Norwegian University of Life Sciences

Master's Thesis 2022 60 ECTS
Department of Animal and Aquaculture Sciences (Arial, regular, 10pt)

Fish welfare and color of skin and fillets of Atlantic Salmon from two genetic lines exposed to repeated hypoxia prior to harvesting

Marte Røsvik
Master of Science in Aquaculture

Fish welfare and color of skin and fillets of Atlantic Salmon from two genetic lines exposed to repeated hypoxia prior to harvesting

Master thesis in aquaculture, 60 credits

by

Marte Røsvik

Supervisors

Dr. Turid Mørkøre

Dr. Dag Inge Våge

Dr. Jacob Seilø Torgersen

Department of Animal and Aquaculture Sciences

The Norwegian University of Life Sciences (NMBU)

Ås, July 2022

Table of contents

Acknowledgement

This thesis was done at Norwegian University of Life Sciences (NMBU), faculty of Animal and Aquacultural Sciences (IHA), in cooperation with AquaGen.

This thesis was funded by the Norwegian University of Life Sciences, the breeding company AquaGen and FHF (Norwegian seafood research fund). AquaGen also provided fish material.

I would first like to thank my main supervisor, Dr. Turid Mørkøre, who has spent her time guiding me to the finish line. Additionally, I would like to thank her for helping me with statistical analyses and for teaching me the ins and outs of practical work in the field.

I also want to say thank you to Turid Mørkøre for including me in the project, Rød laks – genetiske effekter (project number 901642). It has been a rewarding and educational experience with field work and very varied tasks.

I would also like to thank my co-supervisors, Dr. Dag Inge Våge and Dr. Jacob Seilø Torgersen. Dag Inge Våge who was project leader, and Jacob Seilø Torgersen, who invited me along for laboratory work at the fish laboratory at NMBU.

Additionally, I would like to thank everyone at LetSea, especially Oddrun Elise Olsen, for a welcoming and enjoyable experience at their research facility at Dønna.

Lastly, I would like to thank my friends and family for love and support during the writing of this thesis, and for inspiring me and encouraging me through the two years of my master in aquaculture at NMBU.

Ås, July 2022

Marte Røsvik

Abstract

Oxygen is a fundamental necessity for the fitness and survival of Atlantic salmon. Hypoxia is reduced access to oxygen and low levels of oxygen in body tissues, which affects the welfare, immune response, and product quality of Atlantic salmon. The severity of hypoxia is dependent on exposure time, the size of the fish, life stage and genetics. In the present study, two genetic lines that were selected for fillet color alone (RED and PALE), were exposed to repeated hypoxia (one, two or three times) and the effects on biometric parameters, welfare, skin color, fillet color and fillet quality were evaluated.

The body weight, condition factor, and fillet yield of the PALE genetic line was higher compared to the RED genetic line, and hypoxia had no effect on these biometric parameters. The cardiac somatic index of the RED genetic line was higher compared to the PALE genetic line, and hypoxia had no effect. The operational welfare indicators were all affected by exposure to repeated hypoxia. Most of the indicators were not affected by genetics, however the snout damage score was higher for the RED genetic line compared to the PALE genetic line. The fillet color was redder and the astaxanthin content was higher for the RED genetic line compared to the PALE genetic line, but hypoxia did not negatively affect the fillet color. The skin of NQC got darker and less blue with exposure to repeated hypoxia. The skin color of anterior fillets was not affected by hypoxia. The fillet texture got softer as the fish were exposed to repeated hypoxia.

Genetics affect color and biometric traits, and hypoxia negatively affects welfare and darkens the skin color of NQC. There needs to be more research that covers the interaction between heart health and astaxanthin retention. In conclusion, further research is needed to look at the performance and mechanisms behind the differences between the two genetic lines. The astaxanthin retention and disposition could be genetically linked to biometric traits and heart health and could be a potential topic suitable for further research.

Sammendrag

Oksygen er essensielt for Atlantisk laks sin velferd og overlevelse. Hypoksi er redusert tilgang til oksygen og lave nivåer av oksygen i kroppsvev, som påvirker velferd, immunrespons og produktkvalitet hos Atlantisk laks. Alvorlighetsgraden av hypoksi varierer med hvor lenge fisken er eksponert, fiskestørrelse, livsstadiet og genetikk. I denne studien ble to genetisk ulike linjer

som var selektert kun for farge (RED og PALE), eksponert for hypoksi (en, to eller tre ganger) og effekten på biometriske parameter, velferd, hud og fillet farge, og fillet kvalitet ble evaluert.

Kroppsvekten, filletvekten, kondisjonsfaktor, filletutbytte og kardiosomatisk indeks for den bleke genetiske linjen (PALE/BLEK) var høyere sammenlignet med den røde genetiske linjen (RØD/RED), og hypoksi hadde ingen effekt på de biometriske parameterne. Alle de operative velferdsindikatorene ble påvirket av eksponering av gjentakende hypoksi. De fleste operative velferds indikatorene ble ikke påvirket av genetikk, bortsett fra den RØDE genetiske linjen som hadde høyere skåring på snuteskade sammenlignet med den BLEKE genetiske linjen. Fillet fargen var rødere for den RØDE linjen sammenlignet med den BLEKE linjen. Skinnfargen på NQC ble mørkere og mindre blå med mer eksponering for hypoksi. Skinnfargen på den fremre fileten ble ikke påvirket av hypoksi. Filetteksturen ble mykere ved eksponering av gjentakende hypoksi.

Genetikken påvirket både filetfarge og biometriske egenskaper, og hypoksi påvirket velferd negativt og gjorde skinnfargen mørkere på NQC. Det trengs videre forskning som dekker interaksjon mellom hjerte og astaxanthin retensjon. Konklusjonen er at videre forskning er nødvendig for å finne mekanismene som ligger til grunn for forskjellene i prestasjon mellom de to linjene. Astaxanthin retensjon og disposisjon kan være genetisk koblet til biometriske egenskaper og hjerte helse, og dette kan være et potensielt tema for videre forskning.

1. Introduction

Atlantic salmon (*Salmo Salar*) is a fish that is popular for its red fillet and versatile use in cooking. Salmon is the largest fish species commodity by value, and Atlantic salmon production has had the highest growth both in export revenue and technological advancements (FAO, 2020). Because of biological and regulatory restraints, the price of Atlantic salmon has been pushed higher because of high demand in the market. The world production of Atlantic salmon has increased from approximately 1 437 000 tons in 2010 to approximately 2 435 000 tons in 2018 (FAO, 2020). Increased prices and production reflect an increase in demand of Atlantic salmon in the world market.

Atlantic salmon in aquaculture production are throughout their lives exposed to different kinds of stress. Handling should be kept to a minimum because stress affects the welfare, metabolic effect, and production value of Atlantic salmon. Fish can experience stress with all handling such as crowding, vaccination, delousing, and transport. During handling fish can experience hypoxia, however hypoxia can also occur with high temperatures during summer and early autumn (Remen et al., 2013).

Salmon is dependent on oxygen for all activities such as digestion, swimming, and growth, and all these activities are driven by energy (ATP) that is generated through metabolism, which is dependent on oxygen (aerobic) (Noble et al., 2018). Hypoxia is reduced availability, or no access to oxygen. Hypoxia can be fatal if the fish is being exposed over a longer period. If fish is exposed to hypoxia over short periods it results in physical discomfort and leads to gasping and jumping behavior because the fish is desperate for oxygen. Hypoxia can also lead to reduced welfare because it can result in reduced immune response, as well as it can affect seawater tolerance, growth and worst case increase the mortality rate in a population (Einarsdóttir et al., 2000; Iversen et al., 2005). There is therefore an interest in finding the effects of hypoxia on welfare and quality, and to see if some there is a way to reduce these effects through genetics.

The aim of this thesis is to study effects of low oxygen exposure (hypoxia) on fillet quality and fish welfare of Atlantic salmon selected for high or low fillet color intensity.

2. Literature review

2.1. Biometric traits

Harvest parameters are important parts of a breeding program and the economic value of the product. These traits have heritability and breeding values that can be used to optimize the economic outcome for the salmon companies. However, a lot of the traits are closely related and therefore prioritization needs to be closely considered in a breeding program. Traits like body weight, gutted weight and fillet weight have heritability around 0.45 – 0.50 h^2 (Garber et al., 2019; Powell et al., 2008) compared to calculated parameters such as fillet yield and gutted yield, which have low heritability ranging from 0.02 – 0.27 h^2 (Garber et al., 2019; Powell et al., 2008).

The body weight is the weight of the whole fish, measured in kilograms or grams. Body weight and growth rate are traits that are very important in breeding programs for Atlantic salmon. The body weight of harvest ready Atlantic salmon is typically 5 – 6 kg (Garber et al., 2019). The growth of farm raised Atlantic salmon is much more rapid compared to wild salmon and is usually harvest ready after 2 – 3 years (Powell et al., 2008; Quinton et al., 2005), which depends on the genetics as well as environmental effects such as temperature, light, stress and feeding routines. The heritability of body weight is estimated to be around 0.50 h^2 (Powell et al., 2008; Tsai et al., 2015), and heritability of growth rate ranges from 0.35 – 0.40 h^2 (Sae-Lim et al.).

The gutted weight is the weight of the fish where the inner organs have been removed and it is measured in kilograms or grams. The gutted weight gives information about the guts and the rest of the carcass. If the gutted weight is much lower compared to the body weight, that could indicate there is accumulation of fat around the intestines, which is not a desirable trait. The gutted weight of harvest ready salmon is normally about 3 – 5 kg. The gutted yield represents the ratio between the gutted weight and the body weight and is normally around 90% of the body weight (Powell et al., 2008; Tsai et al., 2015). The heritability of gutted weight is around 0.50 h^2 (Powell et al., 2008).

Condition factor is the ratio between body weight and length and is a measurement of the conformation of the fish. The condition factor is an important economic trait that is normally around 1.20 – 1.40 (Mørkøre et al., 2020) and has a heritability ranging from 0.40 - 0.50 h^2 in salmonoid fishes (Garber et al., 2019; Robinson et al., 2008).

The fillet weight is one of the more important traits in commercial aquaculture. High quality fillets are sold for human consumption and are sold per kilogram. The fillet weight is about 2 - 4 kg (about 60 - 70% of the body weight) (Mørkøre et al., 2020; Powell et al., 2008) and is the weight of the fish where guts, head, spine, tail, and all trimmings have been removed. It is common to leave the skin on the fillet. The fillet yield is an important trait for the salmon farmer because fillets are the edible and best paid part of the fish. Fillet yield is also an indirect measurement of how much energy the salmon uses on growing muscle compared to gonads or fat accumulation around intestines.

Cardio somatic index (CSI) is the ratio between heart weight and body weight and is normally around 0.1% of the body weight. The CSI is important for normal heart function, and therefore also the welfare of the fish. Irregularities of the heart can cause weaker contractions of the heart muscle and an increased risk of mortality (Frisk et al., 2020). Stress affects the heart function of salmonoids, and increased levels of cortisol positively correlates with increased CSI (Johansen et al., 2011). This increase in heart size causes a reduction of maximum stroke volume and cardiac output, which reduces the overall oxygen-transport capacity (Johansen et al., 2017).

2.2. Operational welfare indicators

Animal welfare is simply defined as the quality of life perceived by the animal itself. This means that to maintain good animal welfare it is important that the animal is well fed, is not in pain and can live a life as close to their natural environment as possible. The individual need of the animal can be divided into two categories, ultimate and proximate needs. The ultimate needs are the requirements to keep the animal alive, while the proximate needs are not necessarily important for survival but increase the ability to fulfill the ultimate needs (Noble et al., 2018).

Fish collect information about their surroundings using an array of sensory properties. There is large variation of sensory traits and perception of the surroundings for different fish species that specialize them to their environment. To survive it is necessary to be able to remember and learn from earlier experiences, this to be able to execute actions in reaction to an event (Noble et al., 2018). It is therefore important that the fish is healthy and can operate under conditions that are as natural as possible.

The emotional reward system generates feelings that lead the animal's behavior to fulfill its needs. However, in captivity fish have more difficulties fulfilling its needs independently, and the farmers have a responsibility to uphold the welfare of the fish. It is not possible to ask the fish how it is feeling, which is why welfare indicators are used. There are environmental and animal factors that can give an indication to the welfare of the fish. Environmental welfare indicators are the measurement of environmental factors that affect the welfare of the fish, for example temperature and oxygen saturation. Animal welfare indicators are properties of the individual fish that display the level of welfare, for example condition factor and growth rate. It is also possible to determine animal welfare indicators on behavior, like swimming behavior and gill movement frequency. Animal welfare indicators are usually the result of reduced welfare and are only visible after the problem has occurred. Therefore, reduced individual welfare can indicate that the welfare in the population is reduced as well. The welfare indicators (WI) that determine the welfare of an individual or a population are operative welfare indicators (OWI) and laboratory welfare indicators (LABWI). OWI's are used to document or evaluate welfare in the daily operations of the fish farm. WI's that has to be sent to a laboratory for evaluation are LABWI's (Noble et al., 2018).

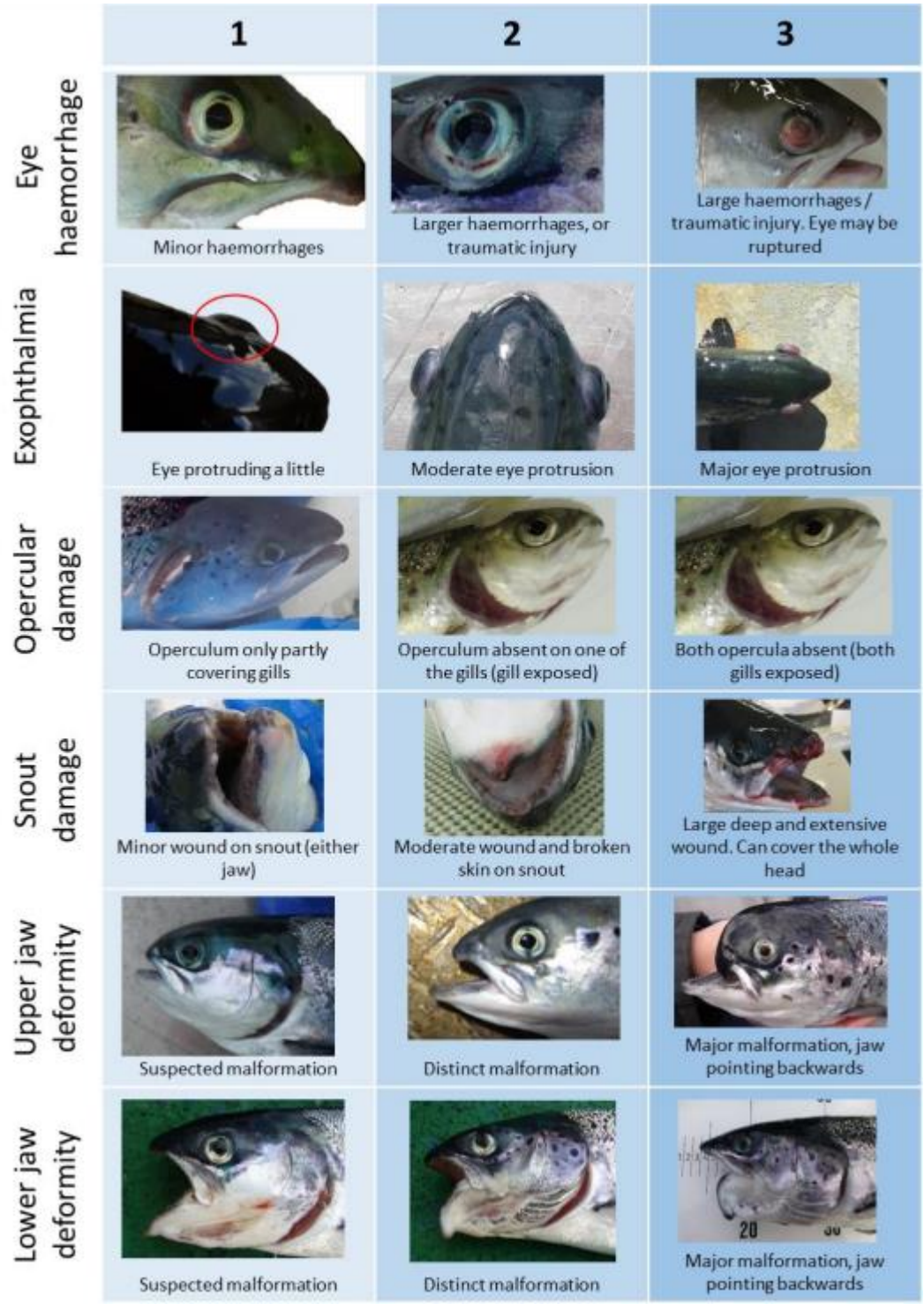


Figure 1. Operational welfare indicators. Picture analysis scoring system of eye hemorrhage, exophthalmia (protruding eyes), opercular damage, snout damage, upper and lower jaw deformities (Noble et al., 2018).

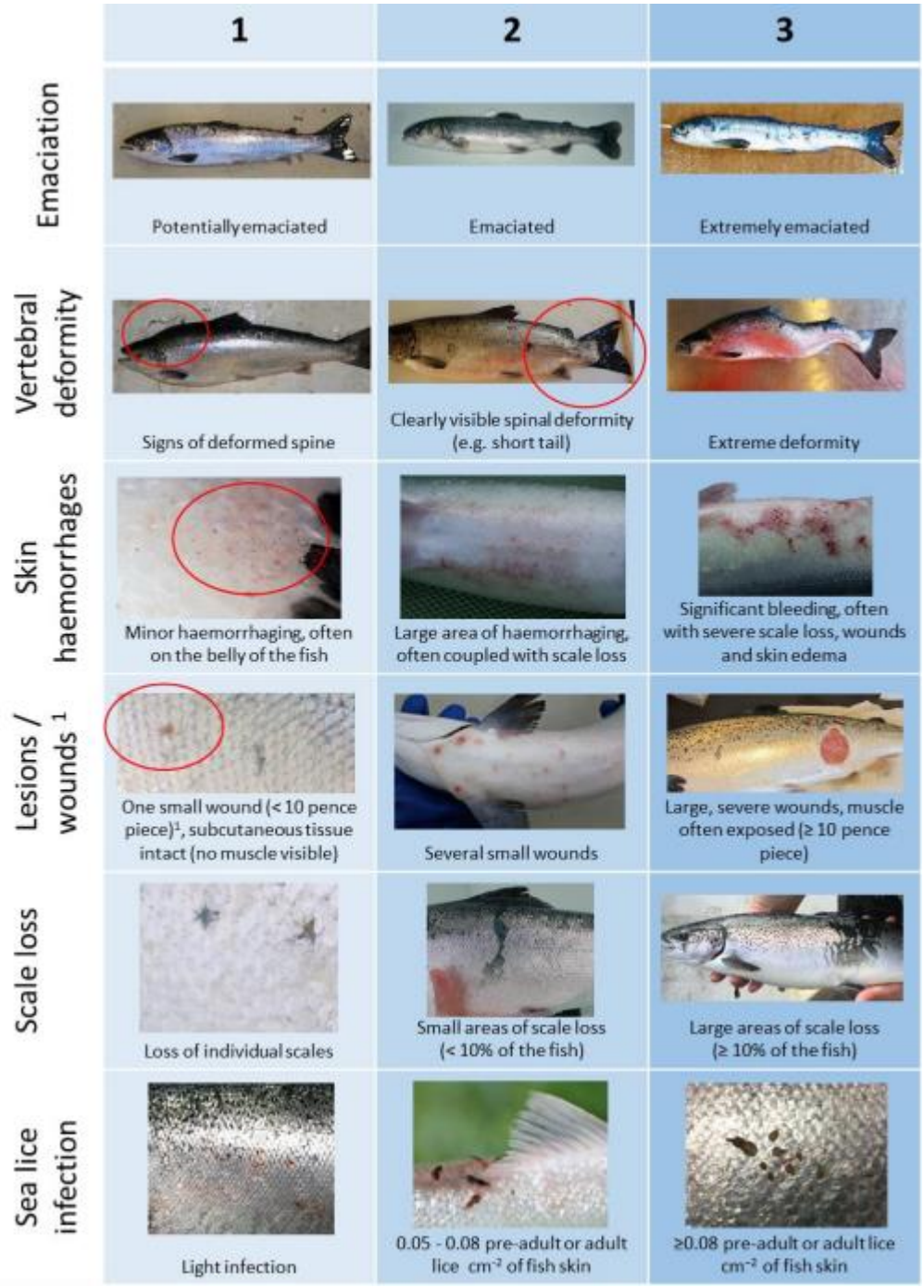


Figure 2. Operational welfare indicators. Picture analysis scoring system of emaciation, vertebral deformities, skin hemorrhages, lesions/wounds, scale loss and sea lice infection (Noble et al., 2018).

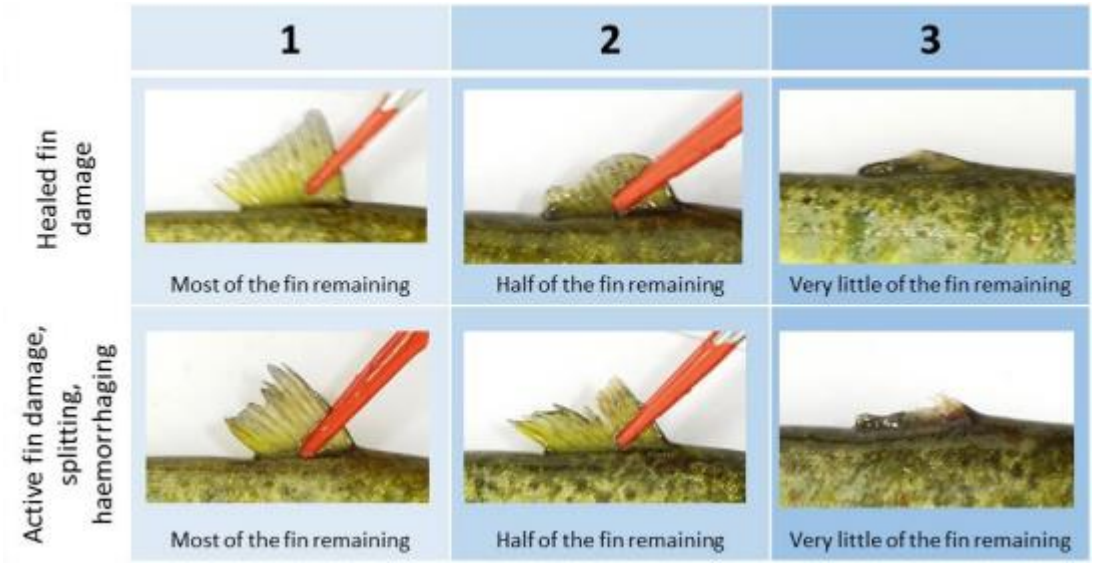


Figure 3. Operational welfare indicators. Picture analysis scoring system of healed and active fin damage (Noble et al., 2018).

The operational welfare indicators are scored on a scale ranging from 0 – 3, where 0 is no observation and 3 is severe. This scoring system was developed to standardize the welfare scoring to be used in the industry and for research (Figure 1, 2 and 3). Eye lens opacity is scored on a scale from 0 – 5, where 0 is a clear and transparent eye lens, while 5 is an eye with opacity that cover over 75% of the lens diameter.

The scales, skin and slime on the fish are their first barrier against infections, and scale loss can lead to a reduced immune defense, hygiene, and difficulties with osmoregulation. This can furthermore lead to possible pain and infections. Skin hemorrhages or skin bleeding are red spots or red flushing under the skin and is epidermal damage that cause pain for the fish. Skin bleeding is usually visible on the belly of the fish, where the skin is lighter. Snout damage can range from minor wounds to severe damage to the whole head and can be painful for the fish. Snout damage can be a sign of aggression in the sea cage or occur during crowding or handling. Jaw deformities can affect the swimming behavior of the fish, as well as the oxygen consumption of the fish (Lijalad & Powell, 2009). Eggs incubated in higher water temperatures (> 8 °C) increase the risk of jaw deformities as the fish grows up. Fin damage can occur with aggression or with handling, and it affects the welfare of the fish. Not only are there pain receptors in fin tissue, but damage or open wounds on fins can cause infections and osmotic imbalance. Loss of fins can also cause problems for swimming behavior in Atlantic salmon. The eyes of the fish are vulnerable to damage, as fish does not have eye lids and the eyes stick slightly out from the skull. A somewhat

common problem in rearing of carnivore fish is “eye-snapping”, which is when fish bite or snap at the eyes of other fish. This can cause bleeding in the eyes or puncturing of the eye lens. Damage to the eyes or increased eye lens opacity reduces the eyesight of the fish and in worst case scenarios eye damage can cause blindness. A fish with negatively affected eyesight will have a reduced ability to feed, grow and has an increased susceptibility to infections and diseases (Noble et al., 2018).

The operational welfare indicators were made to more accurately evaluate the welfare of the fish. The welfare is highly affected by physical damage that can occur with aggression in the net pen and handling, as well as poor water quality and high or low temperatures can also worsen the state of the fish welfare (Noble et al., 2018). It is therefore important to avoid these stressors that leads to lessened welfare and increased risk of mortality.

2.3. Fillet color

Salmon is known for its pink-red flesh. If a fillet is pale, it is less appealing to the consumer and a very pale fillet will be downgraded and sold as cheaper products. Salmon is not naturally red and cannot synthesize carotenoids that give the red pigment, but the flesh becomes red through its diet (Rajasingh et al., 2006). In the wild salmon consume crustaceans that contain carotenoids like astaxanthin and cantastaxanthin, which the crustaceans have consumed through microalgae. However, microalgae are expensive, and harvest of crustaceans is not sustainable to use in the aquaculture industry. Therefore, artificially synthesized astaxanthin is commonly used in aquaculture (Lorenz & Cysewski, 2000).

Carotenoids is a chemical group consisting of more than 1100 pigments that are synthesized in plants, bacteria, algae and fungi (Zia-Ul-Haq et al., 2021). Carotenoids are efficient antioxidants and has health benefits such as cardiovascular disease prevention, immune system improvement and cataract prevention (Higuera-Ciapara et al., 2006). The carotenoids are divided in two groups, carotenes and xanthophyll. Carotenes are hydrocarbons, and xanthophyll is oxygenated and derives from carotenes (Zia-Ul-Haq et al., 2021).

2.3.1. Astaxanthin

Astaxanthin is a carotenoid in the family of xanthophyll and is widely used in feed for Atlantic salmon. This pigment gives a red-orange color to the muscle of Atlantic salmon (Higuera-Ciapara et al., 2006; Rajasingh et al., 2007). Astaxanthin used in salmon feed is mostly artificially synthesized because the process is cheap and efficient, compared to using natural resources like algae and crustaceans. Astaxanthin is a fat-soluble molecule that together with lipoprotein passively diffuse through the intestinal wall (Parker, 1996; Seabra & Pedrosa, 2010).

Carotenoids can exist in different configurations, and carotenoids in nature most commonly are stable trans isomers. There are three configurations of stereoisomers of astaxanthin, a pair of enantiomers (3R,3'R- and 3S,3'S-astaxanthin) and a mesoform (3R,3'S- astaxanthin). 3S,3'S-astaxanthin is the most common isomer in nature. Synthetic astaxanthin, which is most common in the aquaculture industry, consist of a 1:2:1 ratio of 3R,3'R, 3R,3'S and 3S,3'S astaxanthin isomers, respectively (Bjerkeng & Berge, 2000; Seabra & Pedrosa, 2010; Wang et al., 2008).

Astaxanthin is a hydrophobic molecule, same as lipids. The metabolism of carotenoids is therefore closely related to the metabolism of fat, cholesterol and other hydrophobic molecules. About 30-50% of ingested astaxanthin is digestible, however the retention of astaxanthin is usually less than 12% of the intake (Bjerkeng & Berge, 2000; Xu & Ding, 2004). This means that astaxanthin used for muscle pigmentation is limited by other metabolic transformations of astaxanthin. The retention is also species specific. In Atlantic salmon 7.13% of astaxanthin is found in bile and 10.68% is found in liver, muscle and skin (Xu & Ding, 2004).

Astaxanthin also functions as an antioxidant because of the molecular structure. Astaxanthin has hydroxyl- and keto-groups which gives the molecule the ability to be esterified and high electron-donating ability, that can stabilize and terminating free radical chain reactions (Lim et al., 2018). Astaxanthin have shown to increase stress tolerance and increase disease resistance in several aquatic animals, such as rainbow trout and crustaceans (Amar et al., 2001; Angeles Jr et al., 2009), and is therefore a great supplement to add to feed for Atlantic salmon.

The retention and content of astaxanthin depends on the diet (Bjerkeng et al., 1997) and oxidative stress is believed to mobilize astaxanthin to the muscle (Nordgarden et al., 2003). The astaxanthin content in wild Atlantic salmon is 0.61mg/100g. In cultured Atlantic salmon the astaxanthin content depends on the diet, however, salmon fed on synthetic astaxanthin show

lower concentrations (0.20mg/100g) compared to salmon fed on astaxanthin from natural sources like the yeast *Phaffia rhodozyma* (0.26mg/100g) (Bjerkeng et al., 2007). Uptake and retention of astaxanthin can have variations depending on the stereoisomer, concentration of astaxanthin in the diet and fat in the diet. Astaxanthin metabolism is closely related to fatty acid metabolism due to astaxanthins hydrophobic nature (Rajasingh et al., 2006). Uptake and retention of astaxanthin is greater in diets with higher fat content, which implies that fat is important for carotenoid absorption (Bjerkeng et al., 1997).

2.3.2. Visual and colorimetric evaluation methods

Since color is such an important quality trait of Atlantic salmon it is of interest to have a means of measuring the color for breeding programs and feed research alike. One of the methods is visual color measuring, which is commonly used. The fillet color is then measured under standardized light conditions and a DSM SalmoFan^{TM} color scale that ranges from 20 – 34 that is a range of the redness of fillets. Another method is a Minolta chroma meter that is a handheld machine that measure CIE L*a*b*, which is a color system that represents the lightness (L*) and the color hue (a* and b*) of an object. The L* value ranges from 0 – 100, where 0 is black and 100 is white. The a* value ranges from (-120) – 120, and represent a color scale from green to red. The b* value ranges from (-120) – 120 and represents a color scale range from blue to yellow (Erikson & Misimi, 2008). The Minolta chroma meter is a more objective measurement of color compared to SalmoFan, which can be subjectively influenced by perception of color.

2.3.3. Genetics and color

In aquatic species a selection strategy based on individuals and family is commonly used. Atlantic salmon females have about 15 000 offspring which makes it possible to perform this strategy efficiently. When doing family-based selection the heritability value can get as high as 0.71 for full-siblings and up to 0.50 for half-siblings. This is because full-siblings on average share 50% of their gene alleles, and half-siblings on average share 25% of their gene alleles (Gjedrem & Baranski, 2010). Family-based selection is used for traits that have low heritability and for traits like fillet quality where the fish needs to be slaughtered for evaluation. In individual selection phenotypical traits like weight and length are used. The combination of individual and

family-based selection gives high accuracy and genetic gain in the population, also for traits with low heritability. The phenotypic trait of fillet color can only be measured on slaughtered fish, which makes fillet color a trait that need to be evaluated through family-based selection.

The color of Atlantic salmon also be affected by genetics and have varying heritability. For measurements done with SalmoFan color have been found to have a heritability of 0.45 h^2, and for measurements done with Minolta color have been found to have a heritability ranging from 0.42 – 0.58 h^2 (Garber et al., 2019). However, maturity, stress and fat can also affect the color of the fillet negatively, which leaves an environmental aspect that would need to be considered in a breeding program as well (Erikson & Misimi, 2008; Norris & Cunningham, 2004). It is therefore also preferable to select for leaner fish, and late set maturity.

2.4. Melanin

Melanin spots is one of the largest problems in the salmon aquaculture industry, along with pale fillet color. There are many theories of why melanin spots occur, but the main factors believed to be the cause of the issue are vaccination, stress and disease outbreaks (Färber, 2017). Melanin spots are dark spots in the fillet which reduce the quality of the product, which causes downgrading and potentially reduced economic gain and increased waste product. Melanin (absence) has a low heritability of 0.04 h^2 (Garber et al., 2019), which means that to reduce melanin, gentler handling and lowering the risk of disease outbreaks is a more effective solution.

2.5. Fillet quality

The quality of Atlantic salmon fillets is judged on color, flavor, fat, texture, and smell. Some important quality parameters are fat content, composition and distribution, color distribution and intensity, fillet texture and gaping (Sigurgisladottir et al., 1997).

2.5.1. Gaping

Gaping is the separation of the connective tissue between the muscles in raw fillets. This causes splits and holes that is undesirable in the market and lowers the quality of the fillet. Gaping often occurs in areas of the muscle close to the vertebra. Fillets that have a high degree of gaping are

more difficult to skin and cut, which downgrades the fillet to only be used in cheaper products (Pittman & Grigory, 2013). Pre-slaughter stress such as loading, transport and handling result in higher occurrence of gaping. Gaping also positively correlates with fish weight, and larger and faster growing fish tend to be more prone to higher gaping scores (Johnsen et al., 2013). There are different evaluation methods of gaping. One method, that was used in this thesis, is to slide a flat palm under the fillet and look for splits and holes. The fillet is scored on a scale from 0 – 5 on the number and size of the holes, if there are any, where 0 is no holes or splits and 5 is severe gaping where the fillet falls apart (Andersen et al., 1994). Gaping has a low heritability of only 0.15 h^2 (Garber et al., 2019).

2.5.2. Texture

A good quality fillet should have firm texture. Soft fillets are not appealing to the consumer and are therefore downgraded and sold as cheaper products. Stress affects the texture and produce a softer fillet (Sigholt et al., 1997), and higher expression of immune genes is documented in salmon with firmer fillets (Larsson et al., 2012). Higher collagen stability structure in the muscle also gives firmer texture (Moreno et al., 2012). Texture can be evaluated by using the "finger"-test where a finger is pressed down on the fillet with 1 kg pressure to see if the finger goes through the fillet, or if the muscle retracts back to its original state (Botta, 1989). There are other methods like this test, but a machine is used instead which is the same concept but is a more standardized method. Another method is to measure the resistance force or break point of the fillet, which require a machine equipped with a cylinder plunger (Einen et al., 2002). Texture has a low heritability of only 0.16 h^2 (Larsson et al., 2012).

3. Method and materials

The experiment consisted of two genetic lines of Atlantic salmon from AquaGen's selective breeding program, where one group was exclusively genetic selected for red flesh, disregarding all other traits (termed RED). The other genetic group was bred using parents with the lowest possible color score, such as parents with pale flesh (termed PALE).

3.1. Field work

The experiment was conducted at LetSea aquaculture research and research center, located in Dønna municipality in Helgeland (Figure 1). Fish were obtained as 750 PIT-tagged 120g smolts from AquaGen (Kyrkjesæterøra, Norway) September 9th 2020, and distributed into two net pens of 125m^3 (approximately equal number of each genetic line in each net pen). The fish were raised up to an average weight of four kilograms, fed on the same diet for 14 months. The feed used was a standard commercial feed (rapid 80 and rapid 200; Ewos).

Figure 4. Map of Norway. The pin marks the location of LetSea in the municipality of Dønna (Norgeskart).

August 19th 2021, the number of fish per net pen (net pen number 319 and 419) was reduced to 150 fish per net pen; 75 fish from each genetic line.

3.1.1. Stress test

The salmon in net pen number 419 were not disturbed from August 19th 2021 and onwards, while salmon in net pen number 319 were exposed to hypoxia stress three times (Table 1):

1) August 20th, 2021
2) September 29th, 2021
3) November 4th, 2021

The salmon were harvested November 14th – 15th 2021. Starvation time before harvesting was three days before each sampling and harvesting.

The groups are referred to as P0, R0 (control fish, non-stressed), P1, R1 (stressed one time), P2, R2 (stressed two times), P3 and R3 (stressed three times), where P is the PALE genetic line and R is the RED genetic line.

Table 1. Design set up. Stress group, date, sea-cage, and number of fish from each genetic group in each stress group.

	Stress 0 Sea-cage 419	Stress 1 August 20th, 2021 Sea-cage 319	Stress 2 September 20th, 2021 Sea-cage 319	Stress 3 November 4th, 2021 Sea-cage 319
Number of fish stressed	150 fish	150 salmon (0 fish not stressed)	100 salmon (50 fish not stressed)	50 salmon (100 fish not stressed)
Number of fish in each genetic group	Stress 0 Reduced to 25 RED (R0) + 25 PALE (P0)	Stress 1 25 RED (R0) + 25 PALE (P0)	Stress 2 25 RED (R0) + 25 PALE (P0)	Stress 3 25 RED (R0) + 25 PALE (P0)

3.1.2. Procedure, stress test

The first stress test was conducted on August 20th, 2021. All the fish in sea cage 319 were first crowded in a net to make it easier to catch the fish. Fifteen fish were moved from the sea cage to the tank with no oxygen supplied to the tank. Oxygen saturation was measured with a Handy Polaris 2 from OxyGuard. The stress inducing was hypoxia. This was done by filling a tank with 200 liters of seawater with an oxygen saturation estimated to be around 90%. After around 15 - 20 minutes (Table 2) the oxygen-level was reduced to 35% oxygen saturation and the fish were moved over to another tank containing anesthetic (FINQUEL, trikainmesilat) to calm them down, for about one minute. The pit-tag was registered, and the length and weight were measured of the anesthetized fish before they were sent back to the sea cage through a waterfilled pipe. The process was repeated for all 150 fish. The same process was done on the second stress test the

29th of September with 100 fish and third stress test the 4th of November 2021 with 50 fish. This gave 50 fish in each stress group (fish stressed once, twice and three times) (Table 1).

The fish behavior, gasping and jumping, were also registered in the tank during the exposure to hypoxia. This was done in one tank at the time before they were transferred to the tank containing the anesthetic.

Table 2. O_2 range and the number of time gasping, and jumping were registered (means) and the means of time the O_2-level was reached.

O_2 saturation (%)	Gasping	Jumping	Time
90 – 100			
80 – 90			00:01:30
70 – 80	1.3		00:02:50
60 – 70	5.1	2.3	00:04:43
50 – 60	10.8	2.4	00:07:07
40 – 50	26.0	6.5	00:10:03
35 – 40	18.0	7.3	00:13:52
35 (end)			00:16:55

3.1.3. Harvesting

November 14th and 15th 2021 the fish were slaughtered (anaesthetized to death using FINQUEL, trikainmesilat). Thereafter the fish were moved to a separate bleed-out tank after the gills were cut. Pit tag, round weight and length were registered, then a picture of the fish's left side was taken under standardized light condition for later welfare scoring, before the guts were welfare scored. The heart fat and condition, visible visceral fat, liver color and eyes were scored. Then the fish was filleted by hand, and the left fillet was weighed and tagged, before being put on ice for transport to Norwegian University of Life Sciences (NMBU).

3.2. Analysis

Morphological welfare indicators from FishWell (Noble et al., 2018) was used to evaluate the welfare of the fish that went through the stress test and the control fish, based on the pictures taken at LetSea (Figure 5). The scoring system goes from score 0 to 3, where score 0 is low or no sign of a negative welfare indicator and score 1 to 3 is a gradually worse welfare indicator. The welfare indicators that were evaluated included skin bleeding, wounds, scale loss, eye bleeding,

protruding eyes, eye lens opacity, shortened opercular, snout damage, spine deformities, upper and lower jaw deformities, sea lice infection, healed fin damage and active fin damage. Scoring of emaciation was not used, because condition factor is a more precise measurement. Not all welfare indicators were used for further analysis, but indicators that were used included skin bleeding, scale loss, snout damage, upper jaw deformities, active and healed fin damage, eye bleeding and eye lens opacity.

Figure 5. Example of pictures taken of the side and front of the fish. Pictures were used for scoring of operational welfare indicators.

The fillets were analyzed at NMBU for quality parameters. Color was registered with Minolta chroma meter CR-400 slightly above the lateral line in both the anterior cut and the Norwegian quality cut (Figure 6). The visual color of the fillet was measured inside a box with standardized light conditions, using a DSM SalmoFan^{TM} color score (20-34) (DSM). Texture of the fillet was measured by adding one kilo pressure at one spot on the fillet with a finger (Figure 6) and removing the finger to see if the muscle goes back to normal. The texture is scored on a scale from 0 to 5, where score 0 is soft fillet texture and 5 is firm fillet texture (Botta, 1989; Erikson et al., 2009; Mørkøre et al., 2009).

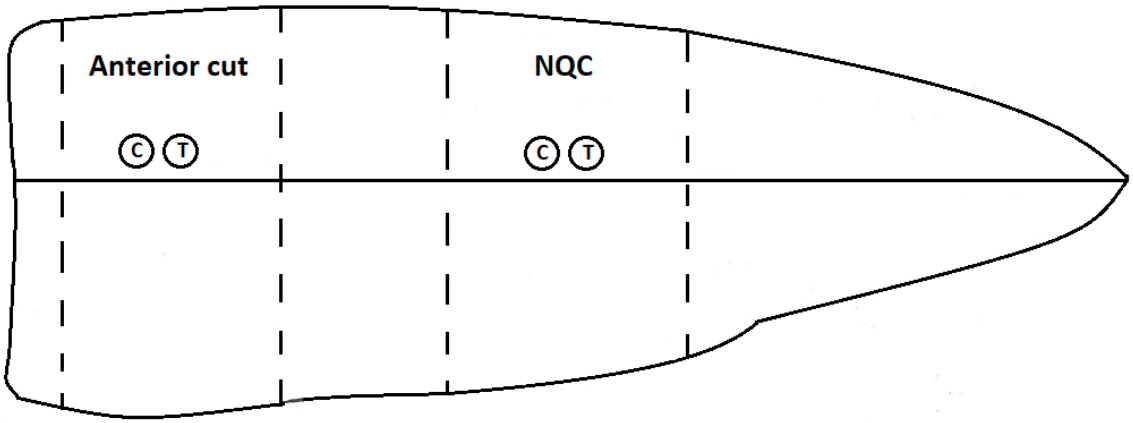


Figure 6. Fillet side of Atlantic salmon. Anterior and Norwegian quality cut (NQC) fillets are marked with the vertical dotted lines. C is where Minolta and Salmofan was measured, T is where texture was measured. The horizontal line represents the lateral line.

Gaping was scored by pulling the hand flat under the fillet to look for holes and splits (score 0-5; where 0 is no gaping and score 5 is extreme gaping) (Andersen et al., 1994). Melanin spots were also registered, and was scored on a scale from 0-8, where 0 is no melanin and 8 is discoloration (melanin spots) on an area larger than 6 cm (Mørkøre, 2012).

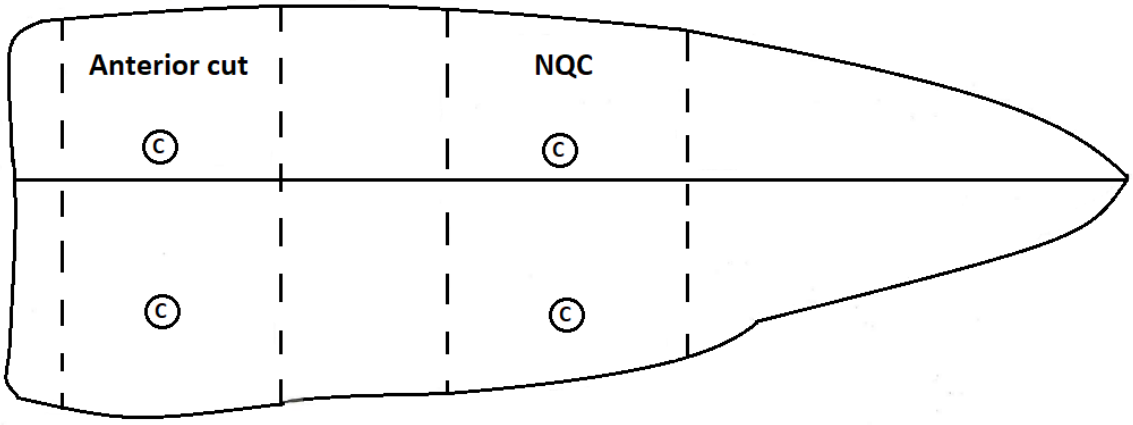


Figure 7. Skin of Atlantic salmon. Anterior and Norwegian quality cut (NQC) fillets are marked with the vertical dotted lines. C is where Minolta was measured. The horizontal line represents the lateral line.

Minolta chroma meter CR-400 (Minolta Sencing, INC Japan) was used to measure the color of the flesh and skin. The skin was measured at four locations, slightly above the lateral line at the

centre of the NQC (Norwegian quality cut) and the anterior fillet, and below the lateral line of NQC and anterior fillets. Minolta measures the L*a*b* values. The L* value measures the lightness of an object on a range from 0 to 100, where 0 is black and 100 is white. Parameter a* measure color from green to red on a scale from -120 to 120, where green is negative values and red is positive values. Parameter b* measure color from blue to yellow and ranges from -120 to 120, where negative values are blue and positive values are yellow (Wu et al., 2012).

Lastly a chemical analysis for free astaxanthin mg/kg was performed. Ten NQC fillets from each group were homogenized, giving 80 samples in total for astaxanthin analysis. The samples were frozen and sent to Nofima's BioLab in Bergen (Nofima, 2021; Zhou et al., 2011).

3.3. Statistics

The model used for most of the measured and calculated values was

$$Y = stress + genetics + (stress * genetics) + gender + body\ weight + \varepsilon$$

Body weight and gender were removed from the model when non-significant.

Gutted yield was calculated from the body weight.

$$Gutted\ yield\ \% = \frac{Gutted\ weight}{body\ weight} * 100$$

Fillet yield was calculated using round weight.

$$Fillet\ yield\ of\ body\ weight\ \% = \frac{fillet\ weight}{body\ weight} * 100$$

Condition factor (CF) was calculated from the body weight in grams and length in cm.

$$Condition\ factor = \left(\frac{body\ weight}{lenght^3}\right) * 100$$

Cardio somatic index (CSI) was calculated from heart weight and body weight.

$$CSI\% = \frac{Heart\ weight}{body\ weight} * 100$$

Effects of genetics and hypoxia on biometric parameters, operational welfare indicators, skin color and fillet color were analyzed by using SAS (statistical analysis software, version 9.4). The method of least squares to fit general linear models was used (the GLM procedure). This method includes analysis of variance, covariance, multivariate analysis of variance, partial correlation and regression (SAS, 2022).

4. Results

The results are presented first with the average range of the parameter/value, the overall effects of stress and genetics with corresponding p-values, trends, the significant differences among groups only, and lastly the p-value and R^2 of the model. The groups are referred to as P (PALE) and R (RED), with corresponding numbers (1, 2 or 3), referring to the number of times the group has been exposed to hypoxia ("stress"). For example, P0 is from the PALE genetic line and has been exposed to hypoxia 0 times.

4.1. Biometric traits

The body weight of the fish groups ranged from 3.65 – 4.36 kg on average (Table 3). The body weight of the PALE genetic line was higher compared to the RED genetic line (p= 0.03). Stress did not have an overall effect on body weight, although repeated exposure to hypoxia tended to reduce body weight increase (p = 0.10). The model had a high p-value (0.0746) and a low R^2 = 0.07. Among groups only, the body weight of P0 was significantly higher than the R groups stressed one (R1), two (R2) and three (R3) times prior to harvesting.

The fork length ranged from 62.5 - 64.7 cm on average (Table 3). The fork length of the RED genetic line was significantly higher than the PALE genetic line (p < 0.0001), but repeated exposure to hypoxia did not have a significant effect (p = 0.07). The RED genetic line showed no significant difference within the line. Among groups the fork length of P3 was significantly higher than P2. The fork length of R0 was not significantly different from P1 and P3. The model had a low P-value (< 0.0001), and a high R^2 (0.89).

The condition factor ranged from 1.5 – 1.6 on average (Table 3). The condition factor for the PALE genetic line was overall significantly higher compared to the RED genetic line (p < 0.0001), but repeated exposure to hypoxia did not have a significant effect (p = 0.053). Among groups the condition factor of the PALE genetic line was significantly higher than the RED genetic line within every stress group, except for stress group one, where there was no significant difference between PALE and RED. The condition factor of P0 and P2 was significantly higher compared to the RED genetic line. P1 and P3 had a significantly higher condition factor compared to R3, but not significantly higher than R0, R1 and R3. The model had a low p-value (< 0.0001) and a moderate R^2 (0.24).

Tabell 1. Means ± SE of body weight, fork length and condition factor (n=193 to 195) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia ("stress") 0, 1, 2 or 3 times prior to harvesting. Different letters within the same row represents significant differences between groups (p<0.05).

		Stress 0		Stress 1		Stress 2		Stress 3	
Biometric parameters	Number of individuals	PALE	RED	PALE	RED	PALE	RED	PALE	RED
Body weight (kg)	195	4.36 ± 0.2 ^{a}	4.10 ± 0.2 ^{ab}	4.10 ± 0.2 ^{ab}	3.75 ± 0.2 ^{b}	4.19 ± 0.2 ^{ab}	3.73 ± 0.2 ^{b}	3.92 ± 0.2 ^{ab}	3.65 ± 0.2 ^{b}
Fork length (cm)	194	62.7 ± 0.3 ^{ab}	64 ± 0.3 ^{cd}	63.4 ± 0.4 ^{abc}	64.5 ± 0.3 ^{d}	62.5 ± 0.4 ^{a}	64.5 ± 0.3 ^{d}	63.5 ± 0.4 ^{b}	64.7 ± 0.3 ^{d}
Condition factor	194	1.6 ± 0 ^{a}	1.5 ± 0 ^{bc}	1.5 ± 0 ^{ab}	1.5 ± 0 ^{bc}	1.6 ± 0 ^{a}	1.5 ± 0 ^{bc}	1.5 ± 0 ^{ab}	1.5 ± 0 ^{c}

The gutted yield ranged from 87.6 – 89.2 % on average (Figure 8a). The gutted yield of the PALE genetic line was not significantly different from the RED genetic line (p = 0.07). There was a trend where stressed fish had a lower gutted yield than fish that were not stressed, but the stress did not have a significant effect (p = 0.12). Among groups only, the gutted yield of P0 was significantly higher than R2 and R3. R1 was significantly higher than R3. The model had a low p-value (0.02) and a low R^2 (0.10).

The fillet yield ranged from 66.4 - 70.1 % on average (Figure 8b). The fillet yield for the PALE genetic line was significantly higher overall compared to the RED genetic line (p < 0.0001). Stress did not have a significant effect in the model (p = 0.16). The filet yield did not differ significantly between groups within the PALE genetic line, and all groups within the PALE genetic line were significantly higher compared to all groups within the RED genetic line. The fillet yield for R0 was significantly lower compared to R1 and R3. R2 were not significantly different from any other groups within the RED genetic line. The p-value of the model was low (< 0.0001) and the R^2 was moderate (0.25).

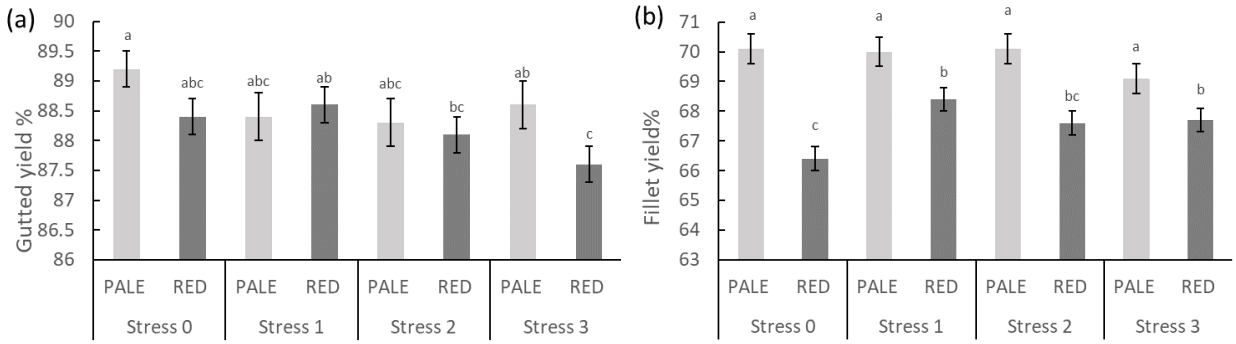


Figure 8. Gutted yield (%, n=193) (a) and fillet yield (%, n=195) (b) of Atlantic salmon, calculated from body weight. The fish are two genetic lines (RED and PALE) exposed to hypoxia ("stress") 0, 1, 2 or 3 times prior to harvesting. Different letters above the standard error bar (SE) represent significant differences between groups (P<0.05).

The cardio somatic index (CSI) ranged from 0.10 – 0.14 % on average (Figure 9). The CSI for the RED genetic line was significantly higher than the PALE genetic line ($p < 0.0001$). Stress did not have a significant effect ($p = 0.63$). No significant differences were observed within the genetic lines. The p-value of the model was low (0.0016) and the R^2 was high (0.56).

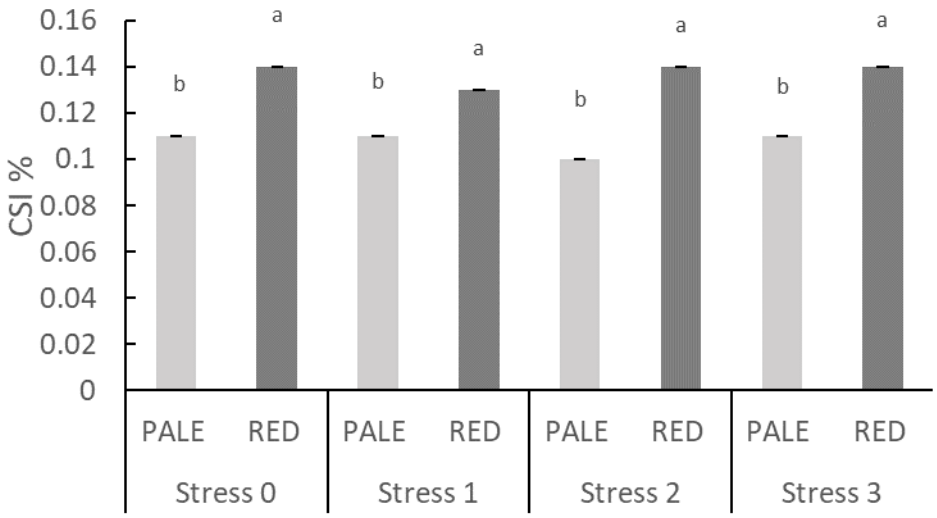


Figure 9. Cardio somatic index % (n=39) calculated from body weight. The fish are two genetic lines (RED and PALE) exposed to hypoxia ("stress") 0, 1, 2 or 3 times prior to harvesting. Different letters above the standard error bar (SE) represent significant differences between groups (P<0.05).

4.2. Operational welfare indicators

The scale loss score ranged from score 1 – 2 (Figure 10a). The genetic lines were not significantly different ($p = 0.62$), but stress had an overall significant effect ($p < 0.0001$). Repeated hypoxia resulted in a higher average score for scale loss. P3 had the highest score and was significantly higher than all other groups, except for R3. P1 had the lowest score and was significantly lower than P2, P3 and R3. The p-value of the model was low (< 0.0001) and the R^2 value was low (0.17).

The skin bleeding score ranged from 0 – 0.25 (Figure 10b). The genetic lines were not significantly different ($p = 0.45$), however stress had an overall significant effect ($p = 0.04$). The fish exposed to repeated hypoxia stress had a higher occurrence of skin bleeding. Among groups only, R0 had the lowest score on skin bleeding and was significantly lower compared to R1, P2 and P3. R1 and P3 had the highest skin bleeding scores and were significantly higher than P0, P1, R0, R2 and R3. The model had a low p-value (0.0004) and a low R^2 value (0.15).

The snout damage score ranged from 0.4 – 1.7 (Figure 10c). The snout damage score was significantly higher for the RED genetic line compared to the PALE genetic line ($p = 0.0021$), and repeated hypoxia resulted in an overall higher score of snout damage ($p > 0.0001$). Among groups R3 had the highest snout damage score and was significantly higher compared to all other groups. P2 had the lowest score and was significantly lower compared to P0, P3, R0, R1, R2 and R3. The model had a low p-value (<0.0001) and a moderate R^2 value (0.20).

The upper jaw deformity score ranged from 0.1 – 0.7 (Figure 10d). The upper jaw deformity score was numerically higher for the PALE genetic line compared to the RED genetic line, but the effect of genetics was not significant in the model ($p = 0.32$). Exposure to repeated hypoxia did have a significant effect on upper jaw deformities ($p < 0.0001$). Among groups only, P1 and R1 had the highest scores and were scored significantly higher than P0, R0 and R2. P3 was significantly higher compared to P0 and R0. Lastly R3 was scored significantly higher compared to R0. The model had a low p-value (0.0057) and a low R^2 (0.12).

The healed fin damage score ranged from 0.9 – 1.6 (Figure 10e). The healed fin damage score was not significantly different between the two genetic lines ($p = 0.88$). Exposure to repeated hypoxia did have a significant effect overall, and repeated stress resulted in an overall increasing scoring of healed fin damage ($p < 0.0001$). P3 and R3 had the highest scores and were scored significantly higher than P0, R0, P2 and R2. P3 were also scored significantly higher than P1. R1 were significantly higher than P0 and R0. P0 had the lowest average score and was scored significantly lower compared to P1, R1, P3 and R3. The model had a low p-value (<0.0001) and a low R^2 (0.19).

The active fin damage score ranged from 1.4 - 2.1 (Figure 10f). The active fin damage score was not significantly different between the two genetic lines ($p = 0.82$). Repeated exposure to hypoxia did have an overall effect, and repeated stress resulted in an overall increasing scoring of active fin damage ($p < 0.0001$). Among groups only, P3 and R3 had the highest average score and were significantly higher than P0, R0, P1, R1 and R2. No other groups were significantly different. The model had a low p-value (< 0.0001) and a low R^2 value (0.17).

The eye bleeding score ranged from 0.1 – 1 (Figure 10g). The eye bleeding score was not significantly different between the PALE and RED genetic line ($p = 0.42$). Exposure to repeated hypoxia had a significant overall effect, and repeated stress resulted in an overall increasing

scoring of eye bleeding ($p = 0.0006$). Among groups only, R3 was significantly higher than stress group 0, 1 and 2. P3 was significantly higher than R1 and P2. No other groups were significantly different. The model had a low p-value (0.0112) and a low R^2 value (0.11).

The eye lens opacity score ranged from 0.4 - 2.2 (Figure 10h). The eye lens opacity score was not significantly different between the PALE and RED genetic line ($p = 0.23$). Exposure to repeated hypoxia did have a significant overall effect, and repeated stress resulted in an overall increasing scoring of the eye lens opacity ($p < 0.0001$). Among groups only, P3 had the highest average score and was scored significantly higher than stress group 0, stress group 2 and P1. R3 was scored significantly higher than stress group 0, P1 and P2. R1 was scored significantly higher than P0, R0 and P2. The model had a low p-value (< 0.0001) and a low R^2 value (0.17).

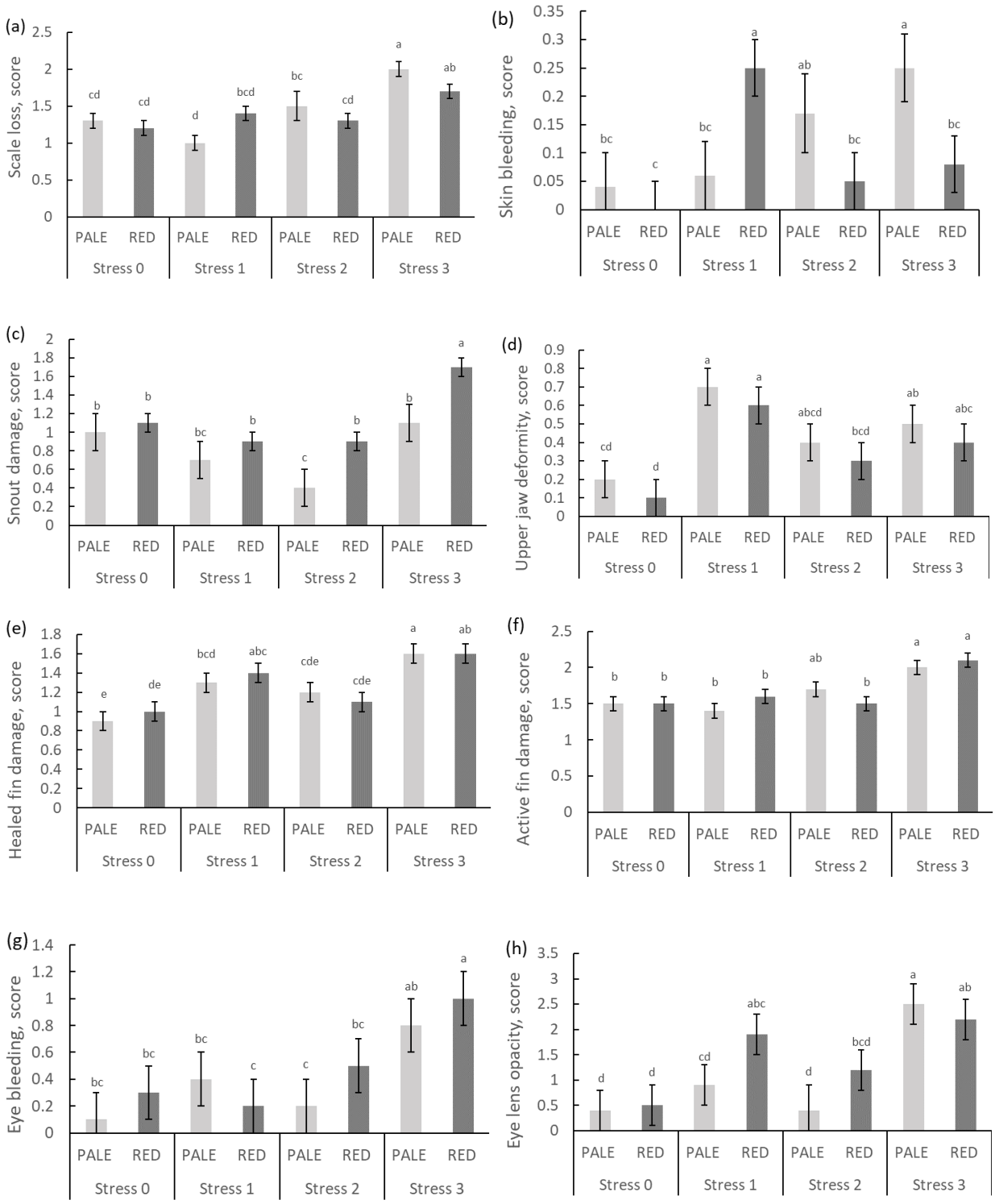


Figure 10. Operational welfare indicators (OWI) for scale loss (a), skin bleeding (b), snout damage (c), upper jaw deformities (d), healed fin damage (e), active fin damage (f) and eye bleeding (g) scored 0-3, and eye lens opacity (h) scored 0-5 on the left side of the fish. The fish are two genetic lines (RED and PALE) in four different stress groups (stressed 0, 1, 2 and 3 times). The different letters represent significant values between groups ($P<0.05$).

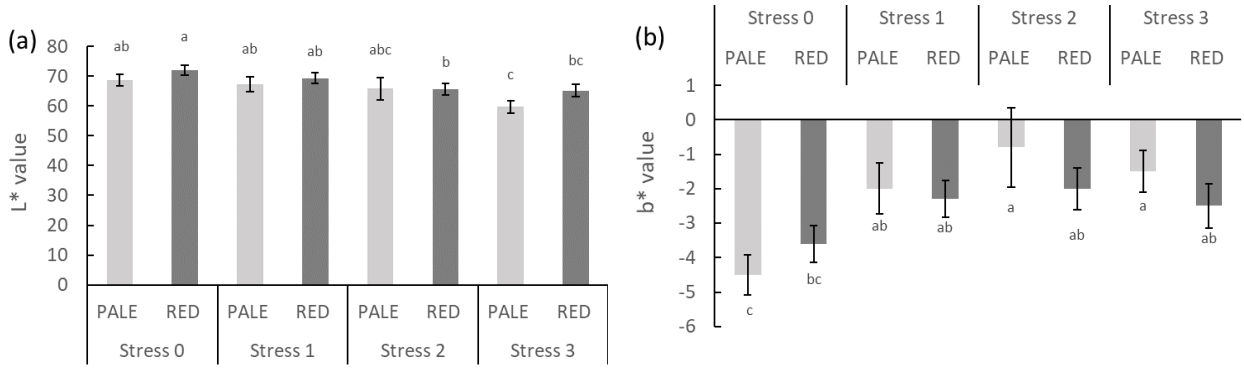
4.3. Skin color

The L* value of the skin over the lateral line on NQC ranged from 59.7 – 72 on average (Figure 11a). The L* value was not significantly different between the PALE and RED genetic line (p = 0.15). Repeated exposure to hypoxia had a significant effect (p = 0.0005), and there seemed to be a trend showing that repeated exposure to hypoxia resulted in darkened skin. Among groups P0, R0, P1 and R1 had a significantly higher L* value compared to P3. R0 was also significantly higher compared to R2 and R3. The model had a low p-value (0.0002) and a moderate R^2 (0.26).

The b* value of the skin over the lateral line on NQC ranged from (-4.5) – (-0.8) on average (Figure 11b). The b* value was not significantly different between the PALE and RED genetic line (p = 0.47). Stress did have a significant effect (p = 0.0003), and repeated exposure to hypoxia resulted in higher b* values. Among groups P0 was significantly lower compared to P1, R1, P2, R2, P3 and R3. R0 was significantly lower than P2 and P3. The model had a low p-value (< 0.0001) and a moderate R^2 (0.30).

The L* value of the skin over the lateral line on anterior fillets ranged from 52.8 – 60 on average (Figure 11c). There was no overall difference between the PALE and RED genetic line (p = 0.8). Repeated exposure to hypoxia resulted in darkened skin color, however, the stress did not have a significant effect (p = 0.08). Among groups P0 had the highest L* value and was significantly higher than P1 and R3. The model had a high p-value (0.08) and a low R^2 (0.08).

The b* value of the skin over the lateral line on anterior fillets ranged from (-1.3) – (-0.5) on average (Figure 11d). The b* value was not significantly different between the PALE and RED genetic line (p = 0.99). Exposure to repeated hypoxia did not have a significant effect (p = 0.67). There was no significant difference among the groups. The model had a low p-value (0.01) and a low R^2 (0.11).



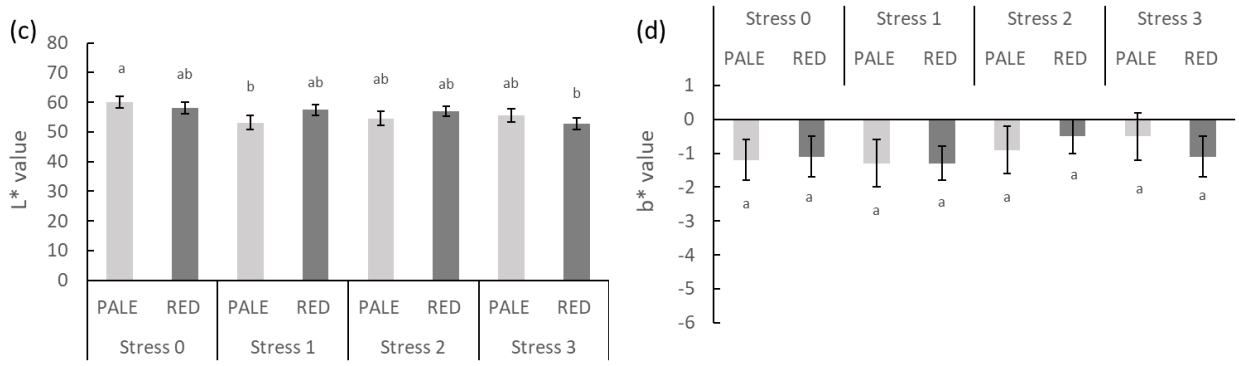


Figure 11. L and b* value on the skin over the lateral line of Norwegian quality cut (a and b) and anterior fillet (c and d) in two genetic lines (RED and PALE) exposed to repeated hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. L* value reflects lightness on a scale from 0 to 100, where 0 is black. b* value is on a scale from -120 to 120, where -120 is blue and 120 is yellow. The different letters represent significant differences between groups (P<0.05). n=114.*

The L* value of the skin below the lateral line on NQC ranged from 83 – 89.6 on average (Figure 12a). The L* value was higher for the RED genetic line compared to the PALE genetic line (p = 0.03). Repeated exposure to hypoxia had a significant effect on the L* value (p < 0.0001). P2 had a significantly lower L* value compared to all other groups. No other groups were significantly different. The model had a low p-value (< 0.0001) and a moderate R^2 (0.36).

The b* value of the skin below the lateral line on NQC ranged from (-4.3) – (0.3) on average (Figure 12b). The b* value was not significantly different between the RED and PALE genetic line (p = 0.25). Stress had a significant effect on the b* value and there seemed to be a trend showing that repeated exposure to hypoxia resulted in an increase of the b* value (p = 0.0003). Among groups only, P2 fish had the highest b* value and was significantly higher compared to all other groups. P0 had the lowest score and had a significantly lower compared to R0, R1, P2, R2, P3 and R3. P1 had a significantly lower b* value compared to R1. The model had a low p-value (0.0005) and a moderate R^2 (0.24).

The L* value of the skin below the lateral line on anterior fillets ranged from 84.6 – 87.6 on average (Figure 12c). There was no significant overall difference between the PALE and RED genetic line (p = 0.40). Exposure to repeated hypoxia did not have a significant effect on the L* value (p = 0.07), but there seemed to be a trend showing that repeated exposure to hypoxia resulted in darkened skin. Among groups P0 and R0 had a significantly higher L* value compared to P2. The model had a low p-value (0.0002) and a low R^2 (0.16).

The b* value of the skin below the lateral line on anterior fillets ranged from (-2.1) – (-0.7) on average (Figure 12d). The b* value was not significantly different between the PALE and RED genetic line (p = 0.97). Repeated exposure to stress had no overall effect (p = 0.27). There were no significant differences among groups. The model had a high p-value (0.81) and a low R^2 (0.03).

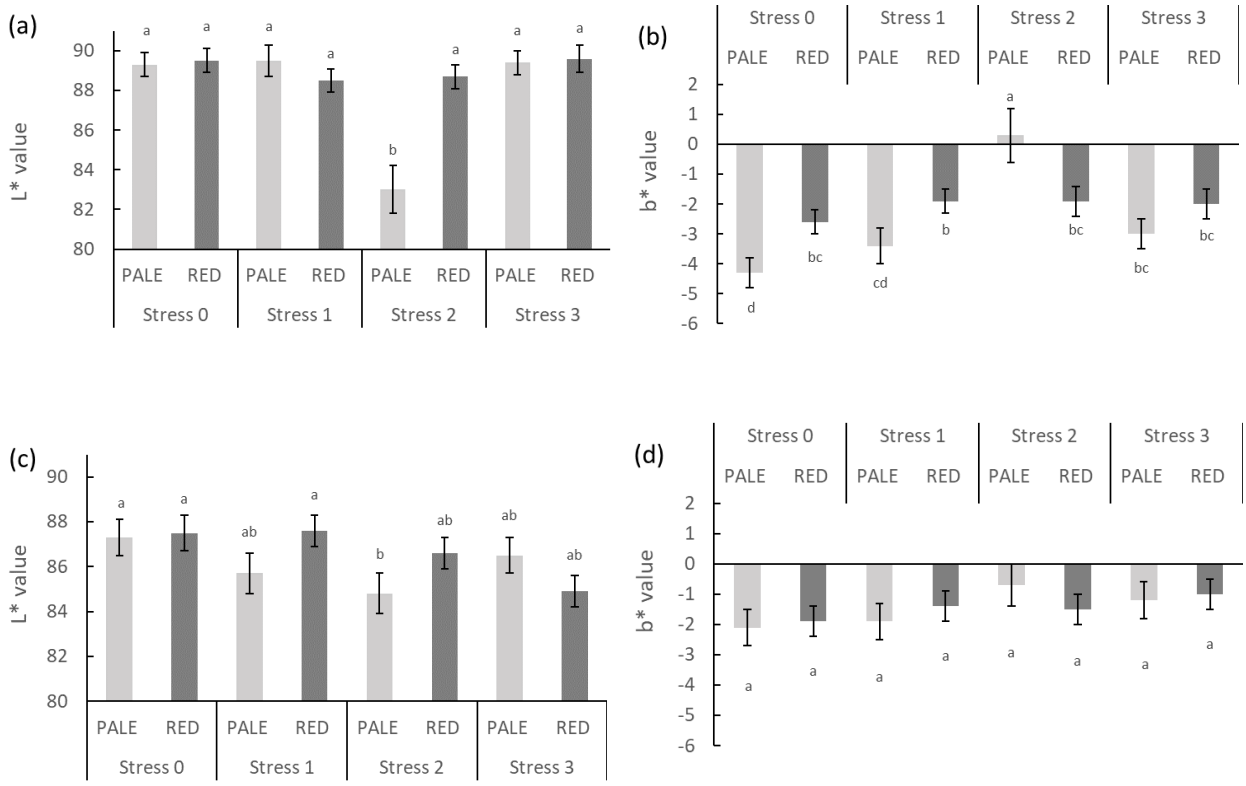


Figure 12. L and b* value on the skin below the lateral line of Norwegian quality cut (a and b) and anterior fillet (c and d) (measured with Minolta chroma meter CR-400) in two genetic lines (RED and PALE) exposed to repeated hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. L* value measure lightness on a scale from 0 to 100, where 0 is black. b* value is on a scale from -120 to 120, where -120 is blue and 120 is yellow. The letters represent significant differences between groups (P<0.05). n=114.*

4.4. Fillet colour

Astaxanthin of NQC ranged from 4 – 7.9 mg/kg (Figure 13). The astaxanthin was higher for the RED genetic line compared to the PALE genetic line ($p > 0.0001$). Exposure to repeated hypoxia did not have a significant effect on astaxanthin concentration in the muscle ($p = 0.56$). The model had a low p-value (< 0.0001) and a high R^2 (0.77).

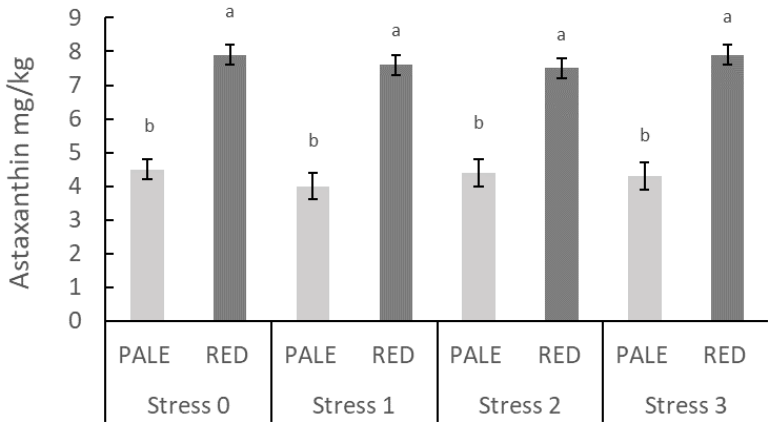


Figure 13. Astaxanthin (mg/kg) (mean±SE) of Norwegian quality cut in two genetic lines (RED and PALE) exposed to repeated hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. The letters represent significant differences between groups (P<0.05). n=80.

4.4.1. Visual colour

The SalmoFan score of NQC fillets ranged from 24.9 – 27.2 on average (Figure 14a). The SalmonFan score was significantly higher for the RED genetic line compared to the PALE genetic line ($p < 0.0001$). Stress had a significant overall ($p < 0.0001$) and repeated exposure to hypoxia resulted in redder flesh of fish that had been stressed compared to the control group (stress 0). Among groups, R1, R2 and R3 were significantly higher scored compared to P0, P1, P2 and P3. R0 was scored significantly higher than P0, P1 and P3. The model had a low p-value (< 0.0001) and a high R^2 value (0.46).

The SalmoFan score on the anterior fillet ranged from 24.4 – 26.9 on average (Figure 14b). The SalmoFan score was significantly higher for the RED genetic line compared to the PALE genetic line ($p < 0.0001$). Stress had a significant effect ($p < 0.0001$) and repeated exposure to hypoxia resulted in a higher SalmoFan score. Among groups, R1, R2 and R3 had a significantly higher SalmoFan score compared to P0, P1, P2 and P3. P0 had a significantly lower SalmoFan score compared to all other groups. R0 was significantly lower compared to the RED genetic line in

other stress groups, but not significantly different to P1, P2 and P3. The model had a low p-value (< 0.0001) and the R^2 was high (0.43).

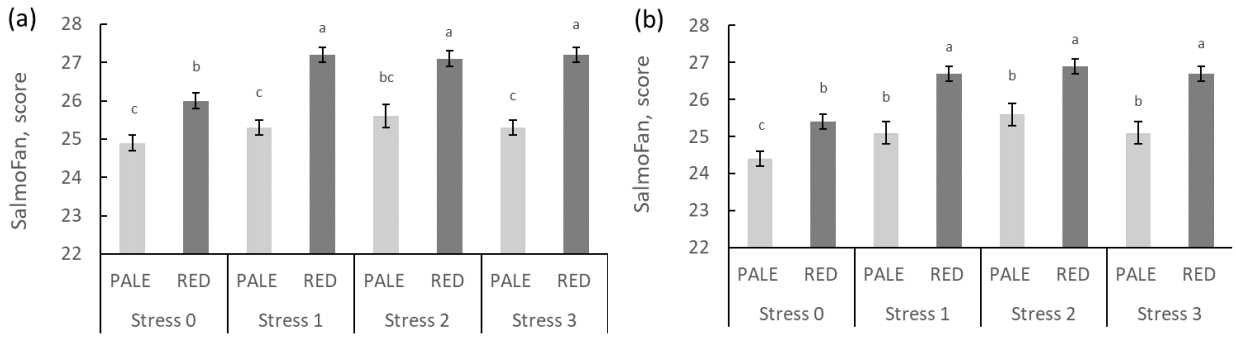


Figure 14. SalmoFan score (mean±SE) of Norwegian quality cut (a) and anterior fillet (b) of two genetic lines (RED and PALE) exposed to repeated hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. The letters represent significant values between groups (P<0.05). n=190

4.4.2. Colorimetric analyses

The L* value of NQC fillets ranged from 38.5 – 41.5 on average (Figure 15a). The L* value of the PALE genetic line was overall higher than for the RED genetic line, but it was not significant (p = 0.07). Exposure to repeated hypoxia did not have a significant effect on the L* value (p = 0.08). Among groups results showed that P0 had the highest L* value and was significantly higher than R1, R2 and R3. The model had a high p-value (0.06) and a low R^2 (0.08).

The L* value of the anterior fillets ranged from 38.3 – 41.3 on average (Figure 15b). The L* value was numerically higher for the PALE genetic line, but it was not significant (p = 0.21). Exposure to repeated hypoxia did not have a significant effect (p = 0.08). Among groups only, P0 and P3 had a significantly higher L* value compared to P2 and R3. P1 had a significantly higher L* value compared to P2. The model had a high p-value (0.08) and a low R^2 (0.08).

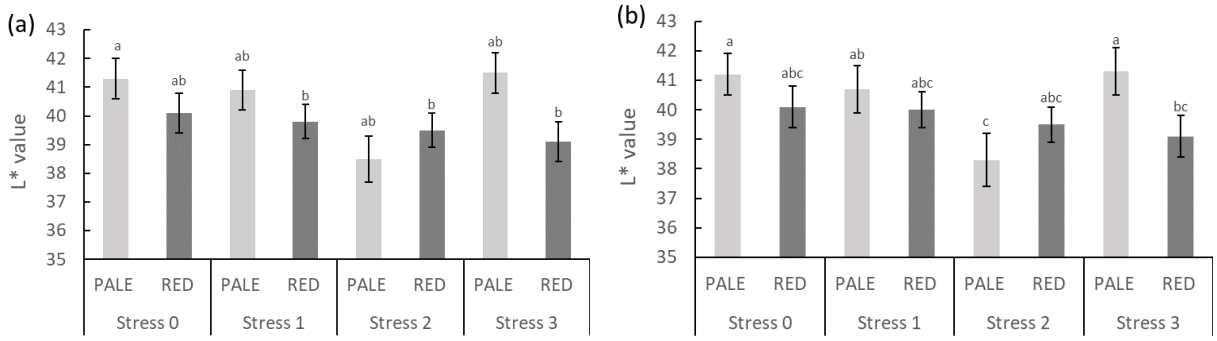


Figure 15. The L value (mean±SE) of NQC (a) and anterior fillet (b) (measured with Minolta chroma meter CR-400) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. L* value reflects lightness on a scale from 0 to 100, where 0 is black and 100 is white. Different letters represent significant differences between groups (P<0.05). n=194.*

The a* value of NQC fillets ranged from 13.8 – 15.9 on average (Figure 16a). The a* value was significantly higher for the RED genetic line compared to the PALE genetic line ($p < 0.0001$). Exposure to repeated hypoxia did not have a significant effect on the a* value ($p = 0.80$). Among groups, R0, R1, R2 and R3 had a significantly higher a* value compared to P0, P1, P2 and P3. The model had a low p-value (< 0.0001) and a high R^2 value (0.32).

The a* value of anterior fillets ranged from 13.9 – 15.9 on average (16b). The a* value of the RED genetic line was significantly higher compared to the PALE genetic line ($p < 0.0001$). Exposure to repeated hypoxia did not have a significant effect on the a* value ($p = 0.80$). Among groups, R0, R1, R2 and R3 had a significantly higher a* value compared to P0, P1, P2 and P3. The model had a low p-value (< 0.0001) and a moderate R^2 (0.32).

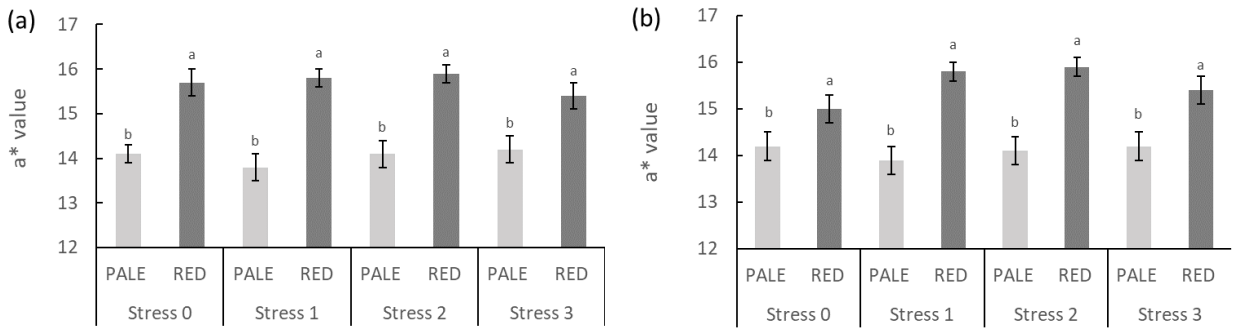


Figure 1516. The a value (mean±SE) of NQC (a) and anterior fillet (b) (measured with Minolta chroma meter CR-400) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. a* value is a chromatic component of the L*a*b* measurement, and is on a scale from -120 to 120, where -120 is green and 120 is red. Different letters represent significant differences between groups (P<0.05). n=194*

The b* value of NQC fillets ranged from 12.8 – 14.2 on average (Figure 17a). Overall the b* value was significantly higher for the RED genetic line overall compared to the PALE genetic line ($p < 0.0001$). Exposure to repeated hypoxia did not have a significant effect on the model ($p = 0.70$). Among groups, R0 and R2 had the highest b* values that were significantly higher compared to P0, P1, P2 and P3. R1 had a significantly higher b* value compared to P1 and P2. The model had a low p-value (0.007) and had a low R^2 (0.11).

The b* value of anterior fillets ranged from 12.8 – 14.2 on average (Figure 17b). The b* value was significantly higher for the RED genetic line compared to the PALE genetic line ($p = 0.002$). Exposure to repeated hypoxia did not have a significant effect on the b* values ($p = 0.70$). Among groups, R0 and R2 had the highest b* value that were significantly higher compared to P0, P1, P2 and P3. R1 had a significantly higher b* value compared to P1 and P2. The model had a low p-value (0.01) and a low R^2 (0.11).

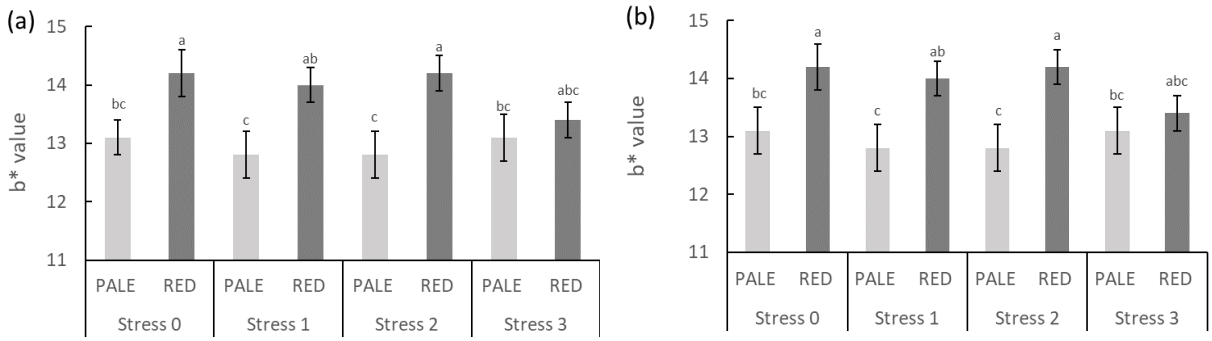


Figure 17. The b value (mean±SE) of NQC (a) and anterior fillet (b) (measured with Minolta chroma meter CR-400) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia (“stress”) 0, 1, 2 or 3 times before harvesting. b* value is a chromatic component of the L*a*b* measurement, and is on a scale from -120 to 120, where -120 is blue and 120 is yellow. Different letters represent significant differences between groups (P<0.05). n=194.*

4.5. Fillet quality parameters

The gaping score ranged from 0 – 0.5 on average (Figure 18). The gaping score was not significantly different between the PALE genetic line and the RED genetic line overall (p = 0.65). Exposure to repeated hypoxia did not have an overall effect on fillet gaping (p = 0.49). Among groups, P2, R2 and R3 had a significantly higher gaping score compared to P0. There were no other significant differences between the groups. The model had a high p-value (0.49) and a low R^2 (0.05).

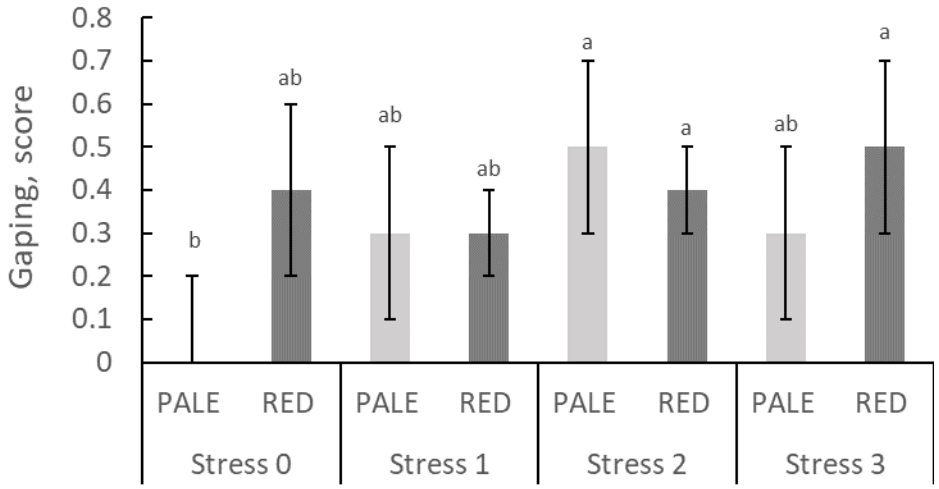


Figure 18. Gaping score (0-5) (mean±SE) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. Different letters represent significant differences between groups (P<0.05). n=186.

The texture score on NQC fillets ranged from 3.1 – 4.2 on average (Figure 19a). The texture score was not significantly different between the PALE genetic line and the RED genetic line (p = 0.93). Exposure to repeated hypoxia did have a significant effect (p = 0.04), and repeated stress resulted in a higher texture score (softer texture). Among groups, P2 had the highest texture score and was significantly higher compared to P0 and R0. There were no other significant differences between groups. The model had a high p-value (0.22) and a low R^2 value (0.10).

The texture score on anterior fillets ranged from 3.3 – 4.3 on average (Figure 19b). The texture score was not significantly different between the PALE genetic line and the RED genetic line overall (0.73). Stress did have a significant effect on the texture (p = 0.004), and repeated exposure to hypoxia resulted in a higher texture score (softer texture). Among groups, R2 had the highest texture score and was significantly higher compared to P0, R0, R1 and P1. P1 had a

significantly higher texture score compared to P0, R0 and P3. The model had a low p-value (0.003) and a low R^2 (0.13).

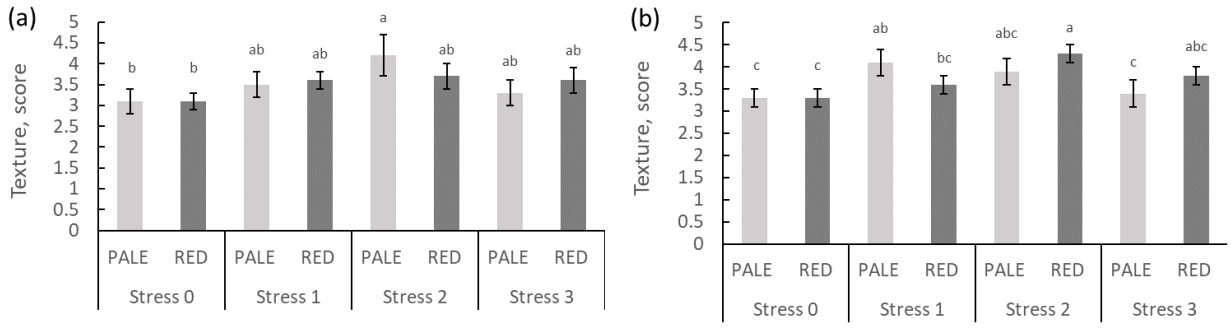


Figure 19. Texture softness (score 1-5) in NQC (a) and anterior fillets (b) of two genetic lines (RED and PALE) of Atlantic salmon exposed to hypoxia ("stress") 0, 1, 2 or 3 times before harvesting. Different letters represent significant differences between groups (P<0.05).

5. Discussion

5.1. Biometric parameters

The range of the average body weight was 3.92 - 4.36 kilograms for the PALE genetic line and 3.65 – 4.10 kilograms on average for the RED genetic line, which is a large range overall when only looking at the average of the groups. The body weight of the groups do however correspond with commercially harvest-ready Atlantic salmon, which is on average around 5 kilograms (SjømatNorge, 2022). The body weight of the PALE genetic line was higher compared to the RED genetic line, which means that the PALE genetic line had a higher growth rate, as all fish were moved to sea at the same time. The genetic lines were only selected for color which resulted in a compromise on other traits like body weight. In a commercial setting the PALE genetic line would likely be preferred over the RED genetic line because it would lower the time spent to reach slaughter size. P1 had the highest body weight and R3 had the lowest body weight, so it seems the body weight was somewhat negatively affected by repeated hypoxia even if stress did not have an overall significant effect. The model had a high p-value and a low R^2 which means the variance from the average was high and the high p-value indicates that the variables used in the model (stress, genetics, and gender) did not fully explain the variance between the groups.

The fork length ranged from 62.5 – 63.5 cm on average for the PALE genetic line and from 64 – 64.7 cm on average for the RED genetic line, which is a small range and within the normal fork length of a harvest ready Atlantic salmon. The fork length was significantly higher for the RED genetic line compared to the PALE genetic line, but the length was not affected by exposure to repeated hypoxia. The body weight was lower for the RED line even if the RED line was longer, which means the RED line was skinnier compared to the PALE line, which is something the condition factor reflects. There were no differences between genetic lines for the gutted yield, which means the RED line had a higher organ weight compared to the PALE line. The model had a low p-value and a high R^2, which in this case means that genetics was a large part of the differences between the groups.

The condition factor (CF) ranged from 1.5 – 1.6, which is a high average CF compared to earlier studies where the CF has been between 1.20 – 1.40 for Atlantic salmon (Garber et al., 2019; Mørkøre et al., 2020). The CF for the PALE genetic line was higher compared to the RED genetic line, and the PALE genetic line was significantly shorter and weighed significantly more, which gives the large difference in CF, between the two lines. This would possibly make the shape of the PALE line more desirable for the aquaculture industry. The CF is an important economic trait and gives information about the girth of the salmon. The high CF and growth rate also indicates that the feed conversion rate could be high for the PALE line and that feeding costs would be lower for the PALE genetic line compared to the RED genetic line. The model had a low p-value and a moderate R^2 which means there was some variation from the average, but the effects from genetics were significant and the null hypothesis saying stress and genetics have no effect can be rejected.

The gutted yield ranged from 87.6 – 89.2%, which is within the normal range. The gutted yield was not significantly different between the PALE genetic line compared to the RED genetic line; however, gutted yield has low heritability of 0.14 h^2 (Garber et al., 2019) which can be one reason for why genetics did not have an overall effect. Repeated hypoxia did not have an overall significant effect, although the difference between P1 and R3 was significant, indicating that stress could have somewhat of an effect. The source of the differences between the groups is likely from fat accumulation around the intestines, but what causes the fat to accumulate is not clear. Other variables that could cause the gutted yield to differ, could be the weight of the

skeleton, head size, fat accumulation around intestine and the weight of the intestine. Temperature can have an effect on the fat accumulation where lower temperatures can cause fat accumulation (Ruyter et al., 2006), hence tolerance of temperature changes could be another factor influencing the gutted yield. The model had a low p-value and a low R^2 which means there is a lot of variation from the average.

Fillet is sold as an end product, and it is desirable to breed a fish that has a high fillet yield. It is likely that the PALE genetic line spent more of its energy growing muscle compared to the RED genetic line, as the fillet yield was higher for the PALE genetic line compared to the RED genetic line. The fillet yield being lower for the RED line could be the consequence of prioritizing color in the breeding process. Hypoxia did not have an overall significant effect on the fillet yield, although the yield was higher for the RED genetic line that had been exposed to repeated hypoxia compared to the control. The model had a low p-value and a moderate R^2, which means the variation from the average was moderate, and there could still be other variables affecting the fillet yield. However, the low p-value indicates that there is a significant effect originating from genetics.

The CSI ranged from 0.1 – 0.14 %, which is within a normal range. The CSI was significantly higher for the RED genetic line compared to the PALE genetic line, which means the RED line had bigger hearts relative to body weight compared to the PALE line. Increased heart size cause a reduction of maximum stroke volume and cardiac output, which reduces the overall oxygen-transport capacity (Johansen et al., 2017) and irregularities of the heart can cause weaker contractions of the heart and an increased risk of mortality (Frisk et al., 2020). The CSI was not affected by exposure to repeated hypoxia. The model had a low p-value and a high R^2 which means the variables in the model explains a large part of the differences between the groups. The genetic mechanisms behind the differences between the lines is unknown, but the genetic differences could potentially be linked to disposition of astaxanthin, which in this study was shown to be genetically different between the lines and should be investigated.

5.2. Operational welfare indicators

The scale loss had a moderate range (1 – 2), which means the severity ranged from loss of a few scales to small areas of scale loss on average. The scales and slime layer of the fish is the first

barrier against infections and scale loss can result in difficulties with osmoregulation and possible pain and infections (Noble et al., 2018). Scale loss and generally bad skin condition also affects the hygiene of the fish. It is therefore important to avoid physical damage to the skin of the fish, which can happen during handling. The PALE and RED genetic line were not significantly different, but exposure to repeated hypoxia did have a significant effect. Before exposure to hypoxia, the fish were crowded and caught with a catch net, hence the handling of fish probably also resulted in a higher scale loss score. Among groups, P3 and R3 had the highest scores, and there was a clear difference between the control group that had not been stressed. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the scale loss score.

The skin bleeding score ranged from 0 – 0.25, which is a small range and an overall low score with smaller bleedings of the abdominal. Skin bleeding comes from physical damage such as handling. There was not a significant difference between the RED and PALE genetic line. However, repeated exposure to hypoxia did have a significant effect, and the fish that was exposed to hypoxia did have a higher occurrence of skin bleeding. This is likely due to the exposure to physical handling during the experiment, which could lead to skin bleeding. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the skin bleeding score.

The snout damage score ranged from 0.4 – 1.7 on average which is a relatively large range and a moderate average score. This means that the snout damage score ranged from damage on the upper or lower jaw to wounds on the snout. Snout damage can happen with any type of handling (Noble et al., 2018), for example during crowding. The snout damage for the RED genetic line was significantly higher compared to the PALE genetic line, which is contradicting to the expectation. The expected outcome was the RED line having a lower scoring because of higher levels of astaxanthin and potentially a higher ability to heal, however this was not the case. The PALE genetic line had a significantly lower score, which means they either had a higher ability to heal, or they were less prone to get wounds. Stress also affected the snout damage score and exposure to repeated hypoxia resulted in a higher score on snout damage, which makes sense because of the exposure to physical handling that could lead to snout damage. The model had a

low p-value and a moderate R^2, which means the variance was moderate, and that there could be other variables affecting the snout damage score.

The upper jaw deformity score ranged from 0.1 – 0.7 on average which is a small range and a low score on average. This means that the average upper jaw deformity score was scored only as suspected deformities because of low scores. Upper jaw deformities are a shortened upper snout and jawbone and can arise with higher temperatures in the egg stage of Atlantic salmon, but this is the first study to document upper jaw deformities in adult fish exposed to hypoxia. The PALE genetic line had a numerically higher upper jaw score compared to the RED genetic line, but it was not significant. This means there could be some differences between the genetic lines on average, and the PALE line could be somewhat more prone to upper jaw deformities. However, because it wasn't significant the differences are likely coincidental. The upper jaw deformity score was affected by exposure to repeated hypoxia and resulted in a higher score. Stress did have a negative effect (increased score on upper jaw deformity) in the trial; however, these results could also be affected by human error when analyzing the pictures. The upper jaw deformity score could have been affected by snout damage and been wrongly considered deformities in the later picture evaluations. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the upper jaw deformity score.

The healed fin damage score ranged from 0-9 – 1.6 which is a large range and moderate scoring on average. This means the healed fin damage ranged from being mostly intact to half of the fin being intact. Healed fin damage limits the swimming capacity of the fish and can come from periods with feed limitations like starving before handling. This is a problem that occurs because of aggression in the sea cages, and limitations to resting periods can also lead to fin damage (Noble et al., 2018; Solstorm et al., 2016). There were no differences between the PALE genetic line and the RED genetic line, but exposure to repeated hypoxia did result in an increased score of healed fin damage. This could be from handling, but another theory is that because of the stress exposure the fish had increased metabolism which could lead to increased aggression during feeding, or limitations to healing with repeated hypoxia. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the healed fin damage score.

The active fin damage ranged from 1.4 – 2.1 which is a large range and a high scoring on average. This means the active fin damage on average ranged from being light splitting and bleeding wounds on only the outer part of the fin to clear splitting and wounds happening down to half the length of the fin. The causes of active fin damage are the same as healed fin damage, which is aggression behavior happening due to limited feed recourses or limited resting periods. There was no difference between the PALE genetic line and the RED genetic line, but exposure to repeated hypoxia resulted in a higher average score. This could be because of the repeated handling, or the hypoxia limited the healing capabilities of the fish. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the active fin damage score.

The eye bleeding score ranged from 0.1 – 1 which is a low score and a low moderate range. This means that the eye bleeding score ranged from not being observed to smaller bleedings. The eyes are very vulnerable to handling that can result in eye bleeding or puncturing of the eye. Eye snapping can also occur as aggression behavior during feeding, or with strong lights going into the sea cage giving light reflections of the eyes that attracts other fish to bite. There were no differences on eye bleeding score between the PALE genetic line and the RED genetic line, but repeated exposure to hypoxia did have a significant effect. The fish were crowded and handled during the hypoxia tests, so the eyes were probably damaged from the physical handling. The repeated hypoxia could also have limited the fish's ability to heal the wounds. The model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the eye bleeding score.

Eye lens opacity ranged from 0.4 – 2.2 which was a low score and a large range. The eye lens opacity score ranged from 0 – 5, and the sum of left and right eye was used. This means that the overall score was low and that the average eye lens opacity ranged from no observation of eye lens opacity to eye lens opacity covering 10 – 50% of the diameter of the eye lens. Eye lens opacity reduces the vision of the fish and higher severities are seen as irreversible and lead to blindness. Reduced vision or blindness can further lead to reduced feeding capacity and therefore reduced growth. Eye lens opacity can be caused by osmotic imbalance, fluctuating water temperatures, infections in the eye, hypoxia, fast growth, genetics, nutritional deficiencies, and toxic environmental factors (Bjerkås & Sveier, 2004). The fish in this experiment was exposed to

repeated hypoxia which influenced the eye lens opacity score, but the model had a low p-value and a low R^2, which means the variance was high, and that there could be other variables affecting the eye lens opacity score, like the ones mentioned before.

5.3. Skin color

The skin of Atlantic salmon above the lateral line is dark and dappled, while the skin on the belly is silver and white. The skin color can give indications of reduced eyesight or increased stress in Atlantic salmon and skin color can therefore be used as an index to evaluate welfare. A study from Kittilsen et al (2009) showed that Atlantic salmon with a more dappled skin had lower physiological and behavioral response to stress. The dappled fish had also a higher feed intake one week after being introduced to a new environment compared to the non-dappled fish. This shows that the individual differences between skin color represent how well adjusted the fish can be to stress, and that there can be genetic differences between how well salmonids handle exposure to stress (like new environments and handling). Additionally, darker dappled fish could have reduced recovery time after being exposed to stress (Kittilsen et al., 2009).

The L* value of the skin above the lateral line on NQC ranged from 59.7 -72, which is a moderate range and a high average L* value. The L* value on the dorsal region can vary but is usually lower (darker) because of the dapples on the skin. Unstressed fish has shown to have a L* value around 41 and stressed fish has been shown to have a slightly higher L* value of 43 (Erikson & Misimi, 2008). The L* value was not significantly different between the PALE genetic line compared to the RED genetic line and repeated exposure to hypoxia had no effect. The model had a low p-value and a moderate R^2 which means there is some variation from the average, and that the null hypothesis that hypoxia and genetics does not affect the skin color cannot be rejected, and that the variance in the model could be random.

The b*value of the skin above the lateral line on NQC ranged from (-4.5) – (-0.8), which is a small range and a normal average. The b* value measures the blue to yellow hue of the skin, which means that in this case the skin was more blue than yellow as the average was negative. There was no significant difference between the PALE genetic line and the RED genetic line. However, repeated exposure to hypoxia did have a significant effect and resulted in a higher b* value, which means that the fish that were stressed several times had less blue skin above the

lateral line on NQC. The model had a low p-value and a moderate R^2 which means there is some variation from the average, and that the null hypothesis that hypoxia and genetics does not affect the skin color cannot be rejected, and that the variance in the model could be random.

The L* value of the skin above the lateral line on anterior fillets ranged from 52.8 – 60 on average, which is a moderate range and a high average. A lower L* value means that the skin is darker, which in turn indicates exposure to stress or reduced eyesight. There were no significant differences between the PALE genetic line and the RED genetic line. Exposure to repeated hypoxia did not have a significant effect on the L* value, which in turn means that the skin color could not give an indication to reduced welfare in this case. The model had a high p-value and a low R^2 which means that the variance in the model was high and that the variance in the model likely came from other variables that were not included in the model. The high p-value means that the null hypothesis that stress and genetics does not affect the skin color cannot be rejected, and that the variance in the model could be random.

The b* value of the skin above the lateral line on anterior fillets ranged from (-1.3) – (-0.5) which is a small range and a normal average. The b* value was on average negative, which means the hue of the skin was more blue than yellow. There was no significant difference between the PALE genetic line and the RED genetic line and exposure to repeated hypoxia did not have a significant effect on the b* value. The model also had a low p-value and a low R^2 which means that the null hypothesis that stress and genetics have no effect can be rejected, however the variance was high.

The L* value of the skin below the lateral line on NQC ranged from 83 – 89.6 on average, which is a small range and a normal average. The skin on the belly of the Atlantic salmon is a silver color and therefore very light compared to the back. The L* value of the RED genetic line was higher compared to the PALE genetic line, which means that the belly of the RED genetic line was lighter than the PALE genetic line. Exposure to repeated hypoxia also influenced the L* value. However, looking at the results it is difficult to see what the effect was. The model had a low p-value and a moderate R^2, which means there is some variation from the average. There was an effect from the variables used in the model, and in this case that was true for both genetics and stress.

The b* value of the skin below the lateral line on NQC ranged from (-4.3) – (-0.3) on average, which is a small range, and a low b* value (Erikson & Misimi, 2008). The b* value was on average negative, which means the hue of the skin was more blue than yellow. The b* value was not significantly different between the PALE and RED genetic line, but exposure to repeated hypoxia resulted in an increased b* value. This means that the skin of unstressed fish had more of a blue hue to the skin on the belly, compared to fish that had been stressed, where repeated hypoxia resulted in a reduced blue hue. The model had a low p-value and a moderate R^2 which means there is some variation from the average, and that the null hypothesis that hypoxia and genetics does not affect the skin color can be rejected, and that the variance in the model could be random. This means that there was an effect from the variables used in the model, and in this case, stress was the variable that had an effect.

The L* value of the skin below the lateral line on anterior fillets ranged from 84.6 – 87.6 on average, which is a small range, and a normal range. The L* value below the lateral line on anterior fillets were not significantly different between the PALE and RED genetic line and exposure to repeated hypoxia had no significant effect on the L* value. The model had a low p-value and a low R^2 which means that the L* value had large variation from the average, but there were significant differences that were from other variables than hypoxia and genetics.

The b* value of the skin below the lateral line on anterior fillets ranged from (-2.1) – (-0.7) on average, which is a small range and a normal range. The b* value below the lateral line on anterior fillets were not significantly different between the PALE and RED genetic line and exposure to repeated hypoxia had no significant effect on the b* value. The model had a high p-value and a low R^2 which means that the variance in the model was high and that the variance in the model likely came from other variables that were not included in the model. The high p-value means that the null hypothesis that stress and genetics does not affect the skin color cannot be rejected, and that the variance in the model could be random.

Overall, there was not much difference between the PALE and RED genetic line on skin color, and only the L* value on the belly of NQC was significantly different between the lines. Exposure to repeated hypoxia did not have an effect the back region (measured over the lateral line) on anterior fillets but did have a significant effect on the back region of NQC for both L* and b* value.

In this study only four spots on the fish skin were measured with a Minolta chroma meter, which could limit the chance to find significant differences because it does not quantify the coloration on the skin, although it is a very precise method. In a study done by Kittilsen et al. (2009) they measured melanin spots per cm^2 above the lateral line from the gills to the dorsal fin, which is a different method that could have been used, however this method is much more time consuming.

The fish were not exposed to stress over a prolonged period and studies have shown that increased cortisol levels might not have a direct effect on skin color (Thorsen, 2019). There is a possibility that stress over a prolonged time with increased glucose would influence skin color, however, this was not measured.

5.4. Fillet color

Color is an important fillet quality trait for Atlantic salmon, which is known for its pink-red flesh. Salmon cannot synthesize astaxanthin in vivo, so pigment must be added in the diet. In the wild salmonoid eat crustaceans that contain astaxanthin. However, capture of crustaceans is not sustainable to use for salmon feed, and synthesized astaxanthin is used instead. Astaxanthin is expensive, so retention of astaxanthin in flesh is important for the aquaculture industry. Visual color is an important measurement, and Atlantic salmon with higher SalmoFan scores are desired and considered to be more fresh, tasty and of higher quality to the consumer. SalmoFan scores are however a more subjective measurement compared to instrumental measurements done with Minolta, but both methods have their advantages. SalmoFan gives an overall impression of the color, whereas Minolta measures a smaller point on the fillet. This means fat and connective tissue, which are lighter than muscle, are included in the Minolta measurement and can affect the score on lightness (L*) and redness (a*) of the fillet.

5.4.1. Pigment

Astaxanthin ranged from 4 – 7.9 mg/kg on average, which is a large range. Astaxanthin retention depends a lot on the diet, environment, and genetics. The RED genetic line had higher concentrations of astaxanthin compared to the PALE genetic line, which means the astaxanthin retention in skeletal muscle was higher for the RED line, even though both lines were fed the same diet. Feed is one of the highest costs in the Atlantic salmon aquaculture industry, and high

utilization of astaxanthin is preferred (Stachowiak & Szulc, 2021). Exposure to hypoxia had no significant effect on the retention of astaxanthin in the muscle. The model had a low p-value and a high R^2 which means there was low variation, and the model highly explains the variation in the population, in other words genetics caused the different levels of free astaxanthin.

5.4.2. Visual color

The SalmoFan score on NQC ranged from 24.9 – 25.6 on average for the PALE genetic line and 26 – 27.2 on average for the RED genetic line. The range for the PALE line was significantly lower compared to the RED genetic line, which means the RED line had a deeper red color and is more favorable for consumers and in the aquaculture industry, because a higher SalmoFan score increase the quality of fillets. The overall range is large and within what is normal for Atlantic salmon. Exposure to repeated hypoxia had a significant effect and resulted in a higher SalmoFan score. However, hypoxia (stress) has other negative effects on welfare and the immune defense, which is not beneficial to the fish and reduce the quality in other ways. The astaxanthin retention was as mentioned not affected by hypoxia, this means that the SalmoFan score was higher for stressed fish likely because of less fat in the fillet and not higher mobilization of astaxanthin in skeletal muscle. Higher fat content dilutes astaxanthin and interferes with visual color perception (Erikson & Misimi, 2008). This could mean that fat could be lower in the groups that were exposed to hypoxia compared to the groups that were not stressed, however, fat content in fillets were not measured. The model had a low p-value and a high R^2 which means there was low variation, and the model highly explains the variation in the population, in other words genetics and stress caused the variation on SalmoFan score on the NQC in the population.

The SalmoFan score on anterior fillets had a lower range compared to NQC, which was expected, and showed the same effects of genetics and hypoxia as NQC. The SalmoFan score of anterior fillets for the PALE line ranged from 24.4 – 25.6, and the RED genetic line ranged from 25.4 – 26.9. The astaxanthin concentration in anterior fillets were not measured, but it is expected to have the same differences between the two genetic lines. However, it would be expected to be a lower concentration of astaxanthin in anterior fillets compared to NQC. Exposure to repeated hypoxia did result in a higher score of SalmoFan, and as mentioned before this is likely due to reduced fat in the fillet and not higher concentrations of astaxanthin. Even though the SalmoFan

score was higher for the fish exposed to hypoxia, exposing salmon to stress to get a higher SalmoFan score is not something that would be beneficial for the overall welfare and health of the fish. Stress results in higher scores of operational welfare scores, and a reduced welfare overall, as well as stress can potentially reduce immune response capacity (Noble et al., 2018). The model had a low p-value and a high R^2 which means there was low variation, and the model highly explains the variation in the population, in other words genetics and stress caused the variation on SalmoFan score on the anterior fillet in the population.

5.4.3. Colorimetric analysis

The L* value of NQC ranged from 38.5 – 41.5 on average for the PALE genetic line and 39.1 – 40.1 on average for the RED genetic line, which is a small range overall and a low average (Garber et al., 2019; Veiseth-Kent et al., 2010; Yagiz et al., 2009). This means that the NQC fillets were darker than averages from earlier studies found. The L* value of NQC could be low because of low fat content, but fillet fat was not measured in this study. Earlier studies have shown that increasing fat in fillets result in increased L* and b* values (Mørkøre et al., 2001). The L* value was not significantly different between the PALE and RED genetic line and was not significantly affected by repeated exposure to hypoxia. The L* value not being affected by hypoxia slightly contradicts the results of the SalmoFan score. Because the SalmoFan score was higher for the fish that had been exposed to repeated hypoxia, it could be expected the L* value would be lower. However, the L* value measures only a small point on the fillet, while the visual color evaluated with SalmoFan gives a more holistic impression of the fillet. The L* value can also be affected by connective tissue in the fillet. The model had a high p-value and a low R^2 which means the L* value had large variation from the average, and there were no significant differences that originated from hypoxia, genetics, or body weight.

The L* value of anterior fillets ranged from 38.3 – 41.3 for the PALE genetic line on average and 39.1 – 40.1 for the RED genetic line on average, which is a small range and a low average. The L* value of anterior fillets was not significantly different between the PALE and RED genetic line and was not significantly affected by repeated exposure to hypoxia. The same points discussed for the L* value of NQC can be made with the L* value of anterior fillets. The L* value is likely darker because of low fat content in the fillet, and not high consentrations of

astaxanthin. As well as the SalmoFan score on anterior fillets were higher with exposure to hypoxia, and the L* value did not get affected by stress could be because of the nature of the methods. The model had a high p-value and a low R^2 which means the L* value had large variation from the average, and there were no significant differences that originated from hypoxia, genetics, body weight or gender.

The a* value of NQC ranged from 13.8 – 14.2 on average for the PALE genetic line and 15.4 – 15.9 on average for the RED genetic line, which is a normal range and a low average (Erikson & Misimi, 2008; Garber et al., 2019). The a* value was significantly higher for the RED genetic line compared to the PALE genetic line, which means the RED line had a redder fillet than the PALE line. A red fillet is considered to be of better quality and consumers desire a red fillet, so the RED genetic line would likely be more preferable to the aquaculture industry and the consumers. Exposure to repeated hypoxia did not have a significant effect, which means stress does not result in a more pale or red fillet. However, this does contradict with the results from the SalmoFan score of NQC fillets, which did get affected by hypoxia. However as discussed before the Minolta only measures a small point on the fillet, which can be affected by connective tissue and fat, whereas the visual color evaluated with SalmoFan tend to be a more overall impression of the fillet. The genetic effects were however significant for both the a* value and the SalmoFan score. The model had a low p-value and a moderate to high R^2 which means the variance from the average was low, and there is a low chance the results would happen if there were no effects of genetics and stress because of the low p-value, which means the null hypothesis can be rejected.

The a* value of anterior fillet ranged from 13.9 – 14.2 on average for the PALE genetic line and from 15 – 15.9 on average for the RED genetic line, which is a normal range and a low average. The anterior fillets are usually lighter and less red compared to the NQC, but this was not the case for this study. The a* value was significantly higher for the RED genetic line compared to the PALE genetic line, which means the RED line had a redder fillet than the PALE line. This coincides with the differences in astaxanthin concentrations between the two genetic lines. A red fillet is of better quality and consumers desire a red fillet, so it would be preferable to select for and sell the RED genetic line in a commercial setting. Exposure to repeated hypoxia did not have a significant effect, which means stress does not result in a more pale or red fillet. The same as

discussed for NQC applies for the a* value of anterior fillets. The redness of the fillet was visually higher when scored with SalmoFan, but not with Minolta. This could be because of connective tissue or fat in the fillet. The model had a low p-value and a moderate to high R^2 which means the variance from the average was low, and there is a low chance the results would happen if there were no effects of genetics and stress because of the low p-value, which means the null hypothesis can be rejected.

The b* value of NQC ranged from 12.8 – 13.1 on average for the PALE genetic line and from 13.4 – 14.2 on average for the RED genetic line which is a normal range and a normal average (Erikson & Misimi, 2008; Garber et al., 2019). The b* value is positive which means the NQC are more yellow than they are blue. The b* value was significantly higher for the RED genetic line compared to the PALE genetic line, which means the NQC of the RED line had a more yellow hue compared to the PALE line. Exposure to repeated hypoxia had no significant effect, but the RED line seemed to have a slightly lower b* value with repeated hypoxia, as R3 had a lower average than R0, R1 and R2 although the b* value was not significantly lower. The b* value and L* value can both increase with higher fat content (Mørkøre et al., 2001), so that there is no significant decrease from hypoxia for either value, means the fat content was likely not significantly decreased with exposure to repeated hypoxia. The astaxanthin concentration was lower in the PALE genetic line compared to the RED genetic line, so the differences between the lines in b* value is likely connected to the astaxanthin concentration and possibly fat. The model had a low p-value and a low R^2 which means the b* value had large variation from the average, but there were significant differences that were from other variables than hypoxia and genetics.

The b* value of the anterior fillet ranged from 12.8 – 13.1 on average for the PALE genetic line and from 13.4 – 14.2 on average for the RED genetic line, which is a normal range and a normal average. The b* value is positive which means the anterior fillets are more yellow than they are blue. The b* value was significantly higher for the RED genetic line compared to the PALE genetic line, which means the anterior of the RED line had a more yellow hue compared to the PALE line. Exposure to repeated hypoxia had no significant effect, but the RED line seemed to have a slightly lower b* value with repeated hypoxia, as R3 had a lower average than R0, R1 and R2 although the b* value was not significantly lower. The same things discussed for the b* value of NQC is likely the same for the anterior fillets, however astaxanthin concentration was not

measured on anterior fillets. The model had a low p-value and a low R^2 which means the b* value had large variation from the average, but there were significant differences that were from other variables than hypoxia and genetics.

5.5. Fillet quality parameters

The gaping score ranged from 0 – 0.5 on average, which is a small range and a low score on average. This means that there was none or close to no gaping on average on the fillets. The gaping score was not affected by genetic or exposure to repeated hypoxia, and there were few differences between the groups. The model had a high p-value and a low R^2 which means the gaping score had large variation from the average, and there were no significant differences that originated from the variables used in the model, such as genetics and exposure to repeated hypoxia.

The texture score on NQC fillets ranged from 3.1 – 4.2 on average which is a moderate range and a high average. This means that the fillets were soft. Genetics did not affect the texture of the fillets, however exposure to repeated hypoxia resulted in a higher texture score, which means the texture got softer with more exposure to hypoxia. Texture is important for the quality of fillet, both as a sensory characteristic for consumers and because firmer fillets are more suited for mechanical processing of fillets. Hypoxia leads to a softer fillet and therefore also reduce the quality of the fillet, which is undesirable. The model had a low p-value and a low R^2 value which means the texture score had large variation from the average, but the p-value indicates that stress and genetics influenced the texture score on NQC.

The texture score on anterior fillets ranged from 3.3 – 4.3 on average which is a moderate range and a high average. The anterior fillets were also soft, and were affected by exposure to repeated hypoxia, but there was no significant difference between the genetic lines overall. The model had a low p-value and a low R^2 value which means the texture score had large variation from the average, but the p-value indicates that stress and genetics influenced the texture score on anterior fillets.

6. Conclusion

In conclusion, further research is needed to look at the performance and mechanisms behind the differences between the two genetic lines. The astaxanthin retention and disposition could be genetically linked to biometric traits and heart health and could be a potential topic suitable for further research.

The body weight, fillet yield and condition factor were higher for the PALE genetic line except for fork length and gutted yield. The reason why the gutted yield was not different between the genetic lines is likely because of the weight of viscera, skeleton, and head. The RED line was longer than the PALE line, and this included the difference in body weight highly contributed to the CF being lower for the RED genetic line compared to the PALE line. This likely means the RED line was growing faster but spent energy on skeletal growth and length instead of muscle growth. The mechanisms behind this are unknown but could be a fruitful topic for further investigation. All the operational welfare indicators (OWI) were affected negatively by being exposed to repeated hypoxia, which could be expected.

However, during the stress-test there was also a lot of handling, so it could be useful to investigate if the hypoxia would affect the OWI scores as much if there was a method to expose the fish with hypoxia with less handling. The only OWI that was affected by genetics was snout damage, where the RED genetic line had a higher score than the PALE genetic line, which could mean that the RED line had a reduced healing capacity. However, this would be opposite to what would be expected since the RED line had a higher concentration of astaxanthin, that has antioxidative properties and positive effects on immune capacity. A potential topic suitable for further research could therefore be to investigate the healing capacity of fish of the RED genetic line, looking at the healing capacity of fish fed different levels of astaxanthin.

Further research is also needed to see if the fat content affected the color perception of fillets, and if hypoxia reduce fat concentration in the fillet. Stress indicators in blood would have been interesting to analyze to see if the repeated hypoxia increased glucose and lactate levels to see if the effects of the stress test affected the fish long term. The stress-test was only done for 15 – 20 minutes three times over a period of a little over three months and there is not a guarantee that the hypoxia alone was the cause of the effects on the OWI's and skin color. The RED genetic line had higher scores on SalmoFan and a* and b* values compared to the PALE genetic line. This

means the fillets of the RED line had a deeper red-pink color, which increases the quality of the fillet. The L*a*b* values were not affected by exposure to stress, however the SalmoFan score was higher for both NQC and anterior fillets for groups that were exposed to repeated hypoxia. This difference could mean that the overall color perception of the fillets was affected by stress. The skin of the anterior fillets was not affected by stress or exposure to repeated hypoxia. However, the NQC, both over and under the lateral line, were affected by exposure to repeated hypoxia. The L* value got lower on the NQC both over and under the lateral line, and the b* value increased. This means that the NQC got darker and less blue with exposure to hypoxia, which also has been shown in earlier research. However, the L* value above the lateral line on NQC was higher for the RED genetic line compared to the PALE genetic line, which means the RED line was darker than the PALE line and there could be differences in response to stress between the lines. The fillet gaping was not affected by exposure to repeated hypoxia or genetics. However, fillet texture got softer with exposure to repeated hypoxia, which is unwanted and not beneficial for fillet quality.

In summary, there were overall differences between the PALE and RED genetic lines when it comes to biometric traits and fillet color, where the PALE line had higher growth rate and fillet yield, which could increase fillet price. However, the RED line had a more red-pink color which gives a higher quality, which could increase willingness to pay more for a fillet. All operational welfare indicators were negatively affected by exposure to repeated hypoxia. This means that handling of the fish should be reduced to a minimum.

7. References

Amar, E. C., Kiron, V., Satoh, S. & Watanabe, T. (2001). Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, Oncorhynchus mykiss (Walbaum). *Aquaculture Research*, 32 (s1): 162-173. doi: https://doi.org/10.1046/j.1355-557x.2001.00051.x.

Andersen, B., Steinsholt, K., Stroemsnes, A. N., Averoey, S. & Thomassen. (1994). *Fillet gaping in farmed Atlantic salmon (Salmo Salar)*.

Angeles Jr, I. P., Chien, Y.-H. & Tayamen, M. M. (2009). Effects of different dosages of astaxanthin on giant freshwater prawn Macrobrachium rosenbergii (De Man) challenged with Lactococcus garvieae. *Aquaculture Research*, 41 (1): 70-77. doi: https://doi.org/10.1111/j.1365-2109.2009.02306.x.

Bjerkeng, B., Refstie, S., Fjalestad, K. T., Storebakken, T., Rødbotten, M. & Roem, A. J. (1997). Quality parameters of the flesh of Atlantic salmon (Salmo salar) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture*, 157 (3): 297-309. doi: https://doi.org/10.1016/S0044-8486(97)00162-2.

Bjerkeng, B. & Berge, G. M. (2000). Apparent digestibility coefficients and accumulation of astaxanthin E/Z isomers in Atlantic salmon (Salmo salar L.) and Atlantic halibut (Hippoglossus hippoglossus L.). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 127 (3): 423-432. doi: https://doi.org/10.1016/S0305-0491(00)00278-9.

Bjerkeng, B., Peisker, M., von Schwartzenberg, K., Ytrestøyl, T. & Åsgård, T. (2007). Digestibility and muscle retention of astaxanthin in Atlantic salmon, Salmo salar, fed diets with the red yeast Phaffia rhodozyma in comparison with synthetic formulated astaxanthin. *Aquaculture*, 269 (1): 476-489. doi: https://doi.org/10.1016/j.aquaculture.2007.04.070.

Bjerkås, E. & Sveier, H. (2004). The influence of nutritional and environmental factors on osmoregulation and cataracts in Atlantic salmon (Salmo salar L). *Aquaculture*, 235 (1): 101-122. doi: https://doi.org/10.1016/j.aquaculture.2003.10.005.

Botta, J. (1989). Method of measuring the firmness of meat. *Canadian Intellectual Property Office* (2004560).

Einarsdóttir, I. E., Nilssen, K. J. & Iversen, M. (2000). Effects of rearing stress on Atlantic salmon (Salmo salar L.) antibody response to a non-pathogenic antigen. *Aquaculture Research*, 31 (12): 923-930. doi: https://doi.org/10.1046/j.1365-2109.2000.00506.x.

Einen, O., Guerin, T., Fjæra, S. O. & Skjervold, P. O. (2002). Freezing of pre-rigor fillets of Atlantic salmon. *Aquaculture*, 212 (1): 129-140. doi: https://doi.org/10.1016/S0044-8486(01)00874-2.

Erikson, U. & Misimi, E. (2008). Atlantic Salmon Skin and Fillet Color Changes Effected by Perimortem Handling Stress, Rigor Mortis, and Ice Storage. *Journal of Food Science*, 73 (2): C50-C59. doi: https://doi.org/10.1111/j.1750-3841.2007.00617.x.

Erikson, U., Bye, G. & Oppedal, K. (2009). *Sluttrapport: Fastere filét - industritest og opplæring*. Fiskeri- og havbruksnæringens forskningsfinansiering. Tilgjengelig fra: https://www.fhf.no/prosjekter/prosjektbasen/900109/ (lest 20.04).

FAO. (2020). The State of World Fisheries and Aquaculture 2020. *Sustainability in action. Rome*. doi: https://doi.org/10.4060/ca9229en.

Frisk, M., Høyland, M., Zhang, L., Vindas, M. A., Øverli, Ø. & Johansen, I. B. (2020). Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (Salmo salar L.). *Aquaculture*, 529: 735615. doi: https://doi.org/10.1016/j.aquaculture.2020.735615.

Färber, F. (2017). *Melanin spots in Atlantic salmon fillets: an investigation of the general problem, the frequency and the economic implication based on an online survey*: Norwegian University of Life Sciences, Ås.

Garber, A. F., Amini, F., Gezan, S. A., Swift, B. D., Hodkinson, S. E., Nickerson, J. & Bridger, C. J. (2019). Genetic and phenotypic evaluation of harvest traits from a comprehensive commercial Atlantic salmon, Salmo salar L., broodstock program. *Aquaculture*, 503: 242-253. doi: https://doi.org/10.1016/j.aquaculture.2019.01.001.

Gjedrem, T. & Baranski, M. (2010). *Selective breeding in aquaculture: an introduction*, b. 10: Springer Science & Business Media.

Higuera-Ciapara, I., Félix-Valenzuela, L. & Goycoolea, F. M. (2006). Astaxanthin: A Review of its Chemistry and Applications. *Critical Reviews in Food Science and Nutrition*, 46 (2): 185-196. doi: 10.1080/10408690590957188.

Iversen, M., Finstad, B., McKinley, R. S., Eliassen, R. A., Carlsen, K. T. & Evjen, T. (2005). Stress responses in Atlantic salmon (Salmo salar L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. *Aquaculture*, 243 (1): 373-382. doi: https://doi.org/10.1016/j.aquaculture.2004.10.019.

Johansen, I. B., Lunde, I. G., Røsjø, H., Christensen, G., Nilsson, G. E., Bakken, M. & Øverli, Ø. (2011). Cortisol response to stress is associated with myocardial remodeling in salmonid fishes. *Journal of Experimental Biology*, 214 (8): 1313-1321. doi: 10.1242/jeb.053058.

Johansen, I. B., Sandblom, E., Skov, P. V., Gräns, A., Ekström, A., Lunde, I. G., Vindas, M. A., Zhang, L., Höglund, E., Frisk, M., et al. (2017). Bigger is not better: cortisol-induced cardiac growth and dysfunction in salmonids. *Journal of Experimental Biology*, 220 (14): 2545-2553. doi: 10.1242/jeb.135046.

Johnsen, C. A., Hagen, Ø., Solberg, C., Björnsson, B. T. H., Jönsson, E., Johansen, S. J. S. & Bendiksen, E. Å. (2013). Seasonal changes in muscle structure and flesh quality of 0+ and 1+ Atlantic salmon (Salmo salar L.): impact of feeding regime and possible roles of ghrelin. *Aquaculture Nutrition*, 19 (1): 15-34. doi: https://doi.org/10.1111/j.1365-2095.2011.00927.x.

Kittilsen, S., Schjolden, J., Beitnes-Johansen, I., Shaw, J. C., Pottinger, T. G., Sørensen, C., Braastad, B. O., Bakken, M. & Øverli, Ø. (2009). Melanin-based skin spots reflect stress responsiveness in salmonid fish. *Hormones and Behavior*, 56 (3): 292-298. doi: https://doi.org/10.1016/j.yhbeh.2009.06.006.

Larsson, T., Mørkøre, T., Kolstad, K., Østbye, T.-K., Afanasyev, S. & Krasnov, A. (2012). Gene Expression Profiling of Soft and Firm Atlantic Salmon Fillet. *PLOS ONE*, 7 (6): e39219. doi: 10.1371/journal.pone.0039219.

Lijalad, M. & Powell, M. D. (2009). Effects of lower jaw deformity on swimming performance and recovery from exhaustive exercise in triploid and diploid Atlantic salmon Salmo salar L. *Aquaculture*, 290 (1): 145-154. doi: https://doi.org/10.1016/j.aquaculture.2009.01.039.

Lim, K. C., Yusoff, F. M., Shariff, M. & Kamarudin, M. S. (2018). Astaxanthin as feed supplement in aquatic animals. *Reviews in Aquaculture*, 10 (3): 738-773. doi: https://doi.org/10.1111/raq.12200.

Lorenz, R. T. & Cysewski, G. R. (2000). Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology*, 18 (4): 160-167. doi: https://doi.org/10.1016/S0167-7799(00)01433-5.

Moreno, H. M., Montero, M. P., Gómez-Guillén, M. C., Fernández-Martín, F., Mørkøre, T. & Borderías, J. (2012). Collagen characteristics of farmed Atlantic salmon with firm and soft fillet texture. *Food Chemistry*, 134 (2): 678-685. doi: https://doi.org/10.1016/j.foodchem.2012.02.160.

Mørkøre, T., Vallet, J. L., Cardinal, M., Gomez-Guillen, M. C., Montero, P., Torrissen, O. J., Nortvedt, R., Sigurgisladottir, S. & Thomassen, M. S. (2001). Fat Content and Fillet Shape of Atlantic Salmon: Relevance for Processing Yield and Quality of Raw and Smoked Products. *Journal of Food Science*, 66 (9): 1348-1354. doi: https://doi.org/10.1111/j.1365-2621.2001.tb15213.x.

Mørkøre, T., Espe, M., Veiseth-Kent, E., Terjesen, B., Koppang, E. & Rørvik, K.-A. (2009). *Bred kartlegging av faktorer som påvirker teksturegenskaper i oppdrettslaks ved multivariat tilnærming*.

Mørkøre, T. (2012). Filet av oppdrettslaks: Kvalitetsavvik og årsakssammenhenger. *Nofima rapportserie*.
Mørkøre, T., Moreno, H. M., Borderías, J., Larsson, T., Hellberg, H., Hatlen, B., Romarheim, O. H., Ruyter, B., Lazado, C. C., Jiménez-Guerrero, R., et al. (2020). Dietary inclusion of Antarctic krill meal during the finishing feed period improves health and fillet quality of Atlantic salmon (Salmo salar L.). *British Journal of Nutrition*, 124 (4): 418-431. doi: 10.1017/S0007114520001282.
Noble, C., Nilsson, J., Stien, L. H., Iversen, M. H., Kolarevic, J. & Gismervik, K. (2018). Velferdsindikatorer for oppdrettslaks: Hvordan vurdere og dokumentere fiskevelferd. 3. utgave.
Nofima. (2021). *BioLab*. Tilgjengelig fra: https://nofima.no/fasilitet/biolab/ (lest 11.07.2022).
Nordgarden, U., Ørnsrud, R., Hansen, T. & Hemre, G.-I. (2003). Seasonal changes in selected muscle quality parameters in Atlantic salmon (Salmo salar L.) reared under natural and continuous light. *Aquaculture Nutrition*, 9 (3): 161-168. doi: https://doi.org/10.1046/j.1365-2095.2003.00236.x.
Norgeskart. Tilgjengelig fra: https://norgeskart.no/#!?project=norgeskart&layers=1002&zoom=3&lat=7173606.58&lon=435806.14&markerLat=7334607.891604986&markerLon=386522.8795795669&p=searchOptionsPanel&sok=Bollh%C3%A5gjen (lest 08.04).
Norris, A. T. & Cunningham, E. P. (2004). Estimates of phenotypic and genetic parameters for flesh colour traits in farmed Atlantic salmon based on multiple trait animal model. *Livestock Production Science*, 89 (2): 209-222. doi: https://doi.org/10.1016/j.livprodsci.2004.02.010.
Parker, R. S. (1996). Absorption, metabolism, and transport of carotenoids. *The FASEB Journal*, 10 (5): 542-551.
Pittman, K. & Grigory, V. (2013). Bridging the gap to sustainable salmon farming: overcoming the gaping problem. *Journal of Fisheries & Livestock Production*.
Powell, J., White, I., Guy, D. & Brotherstone, S. (2008). Genetic parameters of production traits in Atlantic salmon (Salmo salar). *Aquaculture*, 274 (2): 225-231. doi: https://doi.org/10.1016/j.aquaculture.2007.11.036.
Quinton, C. D., McMillan, I. & Glebe, B. D. (2005). Development of an Atlantic salmon (Salmo salar) genetic improvement program: Genetic parameters of harvest body weight and carcass quality traits estimated with animal models. *Aquaculture*, 247 (1): 211-217. doi: https://doi.org/10.1016/j.aquaculture.2005.02.030.
Rajasingh, H., Øyehaug, L., Våge, D. I. & Omholt, S. W. (2006). Carotenoid dynamics in Atlantic salmon. *BMC Biology*, 4 (1): 10. doi: 10.1186/1741-7007-4-10.
Rajasingh, H., Våge, D. I., Pavey, S. A. & Omholt, S. W. (2007). Why are salmonids pink? *Canadian Journal of Fisheries and Aquatic Sciences*, 64 (11): 1614-1627. doi: 10.1139/f07-119.
Remen, M., Oppedal, F., Imsland, A. K., Olsen, R. E. & Torgersen, T. (2013). Hypoxia tolerance thresholds for post-smolt Atlantic salmon: Dependency of temperature and hypoxia acclimation. *Aquaculture*, 416-417: 41-47. doi: https://doi.org/10.1016/j.aquaculture.2013.08.024.
Robinson, M. L., Gomez-Raya, L., Rauw, W. M. & Peacock, M. M. (2008). Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (Oncorhynchus clarki henshawi). *Journal of Thermal Biology*, 33 (6): 363-368. doi: https://doi.org/10.1016/j.jtherbio.2008.05.004.
Ruyter, B., Moya-Falcón, C., Rosenlund, G. & Vegusdal, A. (2006). Fat content and morphology of liver and intestine of Atlantic salmon (Salmo salar): Effects of temperature and dietary soybean oil. *Aquaculture*, 252 (2): 441-452. doi: https://doi.org/10.1016/j.aquaculture.2005.07.014.
Sae-Lim, P., Boison, S., Gonen, S., Baranski, M., Stene, J., Rosenlund, G., Hatlen, B., Ruyter, B., Fontanillas, R. & Rubio, L. M. PIGMENT QTL GENOTYPE BY DIET INTERACTION ON GROWTH IN ATLANTIC SALMON (SALMO SALAR).
SAS. (2022). *GLM procedure*. Tilgjengelig fra: https://support.sas.com/rnd/app/stat/procedures/glm.html (lest 11.07.2022).

Seabra, L. M. A. J. & Pedrosa, L. F. C. (2010). Astaxanthin: structural and functional aspects. *Revista de Nutrição*, 23: 1041-1050.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S. & Seland, A. (1997). Handling Stress and Storage Temperature Affect Meat Quality of Farmed-raised Atlantic Salmon (Salmo Salar). *Journal of Food Science*, 62 (4): 898-905. doi: https://doi.org/10.1111/j.1365-2621.1997.tb15482.x.

Sigurgisladottir, S., ØTorrissen, O., Lie, Ø., Thomassen, M. & Hafsteinsson, H. (1997). Salmon quality: Methods to determine the quality parameters. *Reviews in Fisheries Science*, 5 (3): 223-252. doi: 10.1080/10641269709388599.

SjømatNorge. (2022). *Akvafakta Måned, status per utgangen av mai*. Tilgjengelig fra: https://akvafakta.no/wp-content/uploads/Maned/2022/2206_Akvafakta.pdf (lest 12.07.2022).

Solstorm, F., Solstorm, D., Oppedal, F., Olsen, R. E., Stien, L. H. & Fernö, A. (2016). Not too slow, not too fast: water currents affect group structure, aggression and welfare in post-smolt Atlantic salmon Salmo salar. *Aquaculture Environment Interactions*, 8: 339-347.

Stachowiak, B. & Szulc, P. (2021). Astaxanthin for the Food Industry. *Molecules*, 26 (9): 2666.

Thorsen, D. K. (2019). *Melanin-based skin pigmentation and stress in Atlantic salmon (Salmo salar)*: Norwegian University of Life Sciences, Ås.

Tsai, H. Y., Hamilton, A., Guy, D. R., Tinch, A. E., Bishop, S. C. & Houston, R. D. (2015). The genetic architecture of growth and fillet traits in farmed Atlantic salmon (Salmo salar). *BMC Genetics*, 16 (1): 51. doi: 10.1186/s12863-015-0215-y.

Veiseth-Kent, E., Hildrum, K. I., Ofstad, R., Rørå, M. B., Lea, P. & Rødbotten, M. (2010). The effect of postmortem processing treatments on quality attributes of raw Atlantic salmon (Salmo salar) measured by sensory and instrumental methods. *Food Chemistry*, 121 (1): 275-281. doi: https://doi.org/10.1016/j.foodchem.2009.12.009.

Wang, C., Armstrong, D. W. & Chang, C.-D. (2008). Rapid baseline separation of enantiomers and a mesoform of all-trans-astaxanthin, 13-cis-astaxanthin, adonirubin, and adonixanthin in standards and commercial supplements. *Journal of Chromatography A*, 1194 (2): 172-177. doi: https://doi.org/10.1016/j.chroma.2008.04.063.

Wu, D., Sun, D.-W. & He, Y. (2012). Application of long-wave near infrared hyperspectral imaging for measurement of color distribution in salmon fillet. *Innovative Food Science & Emerging Technologies*, 16: 361-372. doi: https://doi.org/10.1016/j.ifset.2012.08.003.

Xu, Y. & Ding, Z. (2004). Traced studies on metabolism of astaxanthin in Atlantic salmon (salmo salar). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 301A (4): 317-323. doi: https://doi.org/10.1002/jez.a.20036.

Yagiz, Y., Balaban, M. O., Kristinsson, H. G., Welt, B. A. & Marshall, M. R. (2009). Comparison of Minolta colorimeter and machine vision system in measuring colour of irradiated Atlantic salmon. *Journal of the Science of Food and Agriculture*, 89 (4): 728-730. doi: https://doi.org/10.1002/jsfa.3467.

Zhou, J., Bi, W. & Row, K. H. (2011). Optimization and Development of a SPE-HPLC-UV Method to Determine Astaxanthin in Saccharina japonica. *Journal of Food Science*, 76 (3): C441-C446. doi: https://doi.org/10.1111/j.1750-3841.2011.02076.x.

Zia-Ul-Haq, M., Dewanjee, S. & Riaz, M. (2021). *Carotenoids: Structure and Function in the Human Body*: Springer Nature.

8. Appendix

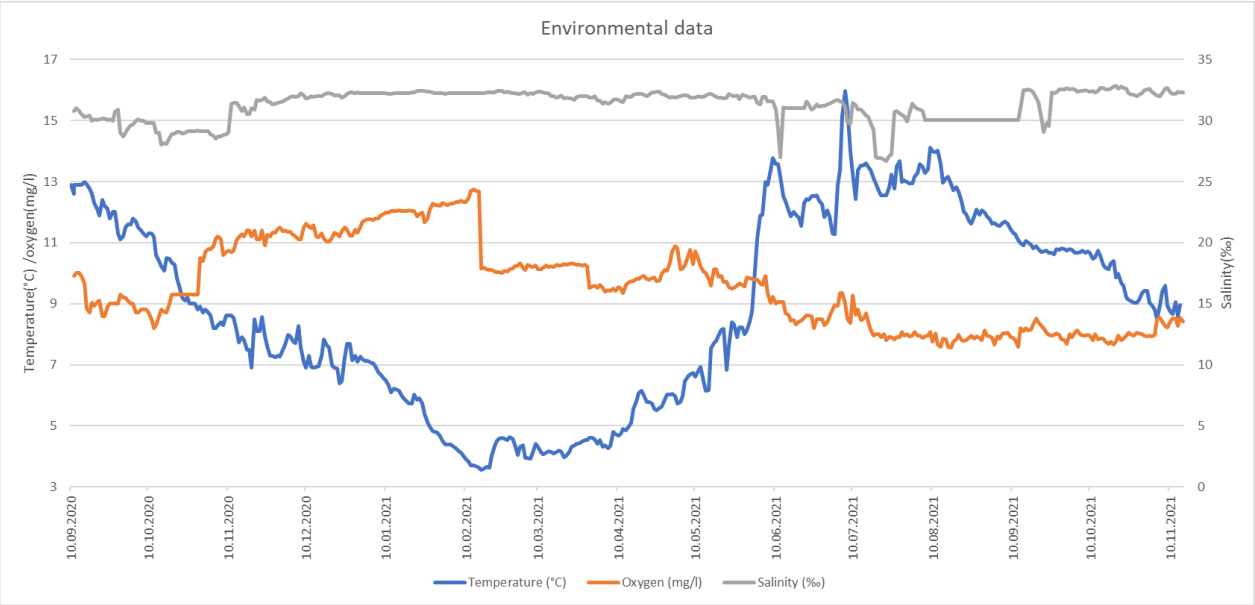


Figure 20. Environmental data. Temperature (℃), oxygen (mg/L) and salinity (‰) measured in the net pens from September 2020 – November 2021.

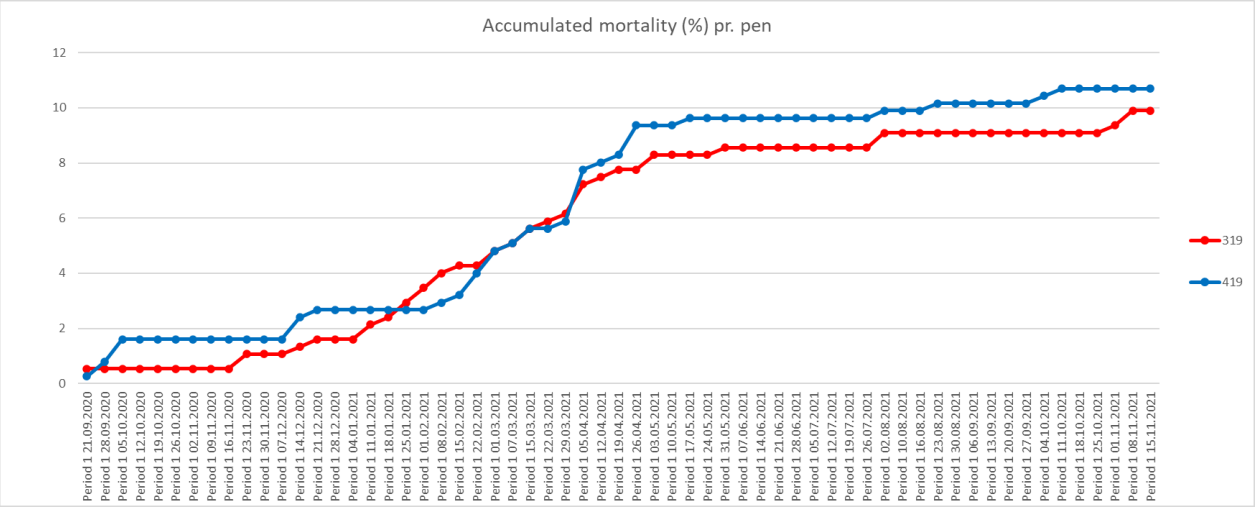
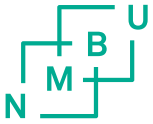


Figure 21. Accumulated mortality (%) in net pen 319 (stress-test) and 419 (control) registered from September 2020 – November 2021.



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway