Master's Thesis

Investigating the Association Two Large Effect Loci *vgll3* and *six6* Have on The Maturation Timing in Wild Atlantic Salmon

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Declaration

I hereby confirm that I am the sole author of this thesis and it is a product of my own academic research.

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Abstract

Maturation timing is a key life history trait in Atlantic salmon (*Salmo salar*), influencing population resilience through variation in age at maturity. Identifying the genetic basis of this trait is a central goal in conservation biology of the species. Two candidate loci, vestigial-like family member 3 (*vgll3)* and SIX homeobox 6 (*six6)*, have previously been identified with a genetic predisposition towards age at maturity due to differences in a single SNP indicating 'early' or 'late; maturation timing. In this project, DNA was extracted from fin clips of wild Atlantic salmon from nine rivers across six regions in Iceland. Genotype frequencies associated with maturation timing were determined using quantitative PCR (qPCR) analysis. Overall, results indicated a genetic predisposition towards earlier maturation at both loci when samples were grouped across all sample sites. Although statistical significance was marginal, an association was detected between sea age and *vgll3*EE* the most frequently observed genotype at the *vgll3* locus. Additional significant relationships were observed between *vgll3* and body length, where *vgll3*EE* individuals were longer than *vgll3*LL* individuals and overall males were shorter than females. A significant association was found between sea age and length indicating 2SW individuals were longer compared to 1SW individuals. Life history traits showed a significant association between *vgll3*EE* and precocious males and a marginally significant association between *vgll3*LL* and iteroparous spawners. When populations were separated by river, a linear model reveled location was a significant determinant of body length compared to genotype and sex at *vgll3* while at *six6* sex and genotype were significant determinants of body length while river showed no statistical significance. Genotype frequencies varied markedly within and among rivers, even after accounting for geographic proximity, possibly due to population differentiation or even local adaptation. Additionally, the genotype frequency of farmed salmon differed significantly from wild salmon at *six6* but not at *vgll3.*These findings are highly relevant in establishing how a gene targeted management approach can inform the conservation and management of Atlantic salmon populations in Iceland.

Útdráttur

Kynþroskaaldur er mikilvægur lífssögueiginleiki hjá Atlantshafslaxinum (*Salmo salar*) og hefur breytileiki hans áhrif á stöðugleika stofna og getur greining á erfðum þessa eiginleika nýst við verndun tegundarinnar. Sýnt hefur verið fram á að breytileiki í tveimur genum, vestigial-like family member 3 (vgll3) og SIX homeobox 6 (six6), sýni sterk tengsl við kynþroskaaldur, þar sem mismunandi samsætur tengjast snemm- og síðkynþroska. Í þessu verkefni var DNA einangrað úr uggasýnum villtra laxa frá níu ám af sex svæðum innan Íslands. Arfgerðir sem hafa verið tengdar við kynþroskatíma voru greindar með magnbundnu PCR (qPCR) og tíðni gerðanna og samsætanna metin. Niðurstöður, sameinaðar frá ólíkum ám, sýna að erfðabreytileiki sem tengist snemmkynþrosaka er algengari en sem valda síðkynþroska. Vísbending var um tengsl aldurs í sjó og breytileika í *vgll3* við kynþroska, *vgll3*EE* greindist frekar hjá löxum sem voru lengri tíma í sjó og þeir voru lengri en *vgll3*LL* og almennt voru hængar minni en hrygnur. V*gll3*EE* reyndist algengari meðal snemmkynþroska laxa og væg vísbending var um aukna tíðni *vgll3*LL* hjá löxum sem æxluðust endurtekið (e. iterparous). Við greiningu á lengd laxanna með línulegu líkani kom í ljós að árnar höfðu meiri áhrif á lengd fiskanna en arfgerð *vgll3* arfgerðarinnar og kyn, hinsvegar þegar lengdin var skoðuð m.t.t. arfgerðar *six6* gensisins var skoðuð reyndist áhrif arfgerðarinnar matktæk auk þess sem kynið hafði áhrif en ekki árnar. Tíðni arfgerðanna meðal villtu laxanna var marktæk frábrugðin arfegrðum eldislaxa í six6 en ekki í vgll3. Þessi rannsókn sýnir hvernig greiningar á breytileika einstakra gena getur veitt upplýsingar fyrir verndun og stjórnun á villtum laxastofnum á Íslandi.

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Units and Acronyms

Units

ºC	Degrees Celsius
cm	Centimeters
g	Grams
kg	Kilograms
ng	Nanogram
mg	miligrams
psu	Practical Salinity Unit
µ	Microliter

Acronyms

1SW: One Sea Winter

2SW: Two Sea Winter

DNA: Deoxyribonucleic Acid

GWAS: Genome Wide Association Study

HWE: Hardy-Weinburg Equilibrium

MSW: Multi-Sea-Winter

MAB: Maximum Allowed Biomass

PCoA: Principal Coordinate Analysis

PCR: Qualitative Polymerase Chain Reaction

qPCR: Qualitative Polymerase Chain Reaction

six6: SIX homeobox 6

SNP: Single-Nucleotide Polymorphism

vgll3: Vestigial-like Family Member 3

Glossary

Terms included in this glossary may be helpful for the reader with no prior background in genetics or fisheries to become more acquainted with terms local to this thesis.

Adaptability: The degree an individual can adjust to a range of environmental conditions by physiological or genetic means

Adaptation: A genetic adjustment to the environment resulting in increased survival

Allele: A DNA sequence of a gene occurring at the same chromosomal location

Gene: The segment of DNA made up of alleles that express a trait

Gene diversity: The variance in allele frequencies in a population at a given locus

Gene flow: The incorporation of genes from one population or species into another through migration and interbreeding

Genetic architecture: Features of the variants influencing a given trait, such as the number of genes, the effect size, and genomic position.

Genetic distance: A measure of the differences in allele types and frequencies

Genotype: the hereditary or genetic constitution of an individual defined by its particular combination of allelic variants possessed at one or more loci, or, in its most general sense, at all loci

Heterozygote: An individual that carries two different alleles at a given locus

Homozygote: An individual with two copies of the same allele in one locus

Life-history trait: A trait which relates to the way an organism structures its life

Locus: Precise location in a DNA sequence; plural loci

Maturation: The developmental process leading to individuals becoming capable of breeding

Microsatellite: Repeated sequences of DNA in coding and non-coding regions, likely to have slippage in base pairs which can led to mutations

Phenotype: The observed trait of an individual
Phenotypic plasticity: The capacity to alter the phenotype from a given genotype in response to variation in environmental conditions

Population: a group of sexually reproducing individuals that constitute a distinct gene pool

Stock: a group of individuals of a species encompassing more than one genetic population

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Published Works

This thesis is based on the following publications:

Barson, N., Aykanat, T., Hindar, K., Baranski, M., Bolstad, GH., Fiske, P., Jacq, C., Jensen, AJ., Johnston, SE., Karlsson, S., Kent, M., Moen, T., Niemelä, E., Nome, T., Næsje, TF., Orell, P., Romakkaniemi, A., Sægrov, H., Urdal, K., Erkinaro, J., Lien, S., Primmer, CR.(2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature. 2015 Dec 17;528(7582):405-8. doi: 10.1038/nature16062. Epub 2015 Nov 4. PMID: 26536110.

Sinclair-Waters, M., Nome, T., Wang, J. et al. (2022).Dissecting the loci underlying maturation timing in Atlantic salmon using haplotype and multi-SNP based association methods. Heredity 129, 356–365. https://doi.org/10.1038/s41437-022-00570-

Sinclair-Waters, M., Ødegård, J., Korsvoll, S.A. et al. (2020). Beyond large-effect loci: large-scale GWAS reveals a mixed large-effect and polygenic architecture for age at maturity of Atlantic salmon. Genet Sel Evol 52, 9. https://doi.org/10.1186/s12711-020-0529-8.

1 Introduction

1.1 Overview

No other group of fish species has received as much combined attention from cultural, commercial, and scientific entities as salmonids (Davidson et al., 2010). Now, once again, it is time for salmon to rule the plotline. Iceland has three native salmonid species, Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), and Arctic charr (*Salvelinus aplinus*) (Olafsson et al., 2016**;** Guðbergsson et al., 2023). Out of all the salmonids, the Atlantic salmon is the most widely studied, due to the expansion of the Atlantic salmon aquaculture industry (Davidson et al., 2010; Menge et al., 2024). The development of open net pen salmon farming in Iceland poses a potential threat, namely genetic introgression, to the already declining populations of wild salmon in the country (Davidson et al., 2010; Saviolidis et al., 2020; Guðbergsson et al., 2023; Bjarnason & Magnusdottir, 2019; Sturlaugsson & Palsson, 2023; Sturlaugsson & Palsson, 2024). The majority of detected genetic introgression in Iceland has been concentrated in the Eastfjords and Westfjords, where most of the open net-pen salmon farming occurs (Sturlaugsson & Palsson, 2023; Sturlaugsson & Palsson, 2024). Ongoing research of the genetic mixing between the strains conducted by Sturlaugsson & Palsson (2024) have shown that, without immediate intervention, the Norwegian farmed salmon genotype could become dominant in salmon rivers in proximity to open net pen salmon farming displacing the native genetic profile. Given that the current trajectory of Icelandic salmon aquaculture production is not undergoing significant changes, it is imperative to conduct research to threatened fish stocks to ensure sustainability of wild salmonids (Sturlaugsson & Palsson, 2023; Sturlaugsson & Palsson, 2024).

Despite opposing dispositions, conservationists and aquaculture producers have a common vested interest, the successful reproduction of Atlantic salmon; a trait vital to the conservation of wild populations and the economic viability to farming salmon (Davidson et al., 2010; Bjarnason & Magnusdottir, 2019). Due to the combined interest between stakeholders, extensive research efforts have gone into understanding the biological mechanisms influencing the reproductive success of Atlantic salmon (Davidson et al., 2010; Ayllon et al., 2019).

Atlantic salmon have evolved a remarkable diversity of life history strategies in order to maximize reproductive success (Fleming, 1996). Diversity in life history strategies can include age at maturity, body size, development of secondary sexual characteristics, and mating tactics (Fleming, 1996). Among these, age at maturity has been established as one of the most principal components in determining successful reproduction as it promotes the stability of populations by buffering them against environmental change and stochasticity (Kuparinen & Hutchings, 2017; Barson et al., 2015; Murzina et al., 2016; Åsheim et al., 2023; Mobley et al., 2021). A crucial step to explaining the variability of an adaptive trait, such as maturation timing is to identify the traits genetic architecture (Andersen et al., 2018; Ghalambor et al., 2007).

In any given trait, the genetic architecture describes the number and genomic location of loci controlling the trait, the loci's effect size, and the degree environmental interaction influences trait expression (Andersen et al., 2018; Ghalambor et al., 2007). The most common technique applied to identifying adaptive traits is a genome wide association study (GWAS) (Uffelmann et al., 2021). GWAS works by scanning the entire genome of a species aiming to identify variations in single-nucleotide polymorphism (SNPS) between individuals who differ phenotypically in order to identify regions showing significant association in the trait of interest (Uffelmann et al., 2021).

Genomic studies have been able to reveal the genetic architecture of age at maturity in Atlantic salmon with a particular focus on the amount of time salmon spend at sea becoming sexually mature (Barson et al., 2015; Mobley et al., 2021; Gibley et al., 2018; Aykanat et al., 2020). It has been identified that the timing of maturation is best explained by two large effect loci, vestigial-like family member 3 (*vgll3)* located on chromosome 25 and SIX homeobox 6 (*six6*) located on chromosome 9 (Barson et al., 2015; Ayllon et al., 2015; Sinclair-Waters et al., 2020; Sinclair-Waters et al., 2022). The variation in age at maturity arises form a single SNP variant in *vgll3* and *six6* genomic regions, indicating either a predisposition towards 'early' or 'late' maturation (Barson et al., 2015; Ayllon et al., 2015; Sinclair-Waters et al., 2020; Sinclair-Waters et al., 2022). The genetic influence between *vgll3* and *six6* explains close to 40% of the total variation in sea age at maturity (Barson et al., 2015; Ayllon et al., 2015; Sinclair-Waters et al., 2020; Sinclair-Waters et al., 2022). Maturation timing is fundamental to a population's overall fitness, as 'late' maturing salmon have increased mating success, higher fecundity, and increased offspring survival but significantly higher rates of mortality pre-spawn compared to 'early' maturing individuals that have lower overall fitness but increased chances of successfully reproducing (Kurko et al., 2020; Ayllon et al., 2015; Debes et al., 2021; Sinclair-Waters et al., 2022; Sinclair-Waters et al., 2021). Understanding a population's genetic predisposition of maturation timing is pivotal to understanding the survival and reproduction rates of wild salmon populations (Raunsgard et al., 2024).

Since the discovery of the association between *vgll3*, *six6* and sea age in Atlantic salmon, a significant series of studies have explored how maturation timing relates to other characteristics linked to age at maturity, such as sex, length, migration timing, spawning success, and mating tactics (Kurko et al., 2019; Ayllon et al., 2015; Debes et al., 2021; Maamela et al., 2025). However, because maturation timing is a highly plastic trait closely influenced by both environmental and genetic factors, the extent *vgll3* and *six6* influence maturation timing varies by population (Kurko et al., 2019; Ayllon et al., 2015; Debes et al., 2021; Sinclair-Waters et al., 2022; Waters et al., 2021; Maamela et al., 2025; Åsheim, et al., 2023).

This thesis investigated the associations between *vgll3* and *six6* genotypes to key life history characteristics associated with age at maturity in wild Atlantic salmon. The primary association being investigated was the influence between sea age and *vgll3* and *six6* genotypes (Barson et al., 2015; Sinclair-Waters et al., 2022; Waters et al., 2021). *Vgll3* and *six6* genotype frequencies were also compared to sex, body size, repeat spawning, and the occurrence of precocious males. Additionally, comparisons of *vgll3* and *six6* genotype frequencies between farmed and wild salmon were conducted to assess genetic diversity at these genomic regions. Overall genetic diversity values for the genes of interest were conducted to assess the extent of inbreeding of the populations included in this project. A major limitation in the effective conservation of wild salmon populations is the lack of genetic data that informs key life history traits (Raunsgard et al., 2024). As GWAS studies have become more standardized this has allowed for genomics to be used as a tool to inform species conservation and management (Aykanat et al., 2016). The findings of this thesis contribute to addressing the knowledge gap of the genetic pre-disposition of maturation timing for several Icelandic populations and demonstrate how a gene targeted approach can be applied to inform the conservation of wild salmon.

Figure 1: Photo taken of a mature male Atlantic salmon in the river Fífustaðadalsá during sample collection. Photo by Sadie Ainsworth.

Figure 2: Photo taken of farmed salmon before being gutted at the Arctic Fish processing plant in Bolungarvik, Iceland. In comparison to Figure 1 salmon have a silvery color associated with the marine phase of their life cycle and noticeably more lesions on their body. Photo by Sadie Ainsworth.

1.2 Research Questions & Aims

This thesis aims to answer the following research question and objectives:

To what extent does the large effect loci vgll3 and six6 explain variation of maturation timing of Atlantic salmon in Icelandic waters?

1. Characterize the fine-scale population structuring of Icelandic Atlantic salmon through association of age at maturity.
2. Determine the associations *vgll3* and *six6* genotypes have on the timing of maturation in Atlantic salmon in Icelandic waters.
3. Formulate management and conservation techniques to conserve the wild Atlantic salmon stock in Iceland using a genomic approach.

The broader objective of this thesis is to contribute to the establishment of knowledge on the genetic diversity of wild salmon populations in Iceland. Specifically, this thesis seeks to advance this foundation by characterizing two major-effect loci in Icelandic salmon populations, with the aim of strengthening the genetic research base and translating the findings into actionable management guidelines.

1.3 Content and Organization of Thesis

Chapter One orients the reader's disposition for the upcoming chapters by providing a general overview of the material discussed in this thesis. Chapter Two establishes relevant background information, beginning with an overview of Atlantic salmon in Iceland, followed by a detailed examination of the mechanisms underlying maturation timing. Chapter Three describes the sample sites, field sampling techniques and the ethics statement. Chapter Four outlines the methodologies employed in the genetic analysis portion of the study and statistical analysis methods. A full report of results for all analyses is provided in Chapter Five and proceeding discussed in Chapter Six. Limitations of the study are addressed in Chapter Seven and Chapter Eight outlines future research directions. The establishment of management recommendations derived from the findings and discussion in Chapters Five and Six is represented in Chapter Nine. The thesis culminates in Chapter Ten, providing the reader with a summary of the study and results and concluding with how a gene targeted approach to management can be incorporated into coastal and marine conservation protocols beyond the scope of this thesis.

2 Background

2.1 Origin of Atlantic Salmon in Icelandic Waters

The origin story of Atlantic salmon inhabiting Icelandic waters begins around 20,000 years ago during the decline of the Last Glacial Maximum (Olafsson et al., 2014, Olafsson et al., 2016). Models of the Last Glacial Maximum indicate the first ice-free environment viable for salmon inhabitation was along the coastline of Breiðafjörður in the northwest of Iceland (Olafsson et al., 2014). The retreat of the glacier started in the north of Iceland and proceeded to recede clockwise around the country until reaching the southwest, consequently opening up viable habitat for salmon to start colonizing all of Iceland's rivers (Olafsson et al., 2014; Guðjónsson 1978; Gilbey et al., 2018). Now at the outskirts of Atlantic salmons distributional range, Iceland presently hosts 80 of the most northern salmon rivers (Olafsson et al., 2014; Guðjónsson 1978; Gilbey et al., 2018). Contemporary populations still reflect deglaciation, with higher genetic diversity observed between northern populations compared to southern populations, as a result of restricted gene flow (Olafsson et al., 2014; King et al., 2021; Danielsdottir et al., 1997; Gudmundsson et al., 2013). However, the genetic variations within populations is most accurately explained by the remarkable diversity of life history strategies (Olafsson et al., 2014).

2.2 Life History Characteristics

The complex lifecycle of Atlantic salmon, throughout all life stages, is well documented in fisheries literature therefore, this text should be considered a general overview of the species' life cycle focusing on the most predominant life cycle characteristics (Mobley et al., 2021; Webb et al., 2007; Guðjónsson et al., 2015). If any aspect of Atlantic salmon life history is of explicit interest to the reader, further reading is encouraged, as this background provides the appropriate context for this thesis there is an enrichment of engaging literature on reproductive strategies and tactics.

The archetypal Atlantic salmon is an anadromous species, migrating between fresh and marine environments throughout its lifetime, spending anywhere between 20-50% of their lifespan at sea (Mobley et al., 2021; Webb et al., 2007). Some Atlantic salmon complete their entire life cycle in freshwater; this resident strategy is generally attributed to entire populations and will not be discussed further, since it does not pertain to populations used in this study. The life cycle of anadromous populations can be divided into two main phases: freshwater and marine. Reproduction and nursery phases occur in freshwater habitats, while predominant feeding and sexual maturation occur in the marine environment.

2.2.1 Freshwater Phase

In Iceland, spawning begins in the fall once river temperatures drop between 2 ºC and 6 ºC (Gudjónsson, 1978). In the early phase of spawning, adult females meticulously select a site and construct a nest, known as a redd (Mobley et al., 2021; Webb et al., 2007; Gudjónsson, 1978). Completion of the redd signals the onset of courtship (Webb et al., 2007). During this period, intraspecific competition is frequently observed between two or more males displaying a series of aggressive behaviors to win access to ovipositing females (Webb et al., 2007; Mobley et al., 2024; Bangura et al., 2022). Males of an increased body size have a fitness advantage to out compete smaller males and are more likely to be selected by a female (Webb et al., 2007; Moreru et al., 2011; Mobley et al., 2024). In cases where populations have a higher proportion of small males lower fertilization rates have been observed due to females choosing to delay spawning in anticipation of a larger male (Järvi, 1990; Webb et al., 2007; Mobley et al., 2024). Body size is an important determinant in breeding success for both sexes (Mobley et al., 2021; Persson et al., 2022). Larger females have an increased ability in redd construction, protecting spawning grounds, higher rates of fecundity and producing larger eggs thus increasing overall juvenile survival rate (Mobley et al., 2021; Persson et al., 2022; Skoglund et al., 2011). Larger males have higher rates of fertilization, owing to the more pronounced development of secondary sexual characteristics that enhance their ability to attract females and establish dominance in male-male competition (Mobley et al., 2021; Persson et al., 2022; Bangura et al., 2022). Altogether, this has resulted in a size dominance hierarchy in Atlantic salmon (Skoglund et al., 2011; Mobley et al., 2021).

After a successful spawn, in the redds, embryos safely develop, hatch in late spring, and form into alevins (Mobley et al., 2021; Gudjónsson, 1978). An Atlantic salmon is considered a fry once it has left the redd to feed (Mobley et al., 2021). Fry in Icelandic waters mostly feed on chironomids (*Chironomidae*), and 1 – 2 year olds predominantly feed on caddis flies (*Trichoptera*) and gastropods (Gudjónsson, 1978). After biological satisfaction, the fry undergoes an ontogenetic shift developing into a parr (Mobley et al., 2021). Parr are physically categorized by darker vertical marks and small red dots on the side of their body (Mobley et al., 2021; Webb et al., 2007). Parr can remain in freshwater between one and eight years but most individuals will have reached the marine environment within four (Mobley et al., 2021; Persson et al., 2022). Some fast growing male parr will mature during their freshwater residency producing viable sperm without ever migrating to sea (Mobley et al., 2021; Ayllon et al., 2015). These mature parr are referred to as precocious males or more commonly “sneakers” (Mobley et al., 2021; Ahi et al., 2022). In a desperate attempt to spawn, precocious males use their small size to an advantage by positioning themselves downstream of larger males in efforts to ‘sneak in’ by depositing milt synchronistical with a dominant male as females deposit eggs (Mobley et al., 2021; Ayllon et al., 2015). Sneakers are important to the genetic diversity of a population and are found in Icelandic populations at varying frequencies (Mobley et al., 2021; Ayllon et al., 2015; Sturlaugsson, 2025a; Sturlaugsson, 2025b).

Smoltification is the transitional phase between the fresh and marine environments, when salmon undergo a physiological shift that enables adaptation to life at sea (Mobley et al., 2021). One particularly interesting shift is the loss of positive rheotaxis (the ability to face upstream) (Mobley et al., 2020). To the human eye, smoltification is marked by an elongation of the body and a transition to a silvery complexation, which persists until salmon start returning to spawn, when coloring shifts back to an earthen hue (Webb et al., 2007). In Iceland smoltification generally occurs in early summer when individuals are three years of age (Gudjónsson, 1978). A universal predictive measurement of ~10 cm has been established as an indicator of smolitfication timing; however, like all other life history traits in Atlantic salmon, variation in smolt timing is ultimately determined by extrinsic environmental factors and their influence on the genetic pathways controlling the transition (Mobley et al., 2021; Mobley et al., 2024).

It should be noted, unlike a majority of salmon species, Atlantic salmon are an iteroparous species and surviving adults can migrate back to sea to develop new gonads post spawn (Mobley et al., 2021; Persson et al., 2022). The frequency of repeat spawners and marine reconditioning time of Atlantic salmon is highly variable between sexes and populations, however, across all populations few will ever spawn more than twice (Persson et al., 2022; Aykanat et al., 2019).

2.2.2 Marine Phase

Upon entry into marine waters, Icelandic Atlantic salmon tend to stay in the epipelagic zone, preferring waters between 8 to 12°C with salinity levels > 35 psu (Guðjónsson, 2015). The species will stay in the sea for one to five years feeding until they have sustained enough mass to develop functional gonads, secondary sexual traits, and account for the energetic cost of spawning (Mobley et al., 2021; Sinclair-Waters et al., 2020; Guðjónsson, 2015). Both sexes are opportunistic eaters preying on a varied diet such as Atlantic herring (*Clupea harengus*), sandeels (*Ammodytes tobianus*), gammarid amphipods (*Gammarida*), and crustaceans (*Crustacea*) with fish constituting the primary prey group (Sturlaugsson, 2000). The conditions influencing nutrient transport and species distribution vary between northern and southern populations resulting in varying migration lengths due to access to suitable feeding grounds and the courses of prominent nearby ocean currents (Guðjónsson, 2015; Gudjonsson et al., 1995). Icelandic salmon have been found as far as the coasts of Greenland and the Faroe Islands (Guðjónsson, 2015). However, the sea is not an endless cornucopia for salmon. The greatest rate of mortality for an Atlantic salmon is during the first 1 – 6 months at sea with total mortality rates reaching as high as 70% (Mobley et al., 2020; Pardo & Hutchings, 2020; Guðjónsson, 2015; Sturlaugsson, 2000).

The length of time spent at sea is dependent on the salmon becoming reproductively capable before it can start the return migration back to its natal stream in safer waters (Åsheim et al.,2023). The directed migration back to the salmons natal stream is called homing (Åsheim et al., 2023; Webb et al., 2007). Homing is the result of an outstandingly complex olfactory system that imprints the chemical characteristics of waterways throughout all life stages (Moustakas-Verho et al., 2020; Haraldstad et al., 2022). The salmon's tendency to home is the precise mechanism segregating Atlantic salmon into distinct populations with restricted gene flow (Miettinen et al., 2021).

It should now be clear, Atlantic Salmon are highly variable in both developmental rates and life history characteristics in both fresh and marine environments (Mobley et al., 2021; Sinclair-Waters et al., 2020). The differences in life history strategies within a species is referred to as intraspecific variation (Mobley et al., 2021). The variation in life history strategies is distinct to the environmental and genetic factors faced on an individual level (Mobley et al., 2021; Sinclair-Waters et al., 2020; Ayllon et al., 2015; Gudjónsson, 1978). Despite the differences in life history strategies, ubiquitously, all life history tactics are oriented towards one thing; successfully reproducing (Mobley et al., 2021; Åsheim et al., 2023). Attaining the reproductive strategy that will produce the most offspring can be ordinarily reduced to this genetic coin toss: later maturing salmon have higher reproductive fitness and stronger offspring but increased mortality due to prolonged exposure in the marine environment in comparison earlier maturing individuals have lower overall fitness but increased chances in successfully reproducing (Verta et al., 2020; Mobley et al., 2021).

2.3 Sea Age Maturity

The main trait being investigated in this thesis is the maturation timing of Atlantic salmon a highly heritable trait commonly referred to as sea age at maturity (Barson et al., 2015). Maturation is measured by an individual's sea age i.e., the period of time spent in the sea measured by the number of winters Atlantic salmon spend becoming sexually mature before returning to freshwater for spawning (Sinclair-Waters et al., 2020; Barson et al., 2015). Salmon maturing after a single winter are referred to as grilse or one sea winter salmon (1SW) (Fraser et al., 2023). Individuals maturing after two winters are classified as two sea winter salmon (2SW) and those spending three years + at sea are referred to as multi-sea winter (MSW) (Fraser et al., 2023).

One of the greatest distinctions between the age classes is the dramatic size difference among the classes (Barson 2015; Waters et al., 2021). 1SW individuals typically weigh 1-3 kg and are 50-60 cm in length compared to MSW salmon weighing in at an average of 10-20 kg and reaching over 100 cm (Barson 2015; Waters et al., 2021). Every year spent in the marine environment the mass of the individual doubles (Mobley et al., 2020).

Sea age at maturity was determined from scale readings, a method that involves analyzing periods of seasonal growth to identify valuable life history information like age of fish, smolt age, current sea age, and spawning participation (Sturlaugsson, 2021). If the sea age was not available from scales of a given fish, fish were grouped by known size thresholds and sex within a given river system to discriminate between one sea winter (1SW-grisle) and two sea winter (2SW) fish based on known data from scale readings. If salmon that entered the rivers showed marks from earlier spawning these individuals were classified as repeated spawners, i.e. the iteroparity part of the stocks (Sturlaugsson, 2021;Sturlaugsson, 2025b).

While age at maturity varies both between and within Atlantic salmon populations some overall trends can be applied to the species as a whole. The mean sea age of Atlantic salmon is 1.6 years (Barson et al., 2015). Females need approximately six times more energy than males to reach maturation resulting in an older average sea age and 2SW and MSW individuals tend to arrive earlier to spawning grounds compared to 1SW salmon (Mobley et al., 2020; Mobley et al., 2021; Debes et al., 2021; Waters et al., 2021).

2.4 Influences on Maturation Timing

2.4.1 Environmental

The environmental conditions encountered throughout all life history phases directly influence the age at maturation of Atlantic salmon (Kuparinen & Hutchings, 2017; Barson et al., 2015; Murzina et al., 2016; Åsheim et al., 2023). Due to the complexity of ecosystem dynamics and the variability of habitats, previous research best explains variances on a population level; therefore this section will summarize the most significant factors known to affect maturation (Åsheim et al., 2023; Baum et al., 2005; Otero et al., 2012). In the freshwater phases, temperature, flow rate, and photoperiod are the three most significant environmental determinants influencing maturation timing (Åsheim et al., 2023; Baum et al., 2005; Otero et al., 2012; Mobley et al., 2020; Ayllon et al., 2015; Korus et al., 2024). In the marine phases water temperature, food availability, and photoperiod are the most significant (Åsheim et al., 2023; Baum et al., 2005; Otero et al., 2012; Mobley et al., 2020; Ayllon et al., 2015; Korus et al., 2024; Ratnarajah). Within existing research there is limited knowledge on the specific environmental factor with the strongest influence on *vgll3* and *six6* genotypes and their effect on maturation (Åsheim et al., 2023; Raunsgard et al., 2024). However, since environments are rarely static, no single phenotype consistently outperforms others across all environments highlighting the value of diversification in life history characteristics in maintaining the overall resilience of populations (Garcia de Leaniz, et al., 2007).

2.4.2 Genetics

The variation in maturation timing in Atlantic salmon reflects the ability of a genotype to produce different phenotypes in response to varying environmental conditions an occurrence known as phenotypic plasticity (Ghalambor et al., 2007). A recent genomic study by Sinclair-Waters et al., (2022) found five genes, *pecam1*, *asap2a*, *six6*, *taar13c*, and *vgll3* were strongly associated with sea age maturity in Atlantic salmon (Sinclair-Waters et al., 2022). Although sea age maturity is an omnigenic trait, influenced by a complex network of genes, two core loci *vgll3* and *six6* explain a large proportion of the variation in sea age resulting in a genetic predisposition toward either earlier or later maturation (Sinclair-Waters et al., 2022; Barson et al., 2015; Ahi et al., 2022). The variation in maturation timing is best explained by the variation of a single nucleotide polymorphisms (SNPs) within the *vgll3* and *six6* regions where the allelic configuration *EE* is associated with earlier maturation as *LL* is to late maturation and *EL* is an intermediary between the two (Barson et al., 2015; Aykanat et al., 2024; Bangura et al., 2022; Sinclair-Waters et al., 2022; Sinclair-Waters et al., 2020; Ayllon et al., 2019). The large effect locus, *vgll3,* has consistently been identified as the principal determinant of maturation timing explaining 20-39% of variation comparative to *six6's* notably smaller effect around 3-9% (Sinclair-Waters et al., 2022; Barson et al., 2015; Sinclair-Waters et al., 2020; Ayllon et al., 2019). Both genes demonstrate a significant role in maturation timing in European Atlantic salmon strains (Sinclair-Waters et al., 2022; Barson et al., 2015; Sinclair-Waters et al., 2020; Ayllon et al., 2019).

The molecular machinery behind *vgll3* and *six6*'s influence on timing of maturation is a complex biochemical process controlled by proteins encoded by *vgll3* and *six6* (Kurko et al., 2020; Murzina et al., 2016). The loci serve as molecular mediators by sensing a combination of stored lipid reserves in the salmon's muscle and environmental cues to regulate the decision of a cellular transition from somatic growth to reproductive development (Kurko et al., 2020; Verta et al., 2024; Barson et al., 2015). The allelic variations, *EE*, *EL*, *LL* are each associated with a predetermined threshold of stored fatty acids required for ontogenesis to proceed (Murzina et al., 2016; Sinclair-Waters et al., 2022; Barson et al., 2015; Sinclair-Waters et al., 2020; Ayllon et al., 2019). If lipid levels are insufficient, gonad development is delayed until adequate reserves are accumulated (Otero et al., 2012; Debes et al., 2021).

2.5 Vestigial-like Family Member 3 and SIX homeobox 6

2.5.1 Vestigial-like Family Member 3

The gene *vgll3* located on chromosome Ssa25 is known as the master regulator having the most pleiotropic effect on life history characteristics in Atlantic salmon (Fraser et al., 2023; Aykanat et al., 2024; Ahi et al., 2022; Ayllon et al., 2019; Kurko et al., 2019; Verta et al., 2024). The SNP with the strongest association to maturation timing is located 10.3 kilobase downstream of *vgll3* near the gene *akap11* (Sinclair-Waters et al., 2021; Barson et al., 2015; Ayllon et al., 2015). The timing of maturation is regulated by *vgll3* due to its relation to other signaling pathways also associated with pubertal development on a molecular level (Boulding et al., 2019; Barson et al., 2015; Kusche et al., 2017; Mobley et al., 2020). *Vgll3* regulates nr5a1 which has been found to regulate gonads, aggressive behavior, and physical activity (Pritchard et al., 2018; Verta et al., 2024). Intriguingly nr5a1 expression has also been linked to timing of puberty in humans (Pritchard et al., 2018; Verta et al., 2024; Boulding et al., 2019; Barson et al., 2015; Sincalir-Waters et al., 2021). Ncoa1, another regulator of *vgll3* has been associated with gamete production in Atlantic salmon and weight gain in mice (Verta et al., 2024). The expression of cyp17a1, also regulated by *vgll3* is strongly tied to testosterone production (Verta et al., 2024; Pritchard et al., 2018; Boulding et al., 2019; Ayllon et al., 2018). The gene *akap11* near the association signal observed in *vgll3* was found to have little effect on maturation timing and its association is primarily driven by encoding A-kinase anchoring protein 11 which regulates cellular processes of the testes during spermatogenesis (Sinclair-Waters et al., 2021). Understanding the molecular role *vgll3* plays in regulating maturation timing provides context for the variety of phenotypic associations associated with age at maturation. Due to the complexity, plasticity, and importance maturation timing has for Atlantic salmon populations researchers have been working on gaining an understanding of *vgll3's* role with age at maturity. Table 1 provides an outline of existing literature highlighting studies most similar to the one in this thesis.

Table 1: A summary of existing literature on association studies between vgll3 and the timing of sexual maturation in Atlantic salmon.

Reference	Genomic Method	Summary of Results
Ahi et al., 2025	PCR	Immature males carrying *vgll3*EE* decrease lipid storage in autumn compared to *vgll3*LL* individuals that do the opposite. Males mature earlier than females. *Vgll3*EE* females compared to *vgll3*EE* males have greater lipid storage.
Allyon et al., 2015	GWAS	Found *vgll3* explains 33 – 36% of phenotypic variation for both wild and domesticated Atlantic salmon males.
Ayllon et al., 2019	qPCR and Sequenom MassArray	Found an association between *vgll3* and sea age in males but not females in the farmed *Mowi* strain.
Aykanat et al., 2019	PCR	*Vgll3*EE* individuals are 2.4 times more likely to spawn a second time compared to *vgll3*LL* individuals.
Bangura et al., 2022	PCR	*Vgll3*LL* individuals were more aggressive than *vgll3*EE* individuals.
Barson et al., 2015	GWAS	- Between sexes *vgll3*EE* was more commonly observed in males and females were more likely to carry *vgll3*LL*. - Mature males with *vgll3*EE* mature on average 0.86 years earlier to *vgll3*LL* males. Mature female with vgll3*EE mature 0.7 years earlier compared to *vgll3*LL* females.

		-*Vgll3*E* is dominant in males and *vgll3*EL* individuals are more similar in sea age to *vgll3*EE* individuals.
Besnier et al., 2024	GWAS	*Vgll3*E* allele is dominant over *vgll3*L*.
Boulding et al., 2019	GWAS	*Vgll3* locus was not significantly associated with early maturation in North American lineage of Atlantic salmon.
Debes et al., 2021	Multiplex PCR	Found no significant correlation between *vgll3* and body length in juveniles. This study tried to confirm the conflicting association results found in Barson et al., 2015 and Fjelldal et al., 2020.
Fraser et al., 2024	GWAS	*Vgll3*EE* was significantly associated with higher grisling rates compared to *EL* and *LL* fish.
Kusche et al., 2017	PCR	Found *vgll3* heterozygous individuals had a 2:1 probability of being female compared to male.
Maamela et al., 2025	GWAS	The additive genetic component of *vgll3* is lower in females compared to males.
Mobley et al., 2024	PCR	Females carrying *vgll3*EL* had 3 times more offspring than homozygous females. Males carrying *vgll3*LL* had two times more off spring than *vgll3*EE* and *vgll3*EL* individuals. Females had a higher sea age compared to *vgll3*LL* individuals.
Raunsgard et al., 2023	GWAS	*Vgll3*LL* carriers had the largest variation in sea age. *Vgll3*LL* and v*gll3*EL* had a similar mean sea age.
Verta et al., 2020	QTL	Male parr carrying *vgll3*EE* were 10 x more likely to mature earlier than *vgll3*LL* males.

2.5.2 SIX homeobox 6

The gene *six6* located on chromosome ssa09 has a considerably smaller effect on maturation timing compared to *vgll3* (Sinclair-Waters et al., 2022; Barson et al., 2015). Unlike *vgll3*, *six6* has been associated with maturation timing in Pacific salmonids (Ahi, et al., 2023*).* As research continues to dissect the underlying mechanisms which *vgll3* influences maturation, comparatively less attention has been directed toward elucidating six6's role as displayed in Table 2 (Aykanat et al., 2019; Sinclair-Waters, et al., 2022; Barson 2015). Similarly to *vgll3,* *six6* has associative roles with adipogenesis (lipid accumulation), spermatogenesis (development of sperm cells), and the hypothalmatic-pituitary gonadal axis (production of gametes and reproductive hormones) in addition to aggressive behavior (Verta et al., 2024; Moustakas-Verho et al., 2020; Kurko et al., 2019; Sinclair-Waters et al., 2022; Barson et al., 2015). One of the regulatory roles more distinctive to *Six6* is the development of photoreceptors namely during the transition periods between fresh and the marine environments (Waters et al., 2021; Sinclair-Waters et al., 2022, Pritchard et al., 2018; Moustakas-Verho et al., 2020; Kurko et al., 2020; Oldham et al., 2023).

Table 2: A summary of existing literature on association studies between six6 and the timing of sexual maturation in Atlantic salmon.

Reference	Genomic Method	Summary of Results
Aykanat et al., 2020	PCR	*Six6*LL* was associated to increased stomach content and larger fish size. Individuals with *six6*LL* have an earlier run timing compared to *six6*EE* individuals.
Aykanat et al., 2024	PCR	No significant correlation between six6 genotypes of a mother and the survival of offspring.
Barson et al., 2015	GWAS	*Six6*LL* individuals were more likely to mature later compared to *six6*EE* individuals. The role of significance between sea age and *six6* genotypes was significantly marginal.
Raunsgard et al., 2023	GWAS	*Six6* had a weaker effect on sea age compared to *vgll3* in both sexes. For every *six6* L* added sea age increased by 0.12-0.05 years in females and 0.09-0.04 years in males.
Sinclair-Waters et al., 2020	GWAS	*Six6* is a significant locus of maturation timing in European Atlantic salmon.
Sinclair-Waters et al., 2022	GWAS	*Six6* is confirmed to be associated with sea age maturity but with a smaller effect size to *vgll3*.

2.6 Sea Age Composition of the Icelandic Stock

The majority of the Icelandic stock spends one winter at sea before spawning (Guðbergsson, 2023; Gudjónsson, 1978; Scarnecchia, 1983; Guðbergsson et al., 2015). The population is mainly comprised of 1SW and 2SW populations, while 3SW individuals have been recorded; it is considered rare (Guðbergsson, 2023). The overall average sea age of the stock is 1.5 sea winters (Guðbergsson, 2023; Gudjónsson, 1978; Scarnecchia, 1983; Guðbergsson et al., 2015). Despite conservation efforts 2SW populations have been declining throughout the country, making up just 19% of the total catch between rod and net fisheries (Guðbergsson, 2023; Guðbergsson, 2014; Þórðardóttir & Guðbergsson, 2023; Guðbergsson et al., 2015). Overall, a higher percentage of 2SW and MSW are found in the north and northeast of Iceland, and a majority are female (Guðbergsson et al., 2015; Scarnecchia, 1983; Guðbergsson, et al., 2015; Þórðardóttir & Guðbergsson, 2022). The specific timing of sea age within Icelandic populations has been attributed to three main factors: historical climatological conditions (Scarnecchia, 1983; Olafsson et al., 2014; Gilbey et al., 2018) prey availability (Guðjónsson et al., 2015), and environmental characteristics (Danielsdottir et al., 1997). Populations from the same region sharing similar climatic and physical characteristics between rivers exhibited the greatest genetic similarity, highlighting that the local adaptations of populations is partly shaped by the environment (Danielsdottir et al., 1997).

2.7 Farmed Atlantic Salmon in Icelandic Waters

Rearing of Atlantic salmon first started in Borgarfjordur, Iceland, in 1944 to increase angling catches (Bjarnason & Magnusdottir, 2019). Since then, salmon production has continued to develop into its current state of aquaculture production producing up to 70,000 tonnes of fish a year (Bjarnason & Magnusdottir, 2019). In regard to this thesis, the most notable development happened in 1984 when Norwegian salmon eggs were first imported into Iceland to begin the ocean farming of Atlantic salmon (Bjarnason & Magnusdottir, 2019). The Norwegian stock was brought to replace the high grilse Icelandic stock that was maturing before market size (Bjarnason & Magnusdottir, 2019). Since 1984, this has continued to be the genetic basis for farmed salmon in Iceland (Bjarnason & Magnusdottir, 2019).

Despite both being European Atlantic salmon from northern latitudes, genetically, the Icelandic and Norwegian stocks are unambiguously distinct (Danielsdottir et al., 1997; Gilbey et al., 2018). A genetic analysis comparing wild Norwegian salmon and farmed Norwegian salmon to four populations of Icelandic wild salmon revealed a significant genetic distance between both the wild and farmed strains, with the greatest genetic dissimilarity to the farmed salmon (Karlsson et al., 2017). A study comparing major regional phylogeographic groups of European Atlantic salmon, the Icelandic strain was genetically more similar to the Baltic strains then the Norwegian (Gilbey et al., 2018). To further exemplify the genetic distance between farmed salmon and wild salmon in Iceland, the river Elliðaár was found to be significantly different genetically compared to other wild populations in Iceland due to a historical influx of escapees in river Elliðaár (Danielsdottir et al., 1997).

Since the 1990's reared salmon have been caught in rivers intermixing with wild Icelandic salmon populations (Gudjonsson, 1991). Escaped farm salmon, escapees, have been identified as the largest threat to wild salmon populations (Karlsson et al., 2017). When an ocean reared salmon escapes, it can spawn with the wild populations traveling up to 250 km away, endangering the genetic integrity of the natural populations (Karlsson et al., 2017). Salmon are adapted to the specific environmental characteristics of their natal waterways and genetic infiltration can "erase" adaptions, resulting in a decrease in long term success to the natural stock if a high proportion of reared salmon are encountered (Gudjonsson, 1991; Danielsdottir et al., 1997; Fleming et al., 2002). Additionally, indirect genetic effects such as parasite and disease interaction also occur between escapees and wild populations even if there is no successful reproduction between the strains (Bjarnason & Magnusdottir, 2019; Gudjonsson, 1991; Sturlaugsson, 2016; Sturlaugsson, 2021). These interactions also reduce the overall success of wild populations (Danielsdottir et al., 1997; Sturlaugsson, 2016; Sturlaugsson, 2021).

2.8 Iceland's Current Management of Salmon Fisheries

The current management structure of Atlantic salmon started with the abolishment of coastal and estuarine salmon fishing in 1932 (Guðbergsson, 2015; Olafsson et al., 2016). Since then, management and exclusive fishing rights has been transferred to the land owners adjacent to the salmon river (Bjarnason & Magnusdottir, 2019. By law, all landowners must form a fishery association which manages the exploitation of the Atlantic salmon population residing in the river adjacent of the homeowner (Guðbergsson, 2015). While the management of the fishery is decided by the fishery association it must comply with the framework established by the Directorate of Fisheries and Institute of Freshwater Fisheries:

- Maximum of 105 days in period from May 20th – September 30th. Permission to extend until October may be granted if approved by the Directorate of Fisheries.
- The fishery must be closed for 84 hours a week with daily fishing not exceeding 12 hours.
- A fixed number of rods can be used in each river at one time.

While these rules apply to all salmon rivers these guidelines were established as a protection for the wild populations in larger salmon fisheries since land owners often lease out their rights to fish (Guðbergsson, 2015). While not mandated by law, a majority of salmon fisheries have mandated the release of salmon > 69 cm in order to conserve the declining MSW stock and have imposed a bag limit on 1SW salmon (Guðbergsson, 2023). Although it is not infrequent to see a blanketed catch and release mandate (Guðbergsson, 2023). All fishing is exclusive to rod and line except for small net fisheries, which are reserved for large glacial rivers with highly turbid waters (Guðbergsson, 2023; Guðbergsson, 2015). In order to monitor salmon fisheries, since 1946, log books have been established for every river and processed by the Institute of Freshwater Fisheries at the end of the fishing season to record the total catch landed, weights, and lengths of fish caught per season (Guðbergsson, 2015).

Ocean reared salmon are a completely different fishery and must comply with an extensive set of rules and regulations established by a network of governmental agencies. In regards to this thesis, the most notable management aspect relative to ocean reared salmon is the regulation of aquaculture production. Currently, the tonnage of farmed salmon that can be produced in one fjord is a limit set by a multifactorial equation largely considering the environmental impact of the salmon farm. The product of the master equation establishes a maximum allowed biomass (MAB) per farm (Ministry of Food, Agriculture, and Fisheries, 2023). The MAB of a fjord cannot be increased if the likelihood of genetic introgression from farmed salmon to wild populations is greater than 4% (Ministry of Food, Agriculture, and Fisheries, 2023).

2.9 A Genomic Approach to Conservation Management

The development of accessible genomic sequencing has elevated population-based conservation management by enabling the use of genomic data to guide the management of threatened species (Onley et al., 2021; Aykanat et al., 2016). Genomic analyses can reveal critical aspects regarding the health of a population such as gene flow, population structure, genetic diversity, and patterns of adaptation that are inaccessible without molecular tools (Onley et al., 2021; Aykanat et al., 2016; Stange et al., 2021). A common application is linking genotypes to phenotypes to understand the adaptive potential of a population and predict how a population may respond to environmental shifts (Waples et al., 2020; Aykanat et al., 2016). This approach is particularly valuable for species such as Atlantic salmon, whose populations often exhibit low genetic diversity, populations are locally adapted and have limited migratory capacity (Waples et al., 2020; Stange et al., 2021). A gene targeted approach is essential if a population is to be stocked or bred in order to not risk losing the population's adaptive history (Waples et al., 2020). If the primary objective in managing Atlantic salmon is to ensure the long term health and resilience of populations by maintaining their biological diversity and natural productivity than understanding the genetic basis of key life history characteristics such as maturation timing is critical to the effective management of Atlantic salmon populations (Waples et al., 2020; Onley et al., 2021; Aykanat et al., 2016).

3 Methods and Materials

3.1 Study Area

This thesis made use of an archive of Atlantic salmon fin clips collected between 2017-2024 primarily from June - October in multiple life history stages. Samples were collected from nine established salmon rivers across six regions in Iceland as shown on Figure 3. The rivers include: Elliðaár, Fífustaðadalsá, Haffjarðará, Laxá í Aðaldal, Miðfjarðará, Mýrarkvísl, Selárdalsá, Sogið, and Stóra Laxá.

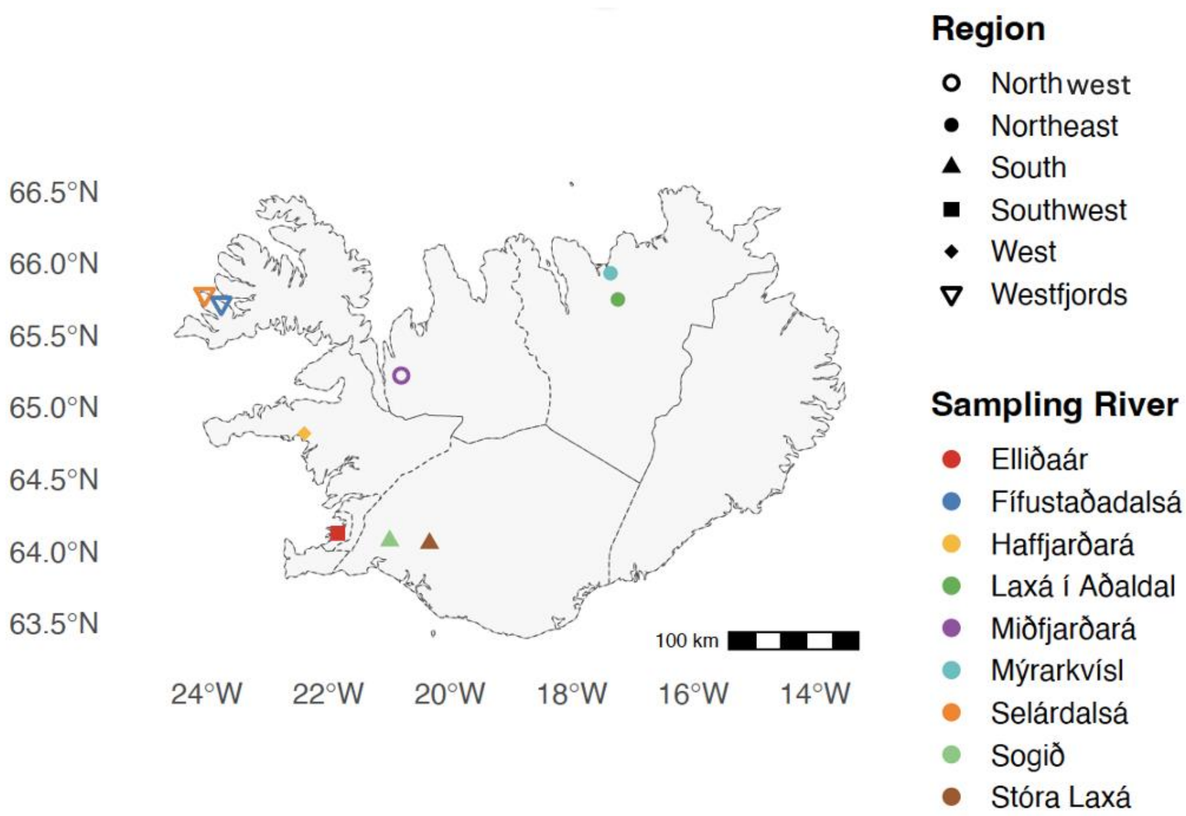

Figure 3: A map of Iceland marked with the approximate location of river sampling sites. Each river is represented on the map by a unique color and shape according to the geographic region of the river. The boundaries of the eight administrative regions of Iceland are also represented on the map. The map was generated in RStudio using ggplot2 (Dunnington, 2023) ggspatial (Wickham, 2016), and rnaturalearthdata (South, et al., 2024) packages.

In addition to fin clips, information if known on sex, length, sea age: 0SW, 1SW, 2SW, repeated spawners, and mature juvenile male data was received. Sea age was classified based on scale readings and the length, sex, and sample site as described in the background section. From the river Fífustaðadalsá, two strains of Atlantic salmon were received; wild and farmed. Farmed salmon were identified based on external characteristics and verified based on genetic markers to infer relatedness (Sturlaugsson & Palsson 2023; Sturlaugsson & Palsson 2024). Any farmed salmon that entered the spawning grounds during monitoring in the autumn were subsequently removed from the river system (Sturlaugsson & Palsson 2023; Sturlaugsson, 2021). Some of the escaped farmed salmon were traced back to fish farms operated by Arnalax in Arnafjörður (Sturlaugsson, 2021). Currently, two companies Arnalax and Arctic Fish have open net pen operating sites in Arnafjörður, the general location of operating sites is shown in Figure 4 (Sturlaugsson, 2016).

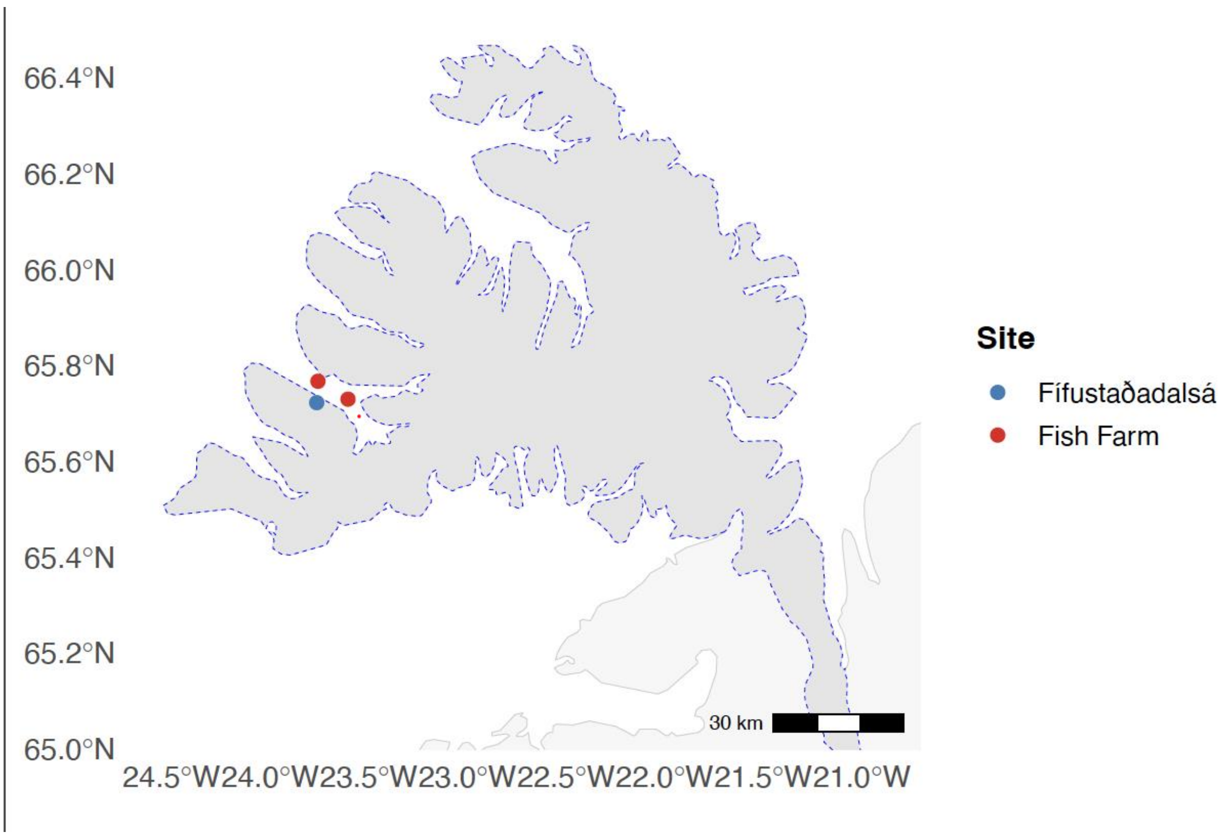

Figure 4: Map of the Westfjords region of Iceland displaying the approximate locations of the sample river Fífustaðadalsá (blue dot) and operation sites of open net pen salmon farms in Arnafjörður (red dots). The map was generated in RStudio using ggplot2 (Dunnington, 2023),ggspatial (Wickham, 2016), and rnaturalearthdata (South, et al., 2024) packages.

3.2 Data Collection

All samples were obtained from the research company Laxfiskar. Data collection was conducted or supervised by Jóhannes Sturlaugsson using either electrofishing, dip net surveys, or rod and line. The author will briefly go over the field methods they employed as a participant data collector during the sampling in 2024 at Selárdalsá (night monitoring) and Fífustaðadalsa (electrofishing and night monitoring) sampling sites.

To collect juvenile samples, backpack electrofishing, a standard method to collect data on fish assemblages in freshwater, was employed (Sturlaugsson & Palsson 2023; Specziár et al., 2012; Thompson et al., 1998). Electrofishing uses a power source to generate a direct current with a voltage set to the relative speed, width, and depth of the river section being sampled (Portt et al., 2006; Thompson et al., 1998). Electrodes attached to the generator create an electric field to produce an attraction zone which fish actively swim toward (Thompson et al., 1998; Specziár et al., 2012). The current momentarily induces fish to the surface to be caught for subsequent processing (Thompson et al., 1998). Electrofishing is a highly effective method reserved best for the collection of juveniles (Specziár et al., 2012). Electrofishing was conducted in three long sections dividing the river by lower, middle, and uppermost sections of the river Fífustaðadalsá were the entire area between riverbanks was sampled.

To obtain data on spawning salmon, night monitoring was carried out a method developed by Sturlaugsson (Sturlaugsson, 2021; Sturlaugsson & Palsson, 2023). The methodology involves the use of both underwater and surface lighting to facilitate the counting and capturing of all salmonid species to be furthered examined, sampled, and tagged. Lights with high light output are used during dark hours and if needed during dusk hours. During the night monitoring participated in 2024, both the rivers, Fífustaðadalsá and Selárdalsá were explored with this method. In both rivers, the monitoring involved wading the full width of the river in waders with lights and dip nets to conduct visual surveys. Monitoring encompassed the entire length of each river, from the ocean estuaries up to impassable sections at the valley bottoms, and was completed within a single night. All salmon spawners were captured using handheld dip nets.

For each sample data collected typically included geographical coordinates, total length (cm), weight (g), sex, and maturation state. Tissue samples for genetic analysis were obtained by clipping a sample of the adipose fin which was then labeled and preserved in 95% ethanol (Hamel et al., 2012). Each individual was visually inspected for previous fin clips and the presence or absence of identification tags. If no marker tag was present, a T-bar anchor tag was inserted adjacent to the dorsal fin to enable future identification. All external features of fish were inspected to discriminate wild salmon from the salmon of farmed origin. All fish with external features that verified its farmed origin were removed from the river (Sturlaugsson, 2021; Sturlaugsson & Palsson, 2023).

3.3 Ethical Considerations

Data collection strictly adhered to the ethical principles and standards as outlined in the University Centre of the Westfjords student handbook and the Icelandic Animal Protection *Act Animal Welfare number 15/1994, article 14* implemented in 1994. Sample collection followed the official Icelandic salmon fishing regulations as outlined in *Law 33 Fisheries and Fish Farming* in conjunction with explicit permission to access sample sites obtained from landowners. Special consideration and forethought was given to the handling of Atlantic salmon ensuring strict adherence to established fisheries sampling methodologies to minimize species destress. Ethics approval from the Master’s Program Committee of the University Centre of the Westfjords is attached in Appendix A.

4.0 Analysis

4.1 Analysis of single nucleotide polymorphisms

The establishment of the Atlantic salmon reference genome has enabled genome wide detection of novel single nucleotide polymorphisms (SNPs) (Barson et al., 2015; Sinclair-Waters et al., 2020, Sinclair-Waters et al., 2022; Aykanat et al., 2016). Previous quantitative trait analyses revealed two major effect loci, *vgll3* and *six6,* which exhibit significant associations with maturation timing in European Atlantic salmon due to variation of a single SNP (Barson et al., 2015; Sinclair-Waters et al., 2020, Sinclair-Waters et al., 2022). This recent progress facilitates the opportunity for efficient genomic analysis of the *vgll3* and *six6* SNP arrays to be analyzed for maturation association studies (Barson et al., 2015; Sinclair-Waters et al., 2020, Sinclair-Waters et al., 2022). The quantitative polymerase chain reaction (qPCR) is a widely adopted technique for gene expression analysis and genotyping across a variety of organisms, including most notable to this thesis teleost fish (Khehra et al., 2023; Guiry et al., 2010; Pabinger et al., 2014; Aykanat, et al., 2016). The qPCR is an especially popular technique due to its rapid processing time and the small amount of sample DNA required (Pabinger et al., 2014; Aykanat, et al., 2016). The technique utilizes a series of thermal cycling steps to denature, anneal, and elongate target DNA. qPCR relies on a chemical reaction in which sample DNA is mixed with a series of reagents such as sequence-specific primers, DNA polymerase, deoxynucleotide triphosphates, and a reaction buffer (Pabinger et al., 2014). As DNA amplifies during qPCR, fluorescent dyes in the reaction mix emit signals proportional to the degree of amplification (Pabinger et al., 2014). These signals are detected by the qPCR machine, enabling efficient and precise genomic analysis to the regions of interest (Khehra et al., 2023; Pabinger et al., 2014).

For this thesis, DNA was extracted from the tissue of the collected fin clip samples and subsequently analyzed using qPCR, targeting SNPs located on chromosome 25 (*vgll3*) and chromosome 6 (*six6*) – the sites which showed the strongest association with maturation timing in Atlantic salmon (Barson et al., 2015; Sinclair-Waters et al., 2020; Sinclair-Waters et al., 2022). DNA sequences from the two genes surrounding the SNPs were obtained from the

Atlantic salmon reference genome, gene bank number GCF_000233375.1, and sent to LGC Biosearch Technologies© (LGC Limited, Middlesex, United Kingdom) which constructed the primers to be applied for the KASP genotyping by qPCR, (as described below). A full summary of the data analyzed is provided in Appendix B.

4.1.1 DNA Extraction

DNA was extracted from the tissue of the fin clip samples using the Monarch® Genomic DNA Purification Kit #T3010L (New England BioLabs, Massachusetts, United States) and Macherey-Nagel NuceloSpin® 740952.50 Bioanalysis Kit (Macherey-Nagel GmbH & Co. Kg, Düeren, Germany), according to the manufacture's protocols. The general principles of DNA extraction can be broken down into two primary parts: lysis and binding. Lysis breaks down cell membranes in order for DNA to be released. In this study, lysis was facilitated by the addition of Tissue Lysis Buffer, proteinase K, and RNase A to 10 mg of tissue sample. The tissue is digested using agitation from a shaking incubator for 60 minutes at 56°C or until fully dissolved. Binding is the process to eluate purified DNA. This occurs when chaotropic salt based Binding Buffer is mixed with the lysate in a sample tube containing a column with a silica membrane. The DNA binds to the silica membrane within two centrifuge cycles. Two final centrifuge cycles are performed to 'wash' the DNA of excess contaminants such as proteins and lipids that could interfere with downstream genomic analysis this is done with the addition of Wash Buffer. Following DNA binding, 100 µl of Elution buffer preheated to 60 °C was added to the microfuge tube and incubated at room temperature for 1 minute and centrifuged for 1 minute (> 12,000 x g) to elute the DNA. The quantity and quality of DNA were determined using Thermo Scientific NanoDrop Spectrophotometer ND-1000.

4.1.2 DNA Amplification

The candidate region for each gene was amplified using qPCR following the Kompetitive Allele Specific PCR (KASP) genotyping V.2 protocol (Kroll, 2022). KASP is a PCR-based genotyping chemistry that distinguishes homozygotes and heterozygotes within SNPs of interest. The mechanism behind the chemistry of KASP utilizes three components per reaction: KASP assay mix, KASP Master mix, and sample DNA (Kroll, 2022). The KASP assay mix contains two competing allele specific forward primers and one common reverse primer. The sequences of these primers are provided in Appendix C. The primers used were based within 50 base pairs of the SNPs. The second component, KASP Master mix is a DNA buffer solution modified for allele specific analysis containing Taq DNA polymerase and fluorescence resonance energy transfer cassettes. The final component is the template DNA previously extracted from the fin clip samples. To array the samples, assay mix (0.14 μL), Master mix (5 μL), sterile water (3.86 μL) and DNA (1 μL at a concentration of 25 ng) were manually pipetted into a 96 well PCR plate, reserving two wells for negative controls (Kroll, 2022). The reagents were sealed into the well plates as the final preparation before thermal cycling.

Thermal cycling was performed in a QuantStudio™ 3 system operating under Design & Analysis Software v2.8.0 with plugin versions Primary Analysis v1.8.1 and Genotyping v1.6.0. The thermal cycling conditions specific to KASP chemistry are comprised of a high temperature phase to denature the DNA and low temperature phase to anneal DNA. The initial denaturation step starts at 94 °C for 15:00 minutes, followed by 10 cycles of 20 second cycles at 94 °C and 60 seconds at 61°C (decreasing 0.6 °C per cycle) (Kroll, 2024). The annealing stage starts with 26 cycles at 94 °C for 20 seconds and 55 °C for 50 seconds followed by a final extension step for 1 cycle of 30 seconds at 60°C (Kroll, 2024). After each run the results were uploaded to Fischer Scientific QuantStudio systems to recall plate readings. The entire genotyping procedure was conducted at the Laboratory of Molecular Genetics, University of Iceland. The aim of the analysis was to genotype each sample two times per assay resulting in successful amplification of twenty individuals per sample site per loci (Kroll, 2024).

4.2 Statistical Tests

A series of statistical tests were used to investigate the role *vgll3* and *six6* have in the sample populations. To quantify the genetic variation of the genotypes within populations, observed heterozygosity (H_O) and expected heterozygosity (H_E) based on Hardy-Weinberg Equilibrium (HWE) were calculated for each river separately by gene. The inbreeding coefficient (F_{IS}) was also calculated to measure deviations from HWE to measure the degree of inbreeding within populations. Statistical significance of deviations from Hardy-Weinberg Equilibrium was assessed by statistical significance of a p-value < 0.05. When results are compared to HWE, the underlying principle is the frequency genotype is expected to remain constant across generations once a population is in HWE (Garnier-Géré & Chikhi, 2013; Weir, 1984; Waples, 2015).

It is standard practice to calculate F-statistics in population genetics studies to revel the general structure of the population as a whole (Waples, 2015). The F-statistics calculated for each gene included: global F_{IS} (total inbreeding within populations), mean F_{IS} (unweighted average of inbreeding across populations), F_{IT} (overall inbreeding across the total population), and F_{ST} (genetic differentiation among populations), following Weir and Cockerham's estimators (Weir, 1984). In addition, Nei's G_{ST} (1973) was calculated as an alternative measure of genetic differentiation (Weir, 1984).

To visualize patterns of genetic similarity among populations, a principal coordinates analysis (PCoA) was performed using Bray-Curtis (1957) dissimilarity calculated from pairwise comparisons of genotype frequencies across rivers for each gene. The Bray-Curtis (1957) distance matrix was chosen because it is widely applied in ecological studies that compare relative abundances or frequencies (Ricotta & Pavoine, 2022). The populations were grouped by region to assess the extent of regional clustering and genetic differentiation among populations.

A series of correlation analyses was performed to test for associations between characteristics and genotype within a respective gene. Association tests were performed using a Pearson's Chi-squared test or a Fishers exact test when the sample size was reduced. These are both association tests that can be applied to categorical data and statistical significance was defined as p-value < 0.05 (Shih & Fay, 2017). Sea age, sex, life history traits: sneakers and repeated spawners, and farmed salmon vs. wild salmon were the variables used. Length, the only continuous variable, failed the Shapiro-Wilk normality test $> p$ 0.05 at both loci. Therefore, a

Kruksal-Wallis test was used to compare length to sea age in an association test (Kurksal & Wallis, 1952). Finally, multiple linear regression models were conducted: sea age (the dependent variable) varies as a function of genotype and river, length (dependent variable) varies as a function of genotype, and length (the dependent variable) varies as a function of genotype, sex, and river. Linear regression was the model of choice as it is a widely applied statistical method when testing for an association between variables (Castro & Ferreira, 2023). Statistical significance was assessed using a threshold of p-value < 0.05 for all tests.

4.3 Statistical Software

All analyses were performed in RStudio using version 4.5.1 Great Square Root 2025. The packages dplyr (Wickham, et al., 2023), tidyr (Wickham, et al., 2024), ggplot2 (Wickham, 2016), and FSA (Ogle, et al., 2025) were used for data manipulation and sorting, hierfstat (Goudet & Jombart, 2022) was used for calculating F-statistics. Data visualizations were performed using ggplot2 (Wickham, 2016), ggspatial (Dunnington, 2023), rnaturearth (South, et al., 2023) and rnaturalearthdata (South, et al., 2023). The sf package (Pebesma, 2018) was used for handling spatial vector data and readxl (Wickham & Bryan, 2025) was used to import Excel files.

5 Results

After completing all qPCR runs for *vgll3* and *six6*, the results were examined for quality control. Samples with undetermined calls, no amplification, or a confidence value of 0.97 or below were excluded from further analysis as outlined by the methodology in Besnier et al., (2024). Out of all the genotyped individuals 38% of *vgll3* and 37% of *six6* samples were supported with a replicate, 58% of *vgll3* and 55% of *six6* samples lacked a replicate sample resulting in only one successful genotype call with all other calls being classified as 'undetermined' or 'unamplified', 4% of *vgll3* and 8% of *six6* calls produced conflicting results and in that case the call with the highest confidence value was retained. The samples with conflicting results are marked with an asterisk (*) preceding the sample name in the dataset provided in Appendix B. Due to limitations in time and material, it was not possible to obtain two successful genotype calls for every sample; therefore, a majority of the results presented in this thesis are based on calls not supported by a replicate sample. After quality control, the total number of samples used in the analysis is shown in Table 3.

Table 3: *The total number of Atlantic Salmon samples successfully genotyped at the vgll3 and six6 loci, grouped by river and region. Columns n_vgll3 and n_six6 indicate the number of individuals used in this analysis for each respective gene. Farmed salmon sampled in Fífustaðadalsá are presented separately.*

River	**Region**	**n_*vgll3***	**n_*six6***
Elliðaár	Southwest	18	13
Fífustaðadalsá	Westfjords	18	13
Haffjarðará	West	19	19
Laxá í Aðaldal	Northeast	9	7
Miðfjarðará	Northwest	0	8
Mýrarkvísl	Northeast	3	0
Selárdalsá	Westfjords	19	22
Sogið	South	9	7
Stóra Laxá	South	20	14
Fífustaðadalsá – Farmed Salmon	Westfjords	5	6

5.1 Reliability of qPCR Method

To assess the reliability of the genomic analysis, summary statistics were calculated for both *vgll3* and *six6* loci. For the *vgll3* SNP assay, 318 qPCR reactions were performed, representing 138 distinct individuals. The overall amplification success rate was 55%, defined as having at least one successful genotype call with a confidence score > 0.97. Among successful calls, the mean confidence score was 0.979 and the mean reliability, defined as the proportion of cases in which two or more successful calls produced the same genotype was 0.912. For the *six6* SNP assay, 502 qPCR reactions were conducted across 160 individuals, yielding an overall amplification success rate of 74%. The variation in the number of individuals genotyped at each locus resulted from the attempt to successfully amplify 20 individuals per site which required differing amounts of resampling across loci. The mean confidence and reliability scores were 0.982 and 0.956, respectively. Analysis of mean confidence and reliability of genotype between *vgll3* and *six6* revealed no significant differences in assay performance across genotype classifications. Nineteen individuals failed to yield a successful genotype for both loci, despite two to four qPCR attempts per sample and locus. Notably, all samples from Miðfjarðará failed amplification at the *vgll3* locus and all samples from Mýrarkvísl failed at the *six6* locus.

5.2 Genotype and Allele Frequencies

To assess genotype frequencies of the wild Icelandic salmon stock, the samples of farmed salmon were excluded from the analysis. For both *vgll3* and *six6,* the *E* allele previously associated with early maturation was the most common across populations (Barson et al., 2015). The homozygous *vgll3*EE* genotype and the heterozygous *six6*EL* genotype were the most frequently observed at their respective loci. The allele frequencies revealed that the *E* allele was more prevalent at *vgll3* than at *six6*. At *six6*, *E* and *L* alleles were more evenly distributed, the *E* allele remained predominant. See Table 4 for the full overview of genotype and allele frequencies.

Table 4: Frequency of genotypes at the genes vgll3 and six6. At the vgll3 locus, the most common genotype among wild populations was EE (43.4%), followed by EL (34.7%) and LL (21.7%). At the six6 locus, the EL genotype was most frequent (36.8%), followed by EE (34.9%), and LL (28.1%).

Gene	**Genotype**	**n_*individuals***	**Frequency**	**Allele**	**Allele Frequency**
vgll3	*EE*	50	0.434	*E*	0.60
	EL	40	0.347	*L*	0.39
	LL	25	0.217		
six6	*EE*	36	0.349	*E*	0.53
	EL	38	0.368	*L*	0.46
	LL	29	0.281		

The genotype frequencies at *vgll3* and *six6* were examined across sample sites and the distribution is represented in Figure 5. For both genes, all three genotypes *EE*, *EL*, *LL* were generally present within each population, although frequencies differed among sites. At the *vgll3* locus, *vgll3*EE* was most predominant in Elliðaár (*n* = 18), Mýrarkvísl (*n* = 3), Sogið (*n* = 9), and Stóra Laxá (*n* = 20). *Vgll3*EL* dominated in Fífustaðadalsá (*n* = 18), Haffjarðará (*n* = 19) and Selárdalsá (*n* = 19). Laxá í Aðaldal (n = 9) was the only river where *vgll3*LL* had the majority. At the *six6* locus, *six6*EE* was most frequent in Fífustaðadalsá (*n* = 13), Haffjarðará (*n* = 19), and Miðfjarðará (*n* = 8). *Six6*EL* dominated in Elliðaár (*n* = 13) and Selárdalsá (*n* = 22). *Six6*LL* was the most prevalent in Laxá í Aðaldal (*n* = 7), Sogið (*n* = 7), and Stóra Laxá (*n* = 14). Notably, Sogið exhibited no genotypic variation at the *six6* locus this was the only occurrence of homogeneity between both genes.

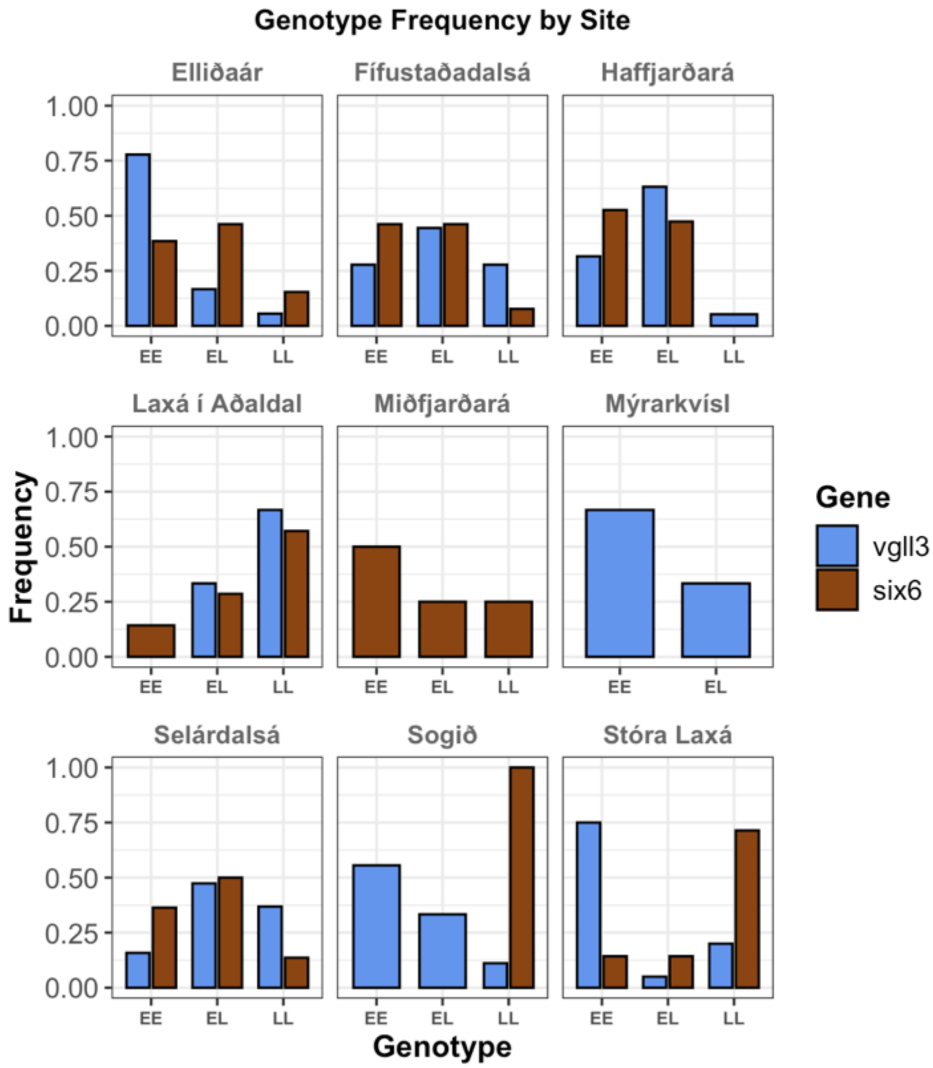

Figure 5: Relative frequencies of genotypes at vgll3 (blue) and six6 (brown) loci for wild Atlantic salmon. Each panel represents a single sample site labeled by the sampling river accompanied by the frequency of the three genotypes EE, EL, LL across all nine sites.

5.3 Genetic Diversity

Observed (H_O) and expected (H_E) heterozygosity, as well as the inbreeding coefficient (F_{IS}) were calculated for each sampling river and the two gene variants. Results are shown in Table 5. In this dataset, per river F_{IS} values for *vgll3* ranged from -0.357 to 0.857, and for *six6* from -0.621 to 0.576. Between loci a majority of the samples indicate a moderate F_{IS} values between 0.10 – 0.40, indicating a general trend toward heterozygote deficiency across the populations. When testing the significance of deviations from the Hardy-Weinberg equilibrium, only the rivers Stóra Laxá (*vgll3* $p = 0$, *six6* $p = 0.031$) and Sogið (six6 $p = 0$) showed statistically significant deviations.

Table 5. The allele frequencies of E, observed (H_O) and expected (H_E) heterozygosity and inbreeding coefficient (F_{IS}) separated by sampling river and gene. Positive values indicate a deficit of heterozygotes compared to Hardy-Weinberg expectations and negative values indicate an excess of heterozygotes compared to Hardy-Weinberg expectations.

Gene	**River**	**Freq E**	**H_o**	**H_E**	**F_{IS}**
vgll3	Elliðaár	0.86	0.167	0.239	0.303
	Fífustaðadalsá	0.50	0.444	0.5	0.111
	Haffjarðará	0.63	0.632	0.465	-0.357
	Laxá í Aðaldal	0.16	0.333	0.278	-0.2
	Mýrarkvísl	0.83	0.333	0.278	-0.2
	Selárdalsá	0.39	0.474	0.478	0.009
	Sogið	0.72	0.333	0.401	0.169
	Stóra Laxá	0.77	0.05	0.349	0.857
six6	Elliðaár	0.61	0.462	0.473	0.025
	Fífustaðadalsá	0.69	0.462	0.426	-0.083
	Haffjarðará	0.76	0.474	0.361	-0.621
	Laxá í Aðaldal	0.28	0.286	0.408	0.3
	Miðfjarðará	0.62	0.286	0.469	0.467
	Selárdalsá	0.61	0.25	0.474	-0.054
	Sogið	0	0	0	NA
	Stóra Laxá	0.21	0.143	0.337	0.576

Global F-statistics and genetic differentiation (G_{ST}) was calculated by pooling the populations by gene. The results are displayed in Table 6. The positive mean and global F_{IS} values (*vgll3:* 0.113, 0.086; *six6:* 0.057, 0.076) indicate a deficit of heterozygotes within individual river populations. The F_{IT} values, which measure the relative proportion of inbreeding to the total population, were also positive (*vgll3*: 0.266; *six6*: 0.258) suggesting evidence that populations are susceptible to inbreeding or population structure within rivers (Garnier-Géré & Chikhi, 2013). Both the genetic differentiation tests, F_{ST} and G_{ST} values were positive indicating there is a moderately high degree of genetic differentiation among populations. Additionally, the global allele frequencies (p and q) values indicate a higher frequency of the *E* allele compared to the *L* allele at both loci, reflecting an overall distribution towards the genotype associated with early maturation across the population.

Table 6: Summary of population genetic statistics for each gene. F_{IS} values represent the inbreeding coefficient within rivers both global (Weir and Cockerham) and unweighted mean values shown. The F_{IT} value is the overall inbreeding coefficient relative to the total population. F_{ST} and Gst (Nei) represent alternative measures of genetic differentiation among populations based on allele frequency variance and gene diversity. Global p and q represent the overall allele frequences of the stock for E and L alleles, respectively. All values are based on a range from 0 -1.

Gene	Global F_{IS}	Mean F_{IS}	F_{IT}	F_{ST}	G_{ST}	Global p	Global q
vgll3	0.113	0.086	0.266	0.172	0.215	0.605	0.395
six6	0.057	0.076	0.258	0.214	0.259	0.534	0.466

5.4 Regional Associations

To test for regional associations, genotype frequencies at the *vgll3* and *six6* loci were compared between rivers and visualized using a Principal Coordinates Analysis (PCoA) seen in Figures 6 and 7. The PCoA plots revealed differing levels of regional structuring between the two loci which could be attributed to the differences in allele frequencies from both genes. Genotypes at the *vgll*3 loci revealed strong regional clustering of populations in the Westfjords with some evidence for a marginal regional association of the southern populations. Genotypes at the *six6* loci similarly displayed pronounced clustering among populations in the Westfjords, with nearby western and southwestern populations grouping nearby. This result suggests a broader regional structure for the western part of the country. The two populations in the southern region once again displayed evidence of a potential regional association.

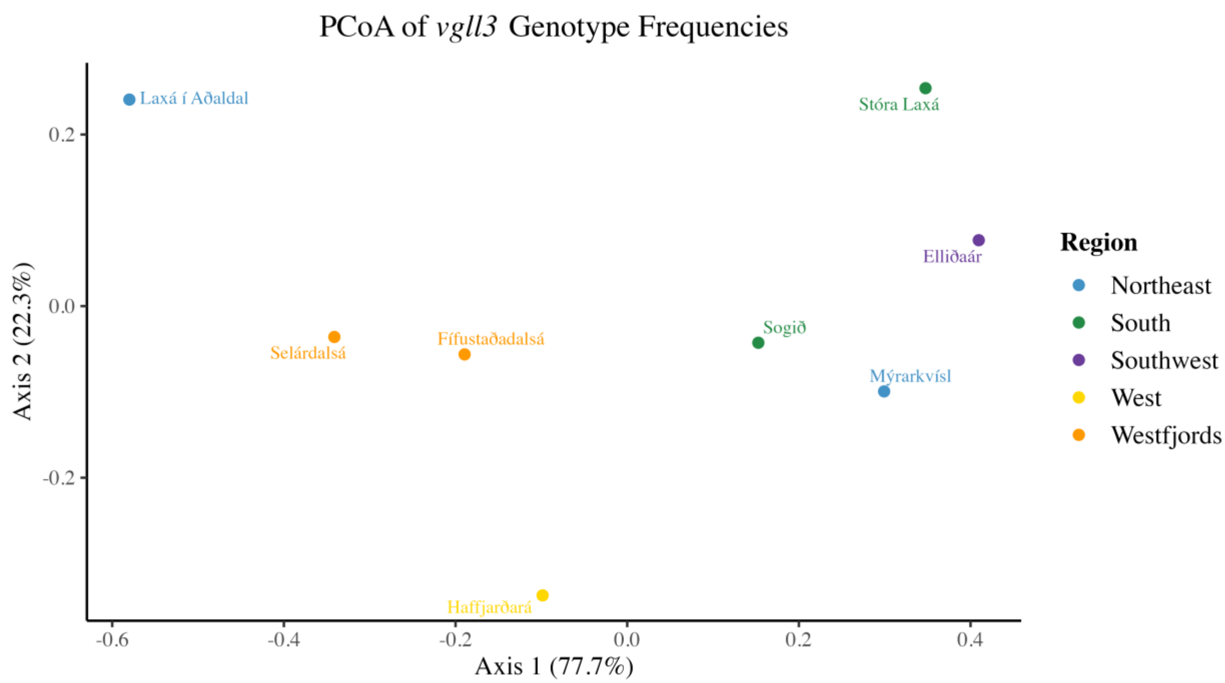

Figure 6: PCoA of wild salmon populations based on river level genotype frequencies at the vgll3 locus. Each point represents a river, colored by region: northeast (blue), south (green), southwest (purple), west (yellow), and westfjords (orange). Axis 1 explains 77.7% of total variance and axis 2 explains 22.3% of variance.

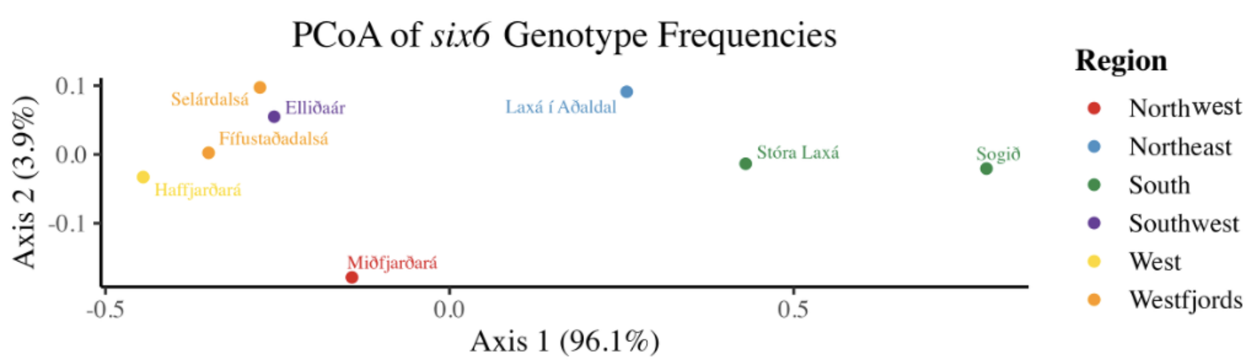

Figure 7: PCoA of wild salmon populations based on river level genotype frequencies at the six6 locus. Each point represents a river, colored by region: northwest (red) northeast (blue), south (green), southwest (purple), west (yellow), and westfjords (orange). Axis 1 explains 96.1% of total variance and axis 2 explains 3.9% of the variance.

5.5 Genotype and Sea Age

To test for an association between genotype and sea age, a chi-squared test of independence was applied to sea age classes (0SW, 1SW, 2SW) and genotypes (*EE, EL, LL),* no significant association was found in either *vgll3* (p = 0.311) or *six6* (p = 0.722). To test for an association between 'early' and 'late' maturation timing to sea age, 1SW and *E* alleles and 2SW and *LL* alleles were tested by applying a series of Fisher's exact tests (Aykanat et al., 2019; Sinclair-Waters et al., 2022; Barson et al., 2015). 0SW was tested for the association between *EE* individuals and non-*EE* individuals (*vgll3*: p = 0.377; *six6*: p = 1) as well as *EE* + *EL* individuals to *LL* individuals (*vgll3*: p = 0.604; *six6*: p = 1). The same series of association tests applied to 0SW individuals was applied to 1SW individuals. The only test showing marginal significance was between *EE* individuals and non-*EE* at the *vgll3* locus (p = 0.087). 2SW individuals carrying the *LL* genotype were compared to non-*LL* individuals and the findings were insignificant (*vgll3*: p = 0.271; *six6*: p = 0.584).

To further explore the relationship between genotype and sea age the distribution of genotype counts to sea age is shown in Table 7 and by river in Figures 8 & 9. Table 7's most notable result is all genotypes are primarily associated with 1SW individuals (*vgll3*EE* 44%; *six6*EE* 54%; *vgll3*EL* 63%, *six6*EL* 52%, *vgll3*LL 60%*; *six6*LL 48%*). Overall, genotypes are fairly evenly distributed across sea ages indicating no clear pattern between sea age and genotype. The lack of association is further displayed in the narrow range of the mean sea age across genotypes *vgll3*: 0.84 -1 and *six6*: 0.88-0.96. To investigate if a potential environmental influence on sea age and genotype, the effect of sampling year was also examined however, no meaningful associations were able to be concluded.

Table 7: Counts of Atlantic salmon grouped by sea age measured in 0, 1, 2 sea winters and genotype EE, EL, and LL presented separately by gene. Mean sea age of genotype is also represented.

Gene	**Genotype**	**0SW**	**1SW**	**2SW**	**Mean Sea Age**
vgll3	*EE*	14	22	13	0.979
	EL	6	24	8	1.05
	LL	7	15	3	0.84
six6	*EE*	10	19	6	0.88
	EL	10	19	7	0.91
	LL	8	14	7	0.96

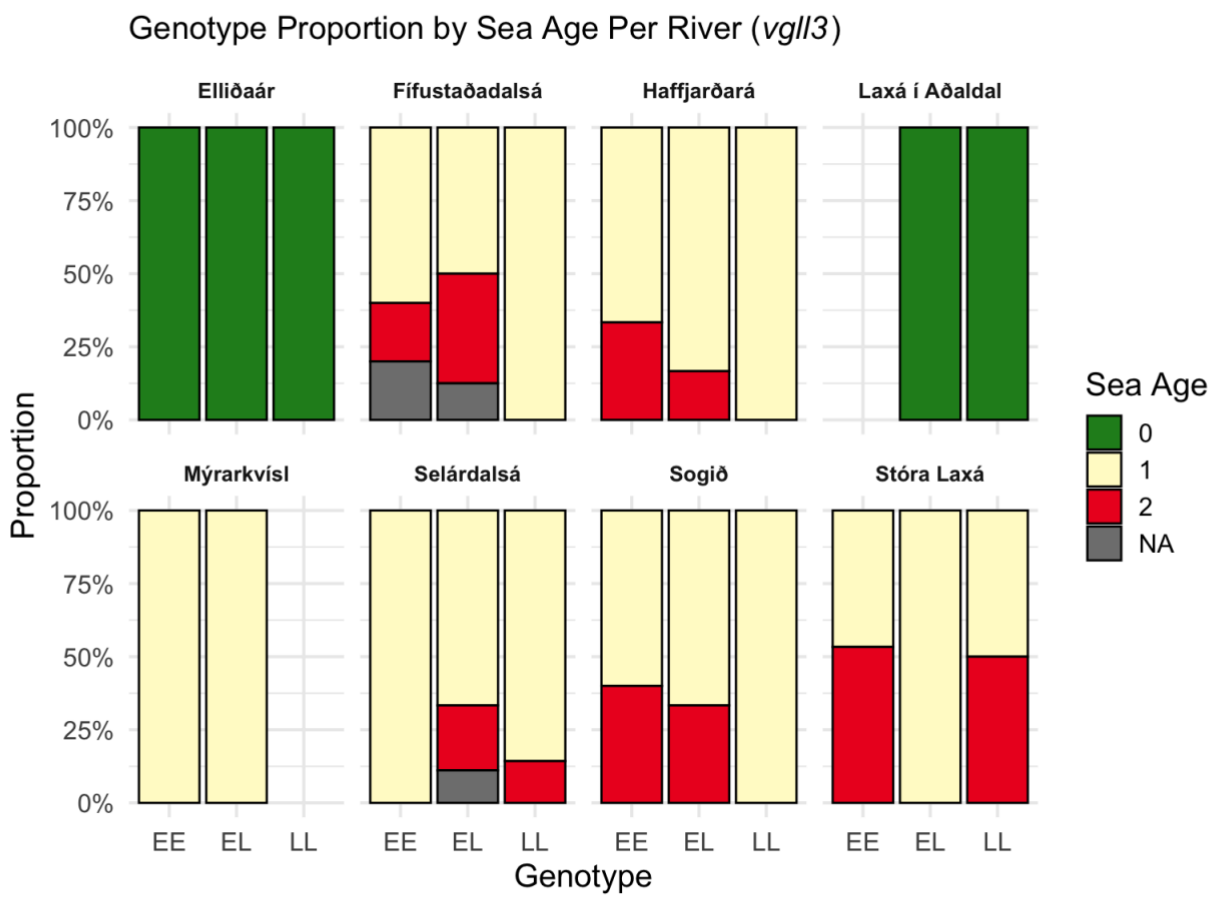

Figure 8: Proportional bar plot of the distribution of genotypes (EE, EL, LL) at the vgll3 locus. Each color represents a sea age (0: green, 1: yellow, 2: red). The NA represented in grey and indicates no sea age data was available. The plots are faceted by river.

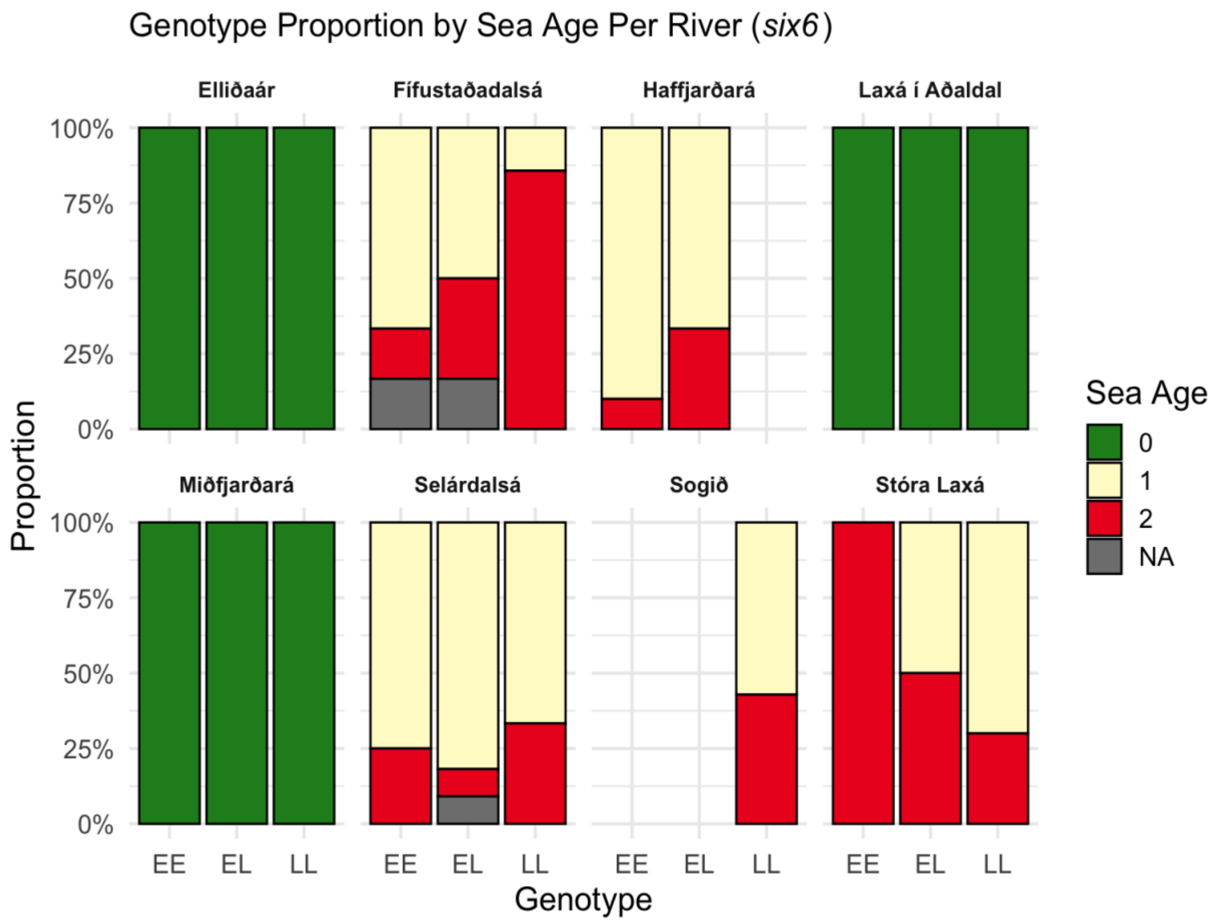

Figure 9: Proportional bar plot of the distribution of genotypes (EE, EL, LL) at the six6 locus. Each color represents a sea age (0: green, 1: yellow, 2: red). The NA represented in grey and indicates no sea age data was available. The plots are faceted by river.

5.6 Characteristic Associations

5.6.1 Sex

A Fisher's exact test found no significant association in *vgll3* ($p = 0.189$) or *six6* ($p = 0.405$) between sex and genotype. These results contrast Barson et al., (2015) but align with Ayllon et al., (2019). See Table 8 for the distribution of genotypes between sexes.

Table 8: Individual counts of male and females between the three genotypes (*EE*, *EL*, *LL*) separated by loci; *vgll3* and *six6*.

Sex	Gene	*EE*	*EL*	*LL*
Male	*vgll3*	37	30	12
	six6	24	28	16
Female	*vgll3*	12	7	7
	six6	6	7	8

5.6.2 Length

To determine whether length is significantly associated with genotype a linear regression model using the formula lm(length ~ genotype) was fitted to both genotypes. For all linear models used, it should be noted that genotype is additive and does not account for any dominance. For *vgll3,* the fitted regression model was: length = 70.142 - 9.764 * genotype. The results predict the average length for *EE* individuals is 70.142 cm and will decrease by an average of 9.76 cm per allele change. The regression was statistically significant, indicating genotype is a significant predictor of body length (p = 0.0001). The model's performance values were: R^2 = 0.15, F (1, 92) = 16.38, residual standard error: 18.2. No significant association was found between *six6* genotypes and length (p = 0.52). Similar results have been found in Fraser et al., (2024) and Debes et al., (2021). The distribution of lengths across genotypes is represented in Figure 10 and the mean length by sex is represented in Table 9.

Table 9: The mean length measured in centimeters separated between sex, genotype, and gene.

Sex	Gene	*EE*	*EL*	*LL*
Male	*vgll3*	37	63.2	69.9
	six6	67.4	66.6	61.2
Female	*vgll3*	72.8	71.1	67.9
	six6	76.2	68.7	68.2

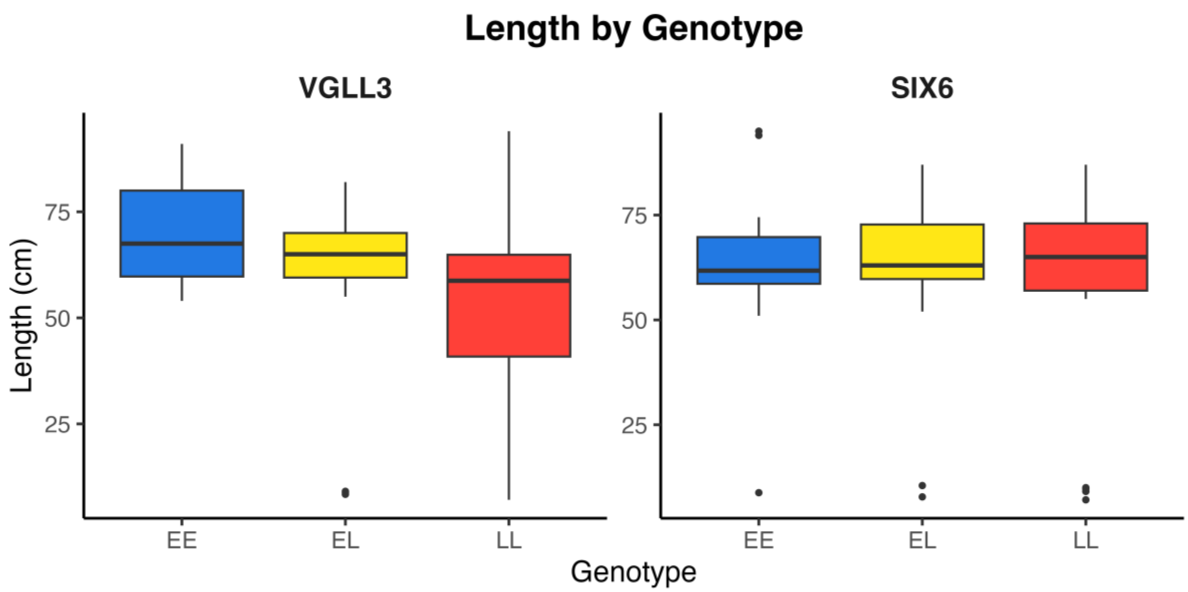

Figure 10: A box-and-whisker plot comparing body length (cm) to genotype EE (blue), EL (yellow), LL (red). The plot displays the median interquartile and outliers for each genotype. The plot is separated by gene, vgll3 (left hand side) and six6 (right hand side).

5.6.3 Length & Sea Age

A Kruskal-Wallis rank sum test was applied to assess whether length differed between sea age. Results indicated highly significant differences as both loci (*vgll3*: p = 1.403e-12; *six6*: p = 1.788e-10). The test statistic in *vgll3* (50.18) is higher compared to *six6* (40.686) indicating the association between length and sea age is slightly stronger in *vgll3* compared to *six6*. These differences are best illustrated in Figure 11, where 2SW fish are notably longer compared to 1SW individuals.

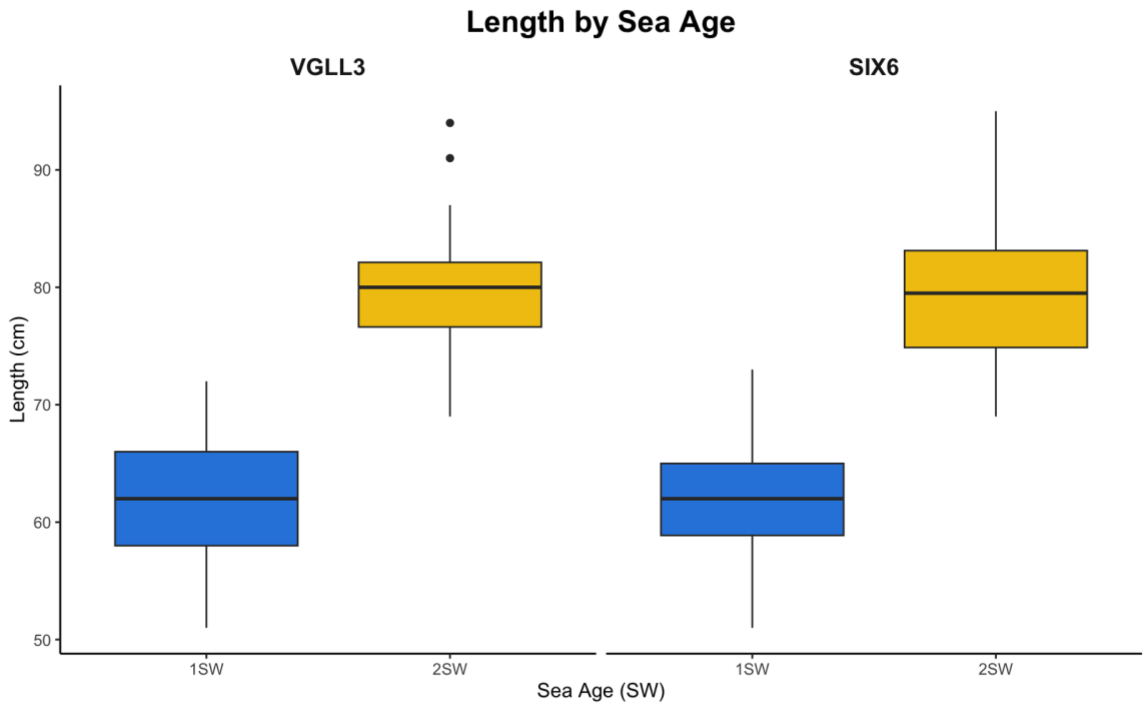

Figure 11. Box-and-whisker plots of body length (cm) by sea age, with individuals classified as either one sea winter (1SW;blue) or two sea winters (2SW; yellow). Data is shown seperatly for vgll3 (left panel) and six6 (right panel). The plot displays the median interquartile range and points representing outliers.

5.6.4 Length & Sex

To test whether genotype, sex, genotype – sex, and river significantly predicted length, a linear regression model was employed following this formula: lm (length ~ genotype * sex + river) and separately applied to both loci. For *vgll3,* the overall model was statistically significant ($p =$ 0.005) explaining 24% of the variation in body length ($R^2 = 0.24$). Genotype ($p = 0.512$), sex ($p =$ 0. 213), nor their interaction ($p = 0.691$) were statistically significant indicating river had the most influence on length among the variables included in this model. While sex was not a significant predictor, the results indicate males are smaller than females overall.

The baseline population referenced by the intercept corresponds to a *vgll3*EE* male from Fífustaðadalsá, with an average length of 59.69 cm. Stóra Laxá was the only river significantly different from the baseline and was longer by 9.23 cm ($p = 0.004$) when compared to the baseline fish. The full output of the regression model is presented in Table

10. Any sample without a recorded length, sex, or sea age 0 was removed from the analysis. All individuals from rivers Laxá í Aðaldal and Elliðaár were removed. A residuals vs fitted plot was used to assess how well the model fits the data upon examination no clear trends indicated the model was unreliable.

*Table 10: Table of the results from the linear regression model for vgll3 genotypes. The overall model fit values were: residual standard error: 9.009, Multiple R-squared: 0.24 Adjusted R-squared:0.16, F-statistic: 3 on 8 and 76 DF, P = 0.005. The level of significance is coded by stars *** representing the highest level of significance.*

Predictor	Estimate	std.error	t-value	p-value	Significance
Intercept	59.698	5.102	11.699	1.13E-18	***
Genotype	-2.577	3.914	-0.658	0.512	
Sex	3.886	3.219	1.207	0.231	
Haffjarðará	3.29	3.212	1.024	0.308	
Mýrarkvísl	-0.082	5.836	-0.014	0.988	
Selárdalsá	-0.395	3.111	-0.127	0.899	
Sogið	3.955	3.831	1.032	0.305	
Stóra Laxá	9.231	3.152	2.928	0.004	**
Genotype:Sex	1.068	2.683	0.398	0.691	

The linear regression model was then applied to *six6* genotypes. The overall model was highly significant (p = 8.55E-11) explaining 27% of the variation in body length (R^2 = 0.275). Genotype (p = 0.017), sex (p = 0.002), and their interaction (p = 0.007) were all statistically significant indicating genotype and sex significantly influence body length, with sex having the largest effect. The results indicate females are larger than males and for every allele change starting from *EE* an increase in 10.8 cm in body length is predicted. No rivers showed statistical significance. The full output of the regression model is presented in Table 11. Any samples without a recorded length and/or sex were removed from the analysis, most notably all individuals from rivers Elliðaár and Miðfjarðará. A residuals vs fitted plot was included to see how well the model fits the data displayed in Figure 12.

*Table 11: Table of the results from the linear regression model for six6 genotypes. Multiple R-squared:0.2755, Adjusted R-squared: 0.1963, F-statistic: 3.478 on 7 and 64 DF, p-value: 0.003215. The level of significance is coded by stars *** representing the highest level of significance.*

Predictor	Estimate	std.error	t-value	p-value	Significance
Intercept	48.449	6.244	7.760	8.55E-11	***
Genotype	10.845	4.413	2.458	0.0167	*
Sex	13.400	4.213	3.181	0.002	**
Haffjarðará	2.985	3.488	0.856	0.395	
Selárdalsá	-2.957	3.313	-0.892	0.375	
Sogið	5.152	5.041	1.022	0.310	
Stóra Laxá	6.732	3.999	1.684	0.097	
Genotype:Sex	-8.383	3.042	-2.756	0.007	**

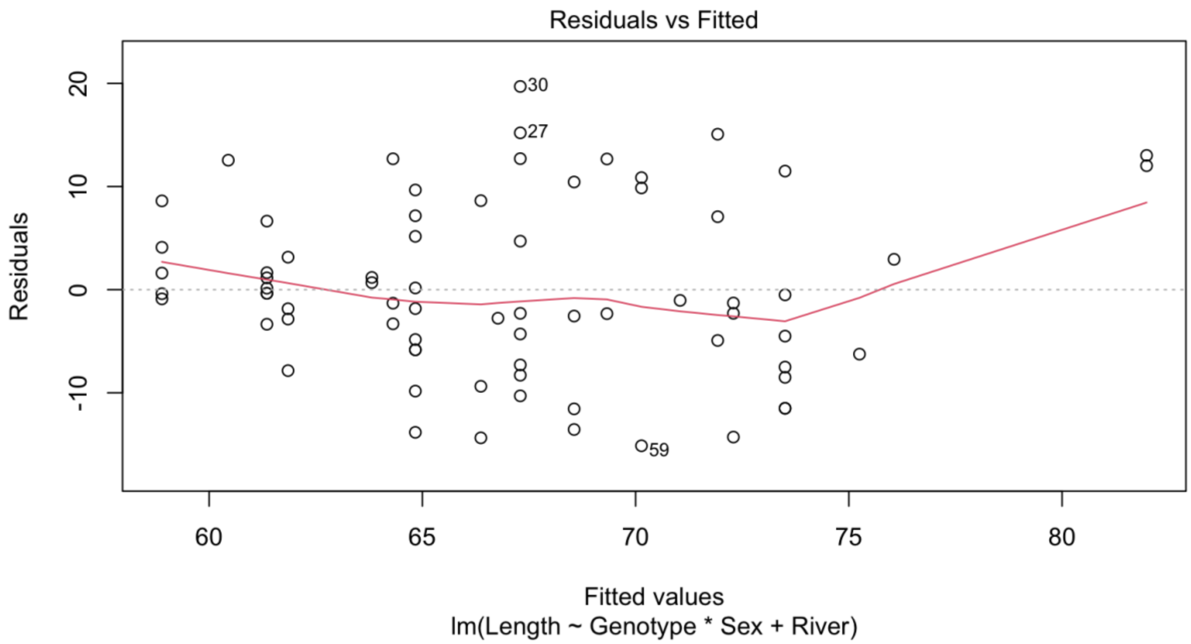

Figure 12: The trend line of the residuals (in red) makes a slight u-curve breaking linearity suggesting a linear model may not capture the full relationship between all predictors. The residuals are predominantly randomly distributed with some clustering occurring between fitted values 62-64 with a few high and low outliers.

A sex specific model of lm (length ~ genotype + river) was applied to both *vgll3* and *six6*. At *vgll3,* the model for males was not significant ($p = 0.080$), but was for females ($p = 0.042$). Genotype was not a significant predictor, but females from Stóra Laxá were significantly longer fish than fish from the reference river, Fífustaðadalsá ($p = 0.034$). At *six6,* the overall model for males was significant ($p = 0.013$), with later maturing genotypes showing a marginal association to longer body length ($p = 0.077$). Among rivers, only Sogið showed marginal significance ($p = 0.083$) of males being longer than those from the reference river, Fífustaðadalsá. The overall model was not significant for females ($p = 0.084$). Additionally, to test for dominance of the *E* allele at both loci, an additional model lm (length ~ genotype + sex + river) was fitted with genotypes coded as *EE/EL* = 1 and *LL* = 0. The effect was not significant in either *vgll3* ($p = 0.147$) or *six6* ($p = 0.294$).

5.6.5 Sneakers

A Fishers Exact Test was conducted to assess the association between *vgll3* and *six6* genotypes and mature male parr, sneakers. The test revealed a significant association in *vgll3* ($p = 0.006$) between genotype and the sneaker reproductive strategy. No significant association was found at the *six6* locus ($p = 0.457$). The contingency table created to run the Fishers test, compared sneakers vs non sneaker; 77% of sneakers carried the *EE* genotype compared to 17% with *EL* and 6% with *LL.* The association between vgll3*EE and sneakers has been found in previous studies (Verta et al., 2020; Barson et al., 2015). For the full genotype distributions of *vgll3* and *six6* of sneakers, see Table 12.

5.6.6 Repeat spawners

A Fishers Exact Test was conducted to assess the association between *vgll3* and *six6* genotypes to repeat spawners. The genotypes of repeat spawners were compared to the genotypes of non-repeat spawners and no significant relationship was found in either gene (*vgll3*: $p = 0.730$, *six6*: $p = 0.276$). For the full genotype distributions of *vgll3* and *six6* of repeat spawners see Table 12. This results conflict with Aykanet et al., (2019) a study that found *vgll3*EE* had a higher probability of repeat spawning and Mobley et al., (2024) found a higher proportion of *vgll3*EL* females to be iteroparous.

Table 12: Genotype frequencies of sneakers, repeat spawners, and farmed salmon, grouped by gene. Values represent the number of individuals per genotype for each characteristic. Frequencies per characteristic separated by a slash (/) and presented in the following order: sneaker, repeat spawner, farmed salmon.

Gene	Genotype	Sneaker	Repeat Spawner	Farmed Salmon	Frequency
vgll3	*EE*	14	4	2	0.77 / 0.33 / 0.40
	EL	3	5	2	0.16 / 0.41 / 0.40
	LL	1	3	1	0.05 / 0.25 / 0.20
six6	*EE*	5	3	0	0.31 / 0.50 / 0.0
	EL	8	3	0	0.50 / 0.50 / 0.0
	LL	3	0	6	0.18 / 0.0 / 1.0

5.7 Farmed and Wild Salmon

The differences of the allelic frequencies between escaped farmed salmon and the wild population in Fífustaðadalsá were compared between the two groups using a Fishers exact test. No significant difference was found at the *vgll3* locus ($p = 1$), suggesting similar allelic frequencies between farmed and wild salmon. When farmed salmon were compared to all samples in the Westfjords sites (Fífustaðadalsá and Selárdalsá), results remained non-significant, countering previous findings of genetic differentiation between farmed and wild salmon strains (Danielsdottir et al., 1997; Olafsson et al., 2014). Interestingly, a significant difference was found at the six6 locus (p = 0.000). Here all farmed salmon exhibited the *LL* genotype, whereas the wild population was composed almost entirely of *EE* and *EL* genotypes. Notably, all farmed salmon genotyped at the *six6* locus were male. For the full genotype distributions of *vgll3* and *six6* of farmed salmon see Table 12.

6 Discussion

The analysis presented in this thesis builds upon the first findings of the associations between *vgll3* and *six6* with age at maturation in Atlantic salmon (Barson et al., 2015; Ayllon et al., 2015; Sincalir-Waters et al., 2020). The findings of this project, indicate *vgll3* and *six6* genotypes, population of origin, and key life history traits influence age at maturity to a certain extent. The most notable result is the apparent dissociation between sea age and genotypes at *vgll3* and *six6,* contradicting the initial hypothesis and findings of the original association studies (Barson et al., 2015; Ayllon et al., 2015; Sincalir-Waters et al., 2020). It is salient to note all previous studies were conducted on Norwegian salmon a strain genetically different from the wild Icelandic samples used in this thesis thus differing results could be attributed to a difference in genomes (Danielsdottir et al., 1997; Gudjonsson, 1991). Additionally, since sea age in Atlantic salmon is controlled by multiple quantitative trait loci in addition to *six6* and *vgll3* it would be beneficial to assess the effect size of all genes influencing maturation timing for the Icelandic strain to see if the effect size differs between Norwegian and Icelandic strains (Sinclair-Waters et al., 2022).

Overall the results are indictive of the highly plastic nature of maturation timing, a trait strongly influenced by a combination of genetic and environmental influences. Similar detections have since been noted by studies succeeding Barson et al., (2015) reflecting a larger environmental influence on maturation timing rather than genetic (Sinclair-Waters et al., 2020; Moustakas-Verho et al., 2020; Besnier et al., 2023). To the authors knowledge, this is the first SNP analysis characterizing the association between maturation timing to *vgll3* and *six6* genotypes of Atlantic salmon in Iceland. All together these results provide new insights between the effects of genotype and maturation timing, highlighting the value of adopting a genetics based approach to guiding the conservation of wild salmon (Onley et al., 2021). It is vital to keep in mind the results being discussed in this thesis are based primarily on a single successful genotype call per sample and further analysis is required to validate and substantiate the findings presented.

6.1 Analysis of Single Nucleotide Polymorphisms

In this thesis, the qPCR method proved to be highly reliable, producing high-confidence calls when a successful genotype was obtained; however, it was not highly effective overall due to the low proportion of successful calls. Conditions contributing to high failure rate of qPCR analysis span from the point of collection to the last step of sample processing and therefore not one variable can be distinguished. All samples were collected between years 2017 – 2024 and therefore age of sample should not have an effect on genotyping success. Failed calls may be attributed to errors made in laboratory methodology or from DNA concentrations out of the range of optimization. High concentrations of DNA were yielded for a large portion of the dataset, even though samples were diluted, concentrations above or below the optimal range can affect the reliability and accuracy of results (Pabinger et al., 2022; Kroll, 2022). Successful genotype calls per sampling site ranged from 0 to 22, with a target of 20 individuals per population for each loci. While a larger sample size would have increased the statistical power of the analyses, the sample range employed here still provides relevant results for conservation managers. The sample size of this study, while on the low range, remains in line with previous genetic studies of Icelandic Atlantic salmon populations, which have drawn conclusive results from samples ranging between 11 – 92 individuals per river (Olafson et al., 2014; Olafson et al., 2016; Danielsdottir et al., 1997). Overall, qPCR analysis has been highly successful among other studies genotyping *vgll3* and *six6* in Atlantic salmon and should still be considered for future studies (Aykanat et al., 2016).

6.2 Sea Age and Genotype Association

Across the sampled populations, there was a higher frequency of the early maturation allele (*E*) at both loci (Barson et al., 2015). Among genotypes, *vgll3*EE* and *six6*EL* were most frequently observed and homozygosity for the late maturation allele was the least prevalent comprising 21.7% of genotypes in *vgll3* and 28.1% at *six6* (Barson et al., 2015). The recorded sea ages indicated a majority of the population matured after a single sea winter, with a mean sea age of 0.956 for *vgll3* and 0.916 for *six6*. These results are consistent with the establishment that the Icelandic stock is largely composed of 1SW individuals (Guðbergsson, 2023; Gudjónsson, 1978; Scarnecchia., 1983; Guðbergsson et al., 2015; Olafsson et al., 2016). Despite the frequency towards early maturation and a high proportion of 1SW individuals, no statistical significance was found between genotype and sea age, aside for a marginal association (p = 0.087) between *vgll3*EE* and 1SW individuals revealed in a Fishers exact test. The absence of any significant association between genotype and sea age suggests that genotype-environmental interactions are playing a greater role in governing maturation timing then genotype alone (Åsheim et al., 2023; Raunsgard et al., 2024). While the result came as a surprise, it is not unexpected, as *vgll3* only explains around 40% of variation in sea age and *six6* to an even lesser extent (Barson et al., 2015; Sinclair-Waters et al., 2020). The marginal association that was found suggests increasing sample size may enhance statistical power in order to detect a more defined genotype-sea age relationship (Barson et al., 2015). Nevertheless, even with greater power, the inherently plastic nature of maturation timing may still not reveal a strong genotype-phenotype interaction found in previous studies (Barson et al., 2015; Sinclair-Waters et al., 2020; Ayllon et al., 2015).

Temporal fluctuations between genotype and sea age associations are expected given that individuals are exposed to variable environmental conditions over time affecting maturation timing (Raunsgard et al., 2024; Barson et al., 2015). The complete absence of association between the *LL* genotype and 2SW individuals may be the clearest indicator environmental factors are strongly influencing age at maturity. A plausible explanation for individuals carrying the allele associated with delayed maturation is an increase in resource availability enabling *LL* individuals, to outride their built in delay system and mature after one sea winter (Barson et al., 2015; Åsheim et al., 2023; Raunsgard et al., 2024). If the shift, in resource abundance is sustained, this could lead to a population structure dominated by smaller earlier maturating individuals. While this may increase the proportion of individuals reaching reproduction due to a temporal reduction in the marine environment thus increasing population size, in the short term it simultaneously reduces body size, a trait strongly associated with offspring fitness and long term population resilience (Mobley et al., 2021). While no definitive conclusions can be drawn regarding the long term health of the stock or the future trajectory of environmental conditions these findings demonstrate the importance of characterizing populations at a fine scale in order to understand a populations dynamics and the impact environmental change may have on a population (Lennox et al., 2021; Ratnarajah et al., 2023).

Even though the results indicate sea age is largely shaped by the ecological regime compared to genotype the marginal association found between *vgll3*EE* should not be completely discounted. This observation can be extrapolated into two important findings: first that *vgll3* may have a stronger effect on maturation age compared to *six6* a finding ubiquitous to maturation studies of Atlantic salmon (Barson et al., 2015; Sinclair-Waters et al., 2020; Ayllon et al., 2015). Second, *vgll3* and *six6* may dictate sea age more significantly when resource availability closely approaches the biological threshold needed to start gametogenesis (Raunsgard et al., 2024; Maamela et al., 2025).

An alternative explanation for the variability between sea age and *vgll3*LL* could be a result of fishing pressure (Raunsgard et al., 2024; Miettinen et al., 2024). Even though fishing in Iceland is strictly recreational it is a highly sought after activity with significant economic value that may inadvertently impact MSW salmon at higher rates due to their earlier spawn time and favorable size (Raunsgard et al., 2024; Olafsson et al., 2016). An increase in pressure from angling on MSW individuals may lead to a natural selection towards *vgll3*LL* individuals with higher maturation plasticity (Raunsgard et al., 2024; Miettinen et al., 2024). However due to a majority of salmon rivers in Iceland enacting catch and release policies further work is required to understand the effects Iceland's recreational fishery may have on MSW salmon.

6.3 Physical Characteristics and Genotype Associations

The significant association found between *vgll3* genotypes and length revealed *vgll3*EE* individuals were, on average, 19.2 cm longer than *vgll3*LL* individuals. At first, this finding came as a surprise as later maturing individuals were expected to have an overall larger body size but when mean body length was compared to mean sea age, *vgll3*LL* individuals exhibited the youngest sea age on average (Bacon et al., 2009). These findings suggest *vgll3* is indirectly affecting growth rate through its impact of the salmon's duration in the marine environment rather than having a regulatory role on growth itself (Fraser et al., 2024; Debes et al., 2021; Mobley et al., 2024). Factors such as foregoing behavior, migrating paths, diet composition and size at outmigration should be compared between *vgll3*LL* and *vgll3*EE* individuals to provide further explanation to the observed result. Length was also found to be significantly associated with sea age. The results indicated 2SW salmon were more likely to be longer than 1SW individuals. This finding aligns with salmonid literature stating 2SW salmon are larger than 1SW individuals due to an extended period of foraging time in the marine environment (Mobley et al., 2020; Sinclair-Waters et al., 2020; Moustakas-Verho et al., 2020; Besnier et al., 2023).

When a linear regression model was performed to determine if genotype, sex, and river can predict length, the two loci revealed opposing results. At the *vgll3* locus, no significance was detected at genotype or sex until the model added sample site indicating a significant association between length and river. While not every river showed significance, the finding indicates ecological conditions have a stronger influence on body length than genotype or sex (Raunsgard et al., 2024; Maamela et al., 2025). In contrast, at the *six6* locus sex, genotype, and their combined effect were significant factors in predicting length while all rivers were insignificant determinants. The opposition in results, beautifully demonstrates the variability of the effect genotype and environment contribute to maturation timing presenting the complexity that is maturation timing displaying how all life factors simultaneously influence multiple biological systems at once within an individual.

Between both loci, a pattern was observed between sex and body length. Males were consistently shorter than females. The size difference observed between males and females is most likely attributed to the high proportion of sneakers within the dataset and the fact females invest more energy in reproduction efforts than males (Maamela et al., 2025). For instance, females need 50% more energy than males in gonad development alone (Maamela et al., 2025). Even though no significant associations were found between genotype and sex in this study, females are more often associated with being 2SW or MSW salmon due to the increased energetic cost of reproduction requiring higher lipid accumulation paired with the reproductive advantage linked to body size (Maamela et al., 2025; Barson et al., 2015).

However, when the population was pooled, there was a significant association between *vgll3*EE* and sneakers indicating a sex specific pattern in *vgll3's* effect on maturation (Barson et al., 2015; Bangura et al., 2024; Ciani et al., 2021). Congruent with these findings, previous studies established that the effect of *vgll3* on age at maturity strongly influences males over females (Maamela et al., 2025; Barson et al., 2015). In addition, males show higher rates of earlier maturation partly due to the fact, when one copy of the early allele is present it overrides *vgll3-L* resulting in early maturation dominance (Barson et al., 2015; Ciani et al., 2021; Maamela et al., 2025; Besnier et al., 2023). These previous findings paired with the significant association between sneakers and *vgll3*EE* can provide important foresight to populations with large proportions of precocious males.

The difference between the expression of *vgll3*EE* in sneakers compared to non-sneakers is an increase in gonadotropin levels (the mechanism regulating testicular function) a process modulated by *vgll3* (Lothian et al., 2024; Ciani et al., 2021; Ayllon et al., 2018). It is not yet known what triggers the increase in gonadotropin levels; however it may be related to water temperature and resource availability, the two major factors shaping maturation pathways in fresh water systems (Lothian et al., 2024; Ciani et al., 2021; Ayllon et al., 2018). In Iceland, higher percentage of grilse were significantly correlated with warmer water temperatures (Gudjonsson et al., 1995). This finding provides evidence that precious male development is strongly influenced by environmental factors if the associated genotype is present (Gudjonsson et al., 1995). Indicating if a fixed level of resources paired with a rise in river temperature, a highly feasible occurrence induced by climate change, there could be a greater influx of mature parr in a population that hosts a considerable number of *vgll3*E* males (Lothian et al., 2024; Korus et al., 2024). The potential for sneaker dominance is further supported by Fleming et al., (2007), who showed when the same female was crossed with sneakers, higher rates of precocious males were observed in the populations compared to when crossed with MSW males. Although this thesis did not detect a significant association between sex and genotype, there was a noticeable underrepresentation of *vgll3*LL* males compared to *EE* and *EL* individuals. This pattern suggests a larger sample size may uncover an association between sex and genotype that has previously been established in the *vgll3* genotype resulting from allele dominance (Barson et al., 2015; Besnier et al., 2023).

6.4 Population Structure

The distribution of genotypes among the subpopulations provides critical insight into the overall fitness and adaptive potential of a population (Olafsson et al., 2014; Besnier et al., 2024, Sinclair-Waters et al., 2022). The moderately low F_{ST} and G_{ST} values at *vgll3* and *six6* indicate genetic variation exists among populations but most remains within the population (Garnier-Géré & Chikhi, 2013). This demonstrates the importance of assessing populations at the local level for more effective conservation management (Jin & Chakraborty, 1995; Webb et al., 2007). The overall variation in genotype distribution within rivers indicates management will be most effective on the local level (Gudmundsson et al., 2013). At the *vgll3* locus, F_{IS} values demonstrated a general trend toward heterozygote deficiency, except in Haffjarðará, Laxá í Aðaldal, and Mýrarkvísl where an excess of heterozygotes was detected. Similarly, the *six6* locus showed a heterozygote deficit majority in all rivers except, Fífustaðadalsá, Haffjarðará, and Selárdalsá. The negative F_{IS} values indicating increased heterozygosity may reflect the presence of unsampled intra-river substructure however additional sampling is needed to assess whether these patterns reflect population structure or are residue of limited sample size (Jin & Chakraborty, 1995; Webb et al., 2007; Putnins & Androulakis., 2021; Ghalambor et al., 2007). The overall heterozygosity deficiency suggests populations have a reduced gene flow due to isolation or population reduction (Jin & Chakraborty, 1995). This is consistent with patterns observed throughout anadromous salmon populations where strong homing behavior tends to generate fine scale genetic differentiation between river systems of small populations (Miettinen et al., 2021; Mobley et al., 2021).

Given the obvious practical challenges of conducting a genetic analysis for all 80 salmon rivers in Iceland grouping genetically similar populations into management units can be an effective management strategy (Danielsdottir et al., 1997; Olafsson et al., 2014; Antonsson et al., 2010). Previous genome wide association studies have revealed the Icelandic stock is aggregated into two distinct genetic units divided between the northern and southern part of the country (Danielsdottir et al., 1997; Olafsson et al., 2014; Antonsson et al., 2010). Clusters of genetically similar units are often found among salmonid populations experiencing parallel evolution which is when environmental conditions are directly correlated to geographical proximity promoting parallel adaptation strategies between populations (Olafsson et al., 2014;

Antonsson et al., 2010). In this thesis there was not a strong indication of genetic similarity between northern and southern populations although the PCoA graph displayed marginal regional structuring between the western and southern parts of the country (Danielsdottir et al., 1997; Olafsson et al., 2014; Antonsson et al., 2010). It is possible the weak evidence of regional structuring observed is a consequence of limited sample size.

6.5 Farmed and Wild Salmon

In salmon aquaculture early maturation is viewed as a serious economic repercussion and strong initiatives are made to effectively eliminate early maturing salmon (Rivera et al., 2021). On top of the quest for early maturation eradication all known instances comparing the genetics of farmed salmon to wild populations in Iceland have shown two genetically distinct strains (Danielsdottir et al., 1997; Gudmundsson et al., 2013; Gudjonsson, 1991). When the two strain's *vgll3* genotypes lacked significance there was initial perplexment until finding the study conducted by Ayllon et al., (2019). Here, the Norwegian farm strain, Mowi, outpowered *vgll3's* effect in an environment with high calorie feed, artificial light, and controlled temperature regimes (Ayllon et al., 2019). Despite, substantial efforts to select against early maturation there was still high genetic variability at *vgll3* in the Mowi strain comparable to the wild population (Ayllon et al., 2019). Since 2022, the company, Mowi, manufacturer of Mowi strain salmon, own 51% of the shares in Arctic Fish (Arctic Fish, 2022). The exact strain being farmed in Arnafjordur is not explicitly stated from Arctic Fish, the association between the two companies compiled with the result from Ayllon et al., (2019) on top of the findings of this study provides an enlightening explanation for an initially puzzling result (Arctic Fish, 2022). The lack of genetic differentiation indicates environmental conditions are bypassing the effect of *vgll3* and therefore selection is not altering genotype frequencies (Ayllon et al., 2019). Previously established genetic factors that influence maturation timing such as sex are found to be lost in aquaculture strains as individuals are strictly targeted for maturation timing thus losing the natural biological variation that may occur in a natural population (Sinclair-Waters et al., 2020; Barson et al., 2015).

When farmed and wild types were compared between *six6* genotypes all farmed salmon exhibited a genetic predisposition to late maturation, at a frequency significantly different to the wild population. The differences in allele frequencies between farmed and wild salmon highlights one of the biggest risks farmed salmon impose on wild salmon populations, genetic introgression (Danielsdottir et al., 1997; Gudmundsson et al., 2013; Moreau et al., 2011). The hybridization between farmed and wild salmon can erode an entire adaptive history of a population or homogenize the population depending on the level of introgression experienced (Gudmundsson et al., 2013; Forseth et al., 2017). Due to the fact hybrid offspring have lower fitness overall, hybrid offspring fail to compensate for losses in wild recruitment levels (Gudmundsson et al., 2013). Additionally, escapees can transfer diseases and parasites to wild populations thus harming the wild population without breeding (Danielsdottir, et al., 1997; Forseth et al., 2017). In all scenarios, escapees reduce the overall fitness and adaptive potential of a population (Gudmundsson et al., 2013; Danielsdottir et al., 1997; Ayllon et al., 2019). The introgression of farmed and wild salmon should be avoided at all costs for the long term viability of wild populations (Danielsdottir et al., 1997; Gudmundsson et al., 2013; Forseth et al., 2017).

7 Limitations

The largest research constraint comes from the limited database of successfully genotyped samples of the qPCR analysis. Despite adhering to all protocol standards the optimal sample size was not produced (Kroll, 2024). The two constraints limiting further genomic analysis to obtain a larger sample set was time and materials. Access to the Molecular Genetic Laboratory at the University of Iceland was regulated by the need for temporary relocation and therefore genotyping analysis was constrained to a one month period. As no external funding was received for the project all materials associated with genomic analysis were covered by Dr. Snæbjörn Pálsson and used until capacity. In addition to low genotyping success variability in river sample sizes, such as Sogið, had low sample representation, with only ten total individuals. These factors combined, resulted in unevenly distributed data between sample sites and may not accurately reflect a population skewing results. While this study did not show as strong associations as previously reported there is variation in genes and connections to life history characteristics results are useful to understanding the maturation timing of populations in Iceland.

Though efforts were taken to reduce bias during sample collection a few biases still remain. There was no way to test for a sibling relationship among samples which could have an effect on the distribution of genotypes and F_{IS} within rivers (Rowe & Thorpe., 1990). A majority of the data being analyzed was collected in one season for each sampling site limiting the scope of the observed genotype variations. The rivers chosen for sampling were restricted to sites with consent from the landowners. This may introduce a selection bias towards rivers with more involved management plans concerning the overall health of a population and may not reflect the regional trend of surrounding populations. Future studies can reduce sampling bias by incorporating more sampling years and sites.

During analysis, the results were limited to references of comparable research outside of Iceland since no studies exist for the Icelandic stock. Frequently, research conducted between Iceland and Norway are used for comparison due to their similarities in climate and species however because genetic variability, environmental influences, and anthropogenic pressures varies greatly between the stocks the reliance in this case is not a reliable reference point

(Mobley et al., 2021; Saviolidis et al., 2020). On the flip side, this provides the exciting opportunity to establish a baseline of the genetic variation in maturation timing for Icelandic populations. The findings reported in this thesis are thus grounded in the observations and patterns found in the data and within existing literature. Due to the high plasticity of maturation timing there is still large discrepancy within the literature especially in regards to the molecular mechanisms and environmental variables effecting maturation making it increasingly challenging to distinguish the implications and reasoning behind some of the observed results (Aykanat et al., 2024). To this effect, when *vgll3* or *six6* is said to have an effect on maturation timing between a characteristic this claim is not accounting for the additional candidate genes linked to maturation timing (Barson et al., 2015; Sincalir-Waters et al., 2022). Given the limited sample size, the analyses was limited to investigating broad patterns of association to maturation timing in *vgll3* and *six6* due to the lack of statistical power to address more refined research questions. Overall, this thesis gives a robust description of *vgll3* and *six6* genotype frequencies of nine populations around Iceland and their associations to physical and life history traits in relation to maturation timing providing a strong baseline for future research to build upon.

8 Future Considerations

To further extend our understanding of the association between *vgll3* and *six6* genotypes and maturation timing in the Icelandic stock follow up studies are necessary to build on the uncertainties found in this thesis. The first recommendation is to replicate the study with the inclusion of more sampling sites, seasons, and size. This will provide a more robust dataset with enhanced statistical power. The effect of *vgll3* and *six6*'s genotypes should be furthered compared to sea age, sex, repeat spawners, and locality testing the variables with either marginal significance or with previous established associations within the literature (Barson et al., 2015; Sinclair-Waters et al., 2022; Aykanat et al., 2024; Gudmundsson et al., 2013; Persson et al., 2023; Aykanet et al.,2019).

During the analysis a number of potential studies revealed themselves that could help contextualize the effect genotype has on age at maturity. First, investigating if *vgll3*EE* and *vgll3*LL* individuals foraging behavior, diet, and migration pathways differ when in the marine environment. A second study could consider the molecular basis behind why farmed salmon in *six6* demonstrate dominance towards later maturation compared to an evenly distributed genotype in *vgll3*. Thirdly, length, sex, and sea age by river should be compared to genotype in order to investigate the prediction power of the current model used to assess sea age in recreational fisheries in Iceland. Lastly, a genomic analysis should be compared between the farmed strains of salmon and wild salmon genotypes to identify the extent of genetic divergence among the farms and between the wild populations.

On multiple occasions the results suggested environmental influences outpowered the genetic influence. It is vital to investigate the underlying environmental factors influencing age at maturation in both the fresh and marine environments (Gudjonsson et al., 1995; Aykanat et al., 2019). To do this, the sea age of populations within a long term dataset should be compared to environmental data to identify environmental factors influencing sea age (Gudjonsson et al., 1995; Aykanat, et al., 2019). Characteristics of rivers should also be compared to genotype frequencies between populations to understand the environmental factors influencing genotype. If significant environmental factors are established future work could use genomic data to model the empirical abundance of Atlantic salmon by predicting how a species maturation timing may respond to different environmental variables (Pardo & Hutchings, 2020). If long term studies were established to monitor and genotype populations this would provide a rare opportunity to observe the evolutionary response of Atlantic salmon. One final consideration, isolating a single environmental variable, that would be of particular interest is comparing the maturation timing of populations inhabiting the same river systems as the newly migrated pink salmon (*Oncorhynchus gorbuscha*) to test if the interspecies dynamic influences sea age (Þórðardóttir & Guðbergsson, 2022).

9 Management Application

This thesis demonstrates how a gene targeted approach can effectively inform conservation management (Onley et al., 2021). The genomic data found in this study can provide resource managers with the genetic variability of sea age maturity to their population of interest. The identification of genetic variation and associated characteristics influencing maturation timing are essential to maintaining the overall health of the population (Sinclair-Waters et al., 2022). Results from this thesis will be conveyed into management recommendations to demonstrate the application of using a gene targeted approach to conservation management.

- Rivers Laxá í Aðaldal, Sogið, Stóra Laxá, Haffjarðará and Mýrakvís host annual rod fisheries, while additional net fishing has been carried out in the glacial waters of the river system Ölfusá-Hvítá were rivers Sogið and Stóra Laxá are tributaries. In recent years, rod fisheries have shifted to landing a majority of the salmon from fly fishing and releasing all salmon in most of the rivers. In systems were not all fish are released it would be advised to change to a mandated catch and release policy.

  *Iceland has declared the need for conservation strategies to protect the declining MSW populations (Guðberrgsson, 2015). Salmon of older sea age are known to return to freshwater earlier in the migration season compared to 1SW salmon (*Maamela et al., 2025*). In order to reduce the stress on MSW individuals reduction in early season fishing would help protect these populations (Aykanat et al., 2019). Rivers Laxá í Aðaldal, Sogið and Stóra Laxá were selected since these rivers are dominant in the LL genotype between both loci. Reducing pressure would ensure protection of populations with a genetic predisposition towards late maturation.*

- Ensure all 2SW salmon and repeated spawners salmon are released if not all salmon and end all netting in Ölfusár-Hvítár river system in order to increase number of spawners in rivers Sogið and Stóra Laxá.

The Sogið population showed no genotypic variation at the six6 locus which could indicate a reduction in the populations adaptive potential (Mobley et al., 2021). It is vital to reduce the fatalities of these populations to help sustain or increase salmon abundance.

- Rivers should be monitored for sites of fragmentation and adequate spawning habitat that may be reducing gene flow to the population.

*The rivers positive F_{IS} values indicate there is a higher chance for inbreeding in the population which may be a result of reduction in gene flow (*Lennox et al., 2021; Garnier-Géré & Chikhi, 2013*). Rivers should be assessed for sites of fragmentation that may account for this reduction (*Lennox et al., 2021*). If fragmentation is found, further efforts should go to restoring connectivity (*Lennox et al., 2021*). A potential cause for a positive F_{IS} value reflects small population size therefore monitoring of adequate spawning habitat should be conducted and if necessary the implementation of spawning site rehabilitation in order to keep the population stable (*Lennox et al., 2021; Garnier-Géré & Chikhi, 2013*).*

- If populations become below replacement levels stocking of the river should be based on the gene bank method (Lennox et al., 2021) ensuring to use only individuals from the river being stocked to keep the local adaptations of populations.

The genotype frequencies reveled to vary considerably between rivers even those close in geographic proximity indicating populations are locally adapted to a particular river system (Pritchard et al., 2018; Gudmundsson et al., 2013).

- Rivers should be increasingly monitored for precocious male abundancies.

Populations with higher rates of mature parr are positively correlated with effective population size and increased genetic diversity (Perrier et al., 2014). Mortality rates are highest in the marine environment and populations hosting a significant portion of *sneakers acts as a freshwater 'sperm bank' that can compensate for fluctuations in anadromous males spawning migrations (Perrier et al., 2014). However, there is potential environmental factors may increase rates of male parr changing the rivers population structure (Ahi et al., 2022).*

- An intermediate strategy for rivers with genetic information unavailable to managers should base protocols on the river with suitable data closest in geographic proximity (Pritchard et al., 2018; Gudmundsson et al., 2013).

  Marginal regional associations were found particularly in the western and southern part of the country indicating populations are genetically similar within regions however remain largely distinct.

- All rivers should increase environmental monitoring efforts (Aykanat et al., 2019).

  Results indicated environmental factors are strongly influencing age at maturation and therefore increased efforts in environmental monitoring within rivers can aid in predicting a populations response to environmental change (Aykanat et al., 2019).

An additional finding in this thesis indicates all efforts should be made to eliminate the introgression of farmed salmon on wild populations. Aquaculture management is out of the scope for Atlantic salmon management regimes supporting initiatives that restrict introgression such as the sterility of farmed populations or land based farming operations would help eliminate the direct genetic impact on wild populations (Gudmundsson et al., 2013; Danielsdottir et al., 1997). Overall, Iceland is the model system for the integration of a genomics based approach to informing conservation management given that populations are already managed on the local level and can be integrated seamlessly (Gudmundsson et al., 2013). Tables 13 and 14 indicate how data collected in this project can be separated by river to help inform specific management strategies. Atlantic salmon managers In Iceland should employ a gene wide approach to conservation as it can identify fine scale characteristics of a population and research efforts can be localized to a single population making it well suited technique for local fisheries management (Lennox et al., 2021; Aykanat et al., 2019; Sturlaugsson & Pálsson, 2023; Sturlaugsson & Pálsson, 2024).

Table 13. A summary table showing frequency of genotype separated by sex, sea age percentage, and percentage of traits sneakers and repeat spawner at the vgll3 locus.

	Male Genotype (*n*)			Female Genotype (*n*)			Sea Age (%)			Trait (%)		Notes
River	EE	EL	LL	EE	EL	LL	0	1	2	Sneaker	Repeat Spawner	
Elliðaár	14	3	1	0	0	0	100%	0%	0%	100%		
Fífustaðadalsá	3	4	3	1	3	2	0%	75%	25%		39%	
Haffjarðará	6	12	1	0	0	0	0%	79%	21%			
Laxá í Aðaldal	0	3	6	0	0	0	100%	0%	0%			No sex data
Mýrarkvísl	2	1	0	0	0	0	0%	100%	0%			
Selárdalsá	2	5	5	1	4	2	0%	83%	17%		21%	
Sogið	2	3	0	3	0	1	0%	67%	33%			
Stóra Laxá	8	1	2	7	0	2	0%	50%	50%		5%	

Table 14. A summary table showing frequency of genotype separated by sex, sea age percentage, and percentage of traits sneakers and repeat spawner at the *six6* locus.

	Male Genotype (*n*)			Female Genotype (*n*)			Sea Age (%)			Trait (%)		Notes
River	EE	EL	LL	EE	EL	LL	0	1	2	Sneaker	Repeat Spawner	
Elliðaár	5	6	2	0	0	0	100%	0%	0%	100%		
Fífustaðadalsá	4	3	1	1	2	0	0%	73%	27%		15%	
Haffjarðará	10	9	0	0	0	0	0%	79%	21%			
Laxá í Aðaldal	1	2	4	0	0	0	100%	0%	0%		5%	No sex data
Mýrarkvísl	0	2	1	0	0	0	100%	0%	0%			
Selárdalsá	5	7	2	3	4	1	0%	81%	19%		9%	
Sogið	0	0	3	0	0	4	0%	57%	43%			
Stóra Laxá	0	1	7	2	1	3	0%	57%	43%		7%	

10 Conclusion

This thesis explored the associations between two major effect loci *vgll3* and *six6* with maturation timing in Atlantic salmon to gain a deeper understanding of the genetic diversity and phenotypic characteristics associated with age at maturity. Age at maturation is a critical life history trait with far reaching implications towards overall population fitness (Barson et al., 2015). The investigation in this thesis started by extracting DNA from fin clip samples that were than analyzed using qPCR analysis following the KASP genotyping protocol to detect genotypic differences at the *vgll3* and *six6* regions.

The findings indicate qPCR sequencing, a previously regarded technique in maturation studies can be used to detect genetic variation associated to maturation timing in Atlantic salmon (Aykanat, et al., 2019). Even though the success rate was lower when compared to similar studies, given its demonstrated reliability and accessibility the application of qPCR to assess *vgll3* and *six6* genotypes is still a recommended methodology (Aykanat et al., 2019). The SNP analysis reveled a majority of the population are genetically predisposed towards early maturation. Despite the lack of significance between genotype and sea age, this finding is reflective of the average sea age recorded for the study population. The lack of significance between sea age and genotype implies sea age is largely being regulated by environmental influences. Length was significantly different at *vgll3* genotypes a finding further reflected in the significant association between length, genotype and sample site. Sex and genotype displayed no significant association but males were smaller then females overall with *vgll3*EE* and *vgll3*LL* individuals differing significantly in length. To see what factors might influence length, genotype and sex and river were modeled. At *vgll3* river was the only significant factor and at *six6* sex and genotype were the only significant factors. This result displays the myriad of influences that must be accounted for when predicting maturation timing of Atlantic salmon. Between life history traits there was a significant association between *vgll3*EE* and sneakers and a marginal association between repeat spawners and *vgll3*LL*. Farmed and wild salmon diverged significantly at the *six6* locus confirming a genetic difference between the two strains. When the sample population was segregated by sampling site, allele frequencies varied within populations indicating an excess of homozygotes for a majority of the population. Surprisingly, only marginal regional relatedness were detected between sites. Taken together, these results are characteristic of distinct populations most effectively managed on a local level. Collectively, the results highlight the variability *vgll3* and *six6* influence age at maturation. This is perhaps one of the greatest challenges in studying maturation timing but the very reason it makes for a deeply fascinating subject.

It is indisputable characterizing the genetic underpinnings of maturation timing is critical to informing the differing management strategies required to ensure effective conservation of Atlantic salmon. The Arctic ecosystem is changing at an accelerated rate and the salmons maturation timing will adapt in response (Raunsgard et al., 2024; Pardo & Hutchings, et al., 2020). Establishing the genetic baseline associated to maturation timing is essential in order to outline future studies that can help predict how a population may respond to changing conditions (Mobley et al., 2021; Pardo & Hutchings, 2020). Applying a gene based approach to conservation management is a pathway to the continued conservation of wild salmon in Iceland. This thesis is just one example of how a gene based approach can inform management but its effectiveness can be transferred to all coastal and marine management protocols associated with diadromous species.

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Appendix

Appendix A

Háskólasetur Vestfjarða
University Centre of the Westfjords

Research ethics training and clearance

University Centre of the Westfjords
Suðurgata 12
400 Ísafjörður, Iceland
+354 450 3040
info@uw.is

This letter certifies that Sadie Ainsworth has completed the following training modules of:

(X) Basic ethics in research
(X) Human subjects research
(X) Animal subjects research

Furthermore, the Masters Program Committee has determined that the proposed masters research entitled "Using Genetics to Investigate the Maturation Timing of Atlantic Salmon (Salmo salar)." meets the ethics and research integrity standards of the University Centre of the Westfjords. Throughout the course of their research, the student has the continued responsibility to adhere to basic ethical principles for the responsible conduct of research and discipline-specific professional standards.

University Centre of the Westfjords ethics training certification and research ethics clearance is valid for one year past the date of issue unless otherwise noted.

Effective Date: 5 September 2024
Expiration Date: 15 September 2025

Reference number: 2024-169-40

Prior to making substantive changes to the scope of research, research tools, or methods, the student is required to contact the Masters Program Committee to determine whether or not additional review is required.

Catherine P. Chambers, Ph.D
Research Manager

Appendix B

Please note an asterisk (*) preceding a sample identification indicates that two differing genotype calls were obtained for that sample; in such cases, the genotype with the highest confidence score was retained for analysis. .

Sample	River	Gene	Genotype	Region	Stage	Sex	Length (cm)	Sea Age 1 = 1SW 2 = SW	Sampling year
MKV-3	Mýrarkvísl	VGLL3	EL	Northeast		Male	62.0	1.0	2024
MKV-4	Mýrarkvísl	VGLL3	EE	Northeast		Male	59.0	1.0	2024
MKV-6	Mýrarkvísl	VGLL3	EE	Northeast		Male	68.0	1.0	2024
LA1	Laxá í Aðaldal	VGLL3	LL	Northeast			8.5	0.0	2020
LA3	Laxá í Aðaldal	VGLL3	LL	Northeast			9.7	0.0	2020
LA6*	Laxá í Aðaldal	VGLL3	EL	Northeast			9.1	0.0	2020
LA9	Laxá í Aðaldal	VGLL3	LL	Northeast			7.8	0.0	2020
LA11	Laxá í Aðaldal	VGLL3	LL	Northeast			7.1	0.0	2020
LA13	Laxá í Aðaldal	VGLL3	LL	Northeast			10.5	0.0	2020
LA20	Laxá í Aðaldal	VGLL3	LL	Northeast			7.3	0.0	2020
LA25	Laxá í Aðaldal	VGLL3	EL	Northeast			8.8	0.0	2020
LA23	Laxá í Aðaldal	VGLL3	EL	Northeast			8.4	0.0	2020
E1-ST2	Elliðaár	VGLL3	EL	Southwest	Sneaker	Male		0.0	2024
E10-A2	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E12-A3	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E13-A3	Elliðaár	VGLL3	EL	Southwest	Sneaker	Male		0.0	2024
E14-A3	Elliðaár	VGLL3	EL	Southwest	Sneaker	Male		0.0	2024
E15-A3	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E16-A4	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E17-A4	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E2-ST2	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E3-ST5	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E4-ST5*	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E5-ST7	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E6-A1	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E7-A2	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E8-A2	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
E9-A2	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2024
EII-ST1-04	Elliðaár	VGLL3	LL	Southwest	Sneaker	Male		0.0	2019
EII-ST4-03	Elliðaár	VGLL3	EE	Southwest	Sneaker	Male		0.0	2019
SL11	Stóra Laxá	VGLL3	EE	South		Male	65.0	1.0	2024
SL13	Stóra Laxá	VGLL3	LL	South		Female	80.0	2.0	2024
SL17	Stóra Laxá	VGLL3	LL	South		Male	70.0	1.0	2024
SL19	Stóra Laxá	VGLL3	EE	South		Female	80.0	2.0	2024
SL21	Stóra Laxá	VGLL3	EE	South		Female	91.0	2.0	2024
SL23	Stóra Laxá	VGLL3	EE	South		Male	65.0	1.0	2024
SL24	Stóra Laxá	VGLL3	EE	South		Female	80.0	2.0	2024
SL29	Stóra Laxá	VGLL3	LL	South		Male	66.0	1.0	2024
SL31	Stóra Laxá	VGLL3	EE	South		Male	69.0	1.0	2024
SL32	Stóra Laxá	VGLL3	EE	South		Male	80.0	2.0	2024
SL33	Stóra Laxá	VGLL3	EE	South		Male	80.0	2.0	2024
SL37	Stóra Laxá	VGLL3	EE	South		Male	62.0	1.0	2024

SL40	Stóra Laxá	VGLL3	EE	South		Female	81.0	2.0	2024
SL42	Stóra Laxá	VGLL3	EE	South		Male	58.0	1.0	2024
SL43	Stóra Laxá	VGLL3	LL	South		Female	94.0	2.0	2024
SL10	Stóra Laxá	VGLL3	EE	South		Female	55.0	1.0	2024
SL34	Stóra Laxá	VGLL3	EE	South		Female	81.0	2.0	2024
SL39	Stóra Laxá	VGLL3	EE	South	Repeat Spawner	Female	87.0	2.0	2024
SL41	Stóra Laxá	VGLL3	EL	South		Male	70.0	1.0	2024
SL16	Stóra Laxá	VGLL3	EE	South		Male	68.0	1.0	2024
SO1	Sogið	VGLL3	EL	South		Male	67.0	1.0	2024
SO2	Sogið	VGLL3	EE	South		Male	62.0	1.0	2024
SO3	Sogið	VGLL3	EE	South		Male	87.0	2.0	2024
SO4	Sogið	VGLL3	LL	South		Female	66.0	1.0	2024
SO5*	Sogið	VGLL3	EE	South		Female	79.0	2.0	2024
SO6	Sogið	VGLL3	EE	South		Female	57.0	1.0	2024
SO8	Sogið	VGLL3	EL	South		Male	66.0	1.0	2024
SO9	Sogið	VGLL3	EL	South		Male	79.0	2.0	2024
SO10	Sogið	VGLL3	EE	South		Female	55.0	1.0	2024
1701	Selárdalsá	VGLL3	EL	Westfjords		Female			2024
1703	Selárdalsá	VGLL3	EE	Westfjords		Male	60.5	1.0	2024
1706	Selárdalsá	VGLL3	EE	Westfjords	Repeat Spawner	Male	63.0	1.0	2024
1707	Selárdalsá	VGLL3	LL	Westfjords	Repeat Spawner	Female	73.0	2.0	2024
1711*	Selárdalsá	VGLL3	EL	Westfjords		Female	57.0	1.0	2024
1712	Selárdalsá	VGLL3	EL	Westfjords		Male	67.5	1.0	2024
1716	Selárdalsá	VGLL3	EL	Westfjords		Female	70.0	2.0	2024
1719	Selárdalsá	VGLL3	EL	Westfjords		Male	58.0	1.0	2024
1721	Selárdalsá	VGLL3	EL	Westfjords	Repeat Spawner	Female	75.0	2.0	2024
1725*	Selárdalsá	VGLL3	LL	Westfjords		Female	52.0	1.0	2024
1726	Selárdalsá	VGLL3	EL	Westfjords		Male	65.0	1.0	2024
1732	Selárdalsá	VGLL3	LL	Westfjords		Male	62.5	1.0	2024
1734	Selárdalsá	VGLL3	LL	Westfjords		Male	61.5	1.0	2024
1735	Selárdalsá	VGLL3	LL	Westfjords		Male	64.5	1.0	2024
1739	Selárdalsá	VGLL3	LL	Westfjords	Repeat Spawner	Male	58.0	1.0	2024
1740	Selárdalsá	VGLL3	EE	Westfjords		Female	58.0	1.0	2024
1741	Selárdalsá	VGLL3	LL	Westfjords		Male	58.5	1.0	2024
1748	Selárdalsá	VGLL3	EL	Westfjords		Male	68.0	1.0	2024
1749	Selárdalsá	VGLL3	EL	Westfjords		Male	63.0	1.0	2024
1151	Fífustaðadalsá	VGLL3	EL	Westfjords		Male	77.0	2.0	2024
1152	Fífustaðadalsá	VGLL3	EE	Westfjords		Male	54.5	1.0	2024
1155	Fífustaðadalsá	VGLL3	EE	Westfjords	Repeat Spawner	Female	69.0	2.0	2024
1156	Fífustaðadalsá	VGLL3	EL	Westfjords	Repeat Spawner	Female	67.0	1.0	2024
1157	Fífustaðadalsá	VGLL3	EL	Westfjords		Male	64.0	1.0	2024
1159	Fífustaðadalsá	VGLL3	LL	Westfjords		Male	60.0	1.0	2024
1161	Fífustaðadalsá	VGLL3	LL	Westfjords		Male	62.0	1.0	2024
1163	Fífustaðadalsá	VGLL3	EL	Westfjords		Male	61.5	1.0	2024
1168	Fífustaðadalsá	VGLL3	LL	Westfjords		Male	59.0	1.0	2024
1169	Fífustaðadalsá	VGLL3	EE	Westfjords		Male	54.0	1.0	2024
1172	Fífustaðadalsá	VGLL3	EL	Westfjords		Male	61.0	1.0	2024

1176	Fífustaðadalsá	VGLL3	EE	Westfjords		Male	66.0	1.0	2024
1178*	Fífustaðadalsá	VGLL3	LL	Westfjords		Female	55.0	1.0	2024
108750*	Fífustaðadalsá	VGLL3	LL	Westfjords	Repeat Spawner	Female	55.0	1.0	2017
109755	Fífustaðadalsá	VGLL3	EL	Westfjords	Repeat Spawner	Female	82.0	2.0	2017
FST-1258	Fífustaðadalsá	VGLL3	EL	Westfjords	Repeat Spawner				2023
FST-1259	Fífustaðadalsá	VGLL3	EE	Westfjords	Repeat Spawner				2023
FST-1274	Fífustaðadalsá	VGLL3	EL	Westfjords	Repeat Spawner	Female	75.5	2.0	2023
Slm-1132	Haffjarðará	VGLL3	EL	West		Male	74.5	2.0	2023
Slm-1135	Haffjarðará	VGLL3	LL	West		Male	51.0	1.0	2023
Slm-1136	Haffjarðará	VGLL3	EE	West		Male	63.0	1.0	2023
Slm-1137	Haffjarðará	VGLL3	EE	West		Male	67.5	1.0	2023
Slm-1141	Haffjarðará	VGLL3	EE	West		Male	82.5	2.0	2023
Slm-1142	Haffjarðará	VGLL3	EL	West		Male	60.0	1.0	2023
Slm-1143	Haffjarðará	VGLL3	EL	West		Male	63.0	1.0	2023
Slm-1147	Haffjarðará	VGLL3	EE	West		Male	87.0	2.0	2023
H1	Haffjarðará	VGLL3	EL	West		Male	72.0	1.0	2024
H2	Haffjarðará	VGLL3	EL	West		Male	65.0	1.0	2024
H3	Haffjarðará	VGLL3	EL	West		Male	60.0	1.0	2024
H5	Haffjarðará	VGLL3	EL	West		Male	59.0	1.0	2024
H6	Haffjarðará	VGLL3	EL	West		Male	65.0	1.0	2024
H8	Haffjarðará	VGLL3	EL	West		Male	57.0	1.0	2024
H9	Haffjarðará	VGLL3	EL	West		Male	59.0	1.0	2024
H10	Haffjarðará	VGLL3	EL	West		Male	80.0	2.0	2024
H11	Haffjarðará	VGLL3	EE	West		Male	59.0	1.0	2024
H12	Haffjarðará	VGLL3	EL	West		Male	55.0	1.0	2024
H13	Haffjarðará	VGLL3	EE	West		Male	70.0	1.0	2024
E10A2	Elliðaár	SIX6	EE	Southwest	Sneaker	Male		0.0	2024
E12A3	Elliðaár	SIX6	EE	Southwest	Sneaker	Male		0.0	2024
E14A3	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2024
E15A3	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2024
E16A4	Elliðaár	SIX6	LL	Southwest	Sneaker	Male		0.0	2024
E1ST2	Elliðaár	SIX6	EE	Southwest	Sneaker	Male		0.0	2024
E3ST5	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2024
E5ST7	Elliðaár	SIX6	EE	Southwest	Sneaker	Male		0.0	2024
E6A1	Elliðaár	SIX6	EE	Southwest	Sneaker	Male		0.0	2024
E7A2	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2024
EIIA3	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2019
EII ST104	Elliðaár	SIX6	LL	Southwest	Sneaker	Male		0.0	2019
EII ST403	Elliðaár	SIX6	EL	Southwest	Sneaker	Male		0.0	2019

1151	Fífustaðadalsá	SIX6	EL	Westfjords		Male	77.0	2.0	2024
1155	Fífustaðadalsá	SIX6	EE	Westfjords		Female	69.0	2.0	2024
1156	Fífustaðadalsá	SIX6	EL	Westfjords		Female	67.0	1.0	2024
1157	Fífustaðadalsá	SIX6	LL	Westfjords		Male	64.0	1.0	2024
1159	Fífustaðadalsá	SIX6	EE	Westfjords		Male	60.0	1.0	2024
1166	Fífustaðadalsá	SIX6	EL	Westfjords		Male	63.0	1.0	2024
1168	Fífustaðadalsá	SIX6	EE	Westfjords		Male	59.0	1.0	2024
1169	Fífustaðadalsá	SIX6	EE	Westfjords		Male	54.0	1.0	2024
1172	Fífustaðadalsá	SIX6	EL	Westfjords		Male	61.0	1.0	2024
1178	Fífustaðadalsá	SIX6	EL	Westfjords					2024
1161	Fífustaðadalsá	SIX6	EE	Westfjords		Male	65.0	1.0	2024
109755	Fífustaðadalsá	SIX6	EL	Westfjords	Repeat Spawner	Female	82.0	2.0	2017
FST-1258	Fífustaðadalsá	SIX6	EE	Westfjords	Repeat Spawner				2023
H1	Haffjarðará	SIX6	EL	West		Male	72.0	1.0	2024
H10	Haffjarðará	SIX6	EL	West		Male	80.0	2.0	2024
H11	Haffjarðará	SIX6	EE	West		Male	59.0	1.0	2024
H12	Haffjarðará	SIX6	EE	West		Male	55.0	1.0	2024
H13	Haffjarðará	SIX6	EE	West		Male	70.0	1.0	2024
H2	Haffjarðará	SIX6	EL	West		Male	65.0	1.0	2024
H3	Haffjarðará	SIX6	EE	West		Male	60.0	1.0	2024
H5	Haffjarðará	SIX6	EL	West		Male	59.0	1.0	2024
H6	Haffjarðará	SIX6	EE	West		Male	65.0	1.0	2024
H7	Haffjarðará	SIX6	EE	West	Repeat Spawner	Male	72.0	1.0	2024
H8	Haffjarðará	SIX6	EL	West		Male	57.0	1.0	2024
H9	Haffjarðará	SIX6	EE	West		Male	59.0	1.0	2024
SLM-1132*	Haffjarðará	SIX6	EE	West		Male	74.5	2.0	2023
SLM-1135	Haffjarðará	SIX6	EE	West		Male	51.0	1.0	2023
SLM-1136	Haffjarðará	SIX6	EE	West		Male	63.0	1.0	2023
SLM-1141	Haffjarðará	SIX6	EL	West		Male	82.5	2.0	2023
SLM-1142	Haffjarðará	SIX6	EL	West		Male	60.0	1.0	2023
SLM-1143	Haffjarðará	SIX6	EL	West		Male	87.0	2.0	2023
SLM1147	Haffjarðará	SIX6	EL	West		Male	87.0	2.0	2023
LA11	Laxá í Aðaldal	SIX6	LL	Northeast			7.1	0.0	2020
LA13	Laxá í Aðaldal	SIX6	EL	Northeast			10.5	0.0	2020
LA17	Laxá í Aðaldal	SIX6	LL	Northeast			10.0	0.0	2020
LA25	Laxá í Aðaldal	SIX6	EE	Northeast			8.8	0.0	2020

LA3	Laxá í Aðaldal	SIX6	LL	Northeast			9.7	0.0	2020
LA6	Laxá í Aðaldal	SIX6	LL	Northeast			9.1	0.0	2020
LA9	Laxá í Aðaldal	SIX6	EL	Northeast			7.8	0.0	2020
M1	Miðfjarðará	SIX6	EL	North	Sneaker	Male		0.0	2024
M12	Miðfjarðará	SIX6	EE	North				0.0	2024
M16	Miðfjarðará	SIX6	EL	North	Sneaker	Male		0.0	2024
M17	Miðfjarðará	SIX6	LL	North	Sneaker	Male		0.0	2024
M19	Miðfjarðará	SIX6	EE	North				0.0	2024
M7	Miðfjarðará	SIX6	EE	North				0.0	2024
MKV5	Miðfjarðará	SIX6	LL	North				0.0	2024
MKV6*	Miðfjarðará	SIX6	EE	North				0.0	2024
1711	Selárdalsá	SIX6	EL	Westfjords		Female	57.0	1.0	2024
1712	Selárdalsá	SIX6	EE	Westfjords		Male	67.5	1.0	2024
1716	Selárdalsá	SIX6	EE	Westfjords		Female	70.0	2.0	2024
1719	Selárdalsá	SIX6	EE	Westfjords		Male	58.0	1.0	2024
1721	Selárdalsá	SIX6	EL	Westfjords		Female	75.0	2.0	2024
1725	Selárdalsá	SIX6	EL	Westfjords		Female	52.0	1.0	2024
1726	Selárdalsá	SIX6	LL	Westfjords		Male	65.0	1.0	2024
1732	Selárdalsá	SIX6	EL	Westfjords		Male	62.5	1.0	2024
1735	Selárdalsá	SIX6	LL	Westfjords		Male	64.5	1.0	2024
1734	Selárdalsá	SIX6	EL	Westfjords		Male	61.5	1.0	2024
1739	Selárdalsá	SIX6	EL	Westfjords	Repeat Spawner	Male	58.0	1.0	2024
1740	Selárdalsá	SIX6	EE	Westfjords		Female	58.0	1.0	2024
1741	Selárdalsá	SIX6	EE	Westfjords		Male	58.5	1.0	2024
1748	Selárdalsá	SIX6	EL	Westfjords		Male	68.0	1.0	2024
1749	Selárdalsá	SIX6	EL	Westfjords		Male	63.0	1.0	2024

1701	Selárdalsá	SIX6	EL	Westfjords		Female			2024
1702	Selárdalsá	SIX6	EL	Westfjords		Male	61.0	1.0	2024
1703	Selárdalsá	SIX6	EE	Westfjords		Male	60.5	1.0	2024
1704	Selárdalsá	SIX6	EL	Westfjords		Male	61.0	1.0	2024
1706	Selárdalsá	SIX6	EE	Westfjords	Repeat Spawner	Male	63.0	1.0	2024
1707	Selárdalsá	SIX6	LL	Westfjords		Female	73.0	2.0	2024
1710	Selárdalsá	SIX6	EE	Westfjords		Female	71.0	2.0	2024
SO1	Sogið	SIX6	LL	South		Male	67.0	1.0	2024
SO10	Sogið	SIX6	LL	South		Female	55.0	1.0	2024
SO3	Sogið	SIX6	LL	South		Male	87.0	2.0	2024
SO4	Sogið	SIX6	LL	South		Female	66.0	1.0	2024
SO5	Sogið	SIX6	LL	South		Female	79.0	2.0	2024
SO6	Sogið	SIX6	LL	South		Female	57.0	1.0	2024
SO9	Sogið	SIX6	LL	South		Male	79.0	2.0	2024
SL10	Stóra Laxá	SIX6	LL	South		Female	55.0	1.0	2024
SL11	Stóra Laxá	SIX6	LL	South		Male	65.0	1.0	2024
SL12	Stóra Laxá	SIX6	LL	South		Male	85.0	2.0	2024
SL13	Stóra Laxá	SIX6	LL	South		Female	80.0	2.0	2024
SL14	Stóra Laxá*	SIX6	EL	South	Repeat Spawner	Female	79.0	2.0	2024
SL15	Stóra Laxá	SIX6	LL	South		Male	62.0	1.0	2024
SL23	Stóra Laxá	SIX6	LL	South		Male	73.0	1.0	2024
SL29	Stóra Laxá	SIX6	LL	South		Male	66.0	1.0	2024
SL31	Stóra Laxá	SIX6	LL	South		Male	69.0	1.0	2024
SL37	Stóra Laxá	SIX6	LL	South		Male	62.0	1.0	2024
SL40	Stóra Laxá	SIX6	LL	South		Female	81.0	2.0	2024

SL41	Stóra Laxá	SIX6	EL	South		Male	70.0	1.0	2024
SL43	Stóra Laxá	SIX6	EE	South		Female	94.0	2.0	2024
SL48*	Stóra Laxá	SIX6	EE	South		Female	95.0	2.0	2024

Sample Id	River	Gene	Genotype	Region	Stage	Sex	Length (cm)	Sea Age	Sampling Year
FST-F6	Fífustaðadalsá	VGLL3	LL	Westfjords	Farmed	Male	80.5	2	2023
FST-F19	Fífustaðadalsá	VGLL3	EL	Westfjords	Farmed	Male	78.0	2	2023
FST-F3	Fífustaðadalsá	VGLL3	EE	Westfjords	Farmed	Male	80.5	2	2023
FST-F1	Fífustaðadalsá	VGLL3	EL	Westfjords	Farmed	Female	79.0	2	2023
FST-F18	Fífustaðadalsá	VGLL3	EE	Westfjords	Farmed	Female	75.0	2	2023
FSTF12	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	80.5	2	2023
FSTF14	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	80.5	2	2023
FSTF19	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	78.0	2	2023
FSTF20	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	82.5	2	2023
FSTF3	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	80.5	2	2023
FSTF6	Fífustaðadalsá	SIX6	LL	Westfjords	Farmed	Male	80.5	2	2023

Appendix C

Assay name: *Vgll3-22*

GAACAGCTCAAATAAGCTCAAATAAGCAAAGAAAAACAACAGTCCATCATTGATTTAAGACATGA
AGGTCAGTAAATACGGAACATTTCAAGAACTTAGTACAGTGACGCAAAAACCAAGCGCTGTGATG
AAACTGGGTCATGAGGGTCATGAAACTGGG[C/T]CATGAGGGTCGCCACAAGAATTGAAGACCCAG
AGTTACCTCTGCTGCCGAGGATAAATTCATTAAAGTTAACCGCACCTTAGATTGCAAGCCAAATAA
ATGCTTCTCAGAGTTCAGGTAACGGACACATCTCAATATCACCTGTTCA

Assay name: *six6*

GAACAGCTCAAATAAGCTCAAATAAGCAAAGAAAAACAACAGTCCATCATTGATTTAAGACATGA
AGGTCAGTAAATACGGAACATTTCAAGAACTTAGTACAGTGACGCAAAAACCAAGCGCTGTGATG
AAACTGGGTCATGAGGGTCATGAAACTGGG[C/T]CATGAGGGTCGCCACAAGAATTGAAGACCCAG
AGTTACCTCTGCTGCCGAGGATAAATTCATTAAAGTTAACCGCACCTTAGATTGCAAGCCAAATAA
ATGCTTCTCAGAGTTCAGGTAACGGACACATCTCAATATCACCTGTTCA