance in two principal applications. **(A)** Researchers can employ indirect CRISPR application in genetic screening processes. **(B)** Researchers can also employ direct CRISPR application, by initiating directed changes to the target genes, giving rise to a gene edited organism exhibiting improved traits, such as enhanced resistance to lice. Images adapted from Houston et al. (2020).

Researchers can apply a method of genome-wide CRISPR knockout (GeCKO), by introducing precise genetic perturbations that can then be screened at scale, in loss-of-function or knockout screens (Shalem *et al.*, 2014; Wang *et al.*, 2014). In this application, a library of sgRNA (single guide RNA) must be generated and each must be cloned into a vector delivery system (usually a lentiviral system). The *in vitro* cells of the chosen cell line are then transduced with the sgRNA library and CRISPR/Cas cargo. Finally, the cells containing the integrated changes/perturbations are selected on an appropriate assay, the DNA is extracted and the recovered sgRNAs are analysed to identify the genes (Figure 6) (Harvey, 2020). The problem is that these types of CRISPR screens are not optimised for salmonid species and requires work at all stages of the genetic screen for future applications to identify gene function (Figure 6) (Reza, 2020).

Figure 6: A genetic screen to identify gene function requires three components. The first is introducing a genetic perturbation. In this case, using a powerful technology like CRISPR/Cas9 system to integrate perturbations. The second requires an optimised model system, that is the choice of relevant cell types that are scalable and technically suitable for the functional testing. The third is the identification of an appropriate assay that enables the physical separation of cells demonstrating the phenotype of interest from those that do not. Image based on the content of the review by Doench (2018).

3. Direct CRISPR for resistance to sea lice

Moving from identified QTL markers to identifying the causative variants of lice resistance requires further investigation. In this endeavour, various projects are funded by the Research Council of Norway (RCN) and the Norwegian Seafood Research Fund (FHF) to identify high priority candidate genes that may later undergo gene editing in salmon embryos (Table 3). Although it is still a way to go, potential application can still be discussed.

Table 3: Examples of funded projects involving Norway that utilise gene editing on Atlantic salmon, to improve the genetic screening protocol, to find causative genes, to introduce lice resistance or a combination of aims. The types of gene edits to be used are classified by 'direct' or 'indirect' edits. The GeneInnovate project is the only project involving both Atlantic salmon and potato. GE is gene editing. This table does not represent an exhaustive list of projects on gene editing in salmon but rather presents examples supplementing the main text of this thesis.

Project Name	Principal Country	Partner Countries	Aim	Types of edits	Budget	Reference
CrispResist	Norway	UK, Australia, Canada, Sweden, USA	Harness cross-species variation from Pacific salmon to achieve sea lice resistance in Atlantic salmon	▪ Direct GE of candidate genes	40 million NOK	(FHF 2020; Nofima AS, 2020b)
GeneInnovate	Norway		Investigate genetic variants that impact production traits in potato, Atlantic salmon and other animals by utilizing and improving gene-editing tools. Large focus areas are to combat late blight in potato and sea lice in Atlantic salmon.	▪ Indirect GE to discover candidate expression QTL ▪ Direct GE to knockout S-genes and introduce R-genes in potato	9 million NOK	(RCN, 2018)
LiceRESIST	Norway		Characterize causative genomic differences underlying differences in host response and resistance to sea lice in seven salmonid species	▪ Indirect GE to discover and validate gene function	12 million NOK	(CIGENE, 2020)
AquaCrispr	France	Norway, Germany	Optimize the CRISPR/Cas9 knock-in technology in zebrafish and establish the protocol for salmon and trout.	▪ Indirect GE to optimize the protocol ▪ Direct GE in model organism for application in farmed organisms	~7 million NOK	(ANR, 2021; RCN, 2021a)
CMSEdit	Norway	Australia	Develop CMS resistant Atlantic salmon using gene editing technologies and design breeding schemes that take advantage of edited genes. CMS resistant fish are better able to survive delousing procedures.	▪ Direct GE of candidate gene	~10 million NOK	(Nofima AS, 2020a)
TUNESAL	Norway	France	Further develop gene editing in salmon with an ultimate aim to increase the robustness of salmon in aquaculture	▪ Indirect GE to understand small nucleotide changes impact disease traits	12 million NOK	(RCN, 2021b)

This thesis identifies four potential strategies where genome editing technology may contribute directly to improving sea lice resistance traits in Atlantic salmon (Table 4). First, however, consider

again the prior knowledge we have on the polygenic nature of the resistance trait together with the number of implicated minor QTL (Tsai *et al.*, 2016; Kjetså *et al.*, 2020). Generally, conventional selection methods can achieve a genetic response between 16 – 23% per generation (4 years) and this rate of genetic gain is affected by numerous variables, like the intensity of selection, the accuracy of selection and the heritability of the trait. Importantly, the response of 16 – 23% is based on all selective pressure applied to sea lice resistance, while in practice multiple traits are included in a selection index. The weighting of sea lice resistance in commercial breeding programs is not reported but it is safe to assume that not all selective pressure will go towards lice resistance and thus will be less than 16 – 23% per generation (Table 4) (Rosendal and Olesen, 2021). Limiting inbreeding depression and linkage drag of other unwanted alleles present additional generational (time) considerations when selecting for desired traits.

Box 6

Paralog A paralogous gene, often referred to as a paralog, arises from a gene duplication in an organism – in Atlantic salmon, there was an entire genome duplication, resulting in a tetraploid organism. The resultant gene copies are paralogs of one another. The paralogs can retain the same function or develop different functions. They can also remain present in the genome or be lost. These patterns make annotation and reference projects of the Atlantic salmon genome particularly complex.

Gene editing can directly introduce favourable alleles where the gene sequence and function is already identified, presenting a powerful tool to target the "low-hanging fruit", those known genes with large effect (Gratacap *et al.*, 2019). Edvardsen *et al.* (2014) thus first demonstrated targeted knockout of two known pigment genes using CRISPR/Cas9 in Atlantic salmon, to give rise to an albino fish phenotype. A further study created sterile Atlantic salmon by using CRISPR/Cas9 to knockout the *dead end* (*dnd*) gene, a gene crucial for germ cell formation (Wargelius *et al.*, 2016). In the latter study, the authors highlight that the *dnd* gene was a suitable target for direct gene editing as it lacked known paralogs (see ***Box 6***), an oft-cited complication brought about by the partial tetraploid genome of Atlantic salmon (Wargelius *et al.*, 2016). These two examples show that desirable traits are capable of being introduced by gene editing where full gene annotation and function are known, a procedure we could refer to as SAGE, the 'standard application of genome editing' (Table 4). Editing one or two genes by SAGE offers rapid, impactful solutions to significant sustainability issues in large-scale production of Atlantic salmon, like creating sterile salmon to prevent interbreeding between farmed escapees and wild salmon populations (Glover *et al.*, 2012; Taranger *et al.*, 2015; Güralp *et al.*, 2020).

Yet, many of the genes associated with disease and resistance traits have not been properly annotated or at least identified via QTL. One example is the cardiomyopathy syndrome (CMS) in Atlantic salmon, a severe cardiac disease caused by the piscine myocarditis virus (often referred to as PMCV) (Garseth *et al.*, 2018). Only recently have researchers discovered that resistance to CMS is highly heritable and influenced by a single large QTL on chromosome 27, this region harbours candidate genes that are related to the immune system (see Figure 7) (Hillestad and Moghadam, 2019; Hillestad *et al.*, 2020). Mechanical delousing methods can be quite stressful for the salmon, causing death in those susceptible to CMS, so salmon demonstrating CMS resistance hold commercial value. Although the QTL is identified to be on chromosome 27, we still don't know the exact sequence or

location of the gene. These results are nevertheless encouraging for later SAGE to introduce favourable alleles where resistance is a monogenic trait (see the CMSEdit project in Table 3).

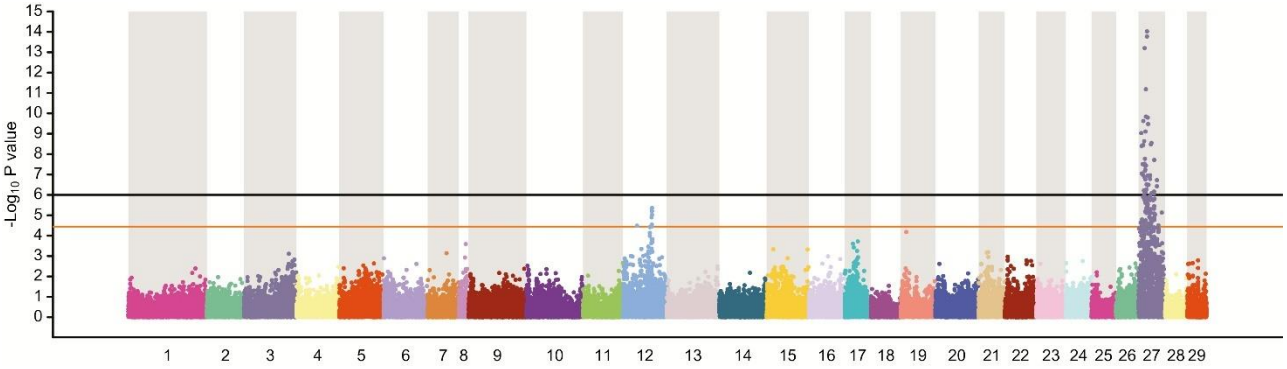

Figure 7: The Manhattan plot of association analysis to CMS resistance in Atlantic salmon, published by Hillestad and Moghadam (2019). Note the strong QTL signal at chromosome 27 associated with CMS resistance. A second signal is suggested on chromosome 12. The black and orange horizontal lines indicate the genome-wide and chromosome-wide significance threshold cut-off levels, respectively.

A second method of introducing favourable alleles into an organism is one proposed by Jenko and colleagues (2015): the 'promotion of alleles by genome editing' (PAGE) (Table 4). The authors tested two hypotheses on the suitability of PAGE for desired traits that are quantitative in nature. The first hypothesizes that many QTL should be edited before a desirable phenotype is observed. This would require large datasets of phenotyped and sequenced individuals to identify the numerous quantitative trait nucleotides (QTN) of the causative genes (Hickey *et al.*, 2016). The second hypothesizes that if the focus of PAGE centers on a small number of QTN, then only a small number of edits is required to observe genetic improvement for the desired trait. In their simulation study, the authors edited 20 loci per animal for a trait defined by 10 000 QTL. The results show a large response to selection between those who underwent genomic selection (GS) only and those where an increasing number of loci were edited (1, 5, 10 and 20 edited loci). It is clear from the model that where 20 edits were made, the response to selection was double that of GS individuals, in other words, a 100% increase in genetic progress compared to GS alone. Further, that when those 20 edits targeted loci with the largest effect on the trait, the authors observed very large differences to the change in allele frequency, without an associated loss of favourable alleles. The authors propose that gene editing brings about a rapid change in the frequency of favourable alleles compared to GS, by increasing the rate of fixation of the allele frequency and by preventing the loss of favourable alleles caused by drift (***Box 7***) and hitchhiking (***Box 4***).

Box 7

Drift This is an evolutionary mechanism, whereby the allele frequency of a given population changes over generations, resulting in the loss or fixation of some alleles.

It must be highlighted nonetheless, that the results from Jenko et al. (2015) are based on a simulation study, to augment a genomic dairy cattle breeding scheme. Secondly, a further simulation study by Simianer, Pook and Schlather (2018) argues that the results reported by Jenko et al. (2015) are overly optimistic, expressing their reservations about the expectations of PAGE. In their simulation study, they found that genetic progress demonstrated was not 100% but rather between 2 and 20% when varying some of the parameters. Particularly, the best genetic progress was observed when the heritability of the trait was higher, with larger mapping populations to better detect QTN, and with a

limited number of QTN. Conclusively, the primary reason for the lowered expected gain is the low success rate of finding true QTN for the gene editing step, in addition to the complexity of polygenic traits (Simianer *et al.*, 2018).

Table 4: An abbreviated summary of the various strategies available to researchers or breeders when targeting specific genes for increased host resistance to sea lice. The table in this case first identifies the methods possible to introduce/change the desired genes (if such information is available). The table also explores the amount of resistance demonstrated by the genetic change as well as the longevity of the resistance conferred (where known). The final column provides an estimation of the time it takes to produce the resistant organism and where known, the time required for market approval.

Strategy	Method	Benefits	Drawbacks	Resistance	Durability	Time	References
Classic breeding	GS/pedigree selection	Low legislative hurdles	Low market incentives related to patenting and commercial interest; limit inbreeding and linkage drag of unwanted alleles	Partial. Possible 16 – 23% per generation but likely lower	Unknown – depends on plasticity of pathogen	4 years per generation; predicted 10 generations to reduced chemical treatments	(Gharbi *et al.*, 2015; Tsai *et al.*, 2016; Greaker, Vormedal and Rosendal, 2020; Coates *et al.*, 2021)
SAGE	Knockout	No new DNA introduced	Single causative gene not identified	Unknown	Unknown	Short time	(Wargelius *et al.*, 2016; Güralp *et al.*, 2020)
PAGE	Genome Editing	Achieve genetic progress in short time	Uncertainty re amount of genetic progress possible; Require improved protocol to edit multiple alleles simultaneously	Unknown – only simulations available	Unknown	4 years per generation	(Jenko *et al.*, 2015; Simianer *et al.*, 2018)
Introgenic resistance	Introgression by editing	Introduce genetic biodiversity not currently found in Atlantic salmon	Possible impact of change in gene expression affecting desired trait	Partial to complete	Unknown	4 years *possibly 20 years for market approval[3]	(Gratacap *et al.*, 2019)
Synthetic DNA	Genome editing	Introduce small insertions or point mutations/ changes to DNA (see **Box 7**)	Unknown genetic and biological mechanism explaining lice resistance	Unknown – not been done	Unknown – not been done	3-6 years	(Sutherland *et al.*, 2017; Bullock *et al.*, 2021)

A third option for breeding quantitative resistance traits could be genome editing to move favourable variants from different populations, strains or species into the genome of Atlantic salmon (Gratacap *et al.*, 2019). In the CrispResist project (Table 3), researchers are attempting to harness the natural biodiversity and increased resistance to sea lice observed in the Pacific salmon species and bring it into Atlantic salmon. By identifying the few loci not present in the Atlantic salmon population, gene editing can facilitate such *introgression-by-editing* (Table 4) – a modern adaptation of traditional introgression (***Box 3***) (Gratacap *et al.*, 2019). Numerous studies corroborate that coho salmon

[3] The AquAdvantage salmon is the only known genetically modified organism to be approved for deliberate release for commercial purposes. The salmon was modified to grow faster by introducing genetic information from a different species (transgenesis). It took over 20 years for release approval in the USA and Canada (Clifford, 2014).

(*Oncorhynchus kisutch*) possess an increased resistance to sea lice: lice infestations are less dense and there is slower maturing of attached lice (Fast *et al.*, 2002; Bravo, 2003; Hamilton-West *et al.*, 2012; Zalcman *et al.*, 2021). If the gene information explaining this resistance can be identified, it may be possible to introduce the gene sequence into Atlantic salmon populations.

Finally, it is also possible to introduce novel or synthetic alleles into the Atlantic salmon genome, based on the biological knowledge of the trait (Table 4). In this case, the biological mechanism conferring resistance may be understood and could lead to a gene edit to introduce that mechanism, thereby not relying on genetic variation existing in the species. Knockout of the *dnd* gene in Atlantic salmon to create sterile fish is such an example, where the sterility trait does not exist in the species but can be introduced (Wargelius *et al.*, 2016). The exploration of the biological mechanism of lice resistance continues (Sutherland *et al.*, 2017; Robledo *et al.*, 2018). The introduction of novel alleles can also be brought about by results of the genomic perturbation screens (GeCKO) covered above.

Chapter 4: Legislation

The second research question of this thesis investigates how the current Norwegian legal framework functions and how potential future frameworks might function. We will consider the present scope of the Gene Technology Act (Genteknologiloven) (hereafter the GTA or the Act) for genome edited organisms as well as the proposal by the Norwegian Biotechnology Advisory Board (hereafter NBAB or the Board). To this end, we briefly explore the international treaty governing movement of modified organisms between states. Thereafter, we explore Norwegian law and how sustainability forms part of the assessment of modified organisms. Since we later consider a genome edited salmon, we must also consider the present Animal Welfare Act (Dyrevelferdloven) and how public perception drives the shaping of what is 'welfare'. The question of sustainability later aids in the third research question whether CRISPR potato and salmon might provide a positive impact on sustainability, specifically environmental health, and fish welfare.

1. Law at the international level

The United Nations (UN) Cartagena Protocol on Biosafety[4] governs the movement of "living modified organisms" (LMOs) between countries. The terms LMO and GMO are generally deemed to have the same meaning and will be treated as one and the same in this thesis (Husby, 2007). The Protocol primarily governs the export and trade of LMOs between states, but it can also be used to justify domestic legislation containing those international trade obligations (Fauchald, 2012). The Protocol is particularly relevant in applying the *precautionary principle* (***Box 8***) when assessing the risk of LMOs to human health and the environment (Martuzzi and Tickner, 2004; Fauchald, 2012). There are four elements underlying the precautionary principle: take preventative action when there is uncertainty; shift the burden of proof to the proponents of the activity; explore alternatives to possibly harmful actions; and increase public participation in decision making (Kriebel *et al.*, 2001). In Norway, the precautionary principle is a core element of the legislation governing the deliberate release of GMOs (Fauchald, 2012). However, Norwegian law also goes beyond the precautionary approach by including elements of sustainability and societal benefit in the assessment for release[5].

Box 8

Precautionary principle A guiding principle widely adopted in national and international instruments, yet without a commonly agreed definition or implementing criteria.

Generally, the principle posits that in cases of serious or irreversible threats to human health or the environment, precautionary steps should be taken even when there is some acknowledged scientific uncertainty.

What constitutes an LMO at the international level has a global impact on national regulatory schemes, particularly for an export-driven Norwegian market. Those same words (or at least similar) are often transposed directly into national legislation, together with the interpretations assigned to those words and the message of inherent risk associated with organisms created by biotechnology. It

[4] Secretariat of the Convention on Biological Diversity (2000). Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes. Montreal: Secretariat of the Convention on Biological Diversity. Available at https: //bch.cbd.int/protocol/text/ [Accessed: October 20, 2020].
[5] §10 of the GTA.

is thus prudent to explore how an LMO is defined. For summary purposes, we present Table 5 on how legislative instruments define what is a 'genetically modified organism' and what methods are deemed 'modern biotechnology'.

Table 5: What organism is defined as a 'genetically modified organism' and what constitutes 'modern biotechnology' per jurisdiction.

Territory	Instrument	'genetically modified organism'	'modern biotechnology'
International Treaty	Cartagena Protocol on Biosafety	**Art 3 (g)** LMO is 'any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology'	**Art 3(i)** the application of: a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;
Norway	Gene Technology Act of 2 April 1993 No. 38 Relating to the Production and Use of Genetically Modified Organisms etc.	**§4(b)** a microorganism, plant or animal in which the genetic material has been altered by means of gene or cell technology	**§4(c)** techniques that involve the isolation, characterisation and modification of heritable material and its introduction into living cells or viruses

A living organism is genetically modified if it meets two requirements: (1) the organism contains a *novel combination* of genetic material, (2) which was introduced by using *modern biotechnology* (Table 5). A *novel combination* encompasses a new combination of nucleic acid containing functional units of heredity not previously existing at the time it was produced (Mackenzie *et al.*, 2003). Here, the novelty results in three ways: (i) via a *novel arrangement* of genes sourced from within and without species, (ii) through the presence of a *novel form* of the gene, i.e. by altering, inserting or deleting one or more nucleotides and (iii) even by the *change* of a single nucleotide (Mackenzie *et al.*, 2003). Notably, a novel combination is limited to the genetic material – in other words, a change to the DNA meets the requirement even if no observable change in the organism's phenotype or behavior occurs.

The second half of the definition refers to *modern biotechnology*, the defining criterion of whether an organism is an LMO or not. Let us consider first how the phrase may be commonly understood. The word *modern* may appear misleading, as the term generally relates to, or is a 'characteristic of the present or the immediate past' (Merriam-Webster, 2021). The term *biotechnology* describes the use of living organisms to improve their suitability for pharmaceutical, agricultural or industrial applications (Rogers, 2021). Indeed, the term is so broad that it does not require gene insertion and encompasses novel scientific fields such as molecular breeding using genomic selection techniques. If we take genomic selection to be a technique developed recently (in the early 2000s) and that uses a living organism to improve a product, it is a great example of modern biotechnology. Yet, we know

that GS is *not* a modern biotechnology under the Protocol. This is because the word 'modern' in the Protocol was actually inserted as a way to exclude LMOs produced by traditional breeding methods and has little to do with *when* the technique is developed (Mackenzie *et al.*, 2003, p. 46). The Protocol thus assigns a narrower definition of *modern biotechnology* (Table 5). It means the application of either *in vitro* nucleic acid techniques (which includes recombinant DNA and direct injection of nucleic acid into cells or organelles) or the fusion of cells beyond the taxonomic family[6]. The text also provides two qualifications: (i) the techniques must "overcome natural physiological reproductive or recombination barriers" and (ii) must not be "techniques used in traditional breeding and selection" (Table 5).

At this stage, contentious discussions continue as to whether certain genome edited organisms are considered an LMO or not per the Cartagena Protocol. The challenge lies in the legal and technical interpretation of what is "modern biotechnology", particularly the focus on overcoming the natural mating or recombination barriers (Keiper and Atanassova, 2020). Some argue that many of the alterations induced by genome editing do not *overcome* what would (or would not) occur during natural mating or recombination. Particularly when considering a deletion or mutation of a few nucleotides of the DNA, a common occurrence in plants and animals during mating or recombination (Custers *et al.*, 2019).

2. The Gene Technology Act

As a member of the European Economic Area (EEA) agreement, Norway remains linked to the EU's internal market and its governing policies. Thus, Norway retains access to the four fundamental freedoms characterising the EU: the free movements of goods, persons, services, and capital. EEA members are not, however, bound by the common EU policies on agriculture and fisheries. That power mostly falls within the purview of the respective territories. The Gene Technology Act[7] regulates the production and use of GMOs in Norway[8] and at this stage, that includes genome edited organisms too (Myskja and Myhr, 2020). Norway is a ratifying member to the Cartagena Protocol but the GTA in fact predates the Protocol, making the country a pioneer in its assessment and regulation of GMOs (Roger, 2015). The definition of what is a *genetically modified organism*[9] thus differs slightly from that contained in the Protocol but the treaty justifies maintaining the current regulations on GMOs (Fauchald, 2012).

In assessing genetically modified organisms, the Act draws a distinction between the process required for contained use (operating in a closed system)[10] and deliberate release[11]. The latter encompasses field experiments, release for commercial purposes like cultivation or farming, greenhouses,

[6] Article 3(i) of the Cartagena Protocol on Biosafety.
[7] Gene Technology Act of 2 April 1993 No. 38 Relating to the Production and Use of Genetically Modified Organisms etc. English version available at https://www.regjeringen.no/en/dokumenter/gene-technology-act/id173031/ [Accessed October 2, 2020]. Norwegian version available at https://lovdata.no/dokument/NL/lov/1993-04-02-38 [Accessed April 6, 2021].
[8] §1 of the Gene Technology Act (*ibid*).
[9] See the definition of 'genetically modified organism' and 'gene technology' in §4 of the GTA (*ibid*).
[10] Chapter 2 Contained use of genetically modified organisms in the GTA.
[11] Chapter 3 Deliberate release of genetically modified organisms in the GTA.

aquaculture or animal facilities that are not approved for contained use, placing a product on the market, import, transport and export[12]. The Act requires an approval process for certain types of deliberate release, which is only granted when the production and use of the organism is in keeping with the purpose of the Act. The purpose of the GTA is to ensure a safety assessment of the organism's effects on health and the environment, as well as a non-safety assessment of the organism's contribution to sustainability, societal benefit, and ethical justifiability[13]. When granting deliberate release, the assessing body must give *considerable weight*[14] to whether the organism "will be of benefit to society and is likely to promote sustainable development"[15]. These *non-safety considerations* are contentious in global discussions about regulating genome-edited organisms, and the arguments for including them in legal frameworks are explored in the discussion of this thesis.

A distinction must be drawn between an assessment for the purposes of making a final decision on release and the assessments conducted by NBAB to facilitate that decision-making process. The former impact assessment is mandated by the Regulations[16] and is generally within the purview of the Norwegian Environment Agency. The role of NBAB is established in §26 of the GTA and §11 of the Regulations, wherein they may give input to the decision-making process based on the documents provided by the applicant. In some cases, the application contained approximately 1600 pages in total (T Hvoslef-Eide, 2021 personal communication 4 June). In their assessment, NBAB primarily considers health, environmental and societal concerns (Rosendal, 2007, 2008). The content of these primary categories can be found in Table 6 below. Certain of the considerations below, such as antibiotic resistance, might no longer be a factor to consider in genome edited products as antibiotic markers are not always necessary. Yet others, such as detectability and tracing might require deeper consideration, particularly when gene edits can be so negligible in size and without an accompanying marker (Duensing *et al.*, 2018; Grohmann *et al.*, 2019).

When it comes to evaluating sustainability and societal benefit, the Regulations require that each be used as an independent evaluating criterion as well as criteria that may result in a less stringent application of the precautionary principle[17]. This is a particularly striking point for this thesis: it implies that we should consider whether the long-term benefits of a CRISPR-edited potato or salmon are strong enough to warrant a less stringent application of the strictly science safety assessment. In this case, the question of a contribution to sustainable benefits must be based on the principles of a cost-benefit analysis[18]. The Regulations provide six broad categories with which to assess sustainability of the product, relating to global impacts, ecology limits, basic human needs, distribution between generations and rich and poor countries, and economic growth impacts[19] - although this list is not exhaustive in nature (see the column *Society* in Table 6). The appendices to

[12] §9 of the GTA.
[13] See §1 of the GTA.
[14] '…vesentlig vekt' in Norwegian.
[15] §10 of the GTA.
[16] Forskrift 16. desember 2005 nr. 1495 om konsekvensutredning etter genteknologiloven. Original Norwegian available at https://lovdata.no/dokument/SF/forskrift/2005-12-16-1495 [Accessed May 18, 2021]. Unofficial English translation available at https://www.regjeringen.no/en/dokumenter/impact-assessment/id440455/ [Accessed May 18, 2021].
[17] Appendix 4 of the 2005 Regulations.
[18] Appendix 4 of the 2005 Regulations.
[19] Part IV (A) of Appendix 4.

the Regulations provide the most important guidance for an impact assessment but NBAB has also published various guiding instruments on specifically assessing the sustainability aspect (NBAB, 2009, 2011, 2013).

Table 6: Considerations by the Norwegian Biotechnology Advisory Board for assessment proceedings of GMO applications. Information from Appendix 4 of the 2005 Regulations combined with the report by Rosendal (2007) (reproduced with permission).

Environment	Health	Society
▪ Cross-pollination and horizontal spread, including resistance in target species and genetic erosion ▪ Effects on non-target species ▪ Tracing and labelling ▪ Precautionary principle ▪ Reduced herbicide use or not ▪ Antibiotic resistance ▪ Liability ▪ Coexistence	▪ Allergies ▪ Digestive effects ▪ Antibiotic resistance ▪ Toxicity	▪ Global impacts on biodiversity, ecosystems and production ▪ Ecological limitations ▪ Basic human needs ▪ Societal utility ▪ Changes/growing social inequity ▪ Economic growth ▪ Reduced opportunity to reuse seeds for farmers ▪ Ethics and sustainable development – effects on use of chemicals when growing GMOs; effects on global agricultural structures and North–South issues of equity ▪ Distribution between generations

Although the GTA was one of the first instruments of its kind, Norway has yet to approve any GMOs for deliberate commercial release, excepting a single species of ornamental purple carnations (Mattilsynet, 2019; ISAAA, 2020). Due to their link to the EU, any GMOs approved for release in the EU automatically take effect for Norway too. However, Article 23(1) of the Directive 2001/18/EC enables EU Member States to restrict or prohibit the release of that GMO in all, or part of their territory. The so-called 'safeguard clause' has resulted in several EU countries or regions prohibiting release of GMOs, thereby creating a *de facto* ban on GMOs – including in Norway[20] (Lombardo and Grando, 2020; Turnbull *et al.*, 2021). In theory, more GMO products should be available for release, including modified maize, oil rapeseed and canola but are in fact prohibited.

Against this backdrop, NBAB wished to acknowledge the significant development and potential of emerging gene technologies to make a positive contribution to not only Norwegian society, but also the EU. The Board thus recently published and delivered a proposal to the government for the relaxation of legislation on the deliberate release of GMOs (NBAB, 2018; Bratlie *et al.*, 2019). NBAB investigated whether the existing legislation and the more restrictive practices sufficiently facilitate and stimulate positive innovation in light of biotechnological advances. The Board thus proposed differentiated requirements for an impact assessment depending on the specific genetic change brought about – in other words, using tiered regulations (see Figure 8). The tiered approach is based

[20] Forskrift 15. desember 2000 nr. 1268 om forbud mot omsetning i Norge av bestemte genmodifiserte produkter. Original Norwegian version available at https://lovdata.no/dokument/SF/forskrift/2000-12-15-1268 [Accessed June 25, 2021].

on two principles: (i) the type of genetic change that occurred and (ii) the principle of equal treatment for similarly altered organisms, irrespective of *how* they were produced. The first principle acknowledges scientific consensus that the size of genetic change does not dictate the extent of the change to the phenotype. Hence, the type of genetic change includes evaluating both the extent of the change and the resulting characteristics from that change (NBAB, 2018, p. 36). Depending on these criteria, the organism (be it plant, animal, or microbe) enters a particular tier with its related requirements.

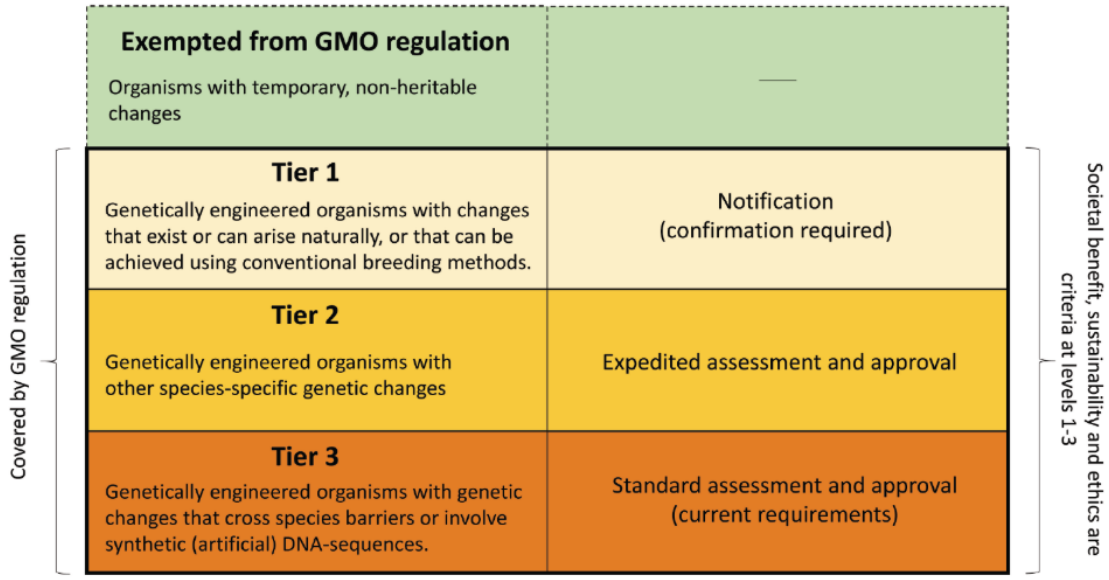

Figure 8: The Norwegian Biotechnology Advisory Board's proposed tiered approach to regulating genetically engineered organisms. The differentiated approach depends on the nature of the change to the genetic material, no matter which organism and method of production. Image from the proposal by the Norwegian Biotechnology Advisory Board (2018, p. 36).

There is uncertainty on how the Norwegian government will amend the GTA to accommodate genome edited organisms in light of NBAB's proposal. Yet, for purposes of this thesis, we shall apply the tier system to the proposed genetic strategies so that we might see the diversity of such a system. It should be noted that NBAB's proposal covers a number of related topics, including labeling, traceability, and monitoring requirements; however, in order to keep the focus of this thesis narrow, we will not discuss these additional topics. Below, a brief account of NBAB's tier system is set out, with a more comprehensive assignment of tiers found in Supplementary Figure 1. First, organisms that demonstrate temporary and non-heritable changes to their DNA enter Tier 0 and are thus exempted from the GTA and associated regulations (depicted by the green tier in Figure 8). An example of such a situation is the use of the veterinary DNA vaccine Clynav on Atlantic salmon to combat a viral-induced pancreatic disease (European Medicines Agency, 2017). The salmon in these cases will not be subject to the GTA regulations because there is no permanent change to the DNA of the salmon[21].

[21] In the course of 2020, the Ministry of Climate and Environment submitted three proposed amendments to the GTA for further consultation. These proposals directly relate to the example of using a DNA vaccine on Atlantic salmon. The third

Organisms entering Tier 1 are those where permanent genetic changes occur, by means of point mutations (***Box 9***) or a substituted allele from the same or a closely-related species (depicted by the light-yellow tier in Figure 8). The genetic changes generated in this case are those that can either exist naturally in the species or are possible using classic breeding strategies (for instance, the mutant *mlo* gene in barley was discovered more than six decades ago as a naturally-occurring resistance) (Pavan *et al.*, 2009). The producer in these circumstances submits a notification of the type of genetic and phenotypic changes that have occurred, also documenting how the product contributes to the three non-safety considerations. Organisms demonstrating large deletions in their DNA, or extra genes copies from the same or closely-related species, might enter Tier 2 and be subject to an expedited assessment and approval process (depicted by the yellow tier in Figure 8). See other types of genetic changes that may result in a Tier 2 assignation in Supplementary Figure 1. A potato containing stacked resistance genes against late blight from a wild potato species provides an example of such an organism (cisgenic organism). Finally, organisms where genetic material is introduced from a different species or derived from novel, synthetic DNA will enter Tier 3 and be subject to the current assessment and approval process set out in the GTA (depicted by the orange tier in Figure 8). At Tiers 1, 2 and 3 the Board emphasises their consistency in requiring an assessment of ethical, sustainable, and societal benefit of the submitted organisms.

Box 9

Point mutations The CRISPR-system offers a single- or double-stranded cut to the DNA coupled with natural repair of the DNA; single, directed nucleotide base changes; or the insertion or deletion of a small number of nucleotide bases. These are referred to as point mutations.

At this stage, the GTA regulates all organisms produced using genome editing as GMOs, which means applicants must seek approval for deliberate release. Since both NBAB and the Ministry of Climate and Environment have proposed changes to the Act, a committee was established in November 2020 to consider amongst others, gene technology, new breeding technologies, and the regulation of these organisms under the Act (Klima- og miljødepartementet, 2020b). While genome editing research continues in Norway on potato and Atlantic salmon (see the list of projects in Table 3), the question which may be considered is whether a product of this research might be approved for release for commercial purposes, in light of previous rejections for other GMOs. In this case, the GTA does provide some flexibility in §10 by providing that the King (that is to say, the government) may by drafting regulations identify specific types of GMOs for release into a specific environment, without undergoing the approval procedure. Such a release would only require the applicant to submit a notification. This certainly leaves room for a more liberal approach to regulating GMOs, whether the government chooses to adapt the GTA to a tiered system as proposed by NBAB or to retain the text of the GTA but enact more lenient regulations as permitted in §10.

proposal calls for an inclusion of a clause to the effect that: regulations may be issued that specific organisms shall not be covered by the Act. In other words, the Ministry seeks to have salmon treated with the vaccine exempted from being considered a GMO (Klima- og miljødepartementet, 2020a).

3. The Animal Welfare Act

Animals, unlike plants, are not simply inert objects but living and sentient organisms (Hoppe, 2018). The way we view animals is no longer purely utilitarian; rather, animals have a moral status, and thus certain activities should be restricted or prohibited (Hoppe, 2018). In addition to the Gene Technology Act, the Animal Welfare Act (AWA)[22] is particularly relevant to our discussion on genome editing in Atlantic salmon. In general, when it comes to production and use of animal biotechnology, three factors must be considered: legal governance, ethical considerations, and public perception.

The AWA has as its object the promotion of good animal welfare and respect for animals[23] (Olesen, 2010). In addition to this, it states that animals have an intrinsic value beyond their utility to man[24] – in other words, the value that man applies to their use (Hoppe, 2018, p. 236). Breeding of animals shall be aimed at encouraging robust animals, that display good function and health[25]. The rest of section 25 is couched in more restraining language: that breeding using gene technology shall not be used to change genes that may alter their inherent 'animal-ness', in other words, the physical, mental or behaviour inherent to that animal; or in a way that causes other ethical reactions. On balance, genome editing for sea lice resistance should not result in a negative change in the physical or mental characteristics of the salmon, or their inherent behavior. Nonetheless, if genome editing causes an ethical reaction (§ 25 litra (c)), its use in animal breeding may be forbidden.

Mejdell (2000) points out that breeding using gene technology is the only circumstance in the AWA in which the general publics' acceptance or rejection (ethical reaction) is given weight[26] (Mejdell, 2000, p. 202). The AWA makes no distinction between traditional breeding practices and gene technology when it comes to ethics. There is a long history of traditional breeding practices in Norwegian production animals for economically valuable traits. Breeding for these traits often exhibited a negative genetic correlation between production effectiveness and health and welfare, across cows, pigs and chickens (Mejdell, 2000). Hence the purpose of the AWA for robust, healthy and well-functioning animals – breeding goals, no matter whether they are achieved by traditional breeding methods or by means of gene technology must balance the pursuit of efficient and profitable production with the wellbeing of the production animals (Olesen, Groen and Gjerde, 2000; Olesen, 2010). Despite the inherent risk to welfare from linkage drag, traditional breeding remains largely uncontroversial, especially compared to more biased perceptions associated with gene technology (Frewer *et al.*, 2013; Van Eenennaam, 2017). This is not the case when using biotechnology, where the main advantage is the absence of linkage drag of those negatively correlated traits due to the technology's precision (Bishop and Van Eenennaam, 2020; Van Eenennaam *et al.*, 2021).

Public perception of animal biotechnology in Norway, like many countries, does not stem from any direct experience with the actual products (Tizard *et al.*, 2016). This leaves evaluating public

[22] Lov 19. juni 2009 nr. 97 om dyrevelferd. Original Norwegian available at https://lovdata.no/dokument/NL/lov/2009-06-19-97 [Accessed April 12, 2021]. Unofficial English translation available at https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/ [Accessed April 12, 2021].
[23] § 1 of AWA.
[24] § 3 of AWA.
[25] § 25 of AWA.
[26] § 25(c) of AWA.

perception by means of surveys and discussions with focus groups, an endeavour undertaken by the GENEinnovate project (Table 3). The survey in this case proposed (amongst others) a scenario of genome edited salmon resistant to sea lice, thus, edited to improve fish health. The results indicate that the majority of Norwegian respondents (just under 60%) were positive about using gene editing to improve fish health (NBAB, 2020b, p. 15). Additionally, the arguments most prevalent in their assessments of gene editing for different purposes were animal welfare, sustainability, and consumer benefits (NBAB, 2020b, p. 16). Similar motivations were found in an earlier Norwegian study where consumers were more willing to pay for farmed Atlantic salmon if it means better resistance to sea lice and thus increased fish welfare (Grimsrud *et al.*, 2013). In an as yet unpublished study by Naab and colleagues, the results indicate that improved animal health could be one 'tipping point' for public acceptance of genome edited animals (Frewer, 2020). The argument for gene editing in Atlantic salmon to avoid current practices to combat sea lice is thus closely linked to Norwegian attitudes to animal (fish) welfare, which is reflected in the law. A more comprehensive discussion of whether we can move to using a CRISPR-edited salmon in Norway continues below.

Chapter 5: Discussion

It is prudent at this stage to return to the research problem initially outlined in the introduction. The goal of this thesis is to fill a knowledge gap about how gene editing technology, current and future regulation, and stakeholder inputs may affect specific industries in Norway. This thesis proposes that breeding for increased host resistance may offer an alternative solution to the current methods employed to combat late blight and sea lice, respectively. To this end, the first research question asks what genetic strategies and methods are available to solve the challenges of pests. The second research question investigates the current legal framework in the Gene Technology Act and the Animal Welfare Act as well as a potential legal framework by a Tiering system that could regulate new products created by CRISPR technology. The final research question asks whether it is possible to move to using new CRISPR technology to provide food that has a positive impact on environmental health and fish welfare. Given the ongoing discussions by the appointed committee on regulating genome edited organisms under the GTA, such an assessment might be viewed as premature but it could nevertheless provide some guidance for future work in anticipation of changes to the law.

The work above reviews the costliest challenges associated with potato and Atlantic salmon production in Norway, brought about by pests that directly impact the sustainable development of those industries (Section 2 of Chapter 1). We then focused on how we can use precision gene technology to target different genes and explore how these options compare in terms of genetic gain, generation interval, durability of the solution, and the advantages and disadvantages of each. As much as genome editing technology holds exciting potential to solve food production issues, we cannot continue to view the science in isolation – those products must be further considered within the existing (and possible future) governance frameworks as well as paying attention to the desires of stakeholders (Figure 9).

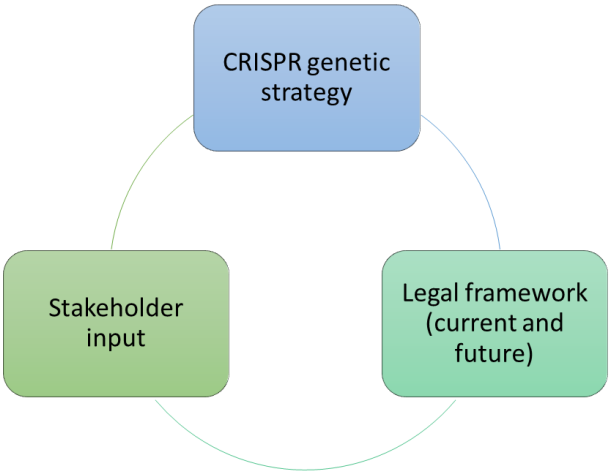

Figure 9: An interconnected, non-hierarchical view of technology, law and stakeholders.

Figure 9 presents a good summary of the work of this thesis. Part of the process requires a discussion on how to use gene technology as a strategy to breed for more resistant organisms (**genetic strategy**). Whichever method we may use, the ultimate aim is to present an organism having demonstrable resistance to their respective pest. To be able to use that resistant organism in production, it must enter a legal framework to assess the risks associated with the new organism, particularly for purposes of deliberate release (**legal framework**). The body assessing those risks examines health and environmental safety criteria as well as non-safety criteria: the societal benefit, and sustainability of the organism. Again, this thesis does not embark on an assessment of the risk criteria, but rather on the non-safety criteria, particularly as it concerns sustainability. When assessing the sustainability of food and agricultural systems, the FAO identifies four dimensions: good governance, environmental integrity, economic resilience and social well-being (FAO, 2014). Environmental integrity and its components (atmosphere, water, land resources, biodiversity, materials and energy, and animal welfare) encompasses the sustainability assessment that is applicable for this thesis (FAO, 2014). In their report, Bardalen, Skjerve, and Olsen (2020) applied these four dimensions to the Norwegian food system, explaining that environmental sustainability means maintaining food production and supply that is crucial to human survival, while minimising negative environmental impacts as well as promoting positive environmental impacts (Bardalen *et al.*, 2020, p. 8). Indicators (such as fungicide use and the presence of sea lice) are just one way to measure the environmental impacts of a specific sector, which informs the progress of that sector's sustainability.

Importantly, stakeholders who are involved and engaged in the development of indicators and their later use are more likely to use and appreciate those results (**stakeholder input**) (Bardalen *et al.*, 2020, p. 138). When considering genome-edited organisms for commercial purposes, we can narrow down certain actors who may inform and contribute to product governance and acceptance. Broadly, those stakeholders include breeders, farmers and growers, regulators, politicians, supermarkets (value chain), and consumers (Bardalen *et al.*, 2020). Stakeholders place varying levels of emphasis on different concerns, particularly as they relate to the environmental impact in potato farming and welfare of farmed salmon, respectively. To avoid cumbersome sentences by trying to include both species, the discussion hereafter will be divided between the two focus organisms. Thereafter, considerations that are common to both species will round off our discussion.

1. Increasingly resistant potato to solve environmental concerns

In Chapter 2 we presented Table 2, comparing potential gene targets and methods when breeding for host resistance against late blight. Table 7 now combines that information with the current legislative framework, as well as NBAB's proposed Tier system. Breeders have depended on classic breeding strategies since the first outbreak of late blight disease in the 1800s, in an attempt to harness complete resistance from wild potato relatives. The main advantage of traditional breeding is its long history of experience, which means it faces fewer legislative hurdles[27] than modern biotechnology techniques

[27] Norway is a ratifying member of the International Convention for the Protection of New Varieties of 1978 (often referred to as UPOV 1978), wherein a novel plant variety must pass the DUS test: the plant must demonstrate distinctness, uniformity and stability. See § 2 litra (a), (b) and (c) in Lov 12. marts 1993 nr. 32 om planteforedlerett. Available in Norwegian at https://lovdata.no/dokument/NL/lov/1993-03-12-32 [Accessed June 21, 2021]. Application in Norway is a relatively simple procedure (Hansen, 2020).

(see Strategy 1 in Table 7). Of course, the major drawbacks include the extraordinary time it takes to introduce those genes from the wild relatives into the domestic species (approximately 50 years) and the additional uncertainty around whether that resistance will be overcome by *P. infestans*.

Although uncertainty of defeat is also linked to genes introduced using traditional GM technology, partial to complete resistance can be achieved much more quickly (about 13 years for commercial R&D) and can use an array of gene targets, if such information is already available (Strategy 2 in Table 7) (Bullock *et al.*, 2021). At this stage, major R genes and Rpi genes are potential gene targets, showing strong resistance in field trials and expecting up to 80% reduced use of fungicide (Strategy 4 in Table 7) (Haverkort *et al.*, 2016; Ghislain *et al.*, 2019). Traditional GM technology to knock down gene expression demonstrates a variety of benefits and drawbacks (Table 2). RNAi may not result in a complete knock out of the target gene possibly due to inaccurate specificity or fluctuation in efficiency of silencing during the plants' development (Mansoor *et al.*, 2006). A product of this technology is also currently defined as a GMO and faces low approval success and low consumer acceptance in Norway (see Strategy 5 in Table 7). Even within the proposed Tier system, the product would likely fall into Tier 3 if it integrates synthetic DNA or DNA from a different species into the organism. Tier 3 evaluates the organism using the standard GMO criteria.

Table 7: Combining available genetic strategies in potato from Chapter 2 with current and proposed legal framework from Chapter 4. Abbreviations: *Admin. Regulations* refers to the regulations that may be issued by the King under § 10 of the GTA. *Not* indicates applicant must submit a *notification* for assessment of genetic and phenotypic changes. *Exp* indicates an *expedited* assessment of the organism and approval for release. *Std* indicates the *standard* assessment and approval procedure for deliberate release in terms of the current Chapter 3 of the Gene Technology Act.

Proposed Strategy	**Gene Technology Act**		**Approval**		**Admin. Regulations**	**Proposed Tier System**		
	GMO	**Non-GMO**	**Safety**	**Non-safety**		**1** *Not.*	**2** *Exp.*	**3** *Std.*
Potato								
Strategy 1: Classic breeding		✓						
Strategy 2: Single R or Rpi gene	✓		✓	✓			✓	
Strategy 3: Stacked R or Rpi genes (somatic hybridisation)		✓					✓	
Strategy 4: Stacked R or Rpi genes (GM tech)	✓		✓	✓			✓	
Strategy 5: S-gene (RNAi)	✓		✓	✓				✓
Strategy 6: S-gene (CRISPR knock out)	✓		✓	✓	✓	✓		
Strategy 7: Multi-edit knock in of R-genes with S-gene knock out	✓		✓	✓	✓	✓		

In an effort to avoid the GMO approval process, somatic hybridisation (SH) may be an option to stack a combination of R-genes and Rpi-genes into the desired cultivar (Strategy 3 in Table 7) (Rakosy-Tican *et al.*, 2020). A product of SH is specifically exempted from the GMO legislation in Annex 1B of the EU Directive 2001/18/EC (Table 5). Interestingly, Norwegian law does not exempt the SH technique itself like the EU legislation. Instead, the product of SH is only exempt if the two species would hybridise in nature[28] – certainly, a consideration worth exploring. If the tiered system were to

[28] § 2(a) of the GTA provides that the GTA does not apply to a genetically modified plant cell where the same result can be achieved by means of traditional breeding methods. In other words, where the plants could naturally hybridise.

apply, a product of SH with DNA from a closely related species would likely fall within Tier 2, requiring an expedited assessment and approval (Strategy 3 in Table 7). If the DNA were from a different species, the organism would enter Tier 3, requiring a standard GMO assessment (not shown in Table 7).

Although the Tier system could treat potato containing DNA from a closely related species as a Tier 2 organism, it must be remembered that it is still a cisgenic potato (Strategy 4 in Table 7). The proposed tiered approach is based on the type of genetic change and the resulting characteristics, with less emphasis on which biotechnological technique was used. This represents a minor shift in focus, acknowledging that some genetic changes are presumed to be less risky than others, especially if they can be achieved through conventional breeding (NBAB, 2018). As a result, incorporating the Tier system into the GTA would shift the law to a hybrid process/product-based framework – where the *process* of using gene technology triggers the GTA but the applicable risk assessment depends on the resulting *product*. It could mean a cisgenic potato faces lower regulatory hurdles than before but whether it necessarily means approval for release is unclear, given previous assessments to use cisgenic potatoes to combat late blight in Norway (Gillund *et al.*, 2015; van Hove and Gillund, 2017).

1.1. Can we move to using a CRISPR potato?

The third research question investigates whether it is possible (at this time) to use CRISPR to breed late blight resistant potatoes, thereby overcoming technical and legal issues associated with traditional breeding and GM technology. Knocking out the S-gene and multi-edit pyramiding using CRISPR technology appear to be the only genetic strategies in which edited potato may face fewer technical and legislative hurdles. As shown in Strategy 6 and 7 in Table 7, the GTA currently compels products of CRISPR technology to enter the GMO approval process, demanding a rigorous safety and non-safety assessment. Should the government choose instead to draft regulations in terms of §10, thereby enabling release of specified GMOs by way of notification, it stands to reason that this flexibility would not apply to GMOs created by classic GM technology. This preliminary conclusion is based on Norway's history of non-approval of GMO products since the GTA came into effect in 1993, as well as the fact that such Regulations have not been drafted during this time. It would almost certainly apply only to genome-edited organisms, in order to accommodate novel CRISPR products while distinguishing them from GM technology. There is accompanying concern though that it would be clearer and stronger to have a legal act by way of amendment to the GTA than an administrative act by way of regulations under §10 of the GTA (GMO-nettverket, 2020; NBAB, 2020a).

Under the Tier system, both a multi-edited potato and one containing no new DNA with a permanent knocked-out gene would likely enter Tier 1 requiring a notification of the resultant genetic and phenotypic changes (Strategy 6 and 7 in Table 7). It is uncertain how the notification process would commence, particularly for pioneering organisms that are the first to enter the Tier system. Let us consider an example: a Norwegian company finds a way to successfully knock out a S-gene in the "Mandel" cultivar (a late blight sensitive cultivar) and submits it under the Tier system for deliberate release. In Tier 1, the applicant company must submit a notification, containing information on the genetic and phenotypic changes, as well as documenting the organism's contribution to sustainable development, societal benefit and ethics. As the first of its kind, would a larger body of evidence be

required compared to subsequent applicant companies submitting organisms with similar genetic changes? The time saved in development R&D to edit and produce a novel, useful cultivar might be completely undone by the evidential requirements of the notification itself: should the applicant provide evidence of field experiments wherein the amount of reduction in fungicide is measured, perhaps across multiple seasons? If the company invests in this type of research for the pioneer potato, will companies with similarly-edited potatoes be excused from investing in this research? These are just a few of the issues that future GTA amendments should address.

1.2. Considering sustainability

Whichever course the government chooses to take, be it fewer or larger amendments to the current GTA, NBAB wishes to preserve the non-safety assessment, including the sustainability aspect. Current agricultural practices rely heavily on fungicide applications to control late blight epidemics. At present, that means several applications every year, costing farmers money and having enormous, accumulative effects on human health and the environment. Numerous measures have been introduced over the years to direct better sustainable use of fungicides in agriculture, such as the late blight forecasting system and banning the most dangerous substances (Tleuova *et al.*, 2020). Nevertheless, sustainability demands that decisions taken now must contribute to the sustainable development of the sector in future generations (Bardalen *et al.*, 2020). The Danish Ethics Committee in 2019 expressed that it would be ethically problematic to reject GMOs that can mitigate or resolve significant issues in agriculture (Det Etiske Råd, 2019). As a result, we can no longer ignore the contribution that a CRISPR potato would make to improving environmental sustainability in Norway. One could say that a decision *against* entertaining a CRISPR potato with increased resistance would be a decision *against* the principles of sustainability.

Yet, when a potential solution to controlling late blight is offered, stakeholders will consider various pivotal aspects on the sustainability of the organism. When considering a cisgenic GM potato resistant to late blight, all stakeholders agreed that breeding potato cultivars with increased resistance is the most sustainable path to controlling late blight disease – whether resistance is introduced by means of biotechnology or by conventional breeding (Gillund *et al.*, 2016). Similarly, Norwegian consumers' attitudes to gene editing depends on the purpose and product – particularly if there is a clear sustainability or societal benefit profile. Almost 70% of respondents were positive to the use of the technology if it meant reduced use of pesticides and crop losses to late blight. Moreover, the survey found that the majority of respondents believe that gene edited crop can be used in organic production if it means cultivation using less pesticide (NBAB, 2020b). Although a sustainable purpose for the edit is crucial for wider acceptance, producers agree that even conventionally bred varieties with increased late blight resistance are not widely adopted because they are not considered to perform well for other agronomic, consumption and processing qualities (Gillund and Myhr, 2016). European producers expressed similar sentiments and would rather choose a better performing quality potato than one that demonstrates better host resistance, precisely because they can rely on the fungicides available (Andrivon *et al.*, 2008).

Stakeholders are also concerned about whether and how the resistant cultivar will affect current agricultural practices – if the cultivar will reduce the need for fungicide, result in less soil

management, result in lower greenhouse gas emissions, or result in a lower need for energy (Gillund *et al.*, 2016). Most actors expect that a biotech potato will reduce the need for fungicides but that more information is needed on this particular topic. Crucially, there is very little scientific literature about the actual fungicide requirements of the GM cultivar in field trials (Gillund *et al.*, 2016, p. 369). Stakeholders want to know that in field trials closely reproducing practices in the potato sector, the fungicide applications were reduced by a measurable amount. They want more information on the extent of reduced use of fungicide (in concentration or numbers of application), consideration of fungicide use in different climatic conditions, and during all phases of the growing season (where earlier studies found that spraying toward the end of the growing season might be required for senescing biotech plants) (Jones *et al.*, 2014). This is a point that should be given serious consideration in future discussions and research for proposed use of gene edited potato in production. If this gap in knowledge is present for a GM potato, then there must also be a gap in the knowledge for a newly developed CRISPR potato that must urgently be addressed. Field trials of genome edited potatoes in the specific climatic conditions of central Norway where most potato production occurs could prove convincing empirical evidence.

Along with a decrease in fungicide input, stakeholders also wish for more information on the durability of the resistant cultivar. Not only will that give a measurable indicator of long-term sustainability, but it may also foster consumer positivity to gene edited products developed for the Norwegian market. Indeed, consumers were more positive if the gene edited product was developed by Norwegian researchers and breeding companies specifically for the Norwegian market rather than by international producers (NBAB, 2020b). Obtaining such information might prove a challenge since breeders would wish to keep the resistance genes confidential to retain their competitive edge. Additionally, even with field trials, it would be difficult to accurately predict the durability of the resistance until such time as the CRISPR cultivar is put into large-scale production. Parallel studies on resistance management strategies would also only give accurate predictions under large-scale production (Gillund and Myhr, 2016). Despite these extensive concerns, most consumers trusted that genome-edited products reaching the market would be for the benefit of society and the environment (NBAB, 2020b).

Breeding resistant potatoes proves a particularly challenging goal, particularly for a pathogen that has plagued farmers for over 180 years. Plenty of researchers or breeders wish to claim their cultivar will see an end to the war waged against late blight (Fry, 2008). For a crop like potato, where traditional breeding methods have not yielded much gain in the last century, GM technology offers a strong alternative. Yet GMOs still face significant barriers for release in Europe and Norway (Turnbull *et al.*, 2021). The hope is that CRISPR/Cas, with its precise ability to generate genetic changes similar to those achieved through conventional breeding, will provide a unique opportunity to combat late blight in a sustainable way. It is unclear at this stage how the GTA will be amended, if at all. However, CRISPR offers both precision and time savings to create a product that could be on the market in 10 years – but in order to truly achieve this, the legal framework and its implementation must not stymie such innovations. Of course, risks to humans and the environment must continue being assessed, and while we cannot possibly assess the minutiae of every single potential risk, we can weigh the benefits of a strong, sustainable option favorably. When we talk about sustainability in potato farming, do we

stick with what we know, fungicides, or do we consider other, technological innovations that could change current unsustainable practices? It seems stakeholders are positive toward and support the technology in achieving these goals while maintaining the quality expected from local varieties. To minimise unsustainable practices and begin implementing sustainable decisions, we must demonstrate empirical and localised evidence of reduced fungicides, as well as the durability and safety of gene edited potatoes. In other words, resistant potatoes not as a temporary solution for companies to make money but for long-term, meaningful use in sustainable potato production.

2. Increasingly resistant Atlantic salmon to solve welfare concerns

In Chapter 3 we presented Table 4, comparing the genetic strategies to breed for host resistance against sea lice in Atlantic salmon. We identified four genome editing methods for introducing desired resistance traits: SAGE, PAGE, introgression-by-editing, and synthetic DNA (Table 4). This approach deviates slightly from the approach taken in Table 2 for potato, due to the dearth of causative gene information for resistance to sea lice. Hence, in Table 8 below, the proposed strategy begins with the method, rather than the genes that may be targeted (like R or S-genes in potato). The four methods give rise to five potential strategies, depending on the type of genetic information that is established through further research efforts.

Table 8: Combining available genetic strategies in Atlantic salmon from Chapter 3 with current and proposed legal framework from Chapter 4. Abbreviations: *Admin. Regulations* refers to the regulations that may be issued by the King under § 10 of the GTA. *Not* indicates applicant must submit a *notification* for assessment of genetic and phenotypic changes. *Exp* indicates an *expedited* assessment of the organism and approval for release. *Std* indicates the *standard* assessment and approval procedure for deliberate release in terms of the current Chapter 3 of the Gene Technology Act.

Proposed Strategy	**Gene Technology Act**		**Approval**		**Admin. Regulations**	**Proposed Tier System**		
	GMO	**Non-GMO**	**Safety**	**Non-safety**		**1** *Not.*	**2** *Exp.*	**3** *Std.*
Atlantic salmon								
Strategy 1: Classic breeding		✓						
Strategy 2: SAGE (point mutations)	✓		✓	✓	✓	✓		
Strategy 3: SAGE (new DNA)	✓		✓	✓				✓
Strategy 4: PAGE	✓		✓	✓	✓	✓		
Strategy 5: Introgress by editing	✓		✓	✓			✓	

Classic breeding strategies in Atlantic salmon aquaculture provide a vivid example of the success of research-based genetic programs for commercial production (see Strategy 1 in Table 8). Over the years, several traits have been included in breeding goals, such as bodyweight at slaughter, increased age for sexual maturity, increased disease resistance, and quality traits (Thodesen and Gjedrem, 2006; Kumar and Engle, 2016). The estimated genetic progress of the first three traits lay in the range of 8 to 10% per generation, with such additive progress underlying the commercial success of salmon aquaculture in Norway (Gjøen and Bentsen, 1997). More recently, breeding companies in Norway have instituted genomic selection as a more effective means to increase lice resistance, with some major companies offering more resistant strains since 2016 (AquaGen, 2016; Coates *et al.*, 2021). After one generation using GS, fish with high resistance to sea lice showed 20-25% difference in lice numbers compared to those with low resistance (AquaGen, 2016). Clearly, there is a documented response of selection for lice resistance (Hillestad *et al.*, 2017; Rosendal and Olesen, 2021). Yet, time

remains a major limiting factor for most classic breeding strategies. It is predicted that after ten generations of selection on the best 1% of the population, treatments to remove sea lice can be eliminated (Gharbi *et al.*, 2015). The simulation in this case focused on one trait alone, and if we assume it takes four years per generation, ten generations of single-trait selection will still require 40 years of breeding, not to mention a high risk of inbreeding.

Genome editing to direct changes to resistance traits in Atlantic salmon includes a number of potential techniques (strategies 2, 3 and 4 in Table 8). At this stage, as with an edited potato, a salmon edited by CRISPR/Cas will be deemed a GMO and must undergo a safety and non-safety assessment. On the one hand, SAGE presents an opportunity to edit genes with a known sequence and function, particularly where one or a few genes underlie the trait. Depending on the desired outcome, the CRISPR-system offers a single- or double-stranded cut to the DNA, single nucleotide base changes or the insertion or deletion of a small number of nucleotide bases (these are all considered point mutations) (Strategy 2 in Table 8). Edited organisms possessing these point mutations will likely enter Tier 1, requiring notification of their genotypic and phenotypic changes, upon which an assessment will be conducted (Strategy 2 in Table 8). PAGE is a form of editing by point mutations, wherein several (up to 20 edits) are made for a highly polygenic trait such as lice resistance (Strategy 4 in Table 8). In this case, although the separate edits may be small individually and initially fall within the Tier 1, it is uncertain whether the total number of small edits may affect the final tier placement of the organism. It is also unclear whether the applicant will be required to provide information for each edit performed, that is to say, the genotype and phenotype for edits 1 through 20. Or would it be an overall assessment, looking at the genotype and phenotype change across the organism as a whole? Particularly for PAGE, the aim is to achieve a certain amount of genetic progress in a short period of time rather than present a wholly resistant organism. Would an edited Atlantic salmon demonstrating better resistance (but not full resistance) be considered enough of contribution to sustainability to overcome the perceived risks of these small edits?

If the strategy is to introduce new DNA into the salmon, either by way of synthetic DNA or derived from a *different* species, then the salmon would enter Tier 3 and be subject to the standard assessment for all GMOs (Strategy 3 in Table 8). The only animal that has ever been approved for deliberate release (in the United States and Canada) is a genetically modified Atlantic salmon called the AquAdvantage salmon (Clifford, 2014). The GM salmon contains a growth hormone-regulating gene from Chinook salmon (*Oncorhynchus tshawytscha*) and a promoter sequence from an ocean pout (*Zoarces americanus*). Should the AquAdvantage salmon request deliberate release in Norway, the organism would likely be subject to the requirements of Tier 3 based on the genes derived from non-closely related species. In any case, the approval process took over 20 years in North America and though this may seem a relatively long time, it still represents half the time required for breeding in lice resistance using traditional breeding methods. Nevertheless, when asked whether AquaBounty would enter the European market, the answer was a resounding no, owing to the legislative barriers and negative consumer attitudes toward GMOs in Europe, which did not make the risk worthwhile (Walton, 2020).

Finally, Strategy 5 in Table 8 offers a thought-provoking situation: an Atlantic salmon possessing targeted, integrated DNA from a closely related species like coho salmon. The GTA deems the cisgenic salmon as a GMO and it would be required to undergo the related assessments. We might consider at this point that there are instances of interspecific hybridisation between Pacific salmon including coho and Atlantic salmon (Blanc and Chevassus, 1979; Noakes, Beamish and Kent, 2000). If we recall the definition of 'modern biotechnology' in the Cartagena Protocol, the technique must be one that overcomes natural reproductive barriers. It seems clear that introducing resistance genes from coho salmon might present an opportunity to circumvent the GTA, although this also depends on the type of technique used. If we recall from somatic hybridisation in potato, Norwegian law does not exempt the technique itself. Instead, the product of SH is only exempt if the two species would hybridise in nature. Under the tier system however, there are two possibilities. If the new DNA introduces an extra copy of the gene, the salmon will enter Tier 2, requiring an expedited assessment (Strategy 5 in Table 8). If the new DNA substitutes a gene variant or allele, then the salmon will enter Tier 1 instead, requiring a notification (not shown in Table 8 but can be found in *Supplementary Figure 1*). Just like with a cisgenic potato, would the tier system necessarily mean a wider acceptance of a cisgenic salmon than is currently enjoyed?

2.1. Can we move to using a CRISPR salmon?

At this point, it is difficult to predict whether Norwegian society will adopt a CRISPR salmon. Based on current genetic data, far more research is needed before using CRISPR: knowing which genes to modify and how to implement and spread the edits in the breeding population are critical (Barrett *et al.*, 2020). Since the research is still in its infancy, there also tends to be a paucity of knowledge on the views of the various stakeholders. What is clear is that there is a strong emphasis on sustainability, including fish welfare, in salmon aquaculture. To maintain the focus on sustainability in research and industry development, we must identify and engage stakeholders in resolving challenges (Olesen, Myhr and Rosendal, 2011).

The aquaculture industry clearly believes that using genetic strategies to breed resistant salmon is a viable option. To illustrate, delousing methods can be quite stressful for the fish, causing death in those susceptible to the cardiac disease CMS (see Figure 7). Norwegian policy currently requires that salmon producers keep lice numbers low but without a genetically resistant line, producers rely more on mechanical delousing. To avoid further losses due to delousing, producers are requesting more CMS-resistant salmon roe, or fish that can 'survive' the delousing treatments (Rosendal and Olesen, 2021). The danger here is that animal breeding dictated by short-term market forces may result in unintended consequences (Olesen *et al.*, 2000). Since there is value in breeding for CMS-resistant salmon, there should be value in breeding for lice resistant lines too. However, this is not the case in all breeding programs, and the selective pressure on sea lice resistance is relatively low when compared to traits such as growth (I Olesen personal communication, 11 June 2021). Faster growth, on the other hand, may imply a shorter time period in sea net pens, giving sea lice less time to attach to hosts.

The low market supply and demand for lice resistant roe can also be partly explained by low market incentives of the trait (Rosendal and Olesen, 2021). Firstly, the polygenic nature of lice resistance makes it an undesirable object for patent protection. Secondly, the trait holds a 'public good' character rather than a commercially-preferred character and thus invites low incentive for private R&D funding (Greaker *et al.*, 2020). Due to the low market incentive, as well as the estimated time it will take to show a demonstrable reduction in lice infestations, and current efficiency in controlling infestations, including sea lice resistance as part of breeding goals is a low priority (Coates *et al.*, 2021). It is also predicted that putting more emphasis on welfare traits will raise production costs, causing product prices to rise (Nielsen *et al.*, 2011; Olesen *et al.*, 2011; Ellingsen *et al.*, 2015). Interestingly, a higher product price does not always deter consumers – there is strong evidence of a willingness to pay for improved salmon welfare through increased resistance to sea lice (Grimsrud *et al.*, 2013).

There is a distinction to be made between interest in a specific trait and use of technology. Although lice resistance is not a high priority for salmon breeders, animal breeders in general are very interested in using gene technology. In European animal breeding research and industry, genome editing for disease resistance in animals appears to be the primary target trait for improvement. In the 'Breeders talk Green' webinar series hosted in March 2021, around 60% of the audience identified improved disease resistance in animals as the trait of most interest (EFFAB, 2021). There was considerably less interest in using genome editing for animal welfare (~20%), protection of genetic biodiversity (~10%), increasing production (~5%) and protecting wild populations (~5%). Remarkably, genome editing for sea lice resistance offers the possibility of improved disease resistance as well as improved animal welfare.

Similar sentiments were expressed by consumers in the survey conducted by the GeneInnovate project (Table 3). Nearly 60% of consumers were positive about gene editing when the purpose for the edits was to improve fish health, like salmon resistant to sea lice (NBAB, 2020b). Contrastingly, only 20% of participants felt positive toward using gene editing for production traits in animals (like high yielding livestock). This number was even less (~10%) if the edit was made for cosmetic traits in the animal product (like salmon fillets with a brighter pink colour). Unlike in plants, using genome editing for production traits like yield entails a consideration of more than the technical challenges and potential solutions. It also raises normative issues about what is desirable, good or justified (Meijboom, 2021). The ethical debate on using genome editing adds a further complicated layer of whether we can move to using a CRISPR salmon, beyond the scope of this thesis (Kramer and Meijboom, 2021).

A distinction must also be made between fish welfare on the one hand and how we go about achieving it on the other. Olesen *et al.* (2000) emphasised breeding goals that are biologically, ecologically, and sociologically sound for the long-term sustainable development of animal production industries. This emphasis extends to the use and development of technology in achieving these goals, in order to make the best use of available resources. The control of sea lice is critical to the sustainability and welfare of salmon aquaculture, but there is a low emphasis on including genetic resistance as a goal. Gene technology resources offer a rapid alternative but with an accompanying skepticism from consumers.

Nonetheless, when technology is used for good, consumers have a more positive attitude. With fewer lice infections, salmon producers may be able to expand their production facilities. Hence, the contribution of such an organism to healthier farmed fish that pose less risk to wild populations may outweigh the perceived safety risks associated with the technology itself.

Part of the concern about a CRISPR potato stems from a lack of evidence about whether and to what extent current pest management practices will change. Such empirical evidence will almost certainly be required from a CRISPR salmon in order to demonstrate the level of impact it may have on an individual, population, and farm pest management level. Evidence of the durability of the resistance will also be required – particularly when host-parasite interactions are influenced by the evolution and adaptation of both organisms. Aquaculture setups may result in strong selective pressure on sea lice due to farmed salmon existing as the primary hosts (Barrett *et al.*, 2020). Evolution depends on amongst others, the genetic variability of the lice population, the heritability of the resistance to the salmon resistance genes, the biological complexity of the salmon resistance genes, the selection intensity, geographic locations, and the prevailing currents and tides (Barrett *et al.*, 2020). The coevolution and adaptation of sea lice will most likely necessitate ongoing breeding of lice-resistant salmon strains to prevent loss of genetic gains.

3. Considerations for both species

Norway pioneered the approach that products of gene technology should be considered in a way that goes beyond the precautionary principle. Norwegian authorities require more than just safety considerations, which proves to be a contentious requirement (Zetterberg and Edvardsson Björnberg, 2017). On the one hand, certain interest groups advocate for a strict science-based risk assessment that considers the extent of DNA changes, the closeness of the relationship between DNA donor and recipient organisms, unintended editing events or off-target effects, and rules that apply to products of conventional breeding (Huang *et al.*, 2016; McHughen, 2016; Scheben and Edwards, 2018). On the other hand, non-safety considerations address the multitude of interests beyond what can be measured, i.e. ethics and religion, aesthetics aspects, and socioeconomic considerations (Roger, 2015; Zetterberg and Edvardsson Björnberg, 2017). What is clear from the preceding discussion is that, in addition to the science evolving and improving, the *purpose* of the science may be the deciding factor in broader public acceptance. Amending the GTA by incorporating our technological knowledge could thus redistribute the regulatory burden based on the risks supported by scientific evidence. However, some evidence of the level of the product's contribution to sustainability may just be enough to 'seal the deal' for commercial release and use.

The two focus species herein provide an interesting reflection of the specific technical, legal and societal acceptance challenges when proposing new breeding technologies as a solution to food production issues. Gene-edited potato may be a potential pioneer organism in Norwegian agriculture, where a much-loved cultivar like ‘Mandel’ not only retains its market quality but also fosters sustainable farming practices. How that resistant potato was bred seems less important than how much it can contribute to sustainable, healthy farming. In this thesis, fungicide use and the presence of sea lice were used as indicators of sustainability. Assessing sustainability, however, requires more than reliance on one indicator. It requires a consideration of how a gene-edited potato or Atlantic salmon

might positively or negatively impact environmental integrity and all its constituent parts (Bardalen *et al.*, 2020). For example, NBAB may be required to assess the spread of this new genetic information to native species. For potato, such spread may be limited as it is a vegetatively propagated crop, not reliant on spread of pollen. Though for gene-edited Atlantic salmon, escapees from farm facilities may interact and spread the gene edits with wild populations in the seas and rivers. Although resistance to sea lice infestations may be an inherently 'good' trait to transfer to wild populations, there would likely be some concern about who should decide which genetic information can and cannot be risked transfer to wild populations. This type of consideration may move the organism from proposed Tier 2 to Tier 3, swinging the assessment back toward the precautionary approach. The focus of this thesis on selected indicators of sustainability is a limitation of this study, and a more thorough evaluation would be beneficial.

A prominent concern in Norway and globally is that GMOs have traditionally been owned and promoted by multinational corporations (MNCs) (Clapp, 2018; NBAB, 2020b). Interestingly, the CRISPR technology itself has already been proposed as a democratising technique (Jackson *et al.*, 2019). Researchers in Argentina showed that with a change to their regulatory framework, more than half of the applications for release of genome edited products was made by local Argentinian companies and research institutes (Whelan, Gutti and Lema, 2020). A third of the applications were submitted by foreign small-to-medium enterprises and less than 10% were submitted by foreign MNCs. CRISPR technology, in conjunction with a lowered regulatory burden, fosters an increase in the number of developers as well as diversification in the products submitted, particularly in niches not previously explored by MNCs (Whelan *et al.*, 2020). In Norway, breeding companies begin their discussions with regulatory authorities before even beginning research into developing new cultivars or employing new technologies. The reason for this is that the company cannot risk losing public trust from producers and consumers by launching new products that are unlikely to succeed in the market. Such dead-end R&D can be harmful to a company's economy as well as its reputation as a breeder, particularly when the company has a local interest in Norway rather than an international market interest. Unfortunately, this means that it is up to MNCs to take such risks. Changes to the status quo cannot be challenged and the industry cannot be disrupted unless a fine balance is struck between redistributing the risk regulated by law and the innovation offered by technology.

Final Remarks

CRISPR/Cas technology offers a powerful tool to breed improved host resistance against harmful pests in Norwegian agriculture and aquaculture. With a sustainable purpose in mind, researchers may be able to (finally) rapidly introgress biodiversity from wild potato relatives, conferring strong, durable resistance and contribute to reduced fungicide use in the food we eat. Sustainable growth of the Atlantic salmon industry may be aided by making small CRISPR edits to salmon DNA to achieve genetic gain against sea lice. We can thus directly cater to improving welfare in farmed fish, while protecting wild fish populations. CRISPR technology has revolutionised how we work with genes, how we find desired traits and study their function, to make the changes we want to see, and to avoid the undesirable effects of other breeding strategies. This opens a wealth of opportunity for creating innovative solutions for the agricultural and aquacultural industries.

CRISPR/Cas technology has not only disrupted the science of genetics and breeding but it has an equally strong domino effect on the way policymakers consider the products of the technology. Norwegian policymakers acknowledge that some genetic changes are presumed to be less risky than others, especially if the same outcome can be achieved through conventional breeding. This thesis shows that amending the Gene Technology Act in line with the Tier system proposed by NBAB presents various opportunities but also some requirement for clarification. It is not just a matter of bringing down the regulatory hurdles but a holistic consideration of the inputs of stakeholders too. A focus on sustainability as part of the non-safety criteria offers the assessment a unique opportunity to go beyond the precautionary principle, to go beyond considering all potential risks and unknown factors. Researchers cannot know every single risk that a technology poses, and this is true too of current practices – where risks to environmental health and fish welfare are constantly being uncovered. The relative simplicity in the genetic technology and the changes it can bring about, coupled with the enormity of the outcomes for sustainable development in food production industries makes genome editing an incredibly attractive solution.

The use of new technology in the development of novel products is not inherently frightening – we readily accept new medical and pharmaceutical innovations that address critical human health issues (the first-ever approved mRNA vaccine against Covid-19 is a great example). Stakeholder wariness to new technologies in food stems from various concerns, mostly related to risks and lack of perceived benefits. Norwegian law attempts to address the latter perception by assessing benefits and drawbacks of a food product produced using new technologies. But we cannot disrupt the status quo unless policymakers can strike a fine balance between regulating the risk and fostering technological innovation.

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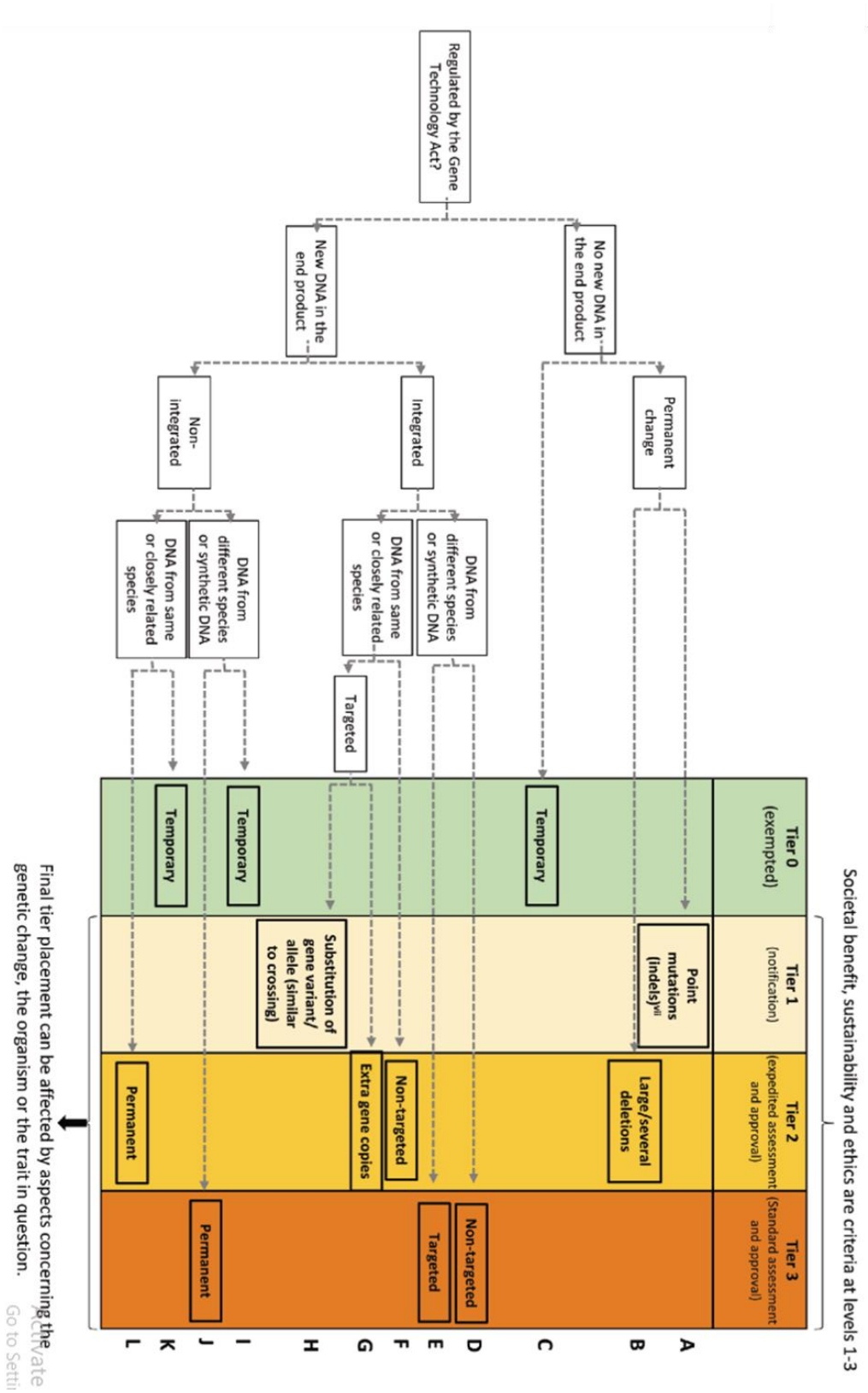
Supplementary information



Supplementary Figure 1: Tiered regulation based on the genetic change induced. This diagram was used throughout the discussion section of the thesis. Image directly from NBAB, 2018.

About the author

Crystal Turnbull was born and raised in South Africa but currently calls Ås, Norway her home, where she lives with her husband and daughter. She completed her BSc Molecular Biology and Biotechnology in 2009 from Stellenbosch University in SA. Following a student exchange opportunity at the University of Antwerp, Belgium, she completed her Bachelor of Law in 2013. Returning to South Africa, she pursued a legal career and was admitted as a practicing Attorney and Notary of the High Court of South Africa in 2015. After moving abroad permanently with her husband, Crystal has pursued many interests, learning Danish, Dutch and Norwegian and being an appointed representative of the Integration Committee in Viborg, Denmark. In March 2018, she graduated from the University of South Africa (UNISA) with a Master of Laws, focusing on refugee children's rights in Europe within the temporary relocation scheme. Her interest in children's rights stemmed from the work she did during her legal career, particularly where she could represent the rights of young children who were living in abject poverty and surrounded by violence and neglect. Since becoming a student at NMBU, Crystal rekindled her love of plant molecular biology and biotechnology, learning new laboratory techniques related to CRISPR/Cas9 and discovering the debate on regulating products of new breeding technologies. With her background in law and plant science, she tackled her first peer-review publication focusing on the legal frameworks on GMOs and the changes to these laws in light of products of genome editing. It is the first publication of its kind to combine known legislative documents on GMOs together with regulations for genome edited plants and crops. During her master degree, Crystal chose several practical subjects related to plant molecular biology, learning how to propagate various plant tissues, somatic embryogenesis, eliminating disease through meristem cultures and protocols for introducing genetic modifications. She also chose additional subjects for experience in genome editing in Atlantic salmon, culturing salmon cell lines, working with heat shock proteins in a genetic screen while helping several PhD students with their cloning protocols. Crystal believes that the covid-19 pandemic provided her with some unique opportunities, and she attended several global webinars on genome editing in plants and animals. After submitting her master thesis, Crystal will remain at NMBU as a research technician at Vollebekk for the summer season, working on scoring disease severity in wheat varieties. Thereafter, she hopes to pursue a PhD in plant science.

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