Doctoral Programme in Wildlife Biology
Organismal and Evolutionary Biology Research Program
Faculty of Biological and Environmental Science
University of Helsinki

FACTORS AFFECTING LIPID PROFILES IN JUVENILE ATLANTIC SALMON

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DOCTORAL DISSERTATION
To be presented for public examination with the permission of the Faculty of
Biological and Environmental Sciences of the University of Helsinki
in lecture room 1041, Biocentre 2, on April 28, at 12:15.

Helsinki 2023

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ISBN 978-951-51-9162-5 (paperback)
ISBN 978-951-51-9163-2 (PDF)

Unigrafia, Helsinki 2023

ABSTRACT

Understanding genotype-phenotype links are important in determining underlying mechanisms involved in the life history of organisms. Gaining insight in early life history strategies and physiological processes enabling optimal progressions between important processes like maturation can help elucidate responses to the environment and how organisms allocate energy. In this thesis, we reared Atlantic salmon during early stages to understand energy allocation strategies and possible *vgll3* genotype effects on its two alleles, E and L associated with early and late maturation, respectively. First, we reared Atlantic salmon with two lipid level diets and looked at muscle lipid profiles between males and females through quantifying important lipid classes. Lipid concentration differences were detected with males having a higher lipid class concentration of TG, C, PC and SM than females showing a sex-specific lipid strategy potentially helping males reach maturation at an early stage. Furthermore, we reared Atlantic salmon for two years until the occurrence of maturation in males under varying temperature treatments to look for links between *vgll3* genotypes, temperature treatment and energy status. We found a seasonal *vgll3* genotype effect on the direction of change of condition factor. The early maturation allele individuals had a more stable body condition over the length of the experiment compared to late maturation allele individuals showing greater seasonal changes. This could be one potential factor allowing for individuals with the early maturation allele to reach maturation during early stages. Lastly, lipidomics methods were used to obtain lipid profiles from a subset of reared individuals from liver and muscle to ascertain physiological status in immature males and females and mature males from a common garden experiment. We found a *vgll3* effect on membrane lipid concentrations, PC and PE, in the liver with early maturation allele individuals showing an increase from spring to autumn while late maturation allele individuals showed a decrease. This *vgll3* effect could be explained through two scenarios 1) *vgll3* specific effect on endoplasmic reticulum volume and 2) *vgll3* specific effect on lipid droplet storage in the liver. Together, these results show sex and *vgll3* effects on early life history lipid strategies. Furthermore, this hints at the mechanisms of early life stages implanting strategies to reach major life history stages and the role of *vgll3* in lipid metabolism in juvenile Atlantic salmon and age-at-maturity.

TIIVISTELMÄ

Organismien genotyypin ja fenotyypin välisten yhteyksien selvittäminen on tärkeässä asemassa määritettäessä eliöiden elinkiertoon liittyviä mekanismeja. Tieto varhaisista elinkiertostrategioista ja fysiologisista prosesseista, jotka mahdollistavat optimaalisen kehityksen tärkeiden prosessien, kuten sukukypsyysiän määräytymisen, taustalla auttaa selventämään eliöiden reak-tioita niiden ympäristöön sekä eliöiden energia-allokointitapoja. Tässä väitöskirjatutkimuksessa kasvatimme Atlantin lohia niiden elämän alku–vaiheessa tutkiaksemme energiaresurssien allokointistrategioita ja *vgll3*-genotyypin varhaiseen (E-alleeli) ja myöhäiseen (L-alleeli) sukukypsyysikään liitettyjen alleelien vaikutuksia tähän allokointiin. Ensiksi kasvatimme lohia kahdessa eri ravintokäsittelyssä, jotka erosivat toisistaan rasvapitoisuudel–taan ja mitta-simme koiras- ja naaraslohien lihasten lipidiprofiilit määrittämällä tärkeim-mät lipidiluokat. Koiraiden ja naaraiden lihaksen lipidipitoisuudessa havait-tiin eroja: koiraiden lihaksissa oli korkeampi TC-, C-, PC- ja SM-pitoisuus verrattuna naaraisiin. Tämä sukupuolisidonnainen lipidistrategia saattaa osaltaan mahdollistaa koiraiden varhaisemman sukukypsyysiän. Lisäksi kasvatimme lohia kahdessa eri lämpötilassa kahden vuoden ajan, kunnes osa koiraslohista saavutti sukukypsyyden tarkastel–laksemme *vgll3*-genotyypin, lämpötilan ja energiastatuksen välisiä vuorov–aikutuksia. Tutkimuksessa ha-vaitsimme vuodenajasta riippuvaisen *vgll3*-genotyypin vaikutuksen kuntokertoimen muutossuunnassa. Varhaiseen sukukypsyysikään liitetyn alleelin omaavien yksilöiden kuntokerroin oli vakaampi tutkimuksen aikana verrat-tuna myöhäisempään sukuky–psyysikään liitetyn alleelin yksilöihin, joiden kuntokertoimessa havaittiin enemmän vaihtelua vuodenaikojen mukaan. Tämä voisi olla yksi selittävä mekanismi, miksi varhaisen sukukypsyysiän alleelin yksilöt saavuttavat sukukypsyyden aikaisemmin. Lopuksi käytimme lipidomiikkaanalyysi–metodeja mitataksemme maksan ja lihaksen lipidiprofiilit osalta kontrolloiduissa laboratorio-olosuhteissa kasvaneilta tutkimuslohilta, jotta pystyimme selvittämään ei vielä sukukypsien koiraiden ja naaraiden sekä sukukypsien koiraiden fysiologisen tilan. *Vgll3*-genotyyppin havaittiin vaikuttavan maksan PC- ja PE-kalvolipidien pitoisuuksiin. Yksilöillä, joilla oli varhaisempaan sukukypsyysikään liitetty alleeli, näiden kalvolipidien pito–isuus kasvoi siirryttäessä keväästä syksyyn, kun taas myöhäisen suku–kypsyysiän alleelin yksilöillä pitoisuudet laskivat. Tämä *vgll3*-genotyypin vaikutus voi selittyä kahden eri mekanismin kautta: 1) *vgll3*-genotyyppistä riippuvainen vaikutus solulimakalvoston tilavuuteen ja 2) *vgll3*-genotyypistä riippuvainen vaikutus maksan lipidipisaravarastoihin. Kokonaisuudessaan

tutkimuksen tulokset osoittavat sukupuolella ja *vgll3*-genotyypillä olevan vaikutuksia lohen elämän alkuvaiheiden lipidistrate-gioihin. Lisäksi tulokset vihjaavat lohen elämän alkuvaiheissa vaikuttavien mekanismien vaikuttavan merkittäviin elinkiertovaiheisiin myöhemmin elämässä sekä *vgll3*-genotyypin vaikuttavan lohenpoikasten lipidiaineen-vaihduntaan ja sukukypsyysikään.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I House, A. H., Debes, P. V., Kurko, J., Erkinaro, J., Käkelä, R., & Primmer, C. R. Sex-specific lipid profiles in the muscle of Atlantic salmon juveniles. Comparative Biochemistry and Physiology - Part D : Genomics and Proteomics. (2021)

II House, A. H., A. H., Debes, P. V., Kurko, J., Erkinaro, J., & Primmer, C. R. Genotype-specific variation in seasonal body condition at a large-effect maturation locus. BioRxiv. (2023)

III House, A. H., Debes, P. V., Ruhanen, H., Holopainen, M., Käkelä, R., Donner, I., Frapin, M., Pashay, E., Kurko, J., & Primmer, C. R. Seasonal and genetic effects on lipid profiles of juvenile Atlantic salmon. BioRxiv. (2023).

The publications are referred to in the text as Chapter 1, Chapter 2 and Chapter 3, respectively.

AUTHOR CONTRIBUTIONS

I PVD, CRP conceived the original idea. PVD, CRP, and **AHH** designed the salmon rearing components of the study. JE gave access to salmon material. **AHH** and PVD carried out the fish rearing and sample collection. **AHH**, JK, CRP and RK planned, and **AHH** conducted, the lipid laboratory work. PVD and **AHH** developed the data analysis methods and performed analyses. **AHH**, PVD, RK and CRP wrote the manuscript, with comments from other authors.

II CRP, PVD **AHH** and JK conceived the original idea. **AHH**, PVD, CRP, and JK designed the study. JE gave access to material. PVD and CRP planned the fish crosses. **AHH** and JK collected the data. PVD and **AHH** developed methods for data analysis. PVD performed the data analyses. **AHH**, CRP and PVD wrote the manuscript, with comments from other authors.

III CRP, PVD **AHH** and JK conceived the original idea. **AHH**, CRP, PVD and JK designed the study design. PVD and CRP planned the fish crosses, and **AHH** and JK coordinated collection of tissue samples and phenotypic data. **AHH**, HR, MH, RK and CRP planned lipid laboratory work and analysis. EP and CRP designed, and EP conducted, the qPCR assay and **AHH**, ID and MF conducted the lipid extractions. HR optimized LC-MS methods and **AHH** conducted LC-MS laboratory work. **AHH** developed and performed data analysis. **AHH** wrote the manuscript with input from CRP, RK, PVD, MH and HR.

ABBREVIATIONS

HPTLC	high performance thin-layer chromatography
LC-MS	liquid chromatography- mass spectrometry
TG	triacylglycerol
PC	phosphatidylcholine
PE	phosphatidylethanolamine
SM	sphingomyelin
CE	cholesterol ester
C	cholesterol
PI	phophatidylinositol
PS	phosphatidylserine
SNP	single nucleotide polymorphism
CF	condition factor
VFI	visceral fat index
MCMC	Markov chain Monte Carlo
REML	residual maximum likelihood

1 INTRODUCTION

Translating genotypes into phenotypes is a fundamental goal in understanding evolutionary outcomes. Developmental and physiological processes are key to understanding genotype functionality and its contribution in the evolution-ecology discipline. Organisms must adequately use energy and resources throughout their entire life history, from birth to death, while navigating changing environments and resource availability to sustain growth, survival and maturation processes (Post & Parkinson, 2001). Age at maturity can be highly variable due to ecological and genetic factors and their interactions within and among species and populations (Reznick et al. 2006). Understanding genetic and environmental influences on phenotypic responses and their change during life-history phases can give a deeper understanding into the underlying mechanisms involved in regulating physiological processes. This thesis gives insight in understanding key topics such as resource allocation strategies used in early development, energy production mechanisms involved in major early life-history stage progressions, environmental effects on lipid phenotypes, and lastly, genotype-phenotype links with lipid resources in indivdiduals. By combining genetic factors with environmental influences, there is a possibility to ascertain interactions and mechanistic understanding contributing to early life-history success.

2 LITERATURE REVIEW

2.1 ENERGY REQUIRMENTS OF FISH

Energy is required for organisms to undergo developmental changes, life-history processes and ultimately survival. Energy, affected by biotic and abiotic factors, is passed down through trophic levels in the environment. Organisms manage energy stores coming from dietary sources or *de novo* synthesis to achieve reproductive success. High-energy demands of mobility and somatic growth can limit physiological and morphological development at the earliest life-history stages (Wieser, 1991; Post & Parkinson, 2001). Trade-offs exist between somatic growth and energy storage, as the production of energy for storage is more physiologically costly than for development of structural components creating a limitation for other energy dependent processes when needing to allocate energy for storage (Brett and Groves, 1979; Mogensen and Post, 2001). However, organisms will allocate surplus energy to maximize survival, reproduction and fitness when resources are limited and energetic costs are conflicting (Mcnamara and Houston, 2008; Varpe et al, 2009). These conflicting demands pose a constraint on an organism's ability to acquire, expend, and store energy from early development to survival to age at maturity and for reproduction.

2.1.1 LIFE-HISTORY TRADE-OFFS AND THE ENVIRONMENT

Resource allocation strategies are the ways in which organisms prioritize and distribute energy. These strategies play a critical role in determining growth, reproduction, and survival throughout one's life history (Manor et al., 2014; Tocher, 2003). Trade-offs in fishes are particularly strong when the two main causes of mortality in juveniles are predation and starvation (Post and Parkinson 2001; Post et al. 1999; Biro et al. 2004). Lipid energy reserves are the main energy source utilized during major physiological and life-history progressions such as sexual maturation (Jonsson Jonsson 2005; reviewed in Wang, Hung, and Randall 2006). Sexual maturation is a dominant physiological process that causes a shift from somatic growth to gonadal growth (Taranger et al., 2010) with visceral fat mobilized first to supply energy for gonadogenesis (Jonsson et al., 1997; Manor et al, 2014; Nassour and Leger, 1989). During times of limited resource availability, mortality risk declines in organisms as lipid reserves increase allowing the individual to meet metabolic requirments. (Mogensen and Post, 2012). Species have a wide range of lipid storage patterns giving rise to a variation in life-history decisions varying within the same species (Kadri et al., 1996; Simpson et al., 1992; Jonsson and Jonsson, 2005). Fish mostly store resources in the muscle myosepta and in the visceral cavity along the intestine as adipose tissue, however, some fish can store lipids in the liver, the organ controlling lipid synthesis and metabolism (Henerson & Tocher, 1987; Jensen-Urstad & Semenkovich., 2012; Morgan et al., 2002; Sheridan, 1988; Sissner et al., 2017; Yeo & Parrish, 2022). Many factors can affect lipid storage and allocation strategies including body size (Simpson, 1992; Sogard & Spencer, 2004; Post and Parkinson 2001), temperature (Morgan et al., 2002; Cunjak, 1988; Metcalfe & Thorpe, 1992), food availability and quality (reviewed in Wang, Hung, and Randall 2006), and life history strategy (Mangel & Satterthwaite, 2008; Morgan et al., 2000; Rinke et al., 2008).

In fish, the physiological processes involved in using and storing energy throughout the body are mainly affected by diet and temperature (Geissinger et al. 2021; Mogensen and Post 2012; Post and Parkinson 2001). For example, fluctuating environmental conditions affect the quantity and quality of the food available for individuals living in seasonal environments, and this variability in the dietary supply of different nutrients influences how energy is allocated within their body (Post & Parkinson, 2001). Individuals and populations inhabiting seasonal environments must solve several problems to survive to reproduction. These organisms must cope with fluctuations in food supply and the often-substantial risk of predation associated with foraging, yet still accumulate sufficient energy stores for reproduction. A key component of energy allocation in pre-reproductive organisms is allocation to energy storage during periods of energy deficit such as metamorphosis (Scott et al. 2007) or low temperatures (Biro et al. 2004; Gurney et al., 2003; Mogensen and Post 2012) which requires pre-allocated lipid stores for survival. Therefore, maintaining optimal allocation of energy between activity, growth and storage in variable environments are crucial for reaching key life stages. Survival to reproduction is one of the primary determinants of success. Moreover, resource allocation patterns affect the probability of survival to reproductive age as well as the probability of maturation at a given age (Post and Parkinson 2001).

2.1.2 MEASURING ENERGETIC STATUS

Measuring energy levels can give an indication of an individual’s physiological status and an estimate of its fitness. Morphometric indices can be used to make these assumptions about energy levels in an organism. The most common is Fulton's condition factor defined as the relative weight of an individual given its length that gives an estimate of the relative lipid stores in an individual. CF has been applied in a range of different species including amphibians (Cogălniceanu et al., 2021), mammals (Bright Ross et al., 2021), birds (Balbontín et al., 2012), and fishes (Haraldstad et al., 2018; Mozsár et al., 2015; Sutton et al., 2000) as proxy for the level of lipid reserves. There have been studies finding a strong positive correlation between CF and total lipid content (Herbinger and Friars 1991; Schulte-Hostede et al., 2005; Sutton et al., 2000), however, this relationship seems to be highly variable among different fish species (Mozsar et al, 2015). Lipid reserves are among the most important energy resources in fish. They are essential for maintaining growth, reproductive fitness and overall survival, thus serving as critical indicators of overall health (Tocher, 2003).

Detailed lipid profiles can allow for gaining a wider scope of an individual's physiological status at given time point (reviewed in Rey et al., 2022). Instead of getting a proxy of the level of lipid reserves, one can obtain specific lipid class levels to infer individual lipid status. Energy is mainly stored in the form of triacylglycerol (TG), which has a higher density of energy than other biomolecules (Sheridan, 1988; Yeo & Parrish, 2022). The structure consists of three fatty acyl chains esterified to the glycerol backbone in which total carbon number, the length and degree of unsaturation of fatty acids, and the position of fatty acyl chains dictate the physical and chemical properties of individual TG species. Further, phospholipids accounts for 60 mol% of lipids in eukaryotic cells playing key roles in maintaining the cellular longetivity (Han and Gross 2001) and in the construction of cell membranes (Yeo & Parrish, 2022). These structurally diverse membrane phospholipids support the functions of integral proteins and serve as precursors for different lipid mediators modulating numerous signaling pathways (Moessinger et al., 2014; Næsje et al., 2006; Sheridan, 1988). In addition to forming the actual energy reserve, lipids have a variety of other important biological roles. When released into circulation, lipids and their derivatives act as signaling molecules informing body organs and tissues on the energy status of the individual for physiological maintenance (Dupont et al., 2013; Koyama et al., 2020; Mangel and Satterthwaite, 2008; Parker and Cheung, 2020; Shalitin and Phillip, 2003; Thorpe et al., 1998) potentially allowing for the progression between life history stages.

Through obtaining lipid profiles using methods such as high-performance thin-layer chromatography (HPTLC) and liquid chromatography-mass spectrometry (LC-MS), one can get a more detailed assessment of detected lipid classes and species, allowing for a more accurate view into the individuals physiological status, to help us understand their contribution to the biological roles on an individual or species level. By determining an individual's lipid profile, inferences can be made into one's physiological capacity and ability at a given time. Overall, measuring total energy levels or specific lipid composition can allow for the assessment of lipid metabolic changes occurring in fishes in addition to the understanding of any changes or alterations with differing temperatures and life-history stages (reviewed in Rey et al., 2022). This could give insight in how successful individuals can be later in life and understanding variation in lipid metabolic capabilities in response to environmental instability such as climate change.

2.2 ATLANTIC SALMON AND MATURATION

Atlantic salmon (*Salmo salar*) are anadromous organisms inhabiting a range of differing environments. Embryonic development takes place in freshwater where juveniles can spend a number of years before making a transition and migrating to saltwater environments. Due to this life cycle, Atlantic salmon must rapidly transition between energy usage and storage to survive necessary physiological changes for the transition from freshwater to saltwater, and for sexual maturation at a variety of ages and sizes demanding sufficient energy (Jonsson et al., 1997). Lipid storage in salmon is mainly located in the muscle and viscera as adipocytes; in the skeletal muscle storage resides in the myospeta, i.e., in the connective tissue between muscle fibers (Zhou et al., 1996). Maturation in salmon has been hypothesized to start soon after fertilization (Thorpe et al., 2007). Therfore, individuals could be undergoing life-history strategies readying for maturation long before an individual has the capability to mature or be reproductively successful. It has been recognized that energy allocation during salmon juvenile phases can have important implications for future sex-specific reproductive strategies (Rowe et al., 1991) requiring different energy resource allocation patterns.

2.2.1 SEX-SPECIFIC STRATEGIES

It is well known that there are sex-specific life-history strategy differ–ences as Atlantic salmon males and females can mature at different sizes and ages (Fleming, 1996). Thus, sex-specific lipid strategies are detected in adult Atlantic salmon. Both males and females use lipid reserves for gamete production and also for other sex-specific reproductive activity: males for mate guarding and/or territory defense and females for nest digging (Jonsson et al., 1997). As the production of large lipid-rich eggs is energetically more costly for females than milt production for male salmon, a sex difference exists in the energy allocated for reproduction. More specifically, males can invest 3-9% of pre-breeding weight while females invest 20-25% (Fleming, 1996; Jonsson et al., 1991). Additionally, females that spend an increased time in freshwater may show reduced pre- and post-smoltification growth (Einum et al. 2002), and a reduction in reproductive success (reviewed in Mobley et al., 2020). This can signify a sex-specific trade-off between time spent in freshwater, growth after marine migration, and reproductive fitness.

Interestingly, the occurrence of maturation in males before smolt–ification and migration adds additional complexity to resource allocation strategies. These individuals need to reach a certain lipid threshold in order to mature and be able to retain and manage energy usage during early development and growth. Male Atlantic salmon parr appear to have a physiological link between lipid accumulation in the body during the spring and the initiation of maturation (Rowe et al., 1991). Additionally, faster growth and larger size at hatching potentially increasing the probability to mature at the parr stage (Aubin-Horth and Dodson 2004; Thorpe 1986). However, growth can decrease by two-fold compared to immature parr once the maturation process is initiated in males start that can lead to delayed smoltification and marine migration (Thorpe, 1986; Whalen & Parrish, 1999). These mature male parr can spawn in the autumn and go through smoltification and migrate to sea during the subsequent spring (Hansen et al. 1989; Jonsson and Jonsson, 2005) showing a need to allocate energy efficiently and maintain a sustained physiological capacity to undergo and survive important life-history stage progressions and times of high energy use.

2.2.2 LARGE-EFFECT MATURATION LOCUS

Atlantic salmon initiating maturation require high energetic demands to go through physiological changes needed to be reproductively viable. While the age at maturity is well characterized, the connection between lipids and energy levels and the maturation process is still unknown. The genetic basis of age at maturity in salmon has been well characterized, with a single genome region, including the *vgll3* gene, explaining 39% of the variation in the age at maturity (Ayllon et al., 2015; Barson et al., 2015; Czorlich et al., 2018) and supported in several common garden experiments associating *vgll3* and salmon maturation timing (Åsheim et al., 2023; Ayllon et al., 2019; Debes et al., 2021; Sinclair-Waters, Nome, et al., 2022; Sinclair-Waters, Piavchenko, et al., 2022). This genomic region encoding *vgll3* has been found to be linked to Atlantic salmon maturation age with two alleles, E and L, associating with earlier or later maturation, respectively. *Vgll3* encodes a transcription cofactor and has been associated with adipocyte differentiation in mice (Halperin et al., 2013) and pubertal timing (Elks et al. 2010), body condition (Tu et al. 2015) and sex-specific autoimmune diseases (Cousminer et al. 2013) in humans. Additionally, it has been found to be play a role in mediating maturation timing via CF (Debes et al., 2021) adding more knowledge of the effect of *vgll3* on lipid storage and metabolism.

Even with progress in understanding mechanisms involved with age at maturity in salmon, more research needs to focus on the functional pathways of *vgll3* and its role in lipid metabolism and storage. By using environmental and seasonal effects during common garden experiments this knowledge gap identifying lipid allocation strategies can be shortened to gain a better understanding of life-history stages and their progression. Additionally, understanding maturation processes, lipid metabolism and any interaction with *vgll3* in juvenile Atlantic salmon.

3 AIMS OF THESIS

1) To identify resource allocation strategies during early life-history stages of Atlantic salmon and implications for maturation

2) To investigate temperature and genetic influences on resource allocation and maturation in Atlantic salmon

3) To characterize lipid profiles and elucidate resource usage and storage capacity in juvenile Atlantic salmon

4 METHODS

4.1 ANIMAL MATERIAL AND EXPERIMENTAL DESIGN

Atlantic salmon juveniles used in this study were derived from a first-generation hatchery stock of Atlantic salmon maintained by the Natural Resources Institute Finland (LUKE) (62°24′50″N, 025°57′15″E, Laukaa, Finland). The parents of the juveniles were caught from the Kymijoki river in southeast Finland and had been crossed in partial factorial manner.

For **Chapter 1**, fertilization took place in November 2016 and eggs were incubated in a flow-through incubation system at the hatchery until hatching in May 2017. Two weeks after hatching, around 2000 individuals were transferred to the Lammi Biological Station (61°04′45″N, 025°00′40″E, Lammi, Finland) for the duration of this common-garden experiment. These juveniles were reared in equal numbers in 10 flow-through tanks (63cm x 63cm x 30cm) under the local natural light cycle at Lammi Biological Station and water was sourced from the nearby Lake Pääjärvi (temperature range: 6.3-17.7 °C).

For **Chapter 2 and 3**, fertilization took place in late October 2017 when unrelated parents with homozygous *vgll3* genotypes were crossed as six 2 × 2 factorials resulting in 24 families. Each factorial included a *vgll3**EE male and female and a *vgll3**LL male and female, where E and L refer to the alleles previously associated with earlier or later maturation, respectively (Barson et al. 2015). Eggs of the 24 families were divided into four batches and incubated in two vertical incubators at two temperatures (2°C difference, hereafter referred to as the warm and cold larval treatments), i.e., with two replicates per family and temperature treatment. A difference of 2°C water temperature was maintained by using a combination of water chillers and room heating. At first feeding, juveniles from the two replicates were pooled and transported to the Lammi Biological Station (61°04′45′′N, 025°00′40′′E, Lammi, Finland) on 10.03.2018 and 24.04.2018 for the warm- and cold-larval treatments, respectively. Half of the individuals of each larval temperature treatment were placed into the same temperature treatment for the juvenile phase (warm and cold juvenile treatments; maintaining a 2°C difference), and the other half of the individuals were transferred to the opposite temperature treatment, thus resulting in a total of four different larval phase-juvenile phase temperature treatment groups, Warm-Warm (WW), Warm-Cold (WC), Cold-Warm (CW), and Cold-Cold (CC) as shown in Figure 1A. Each treatment was replicated in five flow-through circular tanks (diameter 90 cm), and juveniles of each family were allocated to their respective replicate treatment tanks in roughly equal numbers and subsequently reared under a controlled photoperiod set to the local latitude (61.05°N, 25.04°E). Water was sourced from a nearby lake, Lake Pääjärvi, and thus followed the natural annual water temperature cycle with the cold and warm water treatment maintained via a heat-exchange system ranging from 1.30-18.53°C and 1.35-19.04°C, respectively, with an average difference of 1.76°C (Figure 1B).

Fish in **Chapter 1** were fed ad libitum a diet consisting of fine-ground commercial fish food that differed in lipid content (high-lipid:19.9 % and low-lipid 12.6 %; Raisio Baltic Blend; Raisio Oy) and **Chapter 2 and 3** *ad libitum* a diet with commercial fish food pellet size matched for size distribution of the individuals (Raisio Baltic Blend; Raisio Oy).

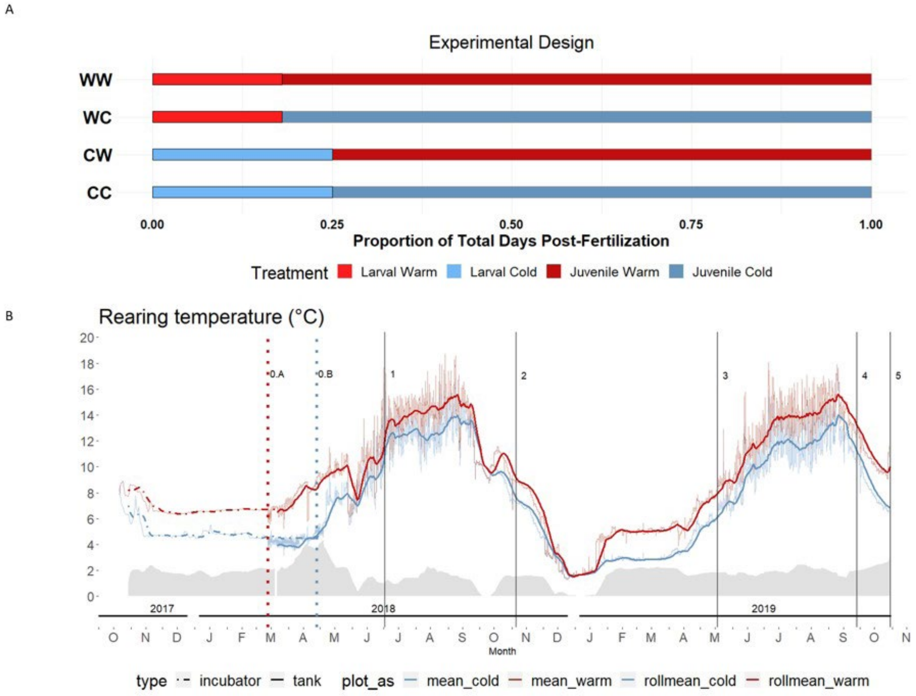

Figure 1: A) Experimental design for temperature treatments for larval and juvenile Atlantic salmon (red = warm temperature, blue = cold temperature) with each temperature group as outlined above (WW, WC, CW, CC) presented as the proportion of total days post-fertilization across the duration of the experiment. B) Temperature curve for the larval (dotted line) and juvenile (solid line) phases of the experiment with the warm temperature treatment water in red and the cold temperature treatment water in blue with gray representing the difference between warm and cold termpartures. 0.A and 0.B indicate transport date of juveniles to Lammi Biological station for the warm and cold larval treatment individuals, respectively. 1, 2 and 3 indicate the times of routine measurements for length and mass of 464-580 individuals at the Summer0, Autumn0 and Spring1 time points, respectively. 4 and 5 indicate the final two time points with routine measurements of length and mass of 464 and ~1205 individuals, respectively, and maturation status check in males.

4.2 MEASUREMENT AND TISSUE COLLECTION

For **Chapter 1**, wet mass (± 0.01 g) and fork length (± 1mm) fish were measured, and fin clips of each individual were taken. During the latter sampling, all fish were euthanized with an overdose of MS-222. A standardized muscle tissue sample from below the dorsal fin and above the lateral line was sampled and weighed for lipid extraction for 49 fish. These 49 samples were flash-frozen in liquid nitrogen and stored in -80 °C until analysis. In addition, the mass of total visceral fat tissue from the body cavity was measured (± 0.001 g) by scraping all visible fat carefully with a scalpel.

For **Chapter 2**, wet mass (± 0.01 g) and fork length (± 1 mm) fish were measured. A fin clip sampled for genetic analysis, for a sub-set of individuals at time points 1-4 (464-580 per time point) and 1205 inviduals at the 5th time point when the experiment was terminated at 24 months post-fertilization (Figure 1B).

For **Chapter 3**, tissue samples from individuals from the CW temperature treatment group at the 3rd and 4th time points (Figure 1B) were dissected. Muscle and liver were dissected and frozen in liquid nitrogen and stored in -80 °C until analysis. Gill tissue was placed in RNAlater all for later laboratory analysis. Weight and length measurements taken at these two time points were also used for later analysis.

4.3 SNP GENOTYPING

For **Chapter 1, 2 and 3**, DNA was extracted from the fin clip samples using standard chelex or salt extraction methods and genotyped for 141 SNPs and a sexing marker as in Aykanat et al., (2016), and assigned the family of origin as outlined in Debes et al. (2021).

4.4 LIPID EXTRACTION, HPTLC AND LC-MS

Total lipids were extracted from 49 muscle tissue samples in **Chapter 1** and from 38 muscle and 30 liver tissue samples in **Chapter 3**. All samples were homogenized in Milli-Q water and the total lipids were extracted into chloroform according to Folch et al. (1957), and the extract solvent was evaporated with nitrogen stream. Immediately, the lipids were dissolved in 1.5 ml chloroform/methanol (1:2 vol/vol) and the sample solution was stored at -80 °C until analysis.

For **Chapter 1**, High Performance Thin Layer Chromatography (HPTLC) was used to separate and quantify concentrations of polar lipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyl–serine (PS) and phosphatidylinositol (PI) and sphingomyelin (SM) eluted with a chloroform/methanol/acetic acid/water (25:17.5:3.8:1.75) solvent, and neutral lipids: cholesterol (C), triacylglycerol (TG), and cholesterol ester (CE) eluted with a hexane/diethyl ether/acetic acid/water (26:6:0.4:0.1) solvent according to Lehti et al. (2018).

For **Chapter 3**, lipidomics was conducted employing Liquid Chromatograph-Mass Spectrometry (LC-MS). The chromatographic separation was conducted in a gradient mode using an Agilent 1290 Infinity HPLC system equipped with a Luna Omega C18 100 Å (50 x 2.1 mm, 1.6 µm) column (Phenomenex), and employing acetonitrile/water/isopropanol-based solvent system (Breitkopf et al., 2017) with the flow rate of 0.200 ml/min and 25°C as the column temperature. Internal standards (TG 14:0/14:0/14:0, PC 14:1/14:1 and PE 14:0/14:0) were spiked into each sample. The column eluent was infused into the electrospray source of an Agilent 6490 Triple Quad LC/MS with iFunnel Technology and spectra were recorded using both positive and negative ionization modes. TG species were detected as [M+NH4]+ ions from MS+ scan. PC species were identified from a Precursor ion 184 scan and detected from an MS+ scan. Additionally, PE species were identified from a Neutral loss 141 scan and detected from an MS- scan. Spectra were extracted from the chromatogram window according to expected TG, PE, and PC elution times for lipids to be identified using LIMSA software on Excel according to Haimi et al. (2006).

4.5 STATISTICAL ANALYSIS

For **Chapter 1**, we fitted a series of multivariate linear animal models for focal traits [PC, PE, PS, PI, SM, C, CE, TG, Condition, Visceral Fat Index (VFI)] as responses to simultaneously test for fixed effects and estimate phenotypic correlations among response traits, adjusted for fixed and random effects, under REML using ASReml-R v. 3 (Butler et al., 2009) in R v. 3.0.2. Animal effects were included to account for the complex relatedness among the hatchery crossed individuals and thus possible genetic correlation for each trait. To test for fixed effects, we used F-test with denominator degrees of freedom approximated according to Kenward and Roger (1997).

For **Chapter 2**, we fitted a generalized animal model with probit-link function to maturation status at age 2 years (coded as binaries) using Bayesian MCMC simulations implemented in MCMCglmm v. 2.32 (Hadfield 2010). To conduct maturation and general animal models with normally distributed residuals for body condition or length records, REML was used as imple–mented in ASReml-R v. 4.1.0.176 (Butler et al. 2018) for growth and condition.

For **Chapter 3**, exploratory principal component analyses (PCA) analyses were carried out first for both tissues separately using molar % of lipid species to assess the relationship between independent variables. Linear mixed effects models were used to test response variable (TG, PC, PE) interactions with fixed effects including sex (male/female), *vgll3* genotype (*vgll3**EE/*vgll3**LL), time point (spring/autumn), maturation status (immature/mature), and fitting random terms for tank and family. All analysis was done using R version 4.2.0 with RStudio 2022.07.2 with lme4, lmertest and factoextra packages.

5 RESULTS AND DISCUSSION

5.1 SEX AND BODY SIZE EFFECTS ON LIPID PROFILES

We identified sex-specific lipid class profiles with HPTLC in **Chapter 1**. The lipid profiles of muscle tissue of juvenile Atlantic salmon were different between males and females and there was an observed general decrease in lipid concentrations with increasing body length (Figure 2). Male juvenile Atlantic salmon had on average a higher concentration of several major lipid classes compared to females. The levels of storage lipids, composed of TG, C, and CE, also decreased with body size. Lipid content can change based on multiple factors including body size, feed and dietary fat level and during major processes such as maturation or smoltification timing (Hillestad and As, 1998; Nordgarden et al., 2003; Rowe et al., 1991; Sheridan, 1989; Storebakken and Austreng, 1987). These factors can help explain the observed sex-specific differences in lipid class concentrations in these individuals. Males and females can mature at different sizes and ages creating sex-specific life-history differences (Fleming, 1996). For example, the potential of maturation before sea migration is increased with a faster growth rate which is much more common in males than in females (Fleming, 1996).

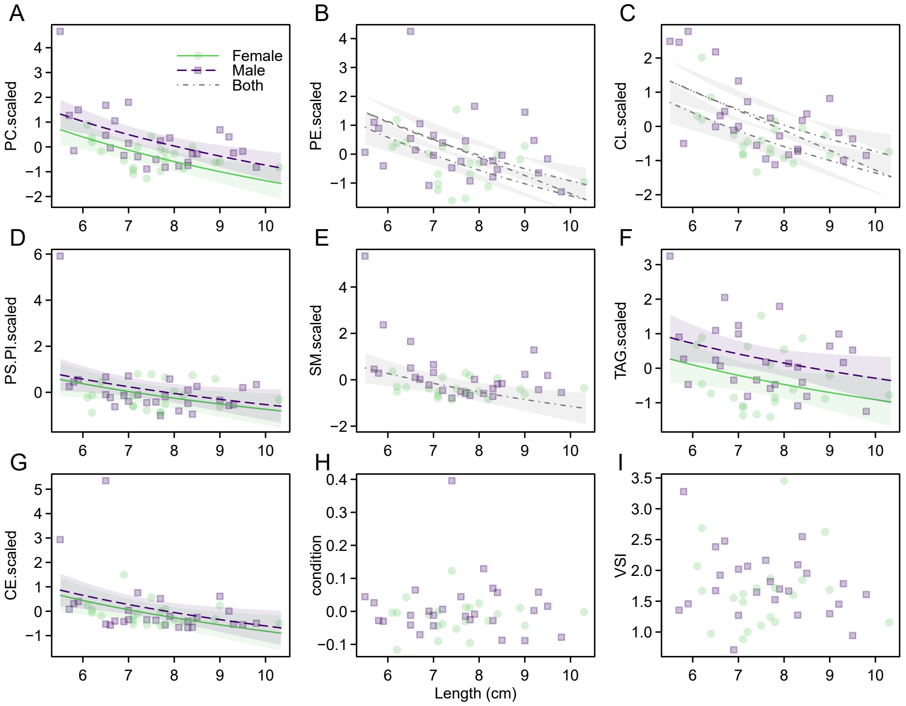

Figure 2. Sex effects and covariance with length for the seven lipid class concentrations (mol/g of tissue), CF and VFI as labelled in the Y-axis vs. individual length (A-I). When sex effects were detected, the regression line is presented for males and females with 95% confidence intervals, otherwise commonly for both sexes.

Maturation and reproductive processes are energetically costly during the juvenile phase (Myers, 1984; Whalen and Parrish, 1999). Thus, the lipid profiles observed in **Chapter 1** could show that juvenile males require larger deposition of slowly mobilized energy such as TG in muscle than juvenile females. This observation could show an early life-history stage resource allocation strategy that is needed to maintain a higher physiological capacity enabling the potential of early maturation. In many salmonid species, including Atlantic salmon, an increase in the β-oxidation capacity in muscle tissues results in lower lipid levels during periods of increased growth (Nordgarden et al., 2003). It has been found that once males start maturing, growth decreases by two-fold compared to immature parr which is due to the energetical costly process of developing mature gonads (Aksnes et al., 1986; Thorpe, 1986; Whalen & Parrish, 1999).

Additionally, fish that fed the high-fat diet had stored slightly more visceral fat as measured by VFI than those on the low-fat diet. This result is in line with precious studies as it has been found that fish store more fat in the viscera with increasing lipid and carbohydrate levels in feed while muscle fat can be reduced with increasing protein levels in feed (Aksnes, 1995; Hillestad and

As, 1998; Weihe et al., 2019). Interestingly, no significant correlation between VFI and any muscle lipid class was detected, nor were there significant VFI differences detected between the diet treatments in **Chapter 1**.

5.2 SEASONAL *VGLL3* SPECIFIC VARIATION IN ENERGY STATUS

In **Chapter 2**, we found a *vgll3* genotype effect on the direction of seasonal change in body condition in both males and females with no difference in length during the 1.5-year study period (seen below in Figure 3). We observed *vgll3**LL individuals having lower body condition than *vgll3**EE individuals in the spring prior to the breeding season, however these individuals had a higher body condition in the autumn. These *vgll3* genotype specific seasonal changes in body condition adds further additions to previous studies that *vgll3* has a potential role in the control of resource allocation (Debes et al. 2021; Halperin et al. 2013). Moreover, *vgll3* effects can express as an effect on average body condition at a given time point but also express as an effect on the direction of body condition change through time. Our *vgll3* genotype findings suggest that the general assumption that individuals with higher body condition are more likely to mature earlier due to having higher lipid levels (Good & Davidson, 2016; Rowe et al., 1991; Taranger et al., 2010) may be more complex. **Chapter 2** adds further evidence backing up previous statements that adequate energy storage are vital at critical life-history timepoints, which for salmon is thought to be in the spring prior to maturation (Rowe et al, 1991). This is indeed the timepoint at which juveniles carrying the *vgll3**EE genotype exhibited higher body condition than individuals carrying other *vgll3* genotypes (Figure 3a), even though body condition was recorded at its lowest point for all genotypes of the five timepoints measured.

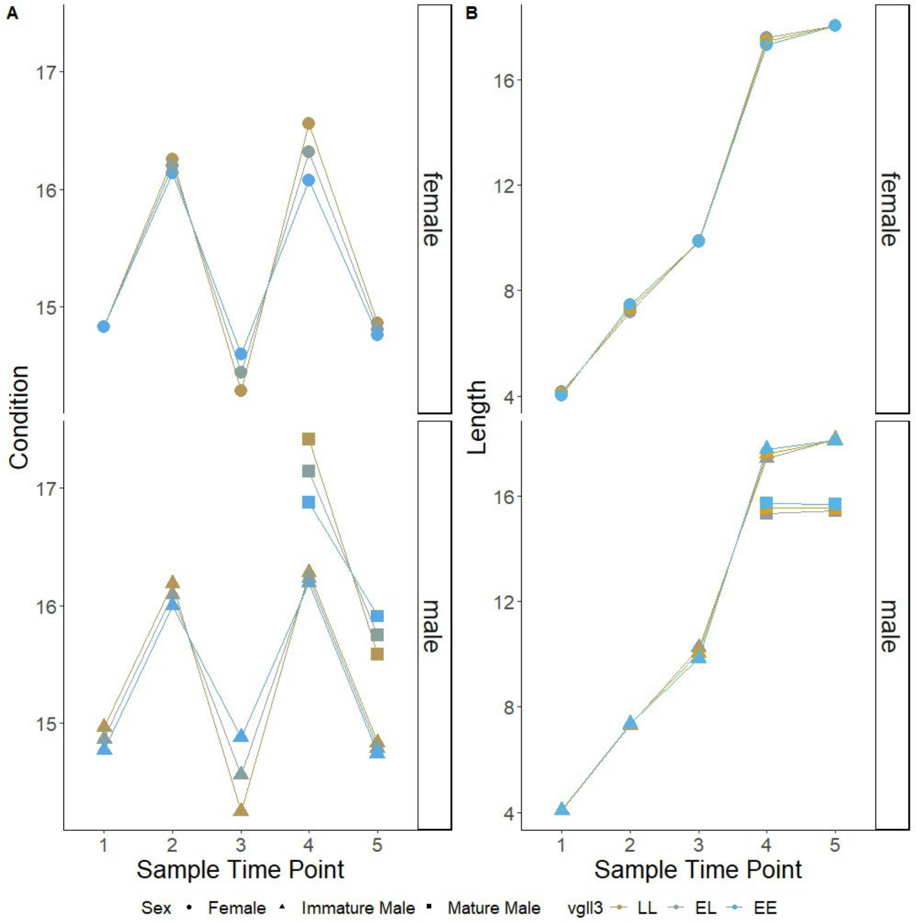

Figure 3. Body condition and length values of *vgll3* genotypes in 3177 juvenile Atlantic salmon across 1.5 years (colors: gold = LL, gray = EL, blue = EE, shape: circle = female, triangle = immature male, square = mature male). Sample time point represent 5 sampling time points throughout the experiment (1 – Summer age 0, 2 – Autumn age 0, 3 - Spring age 1, 4 - Autumn age 1 and 5 – Autumn age 1) with mature males only occurring at time point 4 and 5.

Our finding that the body condition of *vgll3**EE individuals was more stable across seasons than in *vgll3**LL individuals is in line with recent studies investigating the links between juvenile *vgll3* genotypes and phenotypes (Bangura et al. 2022, Prokkola et al. 2022). These studies observed *vgll3* genotype effects with aggressive behavior and aerobic scope, both of which could potentially have an effect on CF in Atlantic salmon. It was found that *vgll3**LL juveniles were more aggressive compared to *vgll3**EE individuals (Bangura et al. 2022). This could result in *vgll3**LL individuals allocating energy for aggressive behaviour instead of allocating energy to lipid storage. Additionally, it was found that *vgll3**EE individuals had higher aerobic scope than *vgll3**LL individuals. Therefore, greater resource acquisition or assimilation via higher aerobic scope can be a potential mechanism by which an increased CF in *vgll3**EE individuals could be achieved (Prokkola et al. 2022). Our findings in **Chapter 2** suggest that this *vgll3* genotype influence may be particularly important during winter months, when *vgll3**LL individuals lost body condition much faster than *vgll3**EE individuals. This observed phenomenon may be derived from *vgll3**EE individuals having higher CF at the critical point in the spring when physiological processes related to maturation are being determined.

Lastly, differing larval or juvenile temperature did not appear to alter growth, length or body condition nor *vgll3* effects on maturation as full growth compensation was detected during the length of the experiment. This leads us to expect a temperature consistency with *vgll3* effects on maturation and a *vgll3*-effect consistency to either natural or artificial selection. This finding is based off a relatively narrow range of temperatures so a broader temperature range should be explored in future research.

5.3 TISSUE-SPECIFIC LIPID PROFILES AND SEASONAL *VGLL3* EFFECTS

In **Chapter 3**, tissue specific lipid profiles were investigated between seasons and between *vgll3* gentotypes. Unlike in **Chapter 1**, no sex-specific differences were detected in lipid profiles in muscle tissue in **Chapter 3** (Figure 4.1). As individuals in **Chapter 3** were year older and mature, they may have potentially utilized the higher lipid concentrations from an earlier life-history stage.

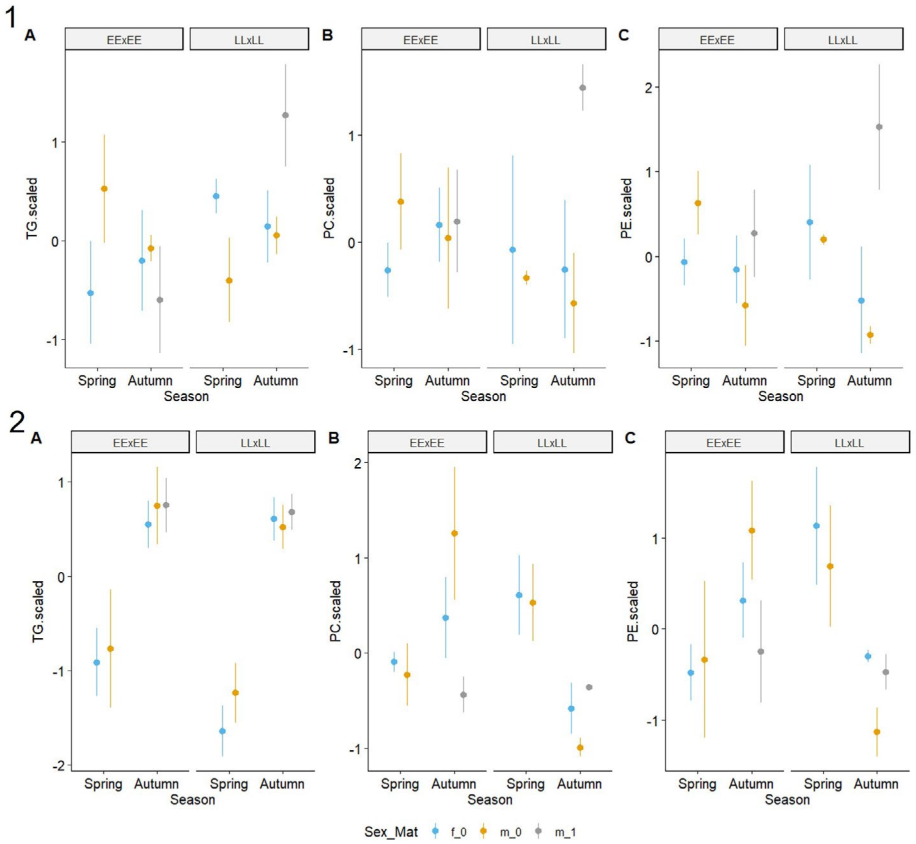

Figure 4: Scaled A) triacylglycerol (TG) B) phosphatidylethanolamine (PE) and C) phsphatidylcholine (PC) concentrations of 1) muscle and 2) liver in immature males, immature females and mature males between the spring and autumn 2019.

Perhaps the most noteworthy finding of **Chapter 3** is the stark contrast in the direction of membrane lipid (PC and PE) concentration changes between seasons in the liver of *vgll3**EE individuals compared to *vgll3**LL individuals (seen above in Figure 4.2). This change between seasons can be explained by two scenarios, one involving genotype-specific differences in lipid synthesis, and another involving genotype- specific differences in lipid storage mechanisms. The first scenario could reflect variation in endoplasmic reticulum (ER) volume in liver of differing *vgll3* genotype individuals. Higher membrane lipid concentrations increasing from spring to autumn in *vgll3**EE individuals could be vital for maintaining a more stable capacity of ER functions compared to the *vgll3**LL individuals showing a decrease in membrane lipid concentrations from spring to autumn. The ER is one of the major sites of protein synthesis and fatty acid and lipid metabolism where de novo synthesis of phospholipids and TG take place. This site also produces lipoprotein particles for transport of diverse biomolecules, including TGs that are largely carried to the main storage sites, muscle myosepta and visceral adipose tissue in salmon (Jensen-Urstad & Semenkovich, 2012; Alves-Bezerra & Cohen, 2017). Therefore, *vgll3**EE individuals showing an increase of membrane lipid concentrations may allow for an increase or retained capability for ER functions across seasons compared to *vgll3**LL individuals that elicit decreasing membrane lipid levles from spring to autumn.

In the second scenario, individuals could be storing lipid droplets in the liver and other tissues in a genotypic-specific manner. This would result in the observed contrast in the membrane lipid (PC and PE) concentration changes from spring to autumn in *vgll3**EE individuals compared to *vgll3**LL individuals. Specifically, the main storage organelles for metabolic energy in most cells are lipid droplets stored in the cytoplasm (Prévost et al., 2018). *vgll3**LL individuals showing a decrease of the membrane lipid concentration from spring to autumn could be due to them storing larger lipid droplets in the liver compared to *vgll3**EE individuals, and thus showing a tradeoff of increasing the mass of storage lipid at the expense of ER network mass. However, additional research is required to gain a more complete understanding of these genotype-based differences. This additional work could include identifying *vgll3* genotype specific differences in transcriptomic data focusing on lipid metabolism genes, such as elongases of very long chain fatty acids and fatty acyl desaturases, known to be involved in fatty acid structural modifications (Colombo et al., 2022; Datsomor et al., 2022; Kabeya et al., 2018), and variation in fatty acid metabolism (Twining et al., 2021). Moreover, the investigation of histological patterns would help to elucidate *vgll3* genotype specific differences in lipid droplets and potentially underlying hyperplasic or hypertrophy through histological patterns (Caballero et al., 2002).

Our findings in **Chapter 3** provide first hints at mechanisms by which *vgll3* contributes to the maintenance of lipid reserves and metabolic capability across seasons in Atlantic salmon. If *vgll3**EE individuals with an increased concentration of membrane lipids from to spring to autumn do indeed have increased ER metabolic activity compared to the opposite trend in *vgll3**LL individuals, it would imply an increased capacity for protein synthesis and thereby a better capability for production of phospholipids and TGs across seasons. This phenomenon would explain why *vgll3**EE individuals maintained a higher body condition in the spring found in **Chapter 2**, which increases the probability to mature in the autumn.

6 CONCLUSION

All together, this thesis shows a potential mechanism in Atlantic salmon that further links *vgll3* with lipid storage and metabolism. Additionally, sex-specific strategies in lipid metabolism seem to exist allowing individuals to go through major physiological processes. The main findings in this thesis are 1) Sex-specific lipid profiles that potentially allow for early life-history stage progressions to be successful, 2) Genotype association with seasonal change in body condition allowing for adequate lipid storage to be achieved at a critical time point for the inititation of maturation and lastly, 3) Genotype association with tissue specific lipid profiles showing mechanistic roles for maintaining improved metabolic capabilities. These results reveals more information why the *vgll3* genotypes may differ in the age of maturation and further linking *vgll3* genotypes in early life stages preparing for maturation processes. We also give insight on of how *vgll3* and environmental effects contribute to maturation probability. This thesis contributes to gaining a better knowledge of energy allocation and physiological capacity allowing for a better understanding of maturation and survival through early life-history stages in juvenile Atlantic salmon.

7 ACKNOWLEDGEMENTS

Sometimes you don't really know where your life is heading until you are years down the road and have a chance to look back and reflect to try to understand how you got to where you are. There are many people who I need to thank for helping me finish my PhD research.

First, I would like to thank Craig for taking a chance on me. I remember the day I sent you a random email about doing a PhD and I got a reply almost immediately. That day and the weeks to come set up the application for the Fulbright and ultimately the move to Finland almost 6 years ago. These past years have been some of the best years of my life and it has been amazing to do my PhD under your supervision. Thanks for all the support and supervision in getting to this stage. Next, I would like to thank Paul Debes. You have been here from my move to Finland, and I want to thank you for all the supervision from fish rearing to statistical analysis and everything in between.

To John, thanks for taking me under your wing when I moved to the station. From you helping me get established in Finland with all the bureaucratic things to the talks over lunch to the rides to and from the station, it has been an honor to get to know you. Thank you for everything. To Suvi, I immediately hit if off with you once I moved to Lammi. From all the support with difficult situations in my life to all the laughs and conversations, thank you for being there for me. To everyone else at Lammi Biological Station, you made me feel at home right away and are one of the main reasons I fell in love with Finland and now call it home. I am so glad I was able to conduct my PhD research there and spend as much time wandering through the forest, sitting by the lake or enjoying my favorite sauna in Finland. You are a special place and will always feel like my home in Finland. To the University of Helsink, everyone in OEB, LUOVA and more specifically, Lipidomics Unit, thank you for the giving me a space to be myself and further my life into becoming a scientist.

To EvolConGen, thank all of you for being there for me and knowing how to party. To Katja, Eirik and Antti, I thank you for all the nice chats and motivation through the years. To Tutku, thanks for being an unofficial supervisor. To Vicky, thanks for all the support during the early years of my PhD! To Johanna, it was incredible to get to work and know you. To Carly, you came in to support me at a critical time and I thank you for being there for me. To Minna, I am glad we became more than just work friends. Thank you for everything. And to Ronan, Carly, Jad and Minna, thanks for proofreading my thesis!

To Jeff, Leslie, Federico and David H, I have to thank you all for helping make this dream and chapter of my life a reality. You all had my back when I

was applying for a Fulbright and helped push me to get out of my comfort zone. To everyone at Fulbright Finland, thanks for making this small-town country boy from Alabama be able to explore a hidden gym and fall in love with a new place. Getting the Fulbright grant changed my life more than most people will every know and being able to meet all of you, enjoy traditional Finnish things and be able to meet many like-minded people have made this process all the better. Thank you, thank you, thank you.

To Jackie, I don't have words to express how much it means to me to have had your support during the PhD. I really couldn't have made it this far without you. Thank you for all the phone calls and zoom work dates. Thank you for helping me survive some of the lowest points of my life. You are a true rockstar! Also, you and Kalle are really like parents to me and I have unlimited love for both of you. To Marion, thanks for the walks, talks, beers, dinners, and support. I loved being able to get to know you and spend so many important moments togethers doing our PhD. I can't wait to continue doing fun things together now that we will both be done. You are truly one of a kind and I miss you like crazy. To Jack, Aina, Miquel, Craig, Unni and Ulla, thanks for all the nice times together and to all the future ones. You really made me feel supported and loved and everyone needs amazing friends like you.

To Michelle, Johanna, Marja, Vilma, Rosa and Karo, thanks for becoming my family. All the girls' nights out have been a dream and I thank you for everything. I have had special moments with each of you and it's been incredible to create my life here in Finland with all of you in it!

To the Groupaaaa, you really were my rock during this PhD. From all the times I needed to get away to all the times I needed a shoulder to cry on. You have all been there every step of the way. To Samuli, it is rare that you connect so deeply soon after meeting. From the night we met on the dancefloor I just knew we would be best friends. I wouldn't be alive today without your love and support through these years. Thanks for being the Thelma to my Louise, the Ariana Grande to my Lady Gaga and the peanut butter to my jelly. Here's to many more all-nighters, dancefloors, saunas and deep talks. Love you babe. To Jad, thanks for supporting me like no other. From checking up on me on the bad days to celebrating together on the good ones. You are a such a genuine human being and loving friend. Love you to the moon and back again. To Petri, I love how connected we are and can't imagine my life without you. To Albi, you are really one of the kindest humans I know. Thanks for all the love you have shared with me over the years. To Itamar, my 'daughter.' You are such a beautiful soul. I am glad to be able to enjoy life together and live our authentic selves. To David S, I have been honored to become a close friend with you over the years, you always gave it to me straight and show me so much love. To Arno, thanks for everything over the years. I have been able to rely on you during difficult times and have only come out the other end a better person because of your support. To Marko, you are the pillar of my life in Finland. Meeting you showed me the person I can be, and you helped forge the friendships and community I have today. I am glad to be alive today and be able to show you how much you mean to me. To Ari, thank you. Thank you for seeing something in me. Thank you for choosing to support me. I owe so much to you being in my life. And to everyone else who I have been able to meet during these years and connect with, I cherish all your friendships and thanks for being a part of my life.

Lastly, to David, thanks for being by my side during these last couple of years. I have always wanted to know how it feels to really love someone the way I love you. It's been a wild ride but having you around has made this finishing process all that much easier. Thanks for all the dinners and beers and cuddles and love. I am so glad I get to have you by my side during these big life changes. I have gone through so much during the last 2 years and knowing that I have been able to have you around and support me has made all the difference. Thanks baby. I love you so much.

REFERENCES

Aksnes, A., Gjerde, B., & Roald, S. O. (1986). Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, Salmo salar. Aquaculture, 53(1), 7–20. https://doi.org/10.1016/0044-8486(86)90295-4

A. Aksnes. (1995). Growth, feed efficiency and slaughter quality of salmon, Salmo salar L, given feeds with different ratios of carbohydrate and protein. Aquaculture Nutrition, 1, 241–248.

Alves-Bezerra, M., & Cohen, D. E. (2017). Triglyceride metabolism in the liver. Comprehensive Physiology, 8(1), 1–8. https://doi.org/10.1002/cphy.c170012

Åsheim, E. R., Debes, P. V., House, A., Liljeström, P., Niemelä, P. T., Siren, J. P., Erkinaro, J., & Primmer, C. R. (2023). Atlantic salmon (Salmo salar) age at maturity is strongly affected by temperature, population and age-at-maturity genotype. Conservation Physiology, 11(1), 1–16. https://doi.org/10.1093/conphys/coac086

Aubin-Horth, N., & Dodson, J. J. (2004). Inlfuence of individual body size and variable thresholds on the incidence of a sneaker male reproudctive tactic in Atlantic salmon. Evolution, 58(1), 136–144.

Aykanat, T., Lindqvist, M., Pritchard, V. L., & Primmer, C. R. (2016). From population genomics to conservation and management: A workflow for targeted analysis of markers identified using genome-wide approaches in Atlantic salmon Salmo salar. Journal of Fish Biology, 89(6), 2658–2679. https://doi.org/10.1111/jfb.13149

Ayllon, F., Kjærner-semb, E., Furmanek, T., Wennevik, V., Solberg, F., Dahle, G., Taranger, G. L., & Glover, K. A. (2015). The vgll3 locus controls age at maturity in wild and domesticated Atlantic salmon (Salmo salar L.) Males. PLOS Genetics, 11(11), 1–15. https://doi.org/10.1371/journal.pgen.1005628

Ayllon, F., Solberg, M. F., Glover, K. A., Mohammadi, F., Kjærner-Semb, E., Fjelldal, P. G., Andersson, E., Hansen, T., Edvardsen, R. B., & Wargelius, A. (2019). The influence of vgll3 genotypes on sea age at maturity is altered in farmed mowi strain Atlantic salmon. BMC Genetics, 20(1), 44. https://doi.org/10.1186/s12863-019-0745-9

Balbontín, J., Møller, A. P., Hermosell, I. G., Marzal, A., Reviriego, M., & De Lope, F. (2012). Lifetime individual plasticity in body condition of a migratory bird. Biological Journal of the Linnean Society, 105(2), 420–434. https://doi.org/10.1111/j.1095-8312.2011.01800.x

Bangura, P. B., Tiira, K., Niemelä, P. T., Erkinaro, J., Liljeström, P., Toikkanen, A., & Primmer, C. R. (2022). Linking vgll3 genotype and aggressive behaviour in juvenile Atlantic salmon (Salmo salar). Journal of Fish Biology, 100(5), 1264–1271. https://doi.org/10.1111/jfb.15040

Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., Jacq, C., Jensen, A. J., Johnston, S. E., Karlsson, S., Kent, M., Moen, T., Niemelä, E., Nome, T., Næsje, T. F., Orell, P., Romakkaniemi, A., Sægrov, H., Urdal, K., … Primmer, C. R. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature, 528(7582), 405–408. https://doi.org/10.1038/nature16062

Biro, P. A., Morton, A. E., Post, J. R., & Parkinson, E. A. (2004). Over-winter lipid depletion and mortality of age-0 rainbow trout (Oncorhynchus mykiss). Canadian Journal of Fisheries and Aquatic Sciences, 61(8), 1513–1519. https://doi.org/10.1139/f04-083

Breitkopf, S. B., Ricoult, S. J. H., Yuan, M., Xu, Y., Peake, D. A., Manning, B. D., & Asara, J. M. (2017). A relative quantitative positive/negative ion switching method for untargeted lipidomics via high resolution LC-MS/MS from any biological source. Metabolomics, 13(3), 30. https://doi.org/10.1007/s11306-016-1157-8

Brett J. R., Groves T. D. D. (1979). Physiological energetics. In: Hoar W. S., Randall D. J., and Brett R. J. (Eds) Fish Physiology. Academic Press, pp 279–352

Bright Ross, J. G., Newman, C., Buesching, C. D., Connolly, E., Nakagawa, S., & Macdonald, D. W. (2021). A fat chance of survival: Body condition provides life-history dependent buffering of environmental change in a wild mammal population. Climate Change Ecology, 2, 100022. https://doi.org/10.1016/j.ecochg.2021.100022

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J., & Thompson, R. (2018). ASReml estimates variance components under a general linear. VSN International Ltd, ASReml-R Reference Manual Version 4, 188.

Butler, D. G., Cullis, B. R., Gogel, B. J., Gilmour, A. R., & Gogel, B. J. (2009). Mixed Models for S language environments. Version 3.

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., & Izquierdo, M. S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchus mykiss. Aquaculture, 214(1–4), 253–271. https://doi.org/10.1016/S0044-8486(01)00852-3

Cogălniceanu, D., Stănescu, F., Székely, D., Topliceanu, T.-S., Iosif, R., & Székely, P. (2021). Age, size and body condition do not equally reflect population response to habitat change in the common spadefoot toad Pelobates fuscus. PeerJ, 9, e11678. https://doi.org/10.7717/peerj.11678

Colombo, S. M., Budge, S. M., Hall, J. R., Kornicer, J., & White, N. (2022). Atlantic salmon adapt to low dietary n-3 PUFA and warmer water temperatures by increasing feed intake and expression of n-3 biosynthesis-related transcripts. Fish Physiology and Biochemistry. https://doi.org/10.1007/s10695-022-01157-2

Cousminer, D. L., Berry, D. J., Timpson, N. J., Ang, W., Thiering, E., Byrne, E. M., Rob Taal, H., Huikari, V., Bradfield, J. P., Kerkhof, M., Groen-Blokhuis, M. M., Kreiner-Møller, E., Marinelli, M., Holst, C., Leinonen, J. T., Perry, J. R. B., Surakka, I., Pietiläinen, O., Kettunen, J., … Widén, E. (2013). Genome-wide association and longitudinal analyses reveal genetic loci linking pubertal height growth, pubertal timing and childhood adiposity. Human Molecular Genetics, 22(13), 2735–2747. https://doi.org/10.1093/hmg/ddt104

Cunjak, R. A. (1988). Physiological consequences of overwintering in streams: The cost of acclimitization? Canadian Journal of Fisheries and Aquatic Sciences, 45(3), 443–452. https://doi.org/10.1139/f88-053

Czorlich, Y., Aykanat, T., Erkinaro, J., Orell, P., and Primmer, C. R. (2018). Rapid sex-specific evolution of age at maturity is shaped by genetic architecture in Atlantic salmon. Nature Ecology & Evolution 2 (11): 1800–1807. https://doi.org/10.1038/s41559-018-0681-5.

Datsomor, A. K., Gillard, G., Jin, Y., Olsen, R. E., & Sandve, S. R. (2022). Molecular regulation of biosynthesis of long chain polyunsaturated fatty acids in Atlantic salmon. Marine Biotechnology, 24(4), 661–670. https://doi.org/10.1007/s10126-022-10144-w

Debes, P. V., Piavchenko, N., Ruokolainen, A., Ovaskainen, O., Moustakas-Verho, J. E., Parre, N., Aykanat, T., Erkinaro, J., & Primmer, C. R. (2021). Polygenic and major-locus contributions to sexual maturation timing in Atlantic salmon. Molecular Ecology, 30(18), 4505–4519. https://doi.org/10.1111/mec.16062

Dupont, J., Reverchon, M., Bertoldo, M. J., & Froment, P. (2013). Nutritional signals and reproduction. Molecular and Cell Endocrinology, 1–11. https://doi.org/10.1016/j.mce.2013.09.028

Einum, S., Thorstad, E. B., & Næsje, T. F. (2002). Growth rate correlations across life-stages in female Atlantic salmon. Journal of Fish Biology, 60(3), 780–784. https://doi.org/10.1111/j.1095-8649.2002.tb01704.x

Elks, C. E., Perry, J. R. B., Sulem, P., Chasman, D. I., Franceschini, N., He, C., Lunetta, K. L., Visser, J. A., Byrne, E. M., Cousminer, D. L., Gudbjartsson, D. F., Esko, T., Feenstra, B., Hottenga, J.-J., Koller, D. L., Kutalik, Z., Lin, P., Mangino, M., Marongiu, M., ... Murray, A. (2010). Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nature Genetics, 42(12), 1077–1085. https://doi.org/10.1038/ng.714

Fleming, I. A. (1996). Reproductive strategies of Atlantic salmon: Ecology and evolution. Reviews in Fish Biology and Fisheries, 6(4), 379–416. https://doi.org/10.1007/BF00164323

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem, 226(1), 497–509. https://doi.org/10.1007/s10858-011-9570-9

Geissinger, E. A., Gregory, R. S., Laurel, B. J., & Snelgrove, P. V. R. (2021) Food and intitial size influence overwinter survival and condition of a juvenile marine fish (age-0 Atlantic cod). Canadian Journal of Fisheries and Aquatic Science, 78(4), 472-482. https://doi.org/10.1139/cjfas-2020-0142

Good, C., & Davidson, J. (2016). A review of factors influencing maturation of Atlantic salmon, Salmo salar, with focus on water recirculation aquaculture system environments. Journal of the World Aquaculture Society, 47(5), 605–632. https://doi.org/10.1111/jwas.12342

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. Journal of Statistical Software, 33(2). https://doi.org/10.18637/jss.v033.i02

Haimi, P., Uphoff, A., Hermansson, M., & Somerharju, P. (2006). Software tools for analysis of mass spectrometric lipidome data. Analytical Chemistry, 78(24), 8324–8331. https://doi.org/10.1021/ac061390w

Halperin, D. S., Pan, C., Lusis, A. J., & Tontonoz, P. (2013). Vestigial-like 3 is an inhibitor of adipocyte differentiation. Journal of Lipid Research, 54, 473–481. https://doi.org/10.1194/jlr.M032755

Han, X., & Gross, R. W. (2001). Quantitative analysis and molecular species fingerprinting of triacylglyceride molecular species directly from lipid extracts of biological samples by electrospray ionization tandem mass Spectrometry. Analytical Biochemistry, 295(1), 88–100. https://doi.org/10.1006/abio.2001.5178

Hansen, L. P., Jonsson, B., Morgan, R. I. G., & Thorpe, J. E. (1989). Influence of parr maturity on emigration of smolting Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences, 46(3), 410–415. https://doi.org/10.1139/f89-054

Haraldstad, T., Höglund, E., Kroglund, F., Lamberg, A., Olsen, E. M., & Haugen, T. O. (2018). Condition-dependent skipped spawning in anadromous brown trout. Canadian Journal of Fisheries and Aquatic Sciences, 75(12), 2313–2319. https://doi.org/10.1139/cjfas-2017-0076

Henderson, J. R., & Tocher, D. R. (1987). The lipid composition and biochemistry of freshwater fish. Progress in Lipid Research, 26(4), 281–347. https://doi.org/10.1016/0163-7827(87)90002-6

Herbinger, C. M., & Friars, G. W. (1991). Correlation between condition factor and total lipid content in Atlantic salmon, Salmo salar L., parr. Aquaculture Research, 22(4), 527–529. https://doi.org/10.1111/j.1365-2109.1991.tb00766.x

Hillestad, M., & As, F. J. B. (1998). Long-term effects of dietary fat level and feeding rate on growth, feed utilization and carcass quality of Atlantic salmon. Aquaculture Nutrition, 4, 89–97.

Jensen-Urstad, A. P. L., & Semenkovich, C. F. (2012). Fatty acid synthase and liver triglyceride metabolism: Housekeeper or messenger? Biochimica et Biophysica Acta, 1821(5), 747–753. https://doi.org/10.1016/j.bbalip.2011.09.017

Jonsson, B., & Jonsson, N. (2005). Lipid energy reserves influence life-history decision of Atlantic salmon (Salmo salar) and brown trout (S. trutta) in fresh water. Ecology of Freshwater Fish, 296–301. https://doi.org/10.1111/j.1600-0633.2005.00098.x

Jonsson, N., Jonsson, B., & Hansen, L. P. (1991). Energetic cost of spawning in male and female Atlantic salmon (Salmo salar L.). Journal of Fish Biology, 39, 739–744.

Jonsson, N., Jonsson, B., & Hansen, L. P. (1997). Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon Salmo salar. The Journal of Animal Ecology, 66(3), 425. https://doi.org/10.2307/5987

Kabeya, N., Yevzelman, S., Oboh, A., Tocher, D. R., & Monroig, O. (2018). Essential fatty acid metabolism and requirements of the cleaner fish, ballan wrasse Labrus bergylta: Defining pathways of long-chain polyunsaturated fatty acid biosynthesis. Aquaculture, 488, 199–206. https://doi.org/10.1016/j.aquaculture.2018.01.039

Kadri, S., Huntingford, F. A., Metcalfe, N. B., & Thorpe, J. E. (1996). Social interactions and the distribution of food among one-sea-winter Atlantic salmon (Salmo salar) in a sea-cage. Aquaculture, 139(1–2), 1–10. https://doi.org/10.1016/0044-8486(95)01163-3

Kenward, M. G., & Roger, J. H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. Biometrics, 53(3), 983–997.

Koyama, T., Texada, M. J., Halberg, K. A., & Rewitz, K. (2020). Metabolism and growth adaptation to environmental conditions in Drosophila. Cellular and Molecular Life Sciences, 77(22), 4523–4551. https://doi.org/10.1007/s00018-020-03547-2

Lehti, S., Nguyen, S. D., Belevich, I., Vihinen, H., Heikkilä, H. M., Soliymani, R., Käkelä, R., Saksi, J., Jauhiainen, M., Grabowski, G. A., Kummu, O., Hörkkö, S., Baumann, M., Lindsberg, P. J., Jokitalo, E., Kovanen, P. T., & Öörni, K. (2018). Extracellular Lipids Accumulate in Human Carotid Arteries as Distinct Three-Dimensional Structures and Have Proinflammatory Properties. American Journal of Pathology, 188(2), 525–538. https://doi.org/10.1016/j.ajpath.2017.09.019

Mangel, M., & Satterthwaite, W. H. (2008). Combining proximate and ultimate approaches to understand life history variation in salmonids with application to fisheries, conservation, and aquaculture. Bulleting of Marine Science, 1(2008), 107–130.

Manor, M. L., Weber, G. M., Cleveland, B. M., & Kenney, P. B. (2014). Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (Oncorhynchus mykiss). Aquaculture, 418–419, 17–25. https://doi.org/10.1016/j.aquaculture.2013.09.023

McNamara, J. M., & Houston, A. I. (2007). Optimal annual routines: Behaviour in the context of physiology and ecology. Philosophical Transactions of the Royal Society B: Biological Sciences, 363(1490), 301–319. https://doi.org/10.1098/rstb.2007.2141

Metcalfe, N. B., & Thorpe, J. E. (1992). Anorexia and defended energy levels in over-wintering juvenile salmon. Journal of Animal Ecology, 61(1), 175–181.

Mobley, K. B., Aykanat, T., Czorlich, Y., House, A., Kurko, J., Miettinen, A., Moustakas-Verho, J., Salgado, A., Sinclair-Waters, M., Verta, J.-P., & Primmer, C. R. (2021). Maturation in Atlantic salmon (Salmo salar, Salmonidae): A synthesis of ecological, genetic, and molecular processes. Reviews in Fish Biology and Fisheries, 31(3), 523–571. https://doi.org/10.1007/s11160-021-09656-w

Moessinger, C., Klizaite, K., Steinhagen, A., Philippou-Massier, J., Shevchenko, A., Hoch, M., Ejsing, C. S., & Thiele, C. (2014). Two different pathways of phosphatidylcholine synthesis, the Kennedy Pathway and the Lands Cycle, differentially regulate cellular triacylglycerol storage. BMC Cell Biology, 15(1), 1–17. https://doi.org/10.1186/s12860-014-0043-3

Mogensen, S., & Post, J. R. (2012). Energy allocation strategy modifies growth-survival trade-offs in juvenile fish across ecological and environmental gradients. Oecologia, 168(4), 923–933. https://doi.org/10.1007/s00442-011-2164-0

Morgan, I. J., McCarthy, I. D., & Metcalfe, N. B. (2000). Life-history strategies and protein metabolism in overwintering juvenile Atlantic salmon: Growth is enhanced in early migrants through lower protein turnover. Journal of Fish Biology, 56(3), 637–647. https://doi.org/10.1111/j.1095-8649.2000.tb00761.x

Morgan, I. J., McCarthy, I. D., & Metcalfe, N. B. (2002). The influence of life-history strategy on lipid metabolism in overwintering juvenile Atlantic salmon. Journal of Fish Biology, 60(3), 674–686. https://doi.org/10.1006/jfbi.2002.1886

Mozsár, A., Boros, G., Sály, P., Antal, L., & Nagy, S. A. (2015). Relationship between Fulton's condition factor and proximate body composition in three freshwater fish species. Journal of Applied Ichthyology, 31(2), 315–320. https://doi.org/10.1111/jai.12658

Myers, R. A. (1984). Demographic consequences of precocious maturation of Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences, 41, 1349–1353.

Næsje, T. F., Thorstad, E. B., Forseth, T., Aursand, M., Saksgård, R., & Finstad, A. G. (2006). Lipid class content as an indicator of critical periods for survival in juvenile Atlantic salmon (Salmo salar). Ecology of Freshwater Fish, 15(4), 572–577. https://doi.org/10.1111/j.1600-0633.2006.00173.x

Nassour, I., & Léger, C. L. (1989). Deposition and mobilization of body fat during sexual maturation in female trout (Salmo gairdneri Richardson). Aquatic Living Resources, 2(3), 153–159.

Nordgarden, U., Torstensen, B. E., Frøyland, L., Hansen, T., & Hemre, G. (2003). Seasonally changing metabolism in Atlantic salmon (Salmo salar L.) II – b -oxidation capacity and fatty acid composition in muscle tissues and plasma lipoproteins. Aquaculture Nutrition, 9, 295-303

Parker, C. G., & Cheung, E. (2020). Metabolic control of teleost reproduction by leptin and its complements: Understanding current insights from mammals. General and Comparative Endocrinology, 292(February), 113467. https://doi.org/10.1016/j.ygcen.2020.113467

Post, J. R., & Parkinson, E. A. (2001). Energy allocation strategy in young fish: Allometry and survival. Ecology, 82(4), 1040–1051. https://doi.org/10.1890/0012-9658(2001)082[1040:EASIYF]2.0.CO;2

Post, J. R., Parkinson, E. A., & Johnston, N. T. (1999). Density-dependent orocesses in structured fish populations: Interaction strengths in whole-lake experiments. Ecological Monographs, 69(2), 155–175. https://doi.org/10.2307/2657235

Prévost, C., Sharp, M. E., Kory, N., Lin, Q., Voth, G. A., Farese, R. V., & Walther, T. C. (2018). Mechanism and determinants of amphipathic helix-containing protein targeting to lipid droplets. Developmental Cell, 44(1), 73-86.e4. https://doi.org/10.1016/j.devcel.2017.12.011

Prokkola, J. M., Åsheim, E. R., Morozov, S., Bangura, P., Erkinaro, J., Ruokolainen, A., Primmer, C. R., & Aykanat, T. (2022). Genetic coupling of life-history and aerobic performance in Atlantic salmon. Proceedings of the Royal Society B: Biological Sciences, 289(1967). https://doi.org/10.1098/rspb.2021.2500

Rey, F., Melo, T., Lopes, D., Couto, D., Marques, F., & Domingues, M. R. (2022). Applications of lipidomics in marine organisms: Progress, challenges and future perspectives. Molecular Omics, 18(5), 357–386. https://doi.org/10.1039/D2MO00012A

Reznick, D., Bryant, M., & Holmes, D. (2006). The evolution of senescence and post-reproductive lifespan in guppies (Poecilia reticulata). PLOS Biology, 4(1), e7. https://doi.org/10.1371/journal.pbio.0040007

Rinke, K., Hu, S., & Mooij, W. M. (2008). Energetic costs , underlying resource allocation patterns , and adaptive value of predator-induced life-history shifts. Oikos, 117, 273–285. https://doi.org/10.1111/j.2007.0030-1299.16099.x

Rowe, D. K., Thorpe, J. E., & Shanks, A. M. (1991). Role of fat stores in the maturation of male Atlantic salmon (Salmo salar) parr. Canadian Journal of Fisheries and Aquatic Sciences, 48, 405–413.

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S., & Hickling, G. J. (2005). Restitution of mass–size residuals: Validating body condition indices. Ecology, 86(1), 155–163. https://doi.org/10.1890/04-0232

Scott, D. E., Casey, E. D., Donovan, M. F., & Lynch, T. K. (2007). Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia, 153(3), 521–532. https://doi.org/10.1007/s00442-007-0755-6

Shalitin, S., & Phillip, M. (2003). Role of obesity and leptin in the pubertal process and pubertal growth F a review. International Journal of Obesity, 27, 869–874. https://doi.org/10.1038/sj.ijo.0802328

Sheridan, M. A. (1988). Lipid dynamics in fish: Aspects of absorption, transportation, deposition and mobilization. Comparative Biochemistry and Physiology Part B: ComparativeBiochemistry, 90(4), 679–690. https://doi.org/10.1016/0305-0491(88)90322-7

Sheridan, M. A. (1989). Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture, 82, 191–203.

Simpson, A. L. (1992). Differences in body size and lipid reserves between maturing and nonmaturing Atlantic salmon parr, Salmo salar L. Canadian Journal of Zoology, 70 (9),

Sinclair-Waters, M., Nome, T., Wang, J., Lien, S., Kent, M. P., Sægrov, H., Florø-Larsen, B., Bolstad, G. H., Primmer, C. R., & Barson, N. J. (2022). Dissecting the loci underlying maturation timing in Atlantic salmon using haplotype and multi-SNP based association methods. Heredity, 129(6), Article 6. https://doi.org/10.1038/s41437-022-00570-w

Sinclair-Waters, M., Piavchenko, N., Ruokolainen, A., Aykanat, T., Erkinaro, J., & Primmer, C. R. (2022). Refining the genomic location of single nucleotide polymorphism variation affecting Atlantic salmon maturation timing at a key large-effect locus. Molecular Ecology, 31(2), 562–570. https://doi.org/10.1111/mec.16256

Sissener, N. H., Torstensen, B. E., Owen, M. A. G., Liland, N. S., Stubhaug, I., & Rosenlund, G. (2017). Temperature modulates liver lipid accumulation in Atlantic salmon (Salmo salar L.) fed low dietary levels of long-chain n-3 fatty acids. Aquaculture Nutrition, 23(4), 865–878. https://doi.org/10.1111/anu.12453

Sogard, S. M., & Spencer, M. L. (2004). Energy allocation in juvenile sablefish: Effects of temperature, ration and body size. Journal of Fish Biology, 64(3), 726–738. https://doi.org/10.1111/j.1095-8649.2004.00342.x

Storebakken, T., & Austreng, E. (1987). Ration level for salmonids I. growth, survival, body composition, and feed conversion in Atlantic salmon fry and fingerlings. Aquaculture, 60, 189–206.

Sutton, S. G., Bult, T. P., & Haedrich, R. L. (2000). Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society, 129(2), 527–538. https://doi.org/10.1577/1548-8659(2000)129<0527:rafwbw>2.0.co;2

Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F. A., Dufour, S., Karlsen, Ø., Norberg, B., Andersson, E., & Hansen, T. (2010). Control of puberty in farmed fish. General and Comparative Endocrinology, 165(3), 483–515. https://doi.org/10.1016/j.ygcen.2009.05.004

Thorpe, J.E., 1986. Age at first maturity in Atlantic salmon, Salmo salar: freshwater period influences and conflicts with smolting. Canadian Special Publication of Fisheries and Aquatic Sciences, 89, 7–14.

Thorpe, J. E. (2007). Maturation responses of salmonids to changing developmental opportunities. Marine Ecology Progress Series, 335(1983), 285–288. https://doi.org/10.3354/meps335285

Thorpe, J. E., Mangel, M., Metcalfe, N. B., & Huntingford, F. A. (1998). Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, Salmo salar L. Evolutionary Ecology, 12(5), 581–599. https://doi.org/10.1023/A:1022351814644

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science, 11(2), 107–184. https://doi.org/10.1080/713610925

Tu, W., Wagner, E. K., Eckert, G. J., Yu, Z., Hannon, T., Pratt, J. H., & He, C. (2015). Associations Between menarche-related genetic variants and pubertal growth in male and female adolescents. Journal of Adolescent Health, 56(1), 66–72. https://doi.org/10.1016/j.jadohealth.2014.07.020

Twining, C. W., Bernhardt, J. R., Derry, A. M., Hudson, C. M., Ishikawa, A., Kabeya, N., Kainz, M. J., Kitano, J., Kowarik, C., Ladd, S. N., Leal, M. C., Scharnweber, K., Shipley, J. R., & Matthews, B. (2021). The evolutionary ecology of fatty-acid variation: Implications for consumer adaptation and diversification. Ecology Letters, 24(8), 1709–1731. https://doi.org/10.1111/ele.13771

Varpe, Ø., Jørgensen, C., Tarling, G. A., & Fiksen, Ø. (2009). The adaptive value of energy storage and capital breeding in seasonal environments. Oikos, 118(3), 363–370.

Wang, T., Hung, C. C. Y., & Randall, D. J. (2006). The comparative physiology of food deprivation: From feast to famine. Annual Review of Physiology, 68(1), 223–251. https://doi.org/10.1146/annurev.physiol.68.040104.105739

Weihe, R., Dessen, J. E., Arge, R., Thomassen, M. S., Hatlen, B., & Rørvik, K. A. (2019). Increased protein-to-lipid ratio in energy dense diets improves slaughter yields and muscle thickness of different weight classes of farmed Atlantic salmon (Salmo salar L.). Aquaculture Reports, 13(September 2018), 100173. https://doi.org/10.1016/j.aqrep.2018.10.001

Whalen, K. G., & Parrish, D. L. (1999). Effect of maturation on parr growth and smolt recruitment of Atlantic salmon. Canadian Journal of Fisheries and Aquatic Sciences, 56, 79–86.

Wieser, W. (1991). Limitations of energy acquisition and energy use in small poikilotherms: Evolutionary implications. Functional Ecology, 5(2), 234–240. https://doi.org/10.2307/2389261

Yeo, J., & Parrish, C. C. (2022). Mass spectrometry-based lipidomics in the characterization of individual triacylglycerol (TAG) and phospholipid (PL) species from marine sources and their beneficial health effects. Reviews in Fisheries Science & Aquaculture, 30(1), 81–100. https://doi.org/10.1080/23308249.2021.1897968

Zhou, S., Ackman, R. G., & Morrison, C. (1996). Adipocytes and lipid distribution in the muscle tissue of Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences, 53, 326–332.