Factors Influencing Development and Eye Migration During Metamorphosis in Commercial Production of Atlantic Halibut (*Hippoglossus hippoglossus* L.*).*

August Borge Harbo

Master's Thesis in Aquaculture

Department of Biological Sciences

The University of Bergen

June 2023

© August Borge Harbo

2023

Factors Influencing Development and Eye Migration During Metamorphosis in Commercial Production of Atlantic Halibut (*Hippoglossus hippoglossus* L.*)*.

Acknowledgments

Finalizing this master's thesis marks the end of my studies at the University of Bergen (UiB) and symbolizes the completion of a five-year master's degree in Aquaculture. This thesis challenged me to implement knowledge acquired over these five years at the "school bench" and implement it within a real-world commercial production setting.

I would like to start by expressing my gratitude to my supervisors at The Institute of Marine Research, Torstein Harboe and Prescilla Perrichon. Prescilla and Torstein, you provided important assistance in establishing the experimental design and plan for this thesis. Your guidance and continuous feedback throughout this past year have been highly appreciated. I have truly enjoyed our collaboration, both the serious aspects and the lighthearted moments filled with jokes, mostly at the cost of your stupidity Torstein. I would also like to extend my appreciation to Jon Vidar Helvik at UiB for your assistance in setting up the project and keeping me on the right track throughout this past year.

I would like to express a special thanks to Børre Erstad and Trond Sandvik from Sterling White Halibut, for granting me this opportunity and your help in creating this master thesis. This master's thesis would not been possible without your interest for such a project. I would like to tank Sterling White Halibut, and especially everyone at the Hatchery in Rørvik. The time spent in Rørvik was incredibly gratifying, and I want to thank everyone for the assistance and for all the good laughs. I would like to express a special thanks to Walter Olsen-Ryum and Gaute Rauø. Walter, thank you for letting me join the Rørvik team during the study and helping me conduct the experiment. Gaute, thank you for teaching me about the start-feeding facility and "life". Your motto "Drikka, Pula, Spy" is something I try to incorporate into my own life as much as I can.

I would then like to extend my appreciation to my fellow students. These five years have been amazing and is something I will always cherish. I would also like to express a special thanks to the sexy gentlemen at my study hall for the good vibes and making the completion of this study a blast.

Lastly, I want to extend my heartfelt appreciation to my tortoise Mashibaba. You rock!

Bergen
June 2023
August Borge Harbo

Abstract

In commercial production of Atlantic halibut an incomplete eye migration in juveniles is a major problem in the production line. Atlantic halibut with abnormalities are removed from production, representing both a welfare issue and a considerable financial loss. This study analyses factors influence development and eye migration in Atlantic halibut.

The study was conducted in the production line of a commercial Halibut producer. Six culturing tanks of different sizes, light distributions, water currents (aeration), larval density and meals size were manipulated. Growth and development were analyzed from the first feeding until post metamorphosis either on an individual or populational level. The impact of the various manipulations on growth rate and eye migration were calculated. The study uses both experimental data and production data to provide a broader and more accurate representation of the results.

Tank size does not show any significant effect on growth rate, whereas a higher meal size (1700+ Artemia per larvae), larval density, light distribution and aeration had a significant effect on growth. A low- larval density and aeration provided better growth. The growth or larval density did not influence the eye migration significantly, however eye migration was significantly affected by an even light distribution and low aeration in the tanks. These results show that the tanks with an even light distribution and low aeration improves the fish welfare significantly and provides commercial producers with more juveniles for further production. This understanding of the different factors provides a strong foundation for a predictable and more sustainable production and may influence an expansion of the industry.

Abbreviations

Asip1 - Agouti signaling protein 1

DPFF – Days post first feeding

Mc1r - Melanocortin 1 receptor

Mc5r - Melanocortin 5 receptor

MH – Myotome height

SF - Start-feeding

SWH – Sterling White Halibut AS

TH – Thyroid hormone

SF tanks:

3.A – 3m in diameter, high-larval density, even spread of light and low aeration.

3.C – 3m in diameter, low-larval density, even spread of light and low aeration.

2.5.C – 2.5m in diameter, low-larval density, even spread of light and low aeration.

4.A – 4m in diameter, medium-larval density, uneven spread of light and low aeration.

4.B – 4m in diameter, medium-larval density, uneven spread of light and high aeration.

4.A – 4m in diameter, medium-larval density, uneven spread of light and high aeration.

Table of Contents

..... I

Acknowledgments II

Abstract III

Abbreviations IV

1. Introduction 1

1.1 Atlantic halibut 1

1.2 Commercial production of Atlantic halibut 1

1.3 Farming conditions of Atlantic halibut 3
1.3.1 Biological development 3
1.3.2 Rearing conditions: from start-feeding to juvenile stages 4

1.4 Larval metamorphosis and influencing factors 4
1.4.1 Morphological and physical remodeling 4
1.4.2 Influence of feeding conditions on metamorphosis 5
1.4.3 Influence of light conditions on metamorphosis 5
1.4.4 Influence of aeration on metamorphosis 7
1.4.5 Influence of rearing density on metamorphosis 7

1.5 Aims and objective 7

1.6 Sterling White Halibut AS 10

2. Materials and methods 11

2.1 Halibut larval production 11
2.1.1 Production of eggs and yolk-sac larvae 11
2.1.2 On-growth facility 11

2.2 Rearing conditions in start-feeding (SF) tanks 12
2.2.1 Production Set-up 12
2.2.2 SF tanks Set-up 13
2.2.3 Light conditions in the SF tanks 14
2.2.4 Aeration conditions in the SF tanks 15
2.2.5 Larval density in the SF tanks 16
2.2.6 Feeding regime in the SF tanks 16

2.3 Biological analysis from start-feeding to settling stage (metamorphosis) 17
2.3.1 Temperature, oxygen and mortality 17
2.3.2 Growth 17
2.3.3 Eye migration after metamorphosis 18

2.4 Data collection 19

2.5 Statistics 19

3. Results 21

3.1 Growth, development and culture conditions in different rearing systems 21
3.1.1 Growth in the 2.5m, 3m, and 4m SF tanks 21
3.1.2 Growth and light distribution in the SF tanks 25
3.1.3 Growth in the different water currents SF tanks 27
3.1.4 Total feeding amount and prey density 29
3.1.5 Gut content 30
3.1.6 Larval population density 31
3.1.7 Degree of eye migration 31

3.2 Possible effects of larval density on growth and development 34
3.2.1 Impact of population density on growth 34
3.2.2 Impact of growth on eye migration 36
3.2.3 Impact of larval density on eye migration 37

3.3 Possible effects of different physical conditions between tanks 40
3.3.1 Impact of surface light distribution on growth 40
3.3.2 Impact of aeration on growth 42
3.3.3 Impact of surface light distribution on eye migration 43
3.3.4 Impact of aeration on eye migration 44

4. Discussion 45

4.1 Method 45

4.2 Growth, development and culture conditions in different rearing systems 45
4.2.1 Growth in the different sized tanks 45
4.2.2 Feeding amount, feed consumption and prey density 46
4.2.3 Feed consumption and growth 46

4.3 Effects of larval density on growth and development 47
4.3.1 Growth and population density in each SF tank 47
4.3.2 Growth rate and eye migration 48
4.3.3 Larval density and eye migration 48

4.4 Effects of different physical conditions 49
4.4.1 Light distribution and growth 49
4.4.2 Aeration and growth 50
4.4.3 Light distribution and eye migration 50
4.4.4 Aeration and eye migration 51

4.5 Conclusion 52

4.6 Future prospects 54

5. Reference list 55

6. Appendix 62

6.1 Normal distribution 62

6.2 Growth, development and culture conditions 64

6.3 Eye migration 65

6.4 Growth and larval density 65

6.5 Growth and eye migration 66

6.6 Larval density and eye migration 67

6.7 Light distribution and growth 67

6.8 Light distribution and eye migration 68

6.9 Aeration and growth 69

6.10 Aeration and eye migration 69

1. Introduction

Aquaculture is currently one of Norway's most important industries and contributes to an exported value of 146.6 billion NOK in 2022 (Statistics Norway -SSB, 2023). This makes it the third largest industry in Norway, behind the oil and gas sector. It is estimated that the aquaculture industry will increase significantly in the coming years. Motivated by the impressive growth in the salmon farming industry, Atlantic halibut (Hippoglossus hippoglossus) is a species that quickly drew interest and was viewed as a promising species for farming in temperate waters (Tilseth, 1990). In 2020, Norwegian halibut producers sold 1 870 000 kg which rose to 2 716 000 kg in 2021 (Directorate of Fisheries, 2022).

1.1 Atlantic halibut

Atlantic halibut (*Hippoglossus hippoglossus*) is a flatfish of the flounder family (Pleuronectidae). It is a cold-water marine fish species and can be found on both sides of the North Atlantic and spawn at depths of 300-700m. Atlantic halibut is the largest teleost in Norwegian waters and females can reach a size of 3.5m and weigh 300kg whereas males reach a much smaller size and a weight of 50kg. Due to its slow growth rate, high age for sexual maturation (females: 5-7 and males: 2-3, (Norberg et al., 2001)) and the high concentration of fish in spawning areas, the halibut is highly vulnerable to overfishing. Consequently, there are limitations and restrictions on catching halibut, including size and fishing season restrictions for this species (Marine Research Institute, 2020). The restrictions have resulted in limited commercial fishing, making the production less exposed to price competition from the fishing industry.

1.2 Commercial production of Atlantic halibut

In Norway, the first hatching and rearing experiments for Atlantic halibut were conducted in 1974 (Solmedal et al., 1974). The first successful attempt to rear Atlantic halibut beyond the metamorphosis took place in 1980, resulting in the survival of two individuals (Blaxter et al, 1983). In the early stages key challenges included limited capital accessibility, technological challenges due to a unique lifecycle and biological challenges (Directorate of Fisheries, 2002). The limited knowledge describing the development, biological requirements and optimal living

conditions of Atlantic halibut led to the commercial industry facing multiple biological challenges in the early stages to establish a more predictable production system. These challenges comprised identification of developmental period of which pigmentation is influenced by nutrient composition in the feed, monitoring the mortality, deformations caused by incomplete metamorphosis, early identification of fast-growing females, and preventing early maturation in males (Directorate of Fisheries, 2002; Pittman, 1996).

Aquareovirus infections (AHRV Atlantic halibut reovirus) were associated with severe liver pathology and massive mortality during the larval stages, that caused a significant decline in the Norwegian production of Atlantic halibut (Blindheim et al., 2014). Today the complete genome of the aquareovirus is known, and a RT PCR test is used to detect the virus in offspring eggs from brood fish (Skoge et al., 2019). Farmers are currently able to control the virus by implementing strict treatment measures and conducting routine inspections of broodstock that may carry the virus. The practices help in producing healthier fry and establishing a more predictable production system. The volume of commercially cultivated halibut sold witnessed a notable escalation, rising from 1,243 tons in 2015 to 1,870 tons in 2020. Considering the extensive production cycle of 3-4 years, fish farmers are witnessing the effects of heightened production numbers from 2019 and continuing thereafter (Directorate of Fisheries, 2023).

The issue of early maturation in males has been solved through the implementation of an “all-female” production system. A collaborative effort between The Institute of Marine Research and Sterling White Halibut AS led to the discovery of a gender marker enabling a faster achievement of an "all-female" fry production system (Erstad, 2014). The female fish grows faster and matures later compared to the male fish. The "all-female" approach is based on the fact that the halibut has an xx/xy genetic system. By indirectly feminizing future broodstock using testosterone or Fadrozole (aromatase inhibitor), a sex reversal occurs in the male fish, resulting in a neo-male. A neo-male has a phenotype of a man but genetically possesses female characteristics. Subsequently, the next generation produced using neo-males will be entirely composed of female fish (Hendry et al., 2003).

High mortality during early life stages and challenges associated with first-feeding, growth, and achieving proper metamorphosis (pigmentation and complete eye migration) remain as major issues in juvenile production of Atlantic halibut (Hamre et al., 2020). In intensive production an incomplete eye migration is a frequent problem, and these abnormal

developments cause a loss in the production of juveniles by 40-50% (Hamre et al., 2002; Næss & Lie, 1998). Halibut with an incomplete eye migration are removed from commercial production due to fish health and consumer non-acceptance of non-qualitative fish, resulting in a major economic loss for the producer. An incomplete eye migration is not only a production issue but also a fish welfare issue. Halibut producers therefore need a broader understanding of which factors influence this biological change in production to have the ability to develop a more sustainable production.

1.3 Farming conditions of Atlantic halibut

1.3.1 Biological development

Atlantic halibut are different from other marine species by having a long yolk-sac period (43 days at 6 °C). During the yolk-sac phase, they rely on the yolk-sac to develop into pelagic larvae that require feed before developing into demersal fry that settle at the bottom (Harboe & Karlsen, 2003). The metamorphosis that occurs during the development from larvae to fry is a complex biological process that makes it challenging to commercially produce Atlantic halibut. Following metamorphosis and the transition to a demersal habitat, Atlantic halibut undergo a process called "weaning" in which they are transitioned to a formulated diet. Once the weaning is successfully completed, the fry is sorted based on optimal growth, and those with incomplete eye migration are removed from production. Thereafter, the focus of production shifts to promoting growth until slaughter.

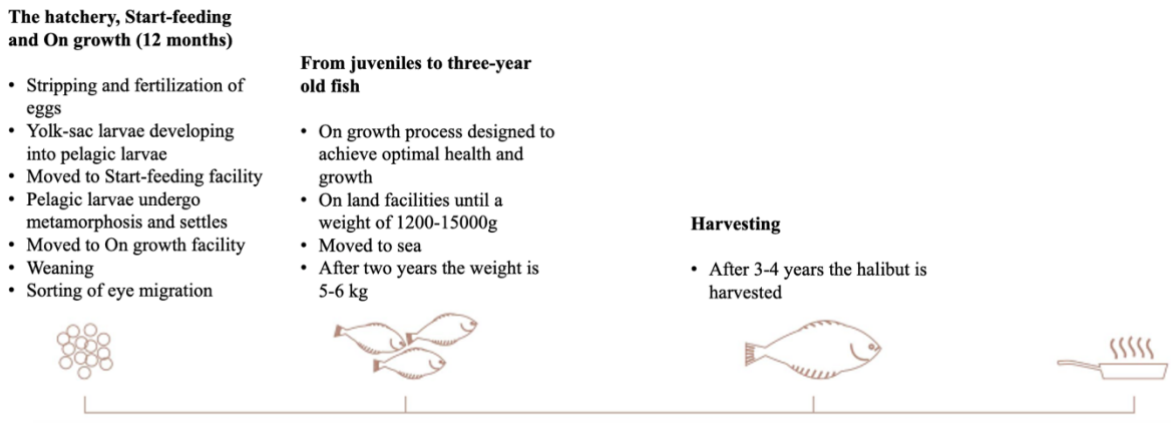


Figure 1: Developmental timeline of the production process. Starting from the hatchery and ending with slaughtered fish after 3-4 years. The complexity of the early life stages compared to later On growth stages. This timeline illustration is acquired from: (Sterling White halibut AS, 2023a).

1.3.2 Rearing conditions: from start-feeding to juvenile stages

The start-feeding larvae are raised in tanks designed for an intensive production (Harboe et al., 1998). Larval surface crowding has been identified as a serious problem in early rearing systems leading a high rate of mortality (Naas et al., 1996). A method developed by Harboe et al., (1998) involves using aeration stones to create a water current that facilitate the dispersion of the larvae into the tank. This method significantly prevents surface crowding and enhances the feeding behavior of the larvae (Harboe et al., 1998).

In the start-feeding facility the water temperature is 11.5°C and there is implemented a diurnal light regime (Harboe et al., 1998, 2009).

Halibut larvae are fed with Artemia nauplii as live feed. Halibut are visual feeders and rely on turbid water to facilitate their capturing of prey. Clay has been increasingly used and added to the water to enhance the prey visibility for halibut. Clay improves the contrast and therefore the prey detection (Harboe & Reitan, 2005; Attramadal et al., 2012). Prior to the use of clay, the green water technique using live micro algae or algae paste were used (Harboe et al., 1998, 2003, 2005; Jones et al., 1981; Naas et al., 1992). However, studies have demonstrated several benefits of using clay instead of algae in halibut production (Harboe & Reitan, 2005; Attramadal et al., 2012). Clay has been found to reduce the levels of bacteria in the water compared to algae. Additionally, clay helps to transport and aggregate the organic matter to the bottom of the tanks, making it easier to remove (Harboe & Reitan, 2005; Attramadal et al., 2012). The practice is now well established in halibut farming.

1.4 Larval metamorphosis and influencing factors

1.4.1 Morphological and physical remodeling

Metamorphosis in flatfish species is the transformation process which the larvae transition from a pelagic habitat to settling at the bottom and adopting a demersal lifestyle. This change in habitat leads to a physiological change in the eye placement causing an eye migration, asymmetrical pigmentation, and a skeleton change (Pittman et al., 1998). The metamorphosis is driven by many internal and external changes that are affected by endogenous thyroid hormone (TH) activity as well as diet and environment (Shao et al., 2017; Zang et al., 2022).

The first indication of hormones influencing the metamorphosis in marine fish larvae was observed in 1930, when an increase in activity in the thyroid tissue was noted (Sclower,

1930). This finding is shown to be essential, and many studies have since researched the effect from TH on metamorphosis and has led to a broad understanding of metamorphosis being mostly hormonally driven (Galay-Burgos et al., 2008; Power et al., 2001; Schreiber et al., 2010; Schreiber & Specker, 2000; Shao et al., 2017; Wang et al., 2011). In a study conducted on Japanese flounder it was demonstrated that retinoic acid signaling with TH and phototransduction pathway are important developmental triggers in the transformation of eye migration and asymmetric pigmentation (Shao et al., 2017)

1.4.2 Influence of feeding conditions on metamorphosis

The dietary composition is an important factor for the larvae culture production. The dietary fatty acid composition has been shown to be a key factor in improving the pigmentation in halibut (Hamre et al., 2007). The halibut larvae are fed with live Artemia during the start-feeding phase. In the natural environment, the larvae feed on copepod nauplii (Moren et al., 2008). Studies have led to Artemia enrichments improving the survival, pigmentation and growth in halibut (Hamre et al., 2007, 2008a, 2008b). The Artemia will during the enrichment take up an emulsion of necessary components by filtration. Enrichment is added to the culture medium and consists mostly of high levels of n-3 fatty acids stimulating a normal pigmentation and provide high energy levels which are shown to be necessary to not limit the eye migration (Hamre & Harboe, 2008a, 2008b). For the Artemia to keep the improved enrichment they are cooled down in the holding Artemia tank.

A study on feed consumption and gut evacuation in Atlantic halibut larvae (van der Meeren, 1995) has been performed. Concluding that there is a maximum level of ingestion.

1.4.3 Influence of light conditions on metamorphosis

Some studies have demonstrated the influence of light conditions on metamorphosis. Halibut are visual feeders and light influence the ability to detect pray. Studies show that halibut under continuous light conditions will continue to capture prey even if the gut is already full, and that this changes the transit time in gut content (Canino, 1995; Harboe et al, 2009). Harboe et al., (2009) performed a study demonstrating that a high gut evacuation results in low digestion and that by implementing a light regime dramatically improves the eye migration.

The influence of light has also been shown to synthesize the retinoic acid gradient on the ocular side. This occurs when rhodopsin in the skin is being exposed to light due to tilted

swimming. This change regulates the asymmetrical physiological transformation and pigmentation during metamorphosis (Shao et al., 2017; Zang et al., 2022).

These findings form the basis for a new study focusing on the influence underwater light intensity has on the metamorphic morphogenesis of Atlantic halibut (Perrichon, pers. comm.). The findings to Perrichon found a clear increase of abnormalities in halibut juveniles exposed to high levels of underwater light during the start-feeding phase. Low under water lighted tanks also had an increase of malformations, however these tanks still produced juveniles without abnormalities. The findings show a clear destruction of a complete metamorphism in the halibut with the use of light (Perrichon, pers. comm.).

The retinoic acid is, as mentioned, important developmental triggers in the transformations of eye migration in Atlantic halibut. Shao et al., (2017) found that the role of retinoic acid in mediating the phototransduction left/right asymmetrical pigmentation in flatfishes are caused by the bodies tilt, which increase the light exposure on the ocular side. This increase of light exposure will during the last stages of metamorphosis case the even distribution of adult chromatophores on the ocular side by instigating the expression of melanocortin 1 receptor (mc1r) or melanocortin 5 receptor (mc5r) or by preventing the expression of agouti signaling protein 1 (asip1) (Figure 2), (Zang et al., 2022).

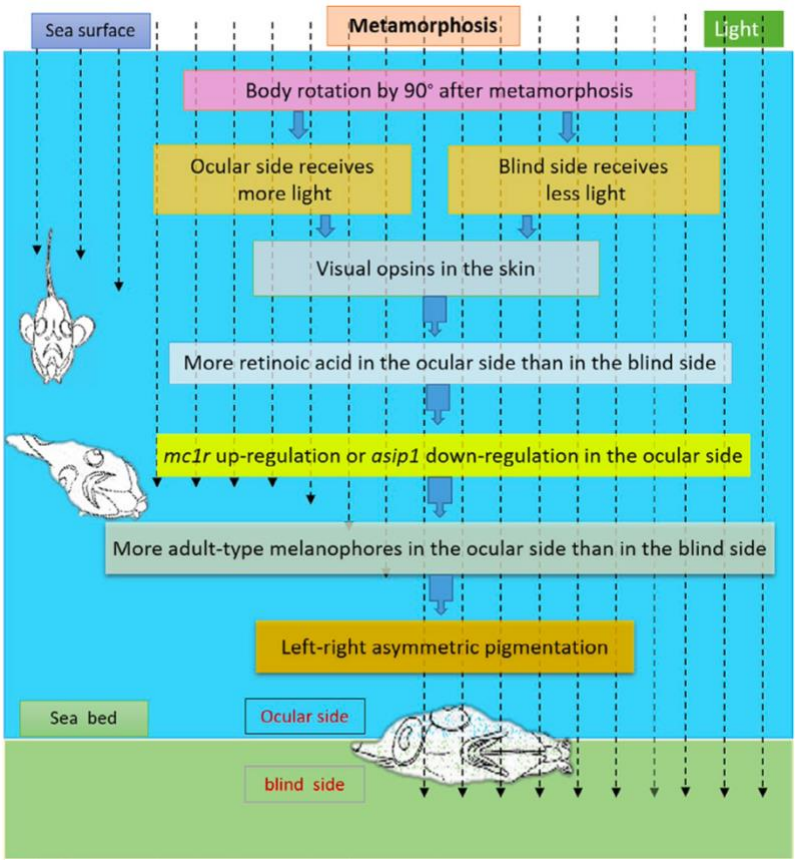


Figure 2: Possible mechanism by which left/right asymmetrical pigmentation is established in flatfishes. Figure 2 is acquired from Zang et al., (2022)

1.4.4 Influence of aeration on metamorphosis

Some studies have shown the influence of aeration conditions on metamorphosis. Halibut larvae are sensitive to mechanical stress and physical stress is shown to create malformations (Opstad & Raae, 1986). A high aeration creates a faster water current, stimulating flatfish to swim more in the water column or moving on the tank bottom. Zang et al., (2022), links hyperactivity of flatfish to a faster water flow in a running water system. The hyperactivity results in a higher water flow across the blind side, which they argue could stimulate pigmentation on the blind side. This stimulation of pigmentation comes from the retinoic acid and the up regulation of mc1r or the downregulations of asip1 (Zang et al., 2022).

These findings were the basis for a study where high aeration and the influence the faster water flow had on eye migration were examined by Perrichon. The high aeration tanks show a clear reduction in fully completed eye migration in the juveniles compared to the standard tanks (Perrichon, pers. comm).

1.4.5 Influence of rearing density on metamorphosis

Study shows that stocking density is a key factor for growth performance and animal welfare in juvenile Olive flounder (Seo & Park, 2023). A high population density increases the stress factor and studies show that stress decrease energy availability for growth (Santos et al., 2010).

Zang et al., (2022) points to density, like for aerations, as a factor for hyperactivity in flatfish. This hyperactivity, as mentioned, could stimulate pigmentation on the blind side (Zang et al., 2022).

1.5 Aims and objective

The main aim of this study was to investigate the factors that influence the rearing conditions and the morphological development of halibut, particularly during the first-feeding phase in a commercial production setting with as little interference as possible, to verify if factors important under laboratory conditions also were critical in a production line. The study examined the potential effects of tank size (2.5-, 3- and 4-m tank) rearing density (low, medium, high), light conditions (intensity and distribution) and water aeration turbulence on the growth,

metamorphosis success (complete eyes migration) and settlement of the juvenile halibut. By improving our understanding of the factors that significantly influence the development and eye migration of Atlantic halibut in commercial production, the industry can become more efficient and produce a larger quantity of healthier fish. This improved knowledge will lay a strong foundation for a sustainable expansion within the industry.

The following hypotheses were considered:

$H0_{1A}$: Different tank size has no significant effect on growth in a commercial production of Atlantic halibut.

HA_{1A}: Different tank size has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{2A}$: Larval density has no significant effect on growth in a commercial production of Atlantic halibut.

HA_{2A}: Larval density has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{3A}$: Growth has no significant effect on eye migration in a commercial production of Atlantic halibut.

HA_{3A}: Growth has a significant effect on eye migration in a commercial production of Atlantic halibut.

$H0_{4A}$: Larval density has no significant effect on eye migration in a commercial production of Atlantic halibut.

HA_{4A}: Larval density has a significant effect on eye migration in a commercial production of Atlantic halibut.

$H0_{5A}$: Surface light distribution has no significant effect on growth in a commercial production of Atlantic halibut.

HA_{5A}: Surface light distribution has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{6A}$: Water current (aeration) has no significant effect on growth in a commercial production of Atlantic halibut.

HA_{6A}: Water current (aeration) has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{7A}$: Surface light distribution has no significant effect on eye migration in a commercial production of Atlantic halibut.

HA_{7A}: Surface light distribution has a significant effect on eye migration in a commercial production of Atlantic halibut.

$H0_{8A}$: Water current (aeration) has no significant effect on eye migration in a commercial production of Atlantic halibut.

HA_{8A}: Water current (aeration) has a significant effect on eye migration in a commercial production of Atlantic halibut.

1.6 Sterling White Halibut AS

This study was conducted in a collaboration with Sterling White Halibut AS (SWH). SWH is a fully integrated halibut farming company with headquarters in Randaberg in Rogaland. SWH has their own hatchery in Rørvik, two rearing facilities in Vindafjord, in Rogaland. Two marine facilities in Hjelmeland, in Rogaland and a Research, Development and Fish Health office in Bergen (Figure 3), (Sterling White Halibut AS, 2023b). This study was performed in their start-feeding facility at their hatchery in Rørvik.

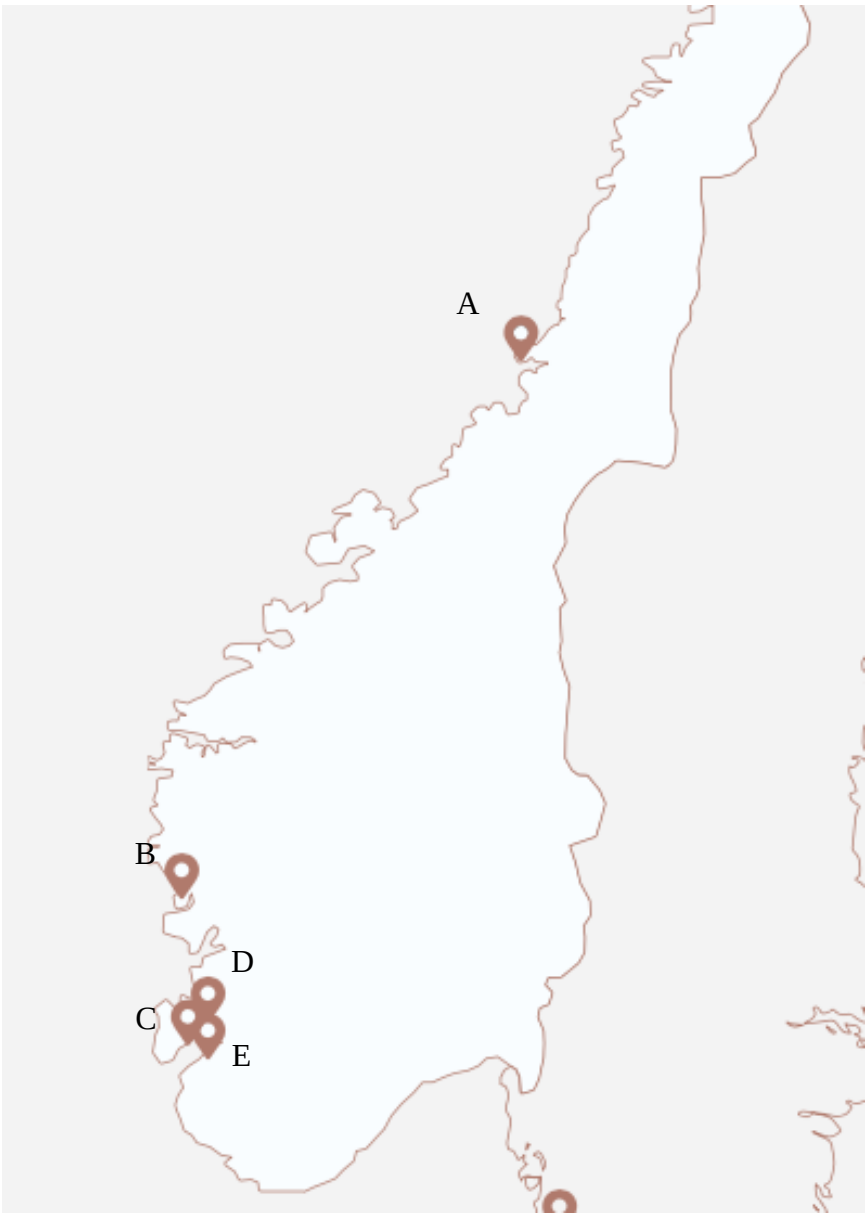


Figure 3: Map showing the locations to SWH. A is the hatchery in Rørvik, B is the Research, Development and Fish Health office in Bergen, C is the headquarters at Randaberg, D are the rearing facilities at Vindafjord and E are the marine facilities at Hjelmeland. Figure acquired from Sterling White Halibut AS. (2023b).

2. Materials and methods

2.1 Halibut larval production

The study took place at Sterling White Halibut AS's hatchery, located at Reipholmen in Rørvik, from June to October 2022. The study was conducted during the standard production cycle of Sterling White Halibut (SWH), which involves three annual spawning groups of halibut, known as C1, C2 and C3. Specifically, this study focused on the C2 spawning group, which represents the second group and is produced during the late spring period. This study was performed on the start-feeding stages of halibut.

2.1.1 Production of eggs and yolk-sac larvae

The production of embryonic and yolk-sac stages followed the standard protocols of SWH. The eggs were obtained by hand-stripping from one female (Norberg et al., 1991), and then fertilized using sperm from a neo-male, before being kept in 600 L incubators and maintained at a temperature of 5.5°C. After 65 degree days (D°), the embryos were moved to a hatching incubator and disinfected using 0.1mL/L pyceze (Mangor-Jensen et al., 1998; Birbeck et al., 2006). Following hatching (around 85 D°), hatched larvae were collected and placed in silos (4500L) up to 265 D° (Harboe et al., 1994). Finally, once the larvae transition from endogenous (yolk-sac stage) to exogenous food (opening-mouth stage) (265 D°), they were transferred to the start-feeding facility to commence the next phase of their development and focus our study objectives.

2.1.2 On-growth facility

In the on-growth facility after the start-feeding facility the halibut undergo "weaning" to a formulated diet (Otohime feed). Otohime has shown to be the best diet for weaning (Hamre et al., 2019). Following the weaning process, the halibut are carefully sorted to promote the best growth and development. A meticulous sorting and quality control measures ensures that only halibut with the desired phenotypes, including appropriate eye migration are retained in the production.

2.2 Rearing conditions in start-feeding (SF) tanks

2.2.1 Production Set-up

The study was based on both experimental and production data. Figure 4 illustrate the tank set up and shows in which tanks experimental data were collected and in which tanks production data were collected and used. Data were collected for the different factors: density, light distribution and aeration were experimental data (Table 1).

It is important to note that data collected before and after the study period of 5 weeks followed SWH's production practices. This applies for the experimental and production tanks. Only the group average for the weight and MH were then recorded for the 20 larvae sampled each week. These group averages represent the production data.

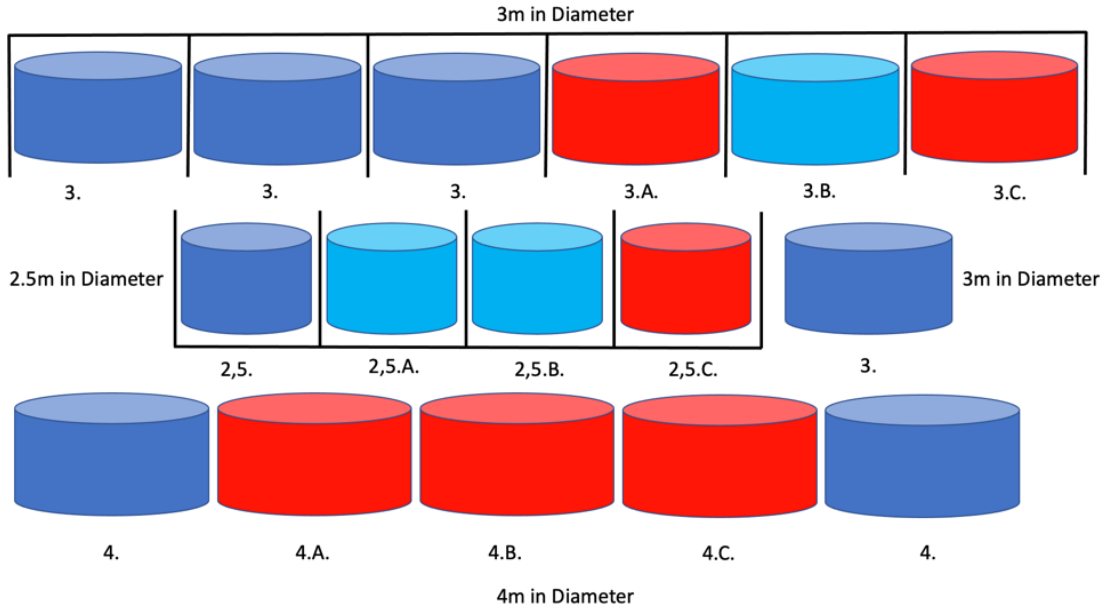


Figure 4: Schematic representation of tank set up in the start-feeding production facility. The tanks used for the study (experimental approach) are highlighted in red, the tanks used for SWH production (production approach) are highlighted in blue. The tanks with a light blue were used to compare the experimental and production approaches. The plastic separation between the SF tanks is shown by the black lines. All thank names used in this study begin with the tanks size followed by a letter (A, B or C).

Table 1: Difference in the factors influencing eye migration between the experimental tanks.

SF Tank	Tank diameter	Larval density	Aeration	Light spread
3.A.	3m	High	Low	Even
3.C.	3m	Low	Low	Even
2.5.C.	2.5m	Low	Low	Even
4.A.	4m	Medim	Low	Uneven
4.B.	4m	Medim	High	Uneven
4.C.	4m	Medim	High	Uneven

2.2.2 SF tanks Set-up

In this study, six start-feeding (SF) tanks of different sizes were tested. These tanks were labeled as follows: one tank with a diameter of 2.5 meters (2.5.C), two tanks with a diameter of 3 meters (3.A and 3.C) and three 4 meter tanks (4.A, 4.B and 4.C) were used (Figure 4). All tanks had a height of 1 meter and filled with approximately 90 cm of seawater. The color of the inside of the tanks was black. The 2.5 m and 3 m tanks (3.A, 3.C and 2.5.C) were made from fiberglass and were regularly used by SWH in their production process. On the contrary, the 4 m tanks (4.A, 4.B and 4.C) were made from plastic and were used for the first time in this study.

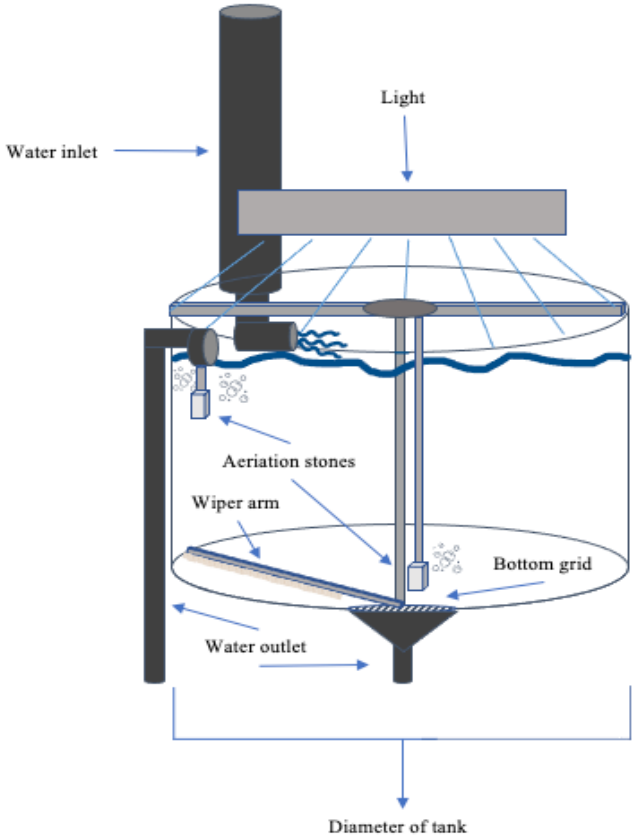


Figure 5: Schematic representation of a standard SF tank set up. Not to scale

Each tank had two outlets: a central drain at the bottom equipped with a 900 µm bottom grid and an overflow strainer at the top (Figure 5). The seawater supply comes from Nærøysundet at a depth of 150 m and was pumped to a facility where it was divided into warm (11.5°C) and cold (5.5°C) water. The cold water was cooled down by passing it though the heating pump (VP-1500) on the cold side while the warm water bypassed it on the warm side. Prior to being pumped to the start-feeding facility, the seawater was sand-filtrated and undergoes an ozone treatment to ensure its quality. In addition, the cold seawater went through a biofilter before being pumped to the start-feeding facility. In each tank the hot and cold water were mixed to achieve the desired temperature (11°C) before being introduced into the tank through a vacuum aerator. The inlet pipe is an elbow-oriented pipe positioned above the water surface (Figure 5). The elbow pipe helped reduce the energy released into the tank, from the water coming through the vacuum aerator. The water renewal rate was set at 20% per hour in the first two days, and then it was increased to 30% per hour thereafter.

Each tank was equipped with a metal wiper arm (Figure 5) designed to collect waste at the bottom of the tank. Every morning, a suction system linked to the wiper arm was used to clean the tanks. Each tank had its own suction system, and the waste was transported to individual buckets. After cleaning, the buckets were inspected to count the number of dead larvae.
The 2.5 m and 3 m tanks (2.5.C, 3.A and 3.C) were separated by plastic sheets to prevent light propagation and minimize the risk of disease transmission between the tanks. However, the 4 m-tanks did not have these plastic sheets installed (Figure 4).

2.2.3 Light conditions in the SF tanks

The light conditions (source, intensity, color and distribution) were tested. The type of light and the number of light sources varied depending on the tank size (Figure 4, Tables 1 and 2). Tank 3.A was equipped with two warm white fluorescent lights, while tank 3.C had two fluorescent light sources emitting blue light. Tank 2.5.C had one light source with a fluorescent blue color light. The tanks 4.A, 4.B and 4.C were all equipped with a single LED light source, emitting white light.

The fluorescent light source consisted of two light rods per source. The different types of light used resulted in varying light spread and intensity within the tanks. The tanks 3.A, 3C and 2.5.C exhibited an even distribution of light, whereas tanks 4.A, 4.B and 4.C had a descending light irradiance from the center and out to the outer periphery (Table 2).

To measure the light intensity Biospherical Instruments Inc., QSL-100 was used.

Table 2: Light condition in SF tanks. The instrument used to measure is a Biospherical Instruments Inc., QSL-100. Measuring quanta sec-1 CM-2 in full scale irradiance (3x1015). The “center” refers to the middle point of the tank, “middle” is a position halfway between the center and the outer periphery, and the “outer periphery” refers to the area near the tank wall. These terms are used to determine the light intensity in the different parts of the tanks. The measurements showing 0 irradiance are wrong, since there is some irradiance, however the spread of light in the tank is the important factor in the table, showing which tanks have an even or uneven spread of light.

SF Tank	Center	Middle	Outer periphery	Light sources	Colour light	Type of light
3.A	0.1	0.5	0.2	2	Warm white	Fluorescent
3.C	0.1	0.2	0.15	2	Blue	Fluorescent
2.5.C	0.1	0.15	0.05	1	Blue	Fluorescent
4.A	1.7	0.4	0	1	White	LED
4.B	1.7	0.4	0	1	White	LED
4.C	1.7	0.4	0	1	White	LED

2.2.4 Aeration conditions in the SF tanks

Different water aeration conditions were also tested in the SF tanks (Figure 4). The tanks 3.A, 3.C and 2.5.C were equipped with one aeration stone placed close to the bottom at the middle of the tank, as well as one aeration stone positioned closer to the surface underneath the water escape outlet. The tanks 4.A, 4.B and 4.C had three aeration sources. Two aeration sources were placed close to the bottom in the middle of the tank to create a circulation current, while one source was positioned closer to the surface underneath the water escape outlet to prevent halibut larvae from getting stuck in the water outlet. The current from the aeration stones move upwards creating a top/turning turbulence in the tank.

To measure the current in the water caused by the water intake and from the aeration stones a light ball was placed on the water and filmed (Smartstudy Viacom 2020). The ball was placed in the center of the tank and the speed of the ball was recorded with an iPhone 13pro, as an indication of the turbulence in the water. The water speeds resulting from the different aeration conditions are reported in Table 3. Although Figure 6 does not show the different water currents caused by the varying number of aeration stones, it is important to note that they contribute to a less homogeneous water current in the tank.

Table 3: Water speed conditions in SF tanks. Low aeration is from 1-6 cm/s, medium is from 6-12 cm/s and high is 12 + cm/s

SF tank	Time in s	Distance	v = cm/s	Aeration
3.A	17.40	30cm	1.72 cm/s	Low
3.C	16.82	35cm	2.08 cm/s	Low
2.5.C	9.21	25cm	2.71 cm/s	Low
4.A	6.79	40cm	5.89 cm/s	Low
4.B	3.43	60cm	17.49 cm/s	High
4.C	5.76	75cm	13.02 cm/s	High

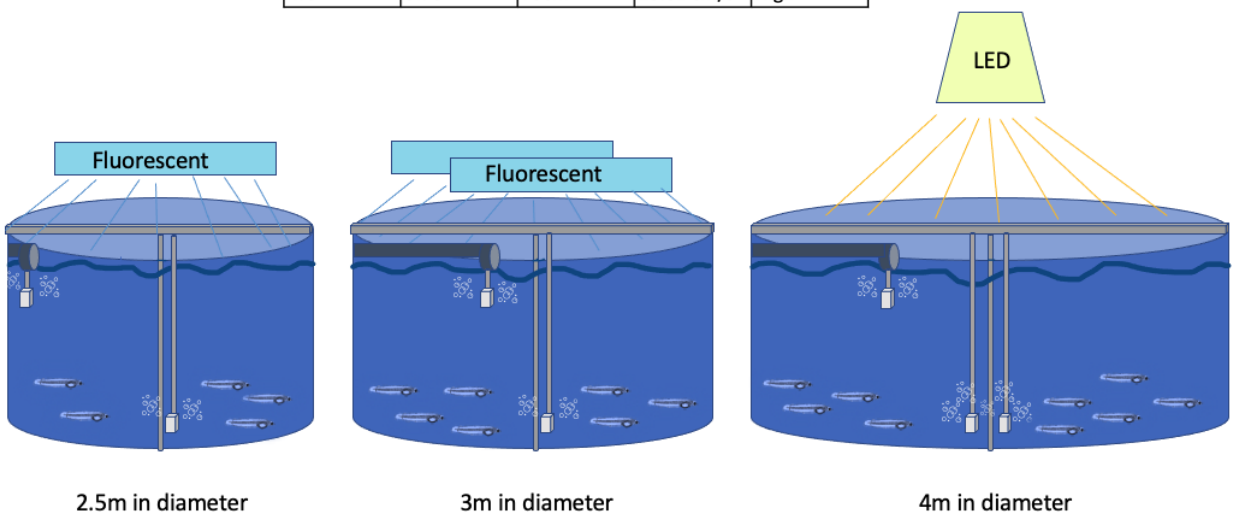


Figure 6: Schematic representation of the difference in diameter, number of aeration stones and number and type of lights in the SF tanks.

2.2.5 Larval density in the SF tanks

The study was conducted using six SF tanks with different densities of halibut larvae. The density estimations of the number of eggs provided by SWH during the production in silos (yolk-sac stage) were used to determine an approximate density difference in the six SF tanks. The calculation of the fertilized egg numbers involved measuring the volume of eggs and applying the fertilization percentage specific to each batch. During the hatchery phase, the mortality numbers were subtracted from the initial egg count. After transferring the larvae from the hatchery to the start feeding facility, a visual check of the tank density was conducted. However, the exact density differences were not known until the final sorting in the on growth facility.

Halibut larvae were transferred in different tanks using a 32 mm PE tube connecting the hatchery and the start-feeding facility. The tanks were divided into 3 densities: high (10+ larvae.L^{-1})-, medium = standard (7-9 larvae.L^{-1})- and low (4-6 larvae.L^{-1})- density tanks (Table 4).

Table 4: Density of halibut larvae distributed in each SF tank.

SF Tank	Number of fish after sorting	Mortality during SF	Fish at the start SF	Number of fish each L	Larval density
3.A	60075	21595	81670	13	High
3.C	21800	8765	30565	5	Low
2.5.C	20900	6715	27615	6	Low
4.A	56706	20215	76921	7	Medium
4.B	62521	18270	80791	7	Medium
4.C	56516	20310	76826	7	Medium

2.2.6 Feeding regime in the SF tanks

During the study, four feeding times per day were implemented, following the standard routine of SWH (10am, 4pm, 10pm and 4am the following day). The larvae were fed live Artemias nauplii. The concentration of Artemia was calculated based on the residual levels from the first feeding time of the day (10am). The meal sizes varied depending on the tank volume. The 3m tanks started with a meal size of 15 million Artemia (approximately 2200 nauplii.L^{-1}). The 2.5m tanks started with 10 million Artemia (approximately 2200 nauplii.L^{-1}) and the 4 m tanks start with 25 million Artemia (approximately 2200 nauplii.L^{-1}). After the first feeding meal, the residual values determined whether the meal size should increase, remain the same, or decrease for the next feeding cycle. Residual values were calculated at 1:30 pm and

2:00 pm. Ideally, the residual values should be between 0.03 and 0.16 nauplii/ml at 2:00 pm. If residual values are too low, the meal size is increased by 2.5 million Artemia for the next feedings. With a 0 residual value at 1:30 pm, the meal is increased by 5 million Artemia for the next feedings. If values exceed 0.2 nauplii/ml the meal is reduced accordingly.

Additionally, clay is continuously added to the SF tanks using a pumping system. This is done to enhance the larval feed uptake and improve larval welfare.

2.3 Biological analysis from start-feeding to settling stage (metamorphosis)

The study was conducted in two developmental phases: i) from start-feeding following the metamorphic stage to weaning (from June to August) and ii) after weaning (in October).

2.3.1 Temperature, oxygen and mortality

Temperature and oxygen in the outlet water of each tank were daily measured using an OxyGuard (Handy Polaris 2). If the oxygen levels dropped below 90%, the water flow in the tanks were increased to improve oxygenation.

Mortality in each SF tank was also monitored on a daily basis. The number of dead larvae was counted after the daily cleaning procedure. The study followed SWH's standard procedures for mortality monitoring.

2.3.2 Growth

For the experimental data, 15-20 larvae per SF-tank were weekly sampled using a hand net for a period of 5 weeks. First, the fish were euthanatized using MS222 (100 mg/L), carefully dried to remove excess water (Figure 7A) and then placed in a plastic Petri dish (Figure 7B). The wet weight (g) and the myotome height (mm) for each individual was measured. The weight was a Mettler Toledo (PG5002-S DeltaRange) and measured from (0.01-5100g). The myotome height (MH) was measured under a Zeiss stereomicroscope (Stemi DV4) with a graduate ruler (Figure 7C).

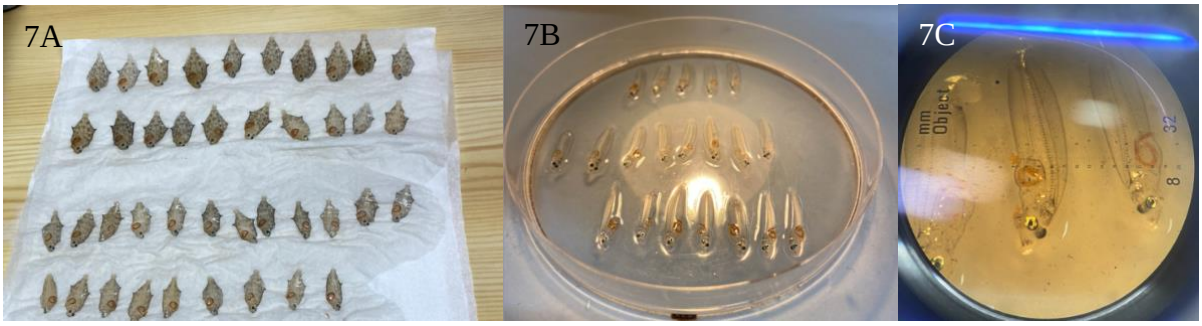


Figure 7. Fish sampling for a morphometric monitoring with the drying step (7A), fish placement in Petri dish (7B) and measurement under stereomicroscope with graduate ruler (7C)

2.3.3 Eye migration after metamorphosis

Eye migration status was recorded for each fish in October 2022, at the end of metamorphosis. Fish from each of the six SF tanks (n = 100+) were placed on a sorting board, and the eye migration was graded on a scale of 0-3 according to the method reported in Næss and Lie (1998): 0 corresponds to no eye migration; 1 corresponds to little eye migration, 2 corresponds to almost complete eye migration and 3 corresponds to complete eye migration (Figure 8). Fish with a score 2 or 3 were selected to be kept in the production process. The halibut with a score 0 or 1 were not retained in the production process and are euthanized with an overdose of MS222.

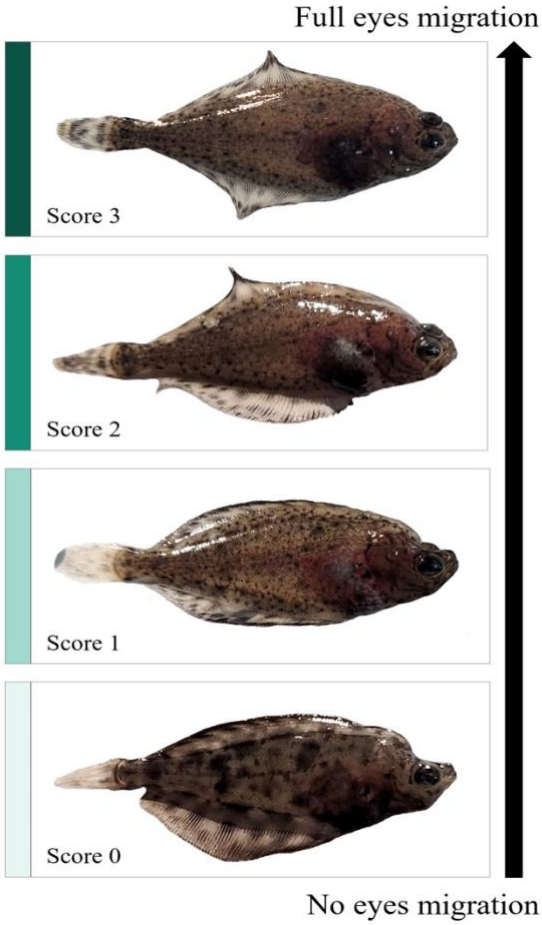


Figure 8: Photo illustration of the different gradings from 0-3 in Atlantic halibut. Figure 8 is acquired from Perrichon, pers. comm.

2.4 Data collection

The study was conducted in a commercial production setting. The data were therefore divided into i) experimental data (collected during the study period) and ii) production data (collected by SWH in the production tanks, and in the experimental tanks before and after the study period). The experimental data were collected from each tank at an individual level. Data are shown as mean ± standard deviation for growth figures and median ± standard deviation for eye migration figures. The production data were defined as the population data. Data from the individual and "population" level were compared to understand the influence of the different factors on growth and the eye migration process.

The halibut were moved to the SF facility at different times. The halibut larvae are therefore not the same age during the study period. To compare and show a broader illustration of the results both the experimental and production data are necessary. When individual data is combined with population data the individual data is transformed to a population data set, which transforms the experimental data to the same as the production data. The data is therefore not combined but separated into two different data sets that are compared to show the findings in a clear and accurate manner.

2.5 Statistics

All statistical tests were performed in Prism 9 (Version 9.5.1(528)) or RStudio (Version 2022.12.0+353) A significant level of 5% was used for all analyses.

The statistics related to growth had to be grouped together by the closest days. Since the halibut larvae are not the same age in the growth samples. The data used for calculating the statistics are grouped together with a maximum of four days between them. Using the oldest individual growth measurements collected during the study period.

The normality and the homogeneity of the variances were first checked with the Shapiro-Wilk test in RStudio. To ensure the homogeneity the skew and kurtosis value were found for growth and eye migration data to all SF tanks (Appendix 1 and 2).

A Student t-test was conducted to compare growth between the different 3m SF tanks (3.A and 3.C).

A one-way ANOVA was conducted for the 4m SF tanks (4.A, 4.B and 4.C), even distribution of light SF tanks (3.A, 3.C and 2.5.C) and low aeration SF tanks (3.A, 3.C, 2.5.C and 4.A) to compare differences in growth (myotome height and weight).

A Chi-square test were performed in Prism 9 and a one-way ANOVA was performed in RStudio to compare eye migration scores influenced by the larval density (high: 3.A and low: 3.C and 2.5.C), aeration (high: 4.B and low: 4.A), the spread of light (even: 3.A and uneven: 4.A) and growth (high: 3.C, 2.5.C and 4.A and low: 3.A, 4.B and 4.C). In Prism it needs to be the same number of measurements for the analysis to work. The two measurements closest to the median were therefore summarized together and divided by two. The high larval density tank had one additional measurement compared to the low larval density tanks, and the high-aeration tank had one additional measurement than the low-aeration tank.

To compare low and high aeration (low: 3.A, 3.C, 2.5.C and 4.A, high: 4.B and 4.C), even or uneven spread of light (even: 3.A, 3.C and 2.5.C, uneven: 4.A, 4.B and 4.C) and larval density (low: 3.C and 2.5.C, medium: 4.A, 4.B and 4.C, and high: 3.A) to growth rate a simple linear regression were performed (in Prism 9) to determine if the growth rate slopes are significantly different. This method is equivalent to an ANCOVA test.

3. Results

3.1 Growth, development and culture conditions in different rearing systems

3.1.1 Growth in the 2.5m, 3m, and 4m SF tanks

The growth measurements were collected to examine the different effects of tank size on growth. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are production data to account for more measurements and clearly show the correlations from the experimental SF tanks to the other production SF tanks.

3.1.1.1 Growth in the 2.5m SF tanks

The three tanks are mostly portraying the same growth pattern for MH. At 12 dpff, the fish from tank 2.5.A exhibited a MH of 1.18mm. Similarly, the fish from tank 2.5.B had a MH of 1.46mm at 10 dpff, while those from tank 2.5.C displayed a MH of 1.59mm at 14 dpff. After these measurements 2.5.B and 2.5.C has an even growth increase, while 2.5.A has a bend from day 19-26 before rapidly increasing and the growth rate stabilizes. The fish from tank 2.5.A demonstrated a remarkable increase in MH, measuring 4.63mm after 33 days. Likewise, the fish from tank 2.5.B displayed a MH of 5.35mm at 31 dpff. Tank 2.5.C on the other hand, showed a MH of 5.59mm at 35 dpff, indicating notable growth across all tanks. Further observations were conducted over an extended period for tanks 2.5.A and 2.5.B. From 47 to 54 dpff, the growth of tank 2.5.A flattens out and exhibited a final MH measurement of 6.25mm. Similarly, the fish from tank 2.5.B had a last MH measurement of 6.93mm at 45 dpff, signifying substantial development. (Figure 9A).

In terms of weight increase, fewer measurements were available for analysis and the growth pattern is less unform as for MH growth. The data indicates that tank 2.5.B had the highest weight measurement, surpassing the other tanks. Tank 2.5.C initially showed slower growth but experienced a rapid increase before reaching a plateau. Tank 2.5.A exhibited steady growth, eventually surpassing tank 2.5.C. Both tank 2.5.A and 2.5.C exhibited a noticeable bend in their growth curve when they reached a weight of around 0.20g (Figure 9A).

There was only one 2.5m SF tank that the study has experimental data on. The P-value was therefore not calculated for these tanks.

3.1.1.2 Growth in the 3m SF tanks

Initially, the MH growth of all tanks had a similar slow growth up to around 10 to 20 dpff. However, from that point onwards, tank 3.C displayed a faster rate of increase compared to tanks 3.A and 3.B. It took tank 3.C 31 days to reach a MH measurement of 5.22mm. Tank 3.A, on the other hand, required 37 days to reach 4.97mm, while tank 3.B took the same duration to reach 5.06mm. Continuing the observations, tank 3.C demonstrated sustained growth and achieved a final MH measurement of 7.04mm by 38 dpff. In contrast, tank 3.B's MH growth began to plateau after 37 dpff, culminating in a final measurement of 5.08mm by 44 dpff. Tank 3.A exhibited a slower growth rate after 44 days, where it starts to flatten out, ultimately reaching a MH measurement of 6.47mm by 51 dpff. (Figure 9B).

The analysis of weight increase in tanks 3.A, 3.B, and 3.C reveals distinct differences, particularly when comparing tank 3.C to tanks 3.A and 3.B. After 38 days, tank 3.C exhibited a weight of 0.19g, surpassing the weights of both tank 3.A and 3.B. Tank 3.A weighed 0.09g after 37 days, while tank 3.B weighed 0.11g after the same duration. Further observations show that tank 3.C had a last weight of 0.30g at 52 dpff, outpacing the weights of both tank 3.A and 3.B, which measured 0.18g after 51 days. Tank 3.B experienced a flattening of growth, maintaining a weight of 0.18g after 58 days. On the other hand, tank 3.A continued with a low increase in weight, reaching 0.22g at 58 dpff (Figure 9B).

The fish growth rate from tank 3.C is significantly faster than the fish raised in tank 3.A (Student t-test performed on experimental data, MH growth had a $p = 6.3x10^{-11}$ and weight had a $p = 5.6x10^{-12}$), (Appendix 3).

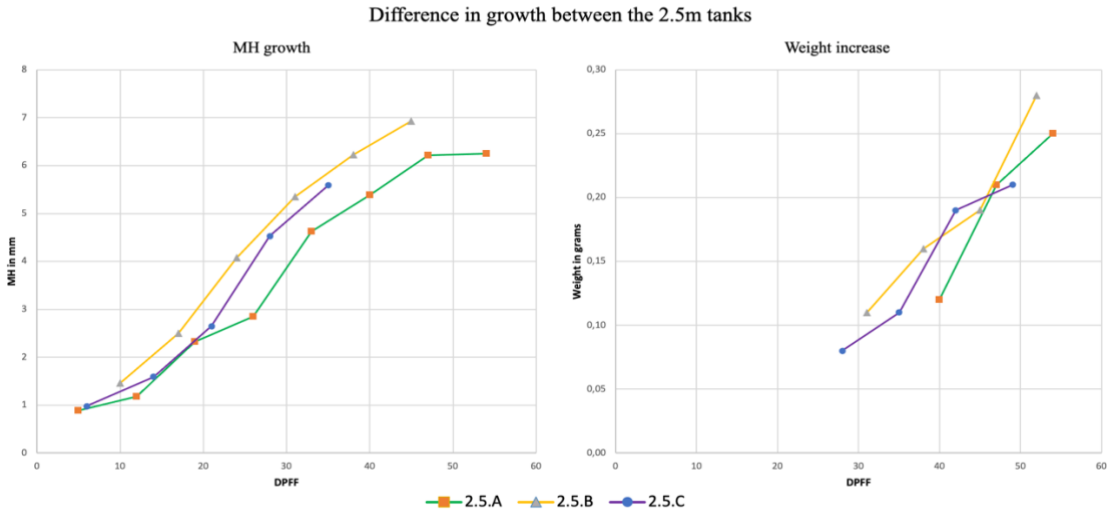
3.1.1.3 Growth in the 4m SF tanks

Initially, the MH growth in all tanks showed a similar growth curve. Up to the first 20 dpff the growth was low and platonic. However, from that point onwards, tank 4.A exhibited a more rapid increase in MH size compared to tanks 4.B and 4.C. Tank 4.A continued its accelerated growth until it stabilized at a MH measurement of 6.5mm before day 40. In contrast, tanks 4.B and 4.C followed a similar growth pattern, with their MH measurements increasing at a comparable rate. Around 40 dpff, the MH growth in all tanks began to flatten. Tank 4.C ended the observation period with a MH measurement of 6.01mm, surpassing tank 4.B, which had a final MH measurement of 5.66mm (Figure 9C).

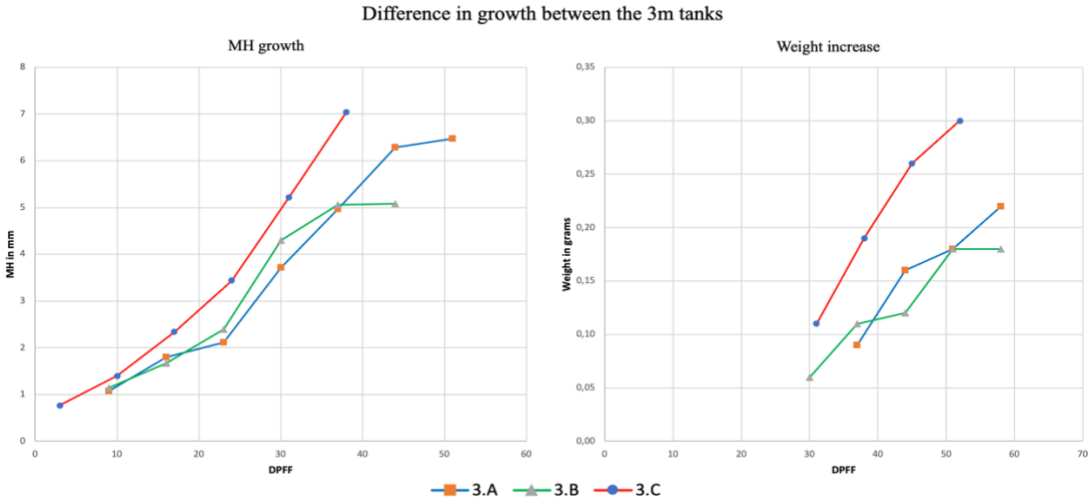
The analysis of weight increase in tanks 4.A, 4.B, and 4.C reveals distinct differences, particularly when comparing tank 4.C to tanks 4.A and 4.B. Tank 4.C has an even growth increase up until the last measurement, where both tanks 4.A and 4.C exhibit a notable bend in their growth curve when they reach a weight of around 0.20g. Tank 4.A reaches a weight of 0.25g in 53 days, showcasing a relatively efficient weight increase. In comparison, tank 4.B requires 64 days to reach a slightly higher weight of 0.26g. Tank 4.C follows a similar trajectory as tank 4.B, also using 60 days to reach a weight of 0.26g (Figure 9C).

The fish growth rate between the tanks is significant, showing that fish in 4.A grows significantly faster than fish raised in 4.B or 4.C (ANOVA: single factor performed on experimental data, MH growth had a $p = 6.7x10^{-9}$ and weight had a $p = 6.7x10^{-6}$), (Appendix 4).

9A



9B



9C

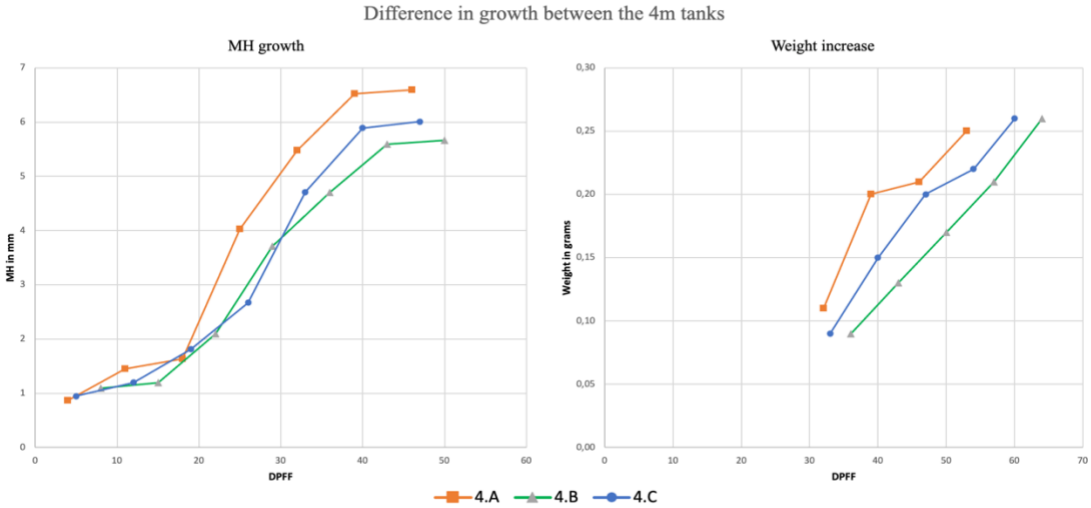


Figure 9: Growth comparison in MH and in weight between the 2.5m tanks (9A), 3m tanks (9B) and 4m tanks (9C). The left figures are the MH growth in mm and the right figure is the weight increase in grams. The data used for the figure is the production data. Data are expressed as mean of 15-20 pool individuals.

3.1.2 Growth and light distribution in the SF tanks

The growth measurements were collected to examine the correlations for growth between the even light distributed tanks. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are production data to include more measurements and clearly show the correlations from the experimental SF tanks to the other production SF tanks.

The growth patterns of the SF tanks exposed to an even spread of light: tanks 3.A, 3.C, and 2.5.C, were examined. Tank 3.C displayed the most rapid increase in MH at the beginning and showed a continuing increase in growth rate through the observation period. Tank 2.5.C also show a steady increase in the growth rate, before it slightly decreases before the final measurement of 5.59mm at 35 dpff. Tank 3.A however exhibit a stagnant growth rate up to day 20+ before having a rapid and even increase of the growth rate up to day 44, where the growth then flattens out. Tank 3.C displayed sustained growth and had a final MH measurement of 7.04mm at 38 dpff. On the contrary, Tank 3.A reached a final MH measurement of 6.47mm at 51 dpff. (Figure 10).

The analysis of weight increase in tanks 3.A, 3.C, and 2.5.C reveals distinct differences, particularly when comparing tank 3.C to tank 3.A and 2.5.C. Tank 3.C displayed the most rapid increase and showed a stable growth through the observation period, except for a slight decrease before the last measurement. Both tank 3.A and 2.5.C experience a bend after day 40 where the growth dramatically decreased. Tank 2.5.C then had its last measurement while tank 3.A experienced an increased growth rate before its last measurement. The final weight measurement for tank 3.C was recorded at 0.30g by 52 dpff, showcasing substantial growth throughout the observation period. Tank 2.5.C, on the other hand, had its last weight measurement at 0.21g on day 49, while tank 3.A reached a weight of 0.22g after 58 days. (Figure 10).

The fish growth rate between the tanks is significant, showing that fish in 3.C grows significantly faster than fish raised in 3.A or 2.5.C (ANOVA: single factor performed on experimental data, MH growth had a $P = 2.1x10^{-15}$ and weight had a $P = 1.4x10^{-15}$), (Appendix 5).

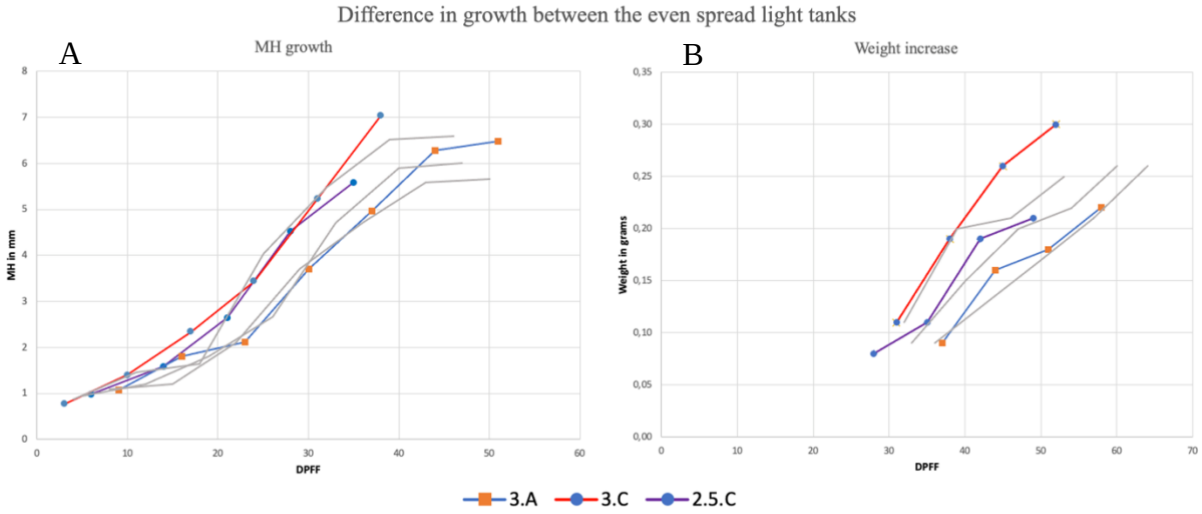


Figure 10: Growth comparison in MH (A, in mm) and weight (B, in g) between the even light distributed SF tanks as a function of the age (dpff) The grey lines show the growth to the uneven light distributed SF tanks (4.A, 4.B and 4.C).

3.1.3 Growth in the different water currents SF tanks

The growth measurements were collected to examine the correlations for growth between the low aeration tanks. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are production data to account for more measurements and clearly show the correlations from the experimental SF tanks to the other production SF tanks.

The growth patterns of the low aeration tanks, including tanks 3.A, 3.C, 2.5.C, and 4.A. Tank 3.C displayed the most rapid increase in MH at the beginning and showed a continuing increase in growth rate throughout the observation period. Tank 2.5.C also show a steady increase in the growth rate, before it slightly decreases before the final measurement of 5.59mm at 35 dpff. Tanks 3.A and 4.A however exhibit a stagnant growth rate up to around day 20. Tank 4.A then has a rapid increase where it crosses 3.A, 3.C and 2.5.C before the growth rate decrease, crossing 3.C again, and flattening out around day 40. Tank 3.A on the other hand exhibit a stagnant growth rate up to day 20+ before having a rapid and even increase of the growth rate up to day 44, where the growth then flattens out. As the study progressed, tank 3.A demonstrated continued growth, reaching a final MH measurement of 6.47mm at 51 dpff. Tank 3.C reached its last MH measurement of 7.04mm at 38 dpff, while tank 4.A ended up with a MH of 6.59mm after 46 days. (Figure 11).

The weight increase among the SF tanks exhibited a more divided pattern when comparing tank 3.C to 3.A, 2.5.C, and 4.A. Tank 3.C displayed the most rapid increase and showed a stable growth rate through the observation period, except for a slight decrease before the last measurement. Tanks 3.A, 2.5.C and 4.A experience a bend after day 40 where the growth rate dramatically decreased. Tank 2.5.C then had its last measurement while tanks 3.A and 4.A experienced an increased in growth rate before its last measurement. Tank 3.C reached its maximum weight of 0.30g by 52 dpff, indicating substantial growth throughout the study. Tank 4.A concluded with a final measurement of 0.25g at 53 dpff. Tank 2.5.C had its last measurement at 49 dpff, weighing 0.21g. In contrast, tank 3.A had a last weight measurement of 0.22g at 58 dpff, representing a slower weight increase compared to the other tanks. (Figure 11).

The fish growth rate between the tanks is significant, showing that fish in 3.C grows significantly faster than fish raised in 3.A, 2.5.C or 4.A (ANOVA: single factor performed on

experimental data, MH growth had a P = $9.9x10^{-20}$ and weight had a P = $3.6x10^{-22}$), (Appendix 6).

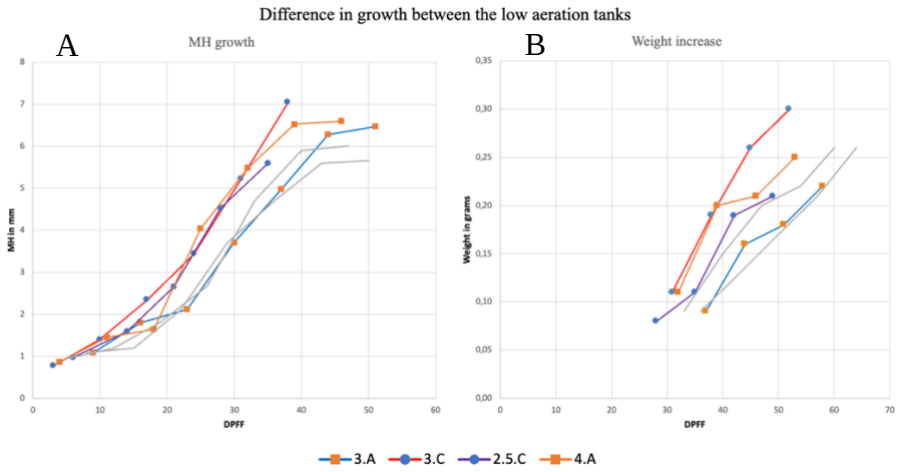


Figure 11: Growth comparison in MH (A, in mm) and in weight (B, in g) between the low aeration SF tanks as a function of the age (dpff). The grey lines show the growth to the high aeration SF tanks (4.B and 4.C).

3.1.4 Total feeding amount and prey density

The data on feeding amount and prey density were collected to compare the meal size to the number of larvae in the tank and use this data to relate with the growth and eye migration data.

The results of the study reveal a clear correlation between the number of Artemia fed per meal and the number of halibut larvae in the SF tanks. Tanks 3.C and 2.5.C demonstrate a distinct separation from the other SF tanks, displaying a faster growing and a higher curve in terms of Artemia's fed each larva.

Tank 3.C reaches its peak at 2290 Artemia fed per larvae at 31 dpff. Similarly, tank 2.5.C reaches its peak at 2268 Artemia fed per larvae, slightly later, after 33 days.

In the middle, tank 4.A reaches its maximum of 1764 Artemia fed per larvae after 41 days, expressing a more gradual but steady progression in feeding habits.

The SF tanks with a slower-growing curve exhibit a later peak of Artemia consumption compared to the faster-growing meal sized tanks. Tank 4.C peaked at 1459 Artemia fed per larvae after 42 days. Tank 3.A, on the other hand, achieves a maximum Artemia meal size of 1290 per larvae after 44 days. Finally, tank 4.B reaches its peak of 1199 Artemia fed per larvae after 48 days (Figure 12).

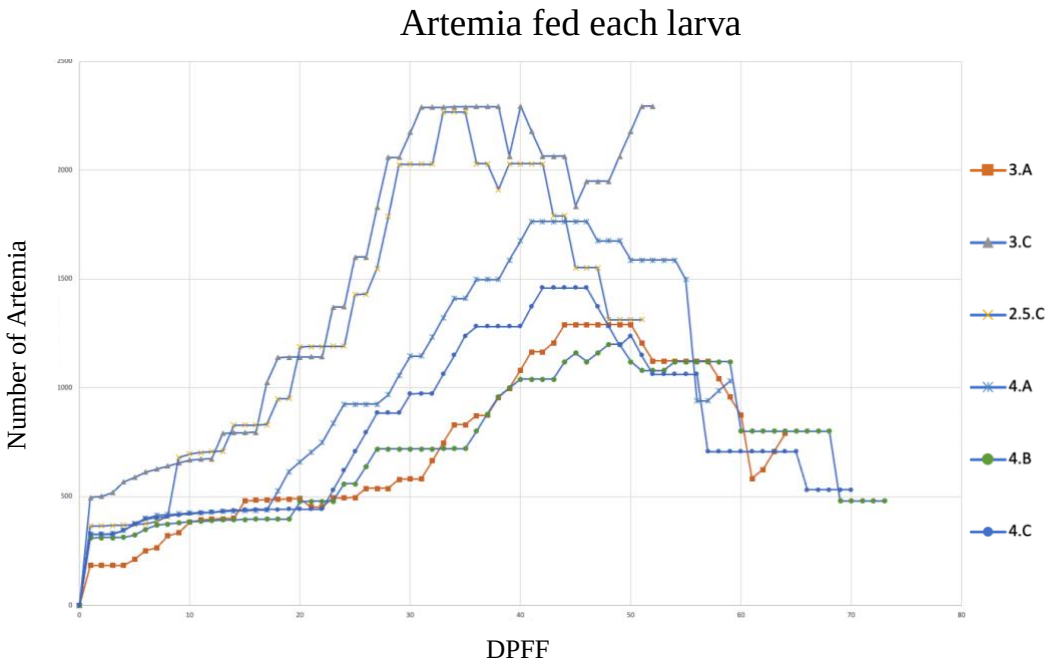


Figure 12: Showing how much Artemia is fed each larva dpff. The starting number of halibut larvae are calculated by using the numbers of halibut from each of the fry tanks after sorting. Adding the number of halibut that has died during the start-feeding phase. Then the number of dead larvae for each day were subtracted to have the correct number of halibut in the tank for each day.

3.1.5 Gut content

The feed consumption data were collected by examining the gut content in the halibut larvae. This data was collected to examine the variability in gut content between the tanks and correlate this to growth and eye migration.

The gut content of the fish in all the SF tanks showed similar patterns during the initial days, with a majority of halibut larvae (between 60-80%) not consuming enough Artemia before transitioning to eating full meals. Subsequently, the percentage of fish with full gut content stabilized at 95% in all tanks (Figure 13).

Tank 3.A exhibited overall stability at 95% full gut content throughout most of the 64-day study period, with a few exceptions and is the tank experiencing the largest drop between the tanks down to 60% full gut content after 30+ days. Tank 3.C maintained a stable proportion until day 53, with occasional decreases below 80% full gut content. Tank 2.5.C remained stable until day 52, with a few instances of decreased gut content. Tank 4.A remained stable until day 60, with one larger decrease. Tank 4.B experienced decreases on four occasions. Tank 4.C showed stability with a few fluctuations, mostly stabilizing at 95% gut content (Figure 13).

Figure 13: The difference in gut content between the SF tanks. Gut content was measured by checking 20 larvae after the first meal each day.

3.1.6 Larval population density

Larval density (larvae.L^{-1})were one of the factors this study examined the effected of on eye migration in the commercial production of Atlantic halibut.

3.A has a high larval density with 13 halibut larvae.L^{-1}. The low larval density tanks are 3.C and 2.5.C, with 5 and 6 halibut larave.L^{-1}. The medium larval density tanks are 4.A, 4.B and 4.C and all have 7 halibut larvae.L^{-1} (Tabell 5).

Tabell 5: Eye migration, number of fish-L^{-1} and the larval density to the different start-feeding tanks. The density for each tank is calculated after final sorting in the fry section. The mortality numbers up to this point are then added. The larval density is determined by the number of fish .L^{-1}.

SF Tank	Not approved (1+2)	Approved (2+3)	Number of fish each L	Larval density
3.A	17 %	83 %	13	High
3.C	1 %	99 %	5	Low
2.5.C	11 %	89 %	6	Low
4.A	39 %	61 %	7	Medium
4.B	60 %	40 %	7	Medium
4.C	83 %	17 %	7	Medium

3.1.7 Degree of eye migration

The data on eye migration were collected to find the degree of eye migration each tank produced and to find the variance between the different SF tanks due to the different factors the tanks were exposed for. The eye migration followed the grading from 0-3, were 0 is no eye migration and 3 is full eye migration. The data are then transformed into approved (grading: 2-3) or not approved (grading: 0-1) for commercial production.

The eye migration data were collected from 19 fry tanks, where n = 100+ from each tank. Comprising a total of 2391 halibut fry. The fry were graded and divided into approved or not approved categories (Appendix 7). The findings highlight the variation in eye migration grading distribution among the different fry tanks

Tank 3.A exhibited a grading where the majority of fry received a grading of 2, with a smaller proportion receiving gradings of 3 and 1.

Tank 3.C had a higher percentage of fry receiving a grading of 3, indicating a relatively higher number of approved individuals.

For tank 2.5.C, the fry showed a more balanced distribution across the grading categories, with a relatively equal representation of gradings 3 and 2.

In tank 4.A, the grading in this tank exhibited a varied distribution across the grading categories, with a substantial proportion receiving gradings 2 and 3.

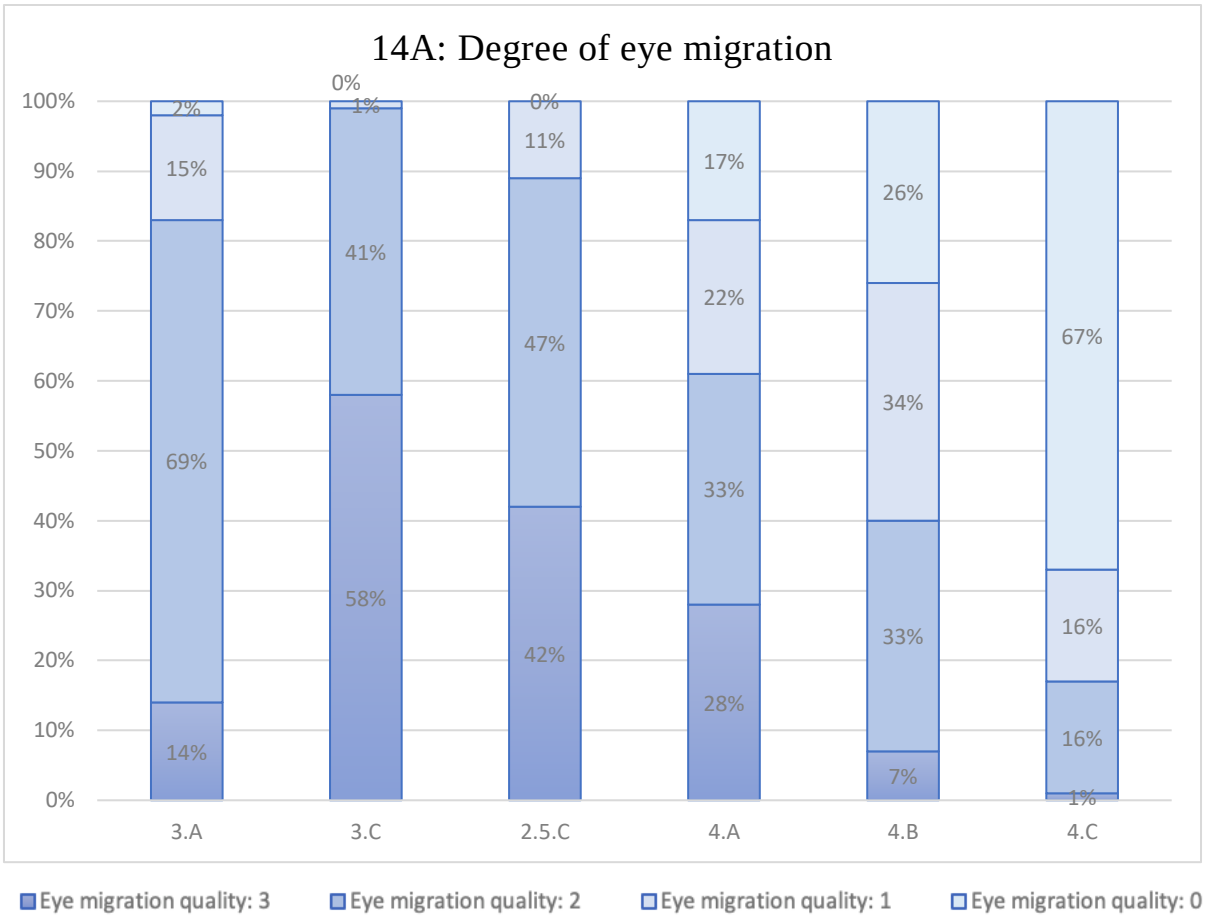
Tank 4.B had a higher percentage of fry receiving gradings 1 and 2, while the proportion of fry receiving grading 3 was relatively low.

Lastly, tank 4.C had the highest percentage of fry receiving grading 0, indicating a lower overall approval rate compared to the other tanks. (Figure 14A).

Among the SF tanks, certain tanks demonstrated higher levels of approved eye migration. The tank with the highest percentage of approved eye migration was tank 3.C at 99%, followed by tank 2.5.C, with 89% of the fry exhibiting approved eye migration. Tank 3.A also displayed a relatively high percentage, with 83% of the fry showing approved eye migration. These tanks showcased favorable eye migration outcomes, indicating successful development in this aspect.

In contrast, tank 4.A exhibited a moderate level of approved eye migration, with 61% of the fry demonstrating the desired eye migration. This places tank 4.A in the middle range compared to the other SF tanks.

On the lower end of the spectrum, tanks 4.B and 4.C displayed the lowest percentages of approved eye migration. Tank 4.B exhibited a relatively lower rate, with only 40% of the fry displaying an approved eye migration. However, tank 4.C had the lowest percentage among all the tanks, with only 17% of the fry demonstrating approved eye migration (Figure 14B).



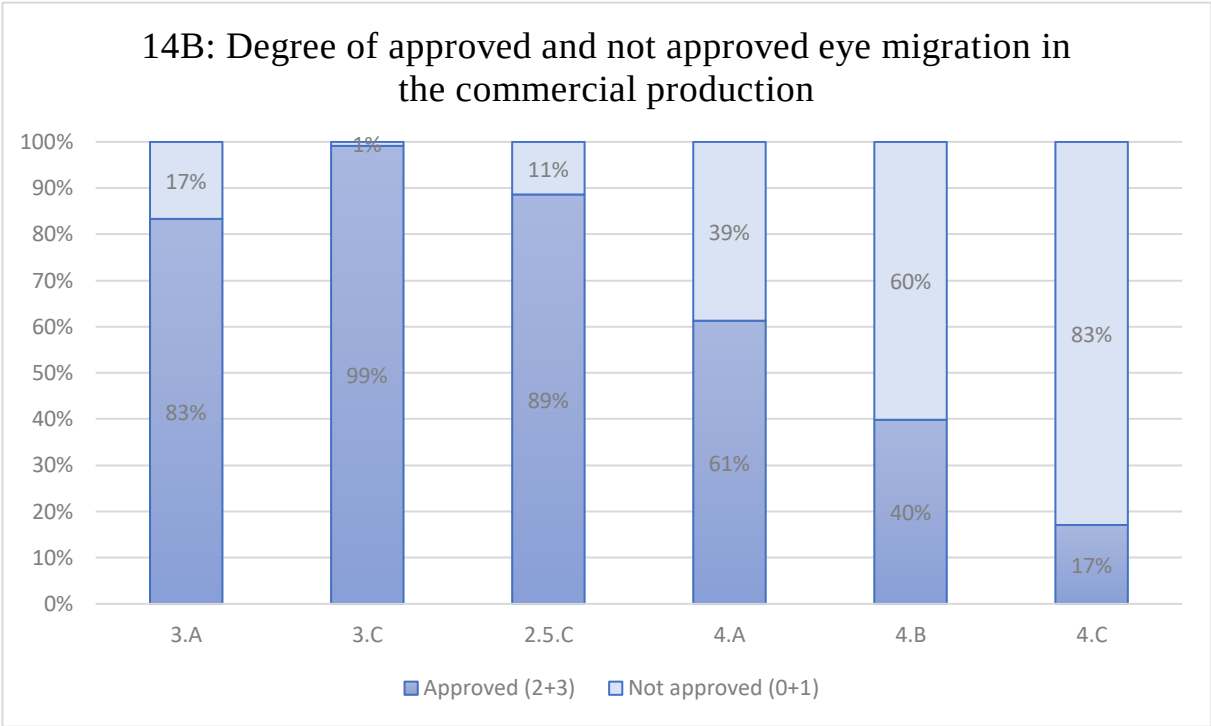


Figure 14: Comparison of eye migration status between the SF tanks. 14A, shows the incidence of different grading in each SF tank, starting with a grading of 3 (complete eyes migration) at the bottom to 0 (no eyes migration) at the top. 14B, the commercial grading is shown. “Approved” halibut includes the grading of 2 and 3 whereas “Not approved” includes the grading 0 and 1. The halibut from the SF tanks used in the study were divided in 19 fry tanks in the weaning facility. More than 100+ halibut fry were graded from each tank.

3.2 Possible effects of larval density on growth and development

3.2.1 Impact of population density on growth

The correlation between growth and the population density data were examined to study if larval density effects the growth rate. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are also experimental data.

The start-feeding tanks were divided into low-, medium- and high-larval density tanks. The low-density tanks have a significant faster growth in MH than the medium- and high-larval density tanks (test of slopes, $p < 0.0001$), (Appendix 8). Medium- and high-density tanks have a similar growth rate, where medium tanks are slightly above the high-density tanks (medium: $y = 0.1489*x-0.3395$ and high: $y = 0.1446*x-0.5831$), (Appendix 9). The black dotted lines show the 95% confidence bands for the simple linear regression line. Some overlapping occurs between the confidence bands to the different larval density slopes (Figure 15).

Few individual measurements for the weight increase had been obtained in this study. The 95% confidence bands are therefore not following the simple linear regression line as firmly and are moving away from the simple linear regression line more from the middle to the last measurements. The low-larval density tanks had a significantly more rapid weight increase and have the least similarities to the other simple linear regression lines (test of slopes and of elevation or interceptions, for slopes: $p = 0.0545$ and for elevation or intercepts: $p < 0.0001$), (Appendix 8). Medium- and high- larval density tanks had a similar weight increase. However medium tanks are slightly above high (medium: $y = 0.006856*x-0.1238$ and high: $y = 0.005854*x-0.1078$), (Appendix 9). Their confidence bands are overlapping one time, in the beginning up to day 40 (Figure 15).

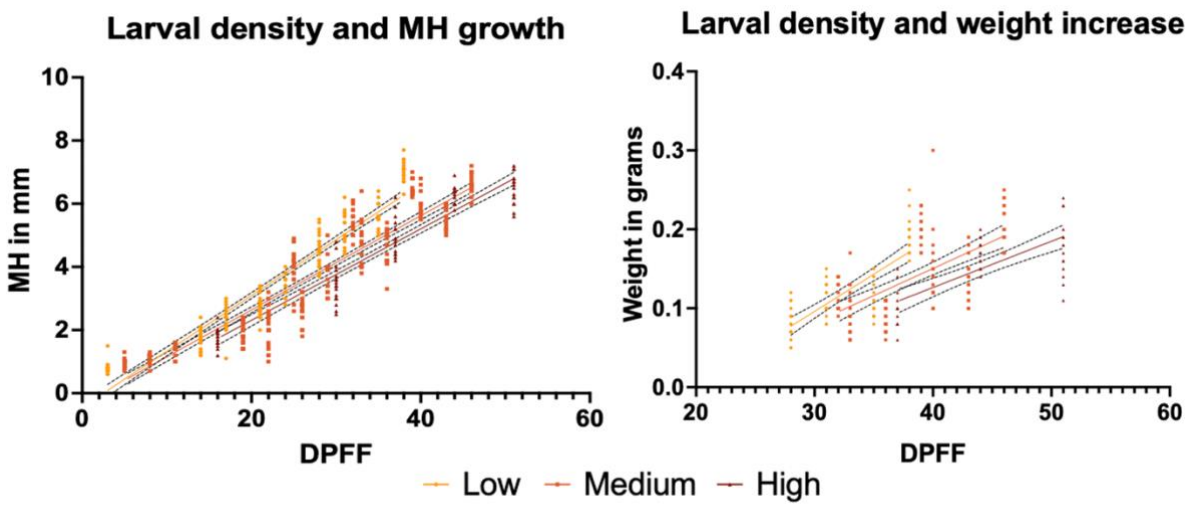


Figure 15: The difference in MH growth (left) and weight increase (right) between the different density start-feeding tanks. Low-density tanks are 3.C and 2.5.C. The medium-density tanks are 4.A, 4.B and 4.C, and the high-density tank is 3.A. The Rsquare and equation for the MH growth were low: Rsquare = 0.91 and equation was y = 0.1767*x-0.4422, medium: Rsquare = 0.86 and equation was y = 0.1489*x-0.3395 and high: Rsquare = 0.91 and equation was y = 0.1446*x-0.5831. The Rsquare and equation for weight increase were low: Rsquare = 0.61 and equation was y = 0.009408*x-0.1862, medium: Rsquare = 0.38 and equation was y = 0.006856*x-0.1238 and high: Rsquare = 0.54 and equation was y = 0.005854*x-0.1078 (Appendix 9).

3.2.2 Impact of growth on eye migration

The connection between growth and the eye migration data were examined to study the effect growth has on eye migration. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are also experimental data.

There were no significant difference between high or low growth and the impact it has on eye migration (ANOVA, p = 0.181), (Appendix 10). High growth had a median of not approved eye migration of 34.3 and for approved eye migration the median was 65.7. For low growth the median of not approved eye migration was 36 and 64 for approved. There were mor variations within the low growth tanks then for the high growth tanks (Figure 16).

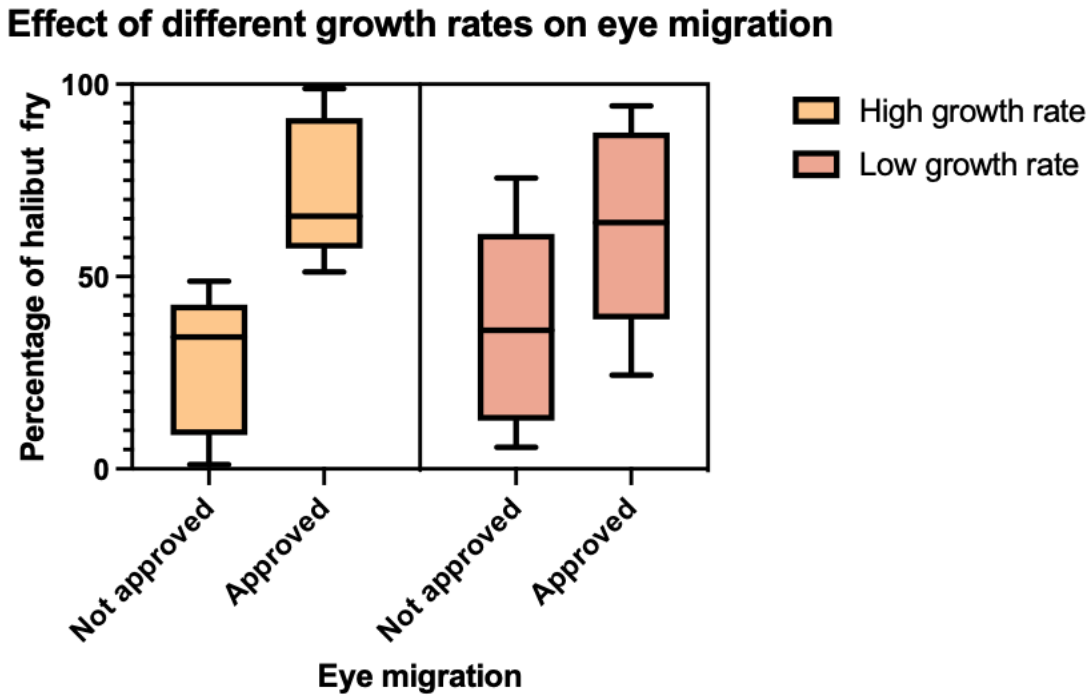


Figure 16: The difference between different growth rate during start-feeding and eye migration. The start-feeding tanks are divided into high growth rate and low growth rate. Showing the number of approved and not approved halibut fry from these tanks. The middle dark line is the median. High growth SF tanks are 3.C, 2.5.C and 4.A. Low growth SF tanks are 3.A and 4.B.

3.2.3 Impact of larval density on eye migration

The correlation between larval density and the eye migration data were examined for variances between the SF tanks. This data was used to find how many larvae each SF tank produced for further production (approved eye migration) and compare this to the other SF tanks, with a focus on the effect of density in the commercial production. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures are also the experimental data.

The low-larval density tanks experienced the best eye migration at 94% approved eye migration. The high-larval density tank has the second highest approved eye migration at 83%. The medium density tanks have the least eye migration at 39% being approved for further production (Figure 17).

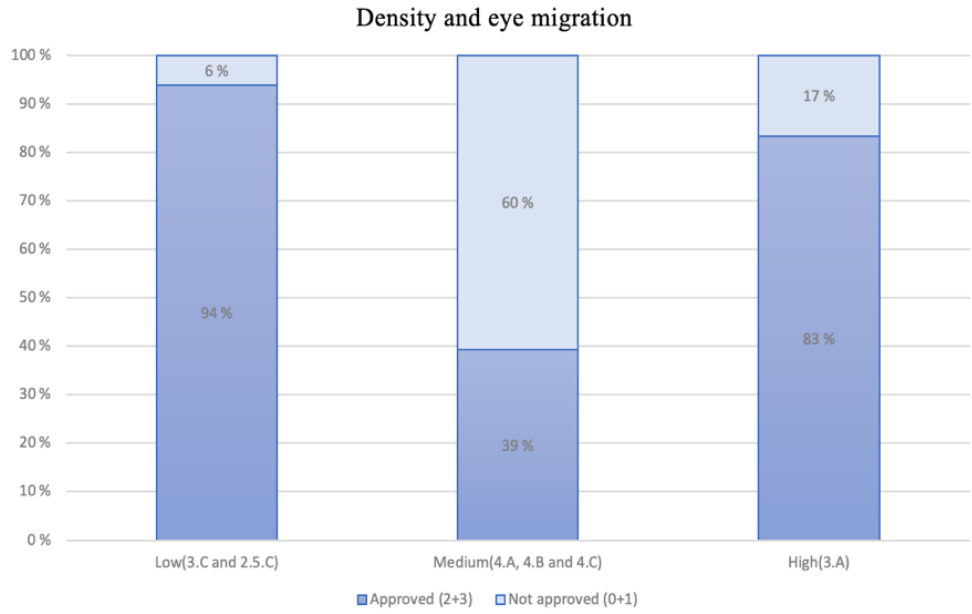


Figure 17: The difference in eye migration between the low-, medium- and high- larval density tanks. Approved eye migration is at the bottom with not approved on top.

After sorting the high-larval density tank produced 11 halibut fry that are left in the production. The two low-larval density tanks both deliver five for further production. Tank 3.C kept every halibut for further production, whereas for tank 2.5.C 1 halibut.L^{-1} were removed from the production. Within the medium tanks larger differences occur. They all started with 7 halibut.L^{-1}. Tank 4.A delivers 4 halibut.L^{-1} for further production, tank 4.B delivers 3 halibut.L^{-1} for further production and tank 4.C delivers 1 halibut.L^{-1} for further production. The high-larval density tank delivers more than double the halibut.L^{-1} for further production than

medium- and low-larval density. Low-larval density tanks are more uniform and delivers more than the medium tanks (Figure 18).

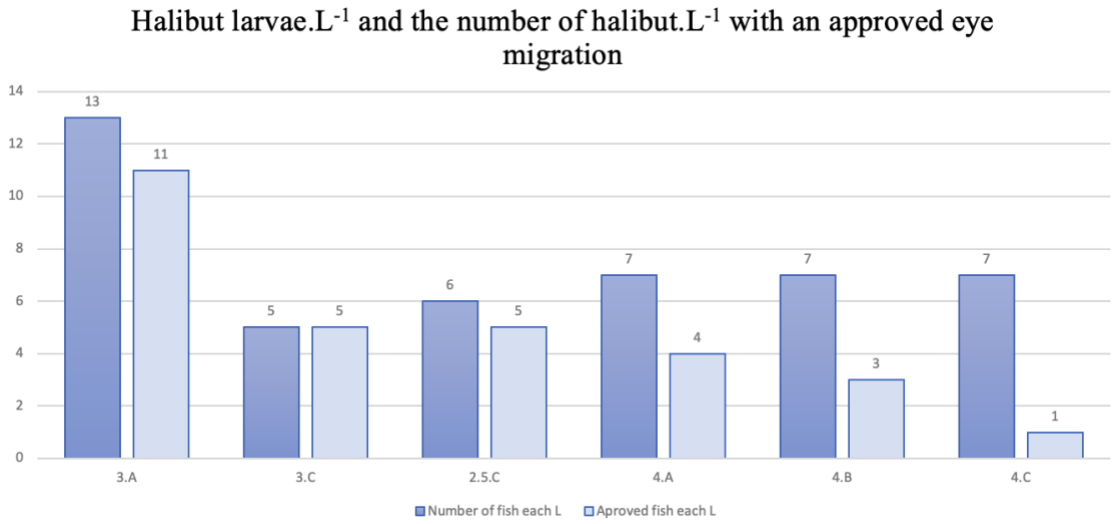


Figure 18: The difference in number of halibut larvae.L^{-1} in each SF tank during the start-feeding phase and the number of approved halibut.L^{-1} for further production. Showing how many halibut each SF tank with different density produce for further production.

The low-larval density tanks had a not approved median of 8.8 and an approved median of 91.2 halibut fry. The high larval density tanks had a not approved median of 14.75 and an approved median of 85.25. The high-larval density tank experienced more variance then the low-larval density tanks (Figure 19). The p-value with performing a Chi-square test for the trend was 0.6799, showing no significant difference between the interaction of low- and high-larval density on eye migration (Appendix 11).

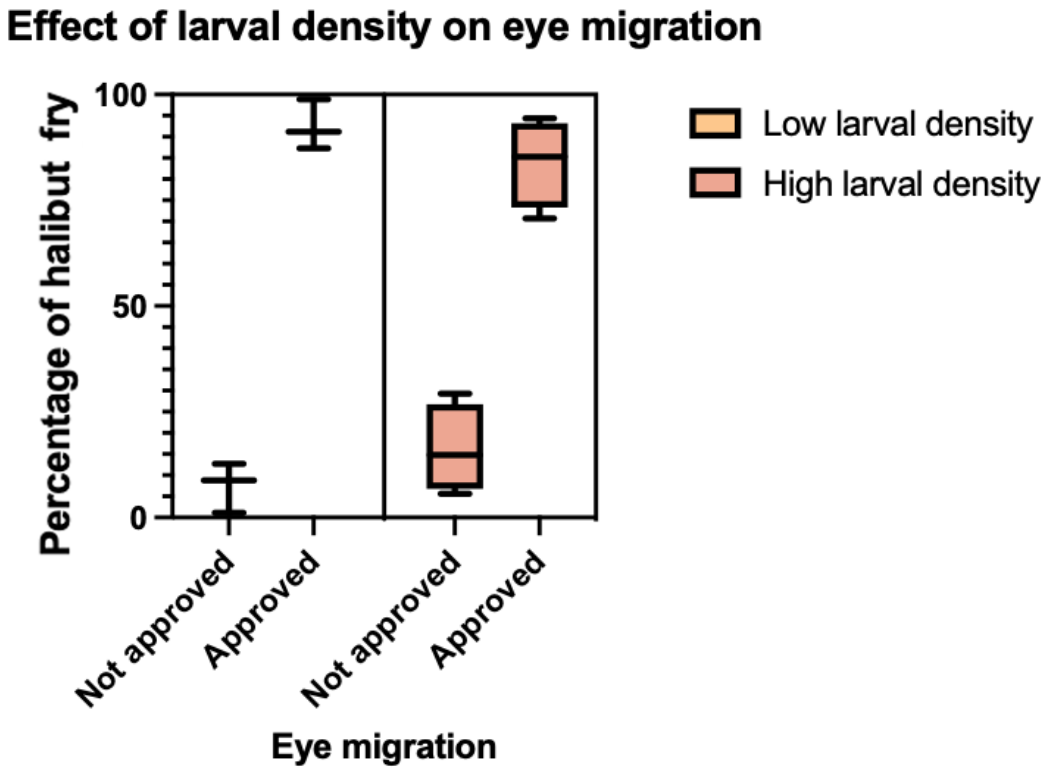


Figure 19: The effect low (3.C and 2.5.C.) and high (3.A) density of halibut larvae in the start-feeding tanks have on eye migration. Low-larval density to the left and high-larval density to the right. Illustrating the percentage of approved and not approved halibut fry from these tanks. The middle dark line is the median.

3.3 Possible effects of different physical conditions between tanks

3.3.1 Impact of surface light distribution on growth

Differences in light distribution are shown in Table 2 and were examined to study the effect of light distribution on growth. The data used for statistical analysis in this chapter were experimental data used to find the variance within each tank and to calculate the P-value. The data used for the figures were also experimental data.

The slopes for even light distribution (3.A, 3.C and 2.5.C) and uneven light distribution (4.A, 4.B and 4.C) when it comes to MH growth were similar (test of slopes and of elevation or interceptions, for slopes: $p = 0.3901$ and for elevation or intercepts: $p = 0.0012$), (Appendix 12). The growth rate to the even light distribution tanks single linear regression is slightly above the uneven tanks (even: $y = 0.1444*x-0.01187$ and uneven: $y = 0.1489*x-0.3395$), (Appendix 13). The 95% confidence bands for the simple regressions are overlapping from day 10 (Figure 20).

The slopes for even light distribution and uneven light distribution when it comes to weight increase were significantly different (test of slopes, $p = 0.0106$), (Appendix 12). The uneven light distributed tanks had the first measurements below the even light distributed tanks before increasing and crossing the single linear regression line for the even light distributed tanks at day 40 (even: $y = 0.004515*x-0.03543$ and uneven: $y = 0.006856*x-0.1238$), (Appendix 13). The single linear regression lines and their 95% confidence bands were overlapping and sharing the same values from day 3 to 45. (Figure 20).

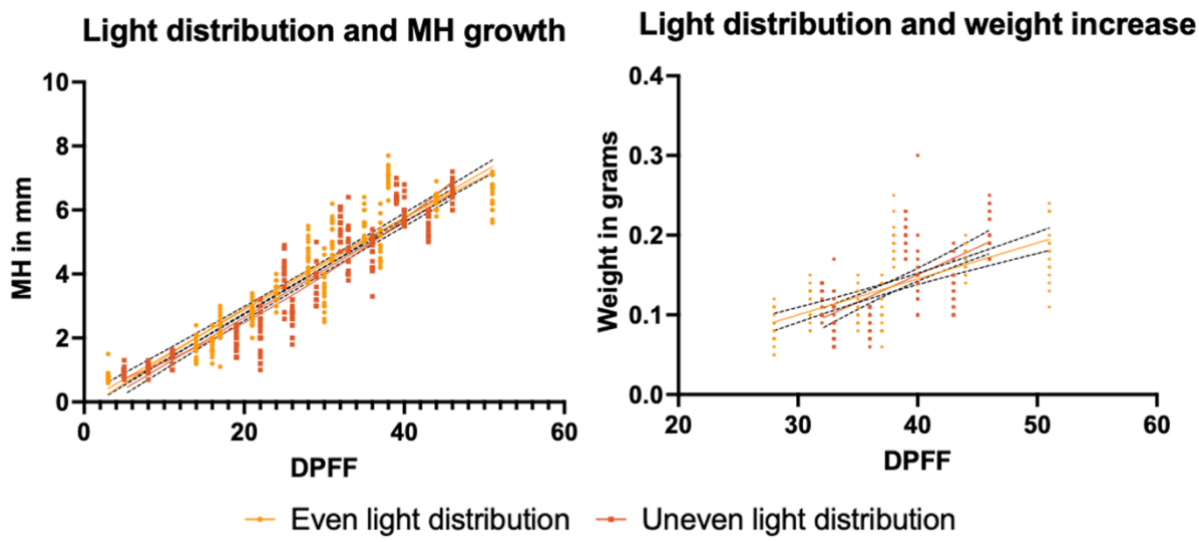


Figure 20: Difference in MH growth (left) and weight increase(right) between the even (3.A, 3.C and 2.5.C.) and uneven (4.A, 4.B and 4.C) light distribution start-feeding tanks. The Rsquare value and equation for the MH growth are even: Rsqaure = 0.85 and equation was y = 0.1444*x-0.01187, uneven: Rsqaure = 0.86 and equation was y = 0.1489*x-0.3395. The Rsquare value and equation for weight increase are even: Rsqaure = 0.47 and equation was y = 0.004515*x-0.03543, uneven: Rsqaure = 0.38 and equation was y = 0.006856*x-0.1238 (Appendix 13).

3.3.2 Impact of aeration on growth

The correlation between aeration and growth were examined to study the effect aeration had on growth. The data used for statistical analysis and figures in this chapter were experimental data.

The is a significant difference between the high and low aeration tanks and MH growth (test of slopes, p = 0.0358), (Appendix 15). The low aeration tanks (3.A, 3.C, 2.5.C. and 4.A) were the fastest growing, whereas the high aeration tanks (4.B and 4.C) had a slower growth (low: y = 0.1485*x-0.03661 and high: y =0.1379*x-0.3078), (Appendix 16). There were some overlapping between the simple linear regression slopes and their 95% confidence intervals. This was however only from day 4-6 and the rest of the slopes have different growth (Figure 21).

The weight increase for the low aeration tanks were the highest and fastest growing (test of slopes and of elevation or interceptions, for slopes: p = 0.7529 and for elevation or intercepts: p < 0.0001), (Appendix 15). The high aeration tanks have a slower growth rate (low: y = 0.005129*x-0.04855 and high: y =0.005512*x-0.09358), (Appendix 16). There is some overlapping between the simple 95% confidence intervals to the linear regression slopes 50 dpff (Figure 21).

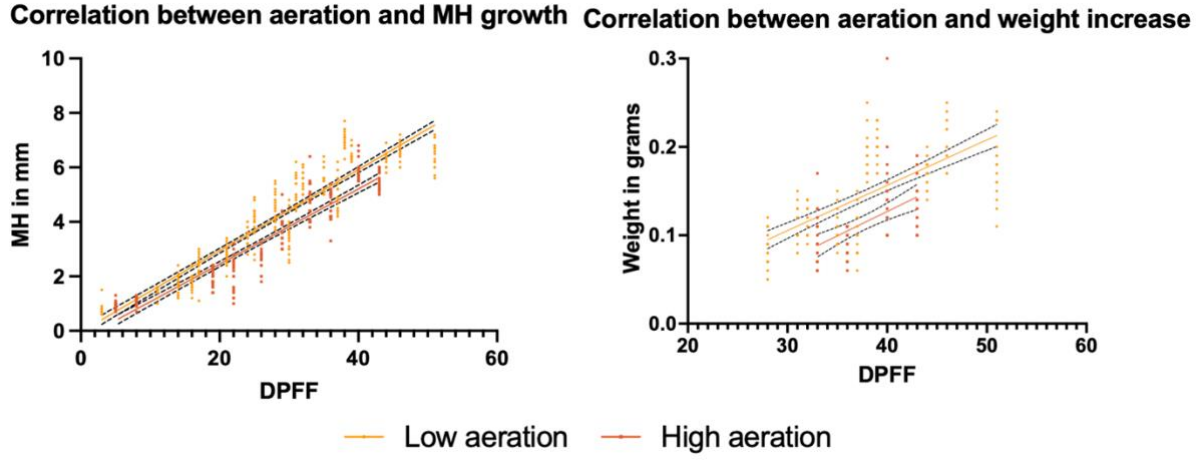


Figure 21: Correlation between low and high aeration in the start-feeding tanks and the MH growth(left) and weight increase(increase). The low aeration tanks are 3.A, 3.C, 2.5.C. and 4.A. The high aeration tanks are 4.B and 4.C. The Rsquare value and equation for the MH growth are low: Rsquare = 0.87 and equation was y = 0.1485*x-0.03661, high: Rsquare = 0.87 and equation was y =0.1379*x-0.3078. The R squared value and equation for weight increase are low: Rsquare = 0.48 and equation was y = 0.005129*x-0.04855, high: Rsquare = 0.27 and equation was y =0.005512*x-0.09358 (Appendix 16).

3.3.3 Impact of surface light distribution on eye migration

The correlation between light distribution and eye migration were examined to study if the spread of light affects the eye migration. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures were also experimental data.

There is a significant effect from light distribution on eye migration (Chi-square test, $p < 0.0001$), (Appendix 14). The even light distribution start-feeding tanks (3.A, 3.C and 2.5.C.) have a low not approved and high approved number of halibut. The median for not approved is 10.4 and for approved the median is 89.6. The uneven light distribution tanks (4.A and 4.B) have almost the same number of not approved and approved eye migration of the halibut. The median for the not approved halibut is 48.8 and for the approved halibut it is 51.2 (Figure 22).

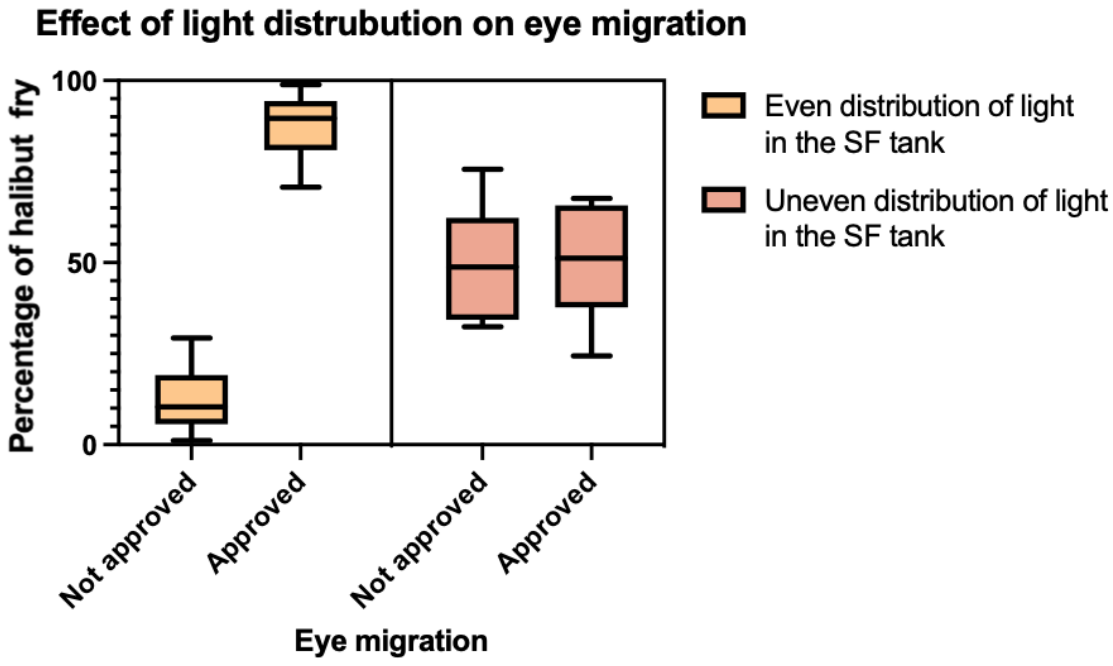


Figure 22: Effect of light distribution in the different start-feeding tanks on the eye migration. The even light distribution start-feeding tanks are 3.A, 3.C and 2.5.C (left). The uneven light distribution tanks are 4.A and 4.B (right). Illustrating the percentage of approved and not approved halibut fry from these tanks. The middle dark line is the median.

3.3.4 Impact of aeration on eye migration

The correlation between aeration and eye migration were examined to study the effect aeration had on eye migration. The data used for statistical analysis in this chapter were experimental data to find the variance within each tank and to calculate the P-value. The data used for the figures were also experimental data.

There is a significant difference between low (4.A) and high (4.B) aeration tanks (Chi-square test, $p < 0.0001$), (Appendix 17). The low aeration tank has a low not approved halibut number with a median of 34.3 and an approved halibut number with a median of 65.7. The high aeration tank has a higher not approved halibut number with a median of 59.95 and a low approved number of halibut with a median of 40.05 (Figure 23).

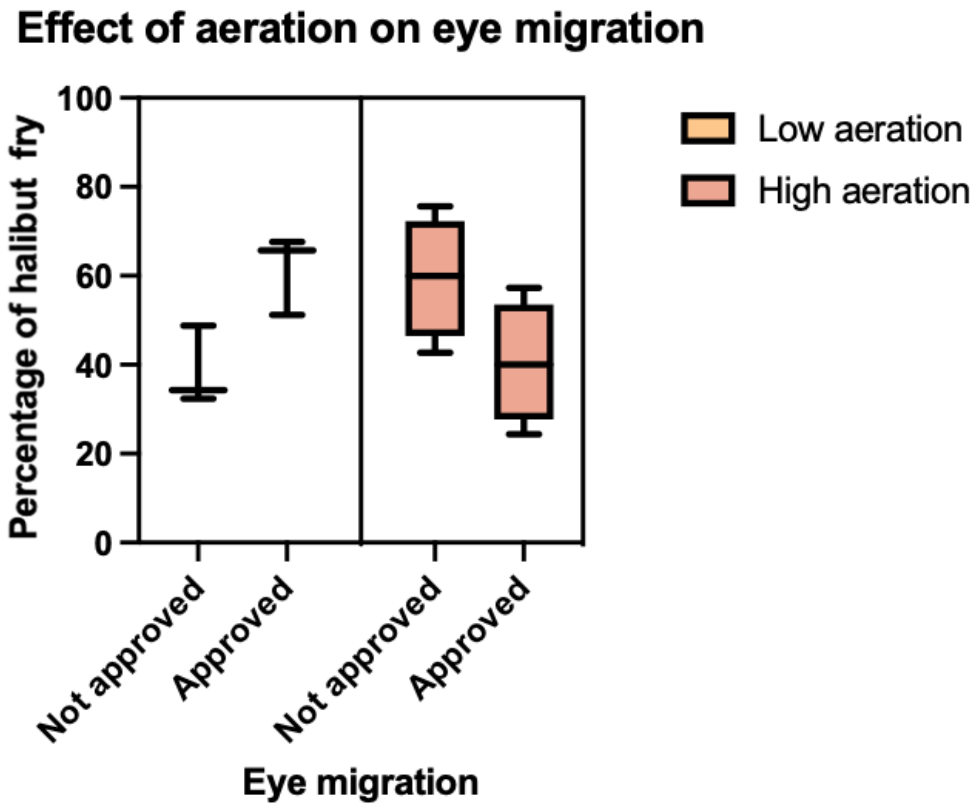


Figure 23: Effect from low and high aeration in the start-feeding tanks and the effect it has on eye migration. The low aeration tank (left) is 4.A and the high aeration tank (right) is 4.B. These two tanks have all the similar factors except for either having a high or low aeration. Illustrating the percentage of approved and not approved halibut fry from these tanks. The middle dark line is the median.

4. Discussion

In commercial production, juveniles that do not conform to a grading of 2 or 3 on the eye migration scale are removed from the production. Incomplete eye migration leads to a loss for the producer and is a fish welfare problem. Cultivating the juveniles up to the point of eye grading takes months and is a complicated part of the production. For an expansion of the industry, better control over the different factors influencing eye migration is required. Eye migration is an important factor for increasing the producing while fish welfare is insured. The factors discussed to influence eye migration and development in this study are: tank size, growth, larval density, light distribution and water current (aeration).

4.1 Method

The factors influencing development and eye migration investigated in this study are conclusions drawn from laboratory experiments. To validate the laboratory findings in a commercial production setting, this study uses both individual data and population data. The individual data refers to the experimental data, which provides depth and required considerable time to collect. On the contrary, the population data pertains to the production data following SWH's standards and provides a broader understanding of the results. However, due to the time-consuming nature of collecting experimental data, the study encountered certain limitations. Consequently, the individual data were only gathered for the six experimental tanks, serving as the basis for the conducted statistical analyses, while the production data offers a broader perspective.

4.2 Growth, development and culture conditions in different rearing systems

4.2.1 Growth in the different sized tanks

The growth in the different sized tanks were not influenced by the tanks size. The tanks have three different sizes in diameter, three different densities, high or low aeration and even or uneven spread of light. Tank 3.C is the overall best growing tank. This tank is 3m in diameter, low density, has an even spread of light and has low aeration. The faster growth could be explained by one or multiple of these factors. 3.A is a tank with slower growth. This tank is also 3m in diameter, high density, even spread of light and low aeration. The two other tanks

with a faster growth rate are tanks 4.A and a 2.5.C. Arguing that tank size should not have influence on the growth rate.

4.2.2 Feeding amount, feed consumption and prey density

The calculations of Artemia fed each larvae show a strong difference between the low-density tanks to the other tanks. This can be related to these two tanks containing less larvae per L than the other tanks resulting in a higher level of Artemia being fed each larva (Figure 12). The observed faster growth in these tanks could potentially be attributed to the higher levels of Artemia provided as compared to the other SF tanks. In a study the feed consumption and gut evacuation in Atlantic halibut were studied. The study shows that feed consumption may optimize commercial production and that there might be maximum levels of ingestion (van der Meeren, 1995).

The gut content is similar between all the SF tanks (Figure 13). There is one major exception in 3.A after 31 days where it drops to a low of 60% full gut content. This is most likely caused by too much salt in the water from the Artemia holding tank. The salt was supposed to destroy potential bacteria by osmosis (Haché et al, 2016). After this drop in gut content in 3.A, SWH stopped adding extra salt to the Artemia holding tank and the results show a quick recovery. Despite this one exception the gut content is similar between the tanks, suggesting that the growth or the eye migration differences in this study are not caused by difference in appetite.

4.2.3 Feed consumption and growth

Comparing the growth rate (Figure 9, 10 and 11) to figure 13, the data correlates with the study conducted by van der Meeren (1995). Tank 3.C and tank 2.5.C are the tanks with the highest meal size and are also best and third best tanks when it comes to growth. Tank 4.A is the second-best tank when it comes to growth, however it has a much lower number of Artemia per halibut larva when compared to tank 3.C and tank 2.5.C. This could indicate over feeding in these two tanks and that the maximal ingestion capacity is around what we see in tank 4.A.

In Atlantic halibut production clay is used instead of algae in the water to improve contrast and prey detection (Harboe & Reitan, 2005; Attramadal et al, 2012;). The study conducted by van der Meeren (1995) was performed before clay was used in commercial halibut production. The study could therefore have a higher number of the Artemia being removed by the water outflow and a higher gut migration time. Van der Meeren (1995) show a result for halibut

maximum ingesting 2554 Artemia. Comparing this to figure 13, shows a higher feeding amount than what we see in the results (Figure 13). However, considering the better prey detection due to the use of clay could explain a more efficient feeding for the Atlantic halibut in today's commercial production.

Van der Meeren (1995) also fed the larvae in excess conditions under a 24-hour stagnant environment. Commercial production today feed in meals and control the light conditions to maximize the feeding regime. Halibut has a diurnal feeding rhythm due to vertical migration of the prey and light conditions. Under continuous light conditions the larvae will therefore continue to capture pray even if the gut is already full (Harboe et al, 2009). Studies have shown that this changes the transit time in gut content (Canino, 1995). Harboe et al., (2009) performed a study demonstrating that a high gut evacuation results in low digestion and that by implementing a light regime dramatically improves the eye migration, however growth was not affected.

All the SF tanks in this study have the same light regime and feed, however the meal size differ. Arguing that a meal with 1700+ Artemia per halibut larvae will have a positive effect on growth. Artemia is a high expense for a commercial producer and preventing overfeeding is therefore an important solution to remove excess expenses. However, 1700+ Artemia each larva is not necessarily the optimum and more studies in this field are required to determine a more exact answer.

4.3 Effects of larval density on growth and development

4.3.1 Growth and population density in each SF tank

There is a significant difference in growth and development between the low-, medium- and high-larval density tanks. The low-density tanks exhibit the highest growth rate (Figure 15). Study shows that stocking density is a key factor for an efficiently production and animal welfare on juvenile Olive Flounder (Seo & Park, 2023). There is a difference between medium- and high-larval density tanks as well. This could indicate a better growth result for halibut larvae up to a certain density. A study conducted on juvenile Olive Flounder indicates a positive effect from an increased stocking density up to a density of 20.02 kg/m^2. A higher density had a decreased effect on the juvenile Olive Flounder (Seo & Park, 2023).

Other studies suggest a negative correlation between stocking density and feed intake due to stress and size differences. A reduced feed intake and increased stress levels could decrease

the feed conversion. Research often report that stocking density and growth rates are related, however the relationships between the two is not always uniformly positive or negative for a given species (Seo & Park, 2023). Stress is another factor which increase with a higher population and studies show that stress can decrease energy availability for growth (Santos et al., 2010). The results in this study show a clear similarity to Seo and Park (2023) and Santos et al., (2010).

The low-larval density tanks demonstrate better growth and the minimal difference between the medium- and high-larval density tanks could indicate that stocking densities higher then 6 halibut larvae.L^{-1} will significantly reduce the growth. There are however multiple other factors differentiating these tanks in addition to density. More studies are therefore required in this field to be certain about the optimal larval density for growth.

4.3.2 Growth rate and eye migration

There is no significant difference between high- and low-growth tanks when it comes to eye migration (Figure 16). Indicating that a high growth does not affect the development of an approved eye migration. The high growth rate tanks are 3.C, 2.5.C and 4.A. Tank 3.C and tank 2.5.C both produced a high number of approved halibut for further production. A high growth rate has shown to indicate good fish welfare. Stress is shown to reduce energy utilization and is a key factor in the development of fish (Santos et al., 2010; Seo & Park, 2023) The low growth rate tanks are 3.A and 4.B. Tank 3.A also had a high number of halibut with an approved eye migration. Eye migration is an indication of the development to halibut juveniles and a complete eye migration require the right energy levels (Hamre & Harboe, 2008a, 2008b). This study does not experience a significant difference between growth rate and eye migration, however more studies should be conducted to further explore this relationship.

4.3.3 Larval density and eye migration

There is no significant difference between the low- and high-density tanks (Figure 17, 19) on eye migration. The low-density tanks exhibit better eye migration then the medium- and high-density tanks. However, the high-density tank had the second highest approved eye migration percentage with more than double the approved rate for medium-density tanks (Figure 17). It has been discussed that a high density affects eye migration due to stress and

hyperactivity in the fish (Santos et al., 2010; Seo & Park 2023; Zang et al., 2022). The results indicate that the larval densities in these tanks did not influence the eye migration.

The results show a larger variance within the high-larval density tank compared to the low-larval density tanks. Moreover, the high-larval density tanks exhibit a higher proportion of grade 2 approved halibut compared to grade 3, unlike the low-larval density tanks. (Figure 14A, 14B and 19). Arguing that the high-density tanks lead to a higher variation of eye migration, however since the difference of halibut for further production is not significant it does not affect the production.

High-larval density has also been suggested to limit space at the bottom when settling, causing later-developing halibut to remain in the water column and potentially affecting the eye migration. A study by Perrichon, pers. comm, indicates that metamorphosis starts around 12 dpff and concludes around 25-30 dpff. This implies that eye migration is determined before day 30, which is prior to the time the majority settle. This suggests that other factors than larval density have a greater influence on the development of eye migration in a commercial setting.

The results also reason that a high-density tank delivers more halibut for further production for farmers (Figure 18). For a producer to find the optimum larval density for an increased production with a high focus on fish welfare are essential. This study argues that this larval density is not affecting the fish welfare negatively however more research is needed to find the optimum density for a commercial producer.

4.4 Effects of different physical conditions

4.4.1 Light distribution and growth

The results show that an even spread of light is significantly better (Figure 18). The growth results for MH shows similarities between the even and uneven light distributed tanks however there were still a significant difference in the elevation of the slopes. The weight increase is arguing a stronger difference between the even and uneven light distributed tanks. The slopes are crossing and divided further then for MH. (Figure 20). Arguing that light distribution affects growth, however a stronger data foundation would provide a more accurate answer to the effect from light distribution on growth.

There are multiple studies arguing the effect light and light spread has on growth in flatfish. Studies show that light plays a critical role in the normal development and feeding process in flatfish (Harboe et al., 2009; Venizelos & Benetti, 1999; Zhang et al., 2022). To capture prey, Halibut larvae are dependent on light since they are visual feeders. An

inappropriate lighting can therefore increase a not approved eye migration and abnormal pigmentation in Atlantic halibut (Harboe et al., 2009). The different start-feeding tanks had the same light regime but not the same spread of light. The results from this study argue that the Halibut larvae due to this uneven spread of light don't have the same visual basis to capture prey.

4.4.2 Aeration and growth

There is a significant difference in growth between the low aeration and high aeration SF tanks (Figure 21). Low aeration tanks are experiencing a better growth than the high aeration tanks. Atlantic halibut larvae have shown to be very sensitive to mechanical stress (Opstad & Raae, 1986) and they argue that larvae that experience high levels of physical stress, use to much of their energy for activity and this will most likely be at the expense of growth and development. High aeration from multiple sources in the tank creates a stronger and less homogeneous water current and is what tanks 4.B and 4.C experience.

In another study, the effect of high aeration on growth and eye migration were examined. The study shows a clear difference in MH growth between the high aeration tanks to the standard tanks (Perrichon, pers. comm). Comparing Perrichon's findings to the results from this study it can be argued that a start-feeding tank with low aeration provide a better environment for growth.

4.4.3 Light distribution and eye migration

The results show a significant difference between the even and uneven light distributed tanks, where an even spread of light creates an improved eye migration (Figure 22). This shows a clear similarity with former studies. Recent studies argue that metamorphosis is the result of a complex interaction between genetic and environmental factors (Shao et al., 2017; Zhang et al., 2022). Metamorphosis is now documented as being driven mostly by hormones (Galay-Burgos et al., 2008; Power et al., 2001; Schreiber et al., 2010; Schreiber and Specker, 2000; Shao et al., 2017; Wang et al., 2011).

Shao et al., (2017) conducted a comparative genomic study that demonstrated that retinoic acid signaling with thyroid hormones and phototransduction pathways are key developmental triggers in the transformation of asymmetric pigmentation and variation of eye migration in

Japanese flounder. Rhodopsin in the skin being exposed to light is argued to be the triggering cause for retinoic acid signaling. This start due to a tilted swimming in the Halibut and therefore regulates the asymmetrical development (Shao et al., 2017; Zhang et al., 2022).

In a study conducted on the effect of underwater lighting on metamorphosis in halibut larvae it was found that both a low underwater light and a high underwater light affected eye migration. With high underwater light levels an eye grading of 3 and 2 were completely removed compared to a standard tank. These high underwater light levels therefore caused a complete lack of eye migration in the halibut. When comparing the low underwater light tank to the standard tank there is also a reduced eye migration in the Halibut larvae (Perrichon, pers. comm). A light source from the bottom will therefore disrupt the effect of tilted swimming on eye migration.

The even spread light provides the same light levels in the whole tank. The tilted swimming will therefore expose the rhodopsin in the skin equally to light anywhere in the tank. An uneven light spread can however cause some areas to be more favorable in the tanks then others depending on the tilt. The larvae move around and therefore expose the ocular side to the light differentially depending on where it is in the tank. If the tilt is leaning towards the light source the ocular side might get less exposed to light something that might affect the eye migration. The presence of clay in the water also causes a reduction in the transmission of surface light to the bottom. This reduction will have a greater impact in a tank with an uneven spread of light compared to an even spread of surface light tank.

The tanks with an even spread of light from the top can therefore be argued to generate a more favorable environment for the halibut larvae. Implementing this into a commercial production can therefore produce better eye migration.

4.4.4 Aeration and eye migration

There is a significant difference between low and high aeration tanks and the effect it has on eye migration. Low aeration has a high number of approved eye migration whereas high aeration has a low number of approved eye migration (Figure 23). Earlier study conducted on this topic had similar results (Perrichon, pers. comm.).

The study conducted by Perrichon shows that halibut exposed to high levels of aeration during the start-feeding phase had a reduction of approved eye migration when compared to the standard tanks. High aeration levels can create a more stressful environment preventing optimal energy utilization for eye migration (Opstad & Raae, 1986).

The stronger water currents created by the aeration could also reduce the tilted swimming from occurring decreasing the amount of light on the ocular side. The increased water flow can also result in the fish becoming more hyperactive. Induced by the heightened water flow across the blind side, staining may be exhibited due to the attribution of the upregulation of mc1r or the downregulation of asip1 on the blind side (Zhang et al., 2022). Malpigmentation on both sides are abnormalities connected to eye migration (Harboe et al., 2009).

The findings in this study can therefore argue that high aeration levels in a tank during the start-feeding phase will decrease eye migration in Atlantic halibut. Implementing lower levels of aeration, creating less water currents, in the commercial production may therefore increase the eye migration and production becomes more sustainable with a high focus on welfare.

4.5 Conclusion

The tanks that exhibited the highest growth rate demonstrated variations in tank diameter, low-larval density, low aeration, and an even spread of light. Moreover, a higher meal size consisting of 1700+ Artemia per larvae resulted in a higher growth rate. Larval density had a significant impact on growth. However, neither the growth rate nor larval density significantly influenced the eye migration. On the contrary, the distribution of light and low aeration had a notable effect on both the growth rate and eye migration.

In intensive production an incomplete eye migration poses challenges in terms of fish health and consumer acceptance, resulting in substantial economic losses for farmers. The findings in this study aim to mitigate the occurrence of incomplete eye migration in commercial production, promoting a more sustainable production and improving fish welfare.

Improving the spread of light and maintaining low aeration contribute to the development of a more sustainable production system, enhancing economic viability and ensuring the overall fish welfare.

The following hypotheses have been answered:

$H0_{1A}$: Different tank size has no significant effect on growth in a commercial production of Atlantic halibut **is accepted**.

$H0_{2A}$: Larval density has no significant effect on growth in a commercial production of Atlantic halibut **is rejected**, and thereby **HA_{2A} is accepted.** Larval density has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{3A}$: Growth has no significant effect on eye migration in a commercial production of Atlantic halibut **is accepted.**

$H0_{4A}$: Larval density has no significant effect on eye migration in a commercial production of Atlantic halibut **is accepted** for a high-larval density up to 13 larvae.L^{-1}.

$H0_{5A}$: Surface light distribution has no significant effect on growth in a commercial production of Atlantic halibut **is rejected**, and thereby **HA_{5A} is accepted.** Surface light distribution has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{6A}$: Water current (aeration) has no significant effect on growth in a commercial production of Atlantic halibut **is rejected**, and thereby **HA_{6A} is accepted.** Water current (aeration) has a significant effect on growth in a commercial production of Atlantic halibut.

$H0_{7A}$: Surface light distribution has no significant effect on eye migration in a commercial production of Atlantic halibut **is rejected**, and thereby **HA_{7A} is accepted:** Surface light distribution has a significant effect on eye migration in a commercial production of Atlantic halibut.

$H0_{8A}$: Water current (aeration) has no significant effect on eye migration in a commercial production of Atlantic halibut **is rejected**, and thereby **HA_{8A} is accepted.** Water current (aeration) has a significant effect on eye migration in a commercial production of Atlantic halibut.

4.6 Future prospects

The present study examines tanks incorporating numerous factors. However, further investigation is necessary to identify the primary factors influencing the growth and eye migration of Atlantic halibut in commercial production. The optimal levels for density, feeding amount, light, and aeration remain unknown, emphasizing the need for future research. Exploring these aspects would contribute to enhancing the sustainability in a commercial production.

5. Reference list

Attramadal, K.J.K., Tøndel, B., Salvesen, I., Øie, G., Vadstein, O., & Olsen, Y. (2012). Ceramic clay reduces the load of organic matter and bacteria in marine fish larval culture tanks. *Aquacultural Engineering, 49*, 23-34. https://doi.org/10.1016/j.aquaeng.2012.02.003.

Birkbeck, T. H., Reid, H. I., Darde, B., & Grant, A. N. (2006). Activity of bronopol (Pyceze®) against bacteria cultured from eggs of halibut, *Hippoglossus hippoglossus* and cod, *Gadus morhua*. *Aquaculture, 254*(1–4), 125-128. https://doi.org/10.1016/j.aquaculture.2005.11.025.

Blaxter, J.H.S., Danielssen, D., Moksness, E., & Øiestad, V. (1983). Description of the early development of the halibut (*Hippoglossus hippoglossus*) and attempts to rear the laravae past first feeding. *Marine Biology, 73*, 99-107. doi: 10.1007/BF00396253

Blindheim, S., Nylund, A., Watanabe, K. et al. (2015). A new aquareovirus causing high mortality in farmed Atlantic halibut fry in Norway. *Arch Virol 160*, 91–102 https://doi.org/10.1007/s00705-014-2235-8

Canino, M.F. & Bailey, K.M. (1995). Gut evacuation of walleye Pollock larvae in response to feeding conditions. *Journal of Fish Biology, 46*: 389-403.

Directorate of Fisheries. (2002). Evaluering av utviklingsprogrammet for marine arter, NOMARO, 21-26. Available from: https://www.regjeringen.no/globalassets/upload/kilde/fid/rap/2003/0003/ddd/pdfv/171239-numario_2003.pdf

Directorate of Fisheries. (2023), Other fish species, Grow out production, Sale 2007-2022 (xlsx, 64.9kB). Available from: https://www.fiskeridir.no/English/Aquaculture/Statistics/Other-fish-species

Directorate of Fisheries.(2022). Økt salg av oppdrettsfisk i 2021. Avaliable from: https://www.fiskeridir.no/Akvakultur/Nyheter/2022/okt-salg-av-oppdrettsfisk-i-2021

Erstad, B. (2014). Sterling White Halibut, Kveiteoppdrett – ett skritt nærmere suksess. [online] Docplayer, 16-22. Available from: https://docplayer.me/11315206-Kveiteoppdre-et-skri-naermere-suksess-borre-erstad.html

Galay-Burgos, M., Power, D.M., Llewellyn, L., & Sweeney, G.E. (2008) Thyroid hormone receptor expression during metamorphosis of Atlantic halibut (*Hippoglossus hippoglossus*). *Molecular and Cellular Endocrinology, 281*, 56-63. doi: 10.1016/j.mce.2007.10.009.

Haché, R., Lanteigne, C., & Hébert, Y. (2016). Salt as a decontamination agent to control bacterial load in Artemia salina cultures. *Aquaculture 452*, 24-27. https://doi.org/10.1016/j.aquaculture.2015.10.017.

Hamre, K., Erstad, B., de Kok, J., Norberg, B., & Harboe, T. (2020). Change in nutrient composition of Artemia grown for 3–4 days and effects of feeding on-grown Artemia on performance of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture Nutrition, 26*, 1542-1554. https://doi.org/10.1111/anu.13101.

Hamre, K., Erstad, B., & Harboe, T. (2019). Early weaning of Atlantic halibut (*Hippoglossus hippoglossus*) larvae. *Aquaculture, 502*, 268-271. https://doi.org/10.1016/j.aquaculture.2018.12.060.

Hamre, K., & Harboe, T. (2008a). Critical levels of essential fatty acids for normal pigmentation in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture, 277*(1-2), 101-108. https://doi.org/10.1016/j.aquaculture.2008.02.020.

Hamre, K., & Harboe, T. (2008b). Artemia enriched with high n-3 HUFA may give a large improvement in performance of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture, 277*(3–4), 239–243. https://doi.org/10.1016/j.aquaculture.2008.02.028

Hamre, K., Holen, E., & Moren, M. (2007). Pigmentation and eye migration in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae: new findings and hypotheses. *Aquaculture Nutrition, 13*, 65-80. https://doi.org/10.1111/j.1365-2095.2007.00467.x.

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G. I., & Pittman, K. (2002). Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural Zooplankton or Artemia. *Aquaculture Nutrition, 8,* 139–148. https://doi.org/10.1046/j.1365-2095.2002.00201.x

Harboe, T., Mangor-Jensen, A., Naas, K.E. & Næss, T. (1998), A tank design for first feeding of Atlantic halibut, *Hippoglossus hippoglossus* L., larvae. *Aquaculture Research, 29*: 919-923. https://doi.org/10.1046/j.1365-2109.1998.29120919.x

Harboe, T., Huse, I., & Øie, G. (1994). Effects of egg disinfection on yolk sac and first feeding larvae of halibut (*Hippoglossus hippoglossus*) larvae. *Aquaculture, 119*, 57–65. https://doi.org/10.1016/0044-8486(94)90172-4.

Harboe, T., & Karlsen, Ø. (2003). Scientific evaluation of potential of new species in Norwegian aquaculture. *Fisken og havet, 6*, 8-11. Available from: https://www.hi.no/resources/publikasjoner/fisken-og-havet/74735/skiftesvik_et_al_2003_fisken_og_havet-nye_arte_i_oppdrettt_1_.pdf

Harboe, T., Mangor-Jensen, A., Moren, M., Hamre, K., & Rønnestad, I. (2009). Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles. *Aquaculture, 290*, 250-255. doi: 10.1016/j.aquaculture.2009.02.032

Harboe, T., & Reitan, K. I. (2005). Halibut fry production. *Larvi 05*, 205-206.

Hendry, C. I., Martin-Robichaud, D. J., & Benfey, T. J. (2003). Hormonal sex reversal of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture, 219*(1-4), 769-781. doi: 10.1016/S0044-8486(02)00344-7.

Jones, A., Prickett, R.A., & Douglas, M.T. (1981). Recent developments in techniques for rearing marine flatfish larvae, particularly turbot (*Scophthalmus maximus* L.), on a pilot commercial scale. *Rapp. P.-V. Réun. Cons. Int. Explor. Mer, 178*, 518-521.

Mangor-Jensen, A., Harboe, T., Hennø, J.S., & Troland, R. (1998). Design and operation of halibut egg incubator. *Aquaculture Research 29*, 887–893. http://dx.doi.org/10.1046/j.1365-2109.1998.29120887.x

Marine Research Institute. (2020). Halibut. Retrieved from: https://www.hi.no/en/hi/temasider/species/halibut

Moren, M., Sloth, J. J., & Hamre, K. (2008). Uptake of iodide from water in Atlantic halibut larvae (*Hippoglossus hippoglossus* L.). *Aquaculture, 285*(1–4), 174-178. https://doi.org/10.1016/j.aquaculture.2008.08.007.

Naas, K.E., Næss, T., & Harboe, T. (1992). Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus* L.) in green water. *Aquaculture, 105*(2), 143-156. https://doi.org/10.1016/0044-8486(92)90126-6.

Norberg, B., Valkner, V., Huse, J., Karlsen, I., & Grung, G. L. (1991). Ovulatory rhythms and egg viability in the Atlantic halibut *(Hippoglossus hippoglossus*). *Aquaculture, 97*(4), 365-371. https://doi.org/10.1016/0044-8486(91)90328-5.

Norberg, B., Weltzien, F.-A., Karlsen, Ø., & Holm, J. C. (2001). Effects of photoperiod on sexual maturation and somatic growth in male Atlantic halibut (*Hippoglossus hippoglossus*, L.). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 129*(2-3), 357-365. doi: 10.1016/S1096-4959(01)00320-7.

Næss, T. & Lie, Ø. (1998). A sensitive period during first feeding for the determination of pigmentation pattern in Atlantic halibut, *Hippoglossus hippoglossus* L., juveniles: the role of diet. *Aquaculture Research, 29*: 925-934.
https://doi.org/10.1046/j.1365-2109.1998.29120925.x

Opstad, I., & Raae, A. J. (1986). Physical stress on halibut larvae. *International Council for Exploration of the Sea, Mariculture Committee*.

Pittman, K. (1996). Rearing Halibut in Norway: Present Practices and Challenges. *Marine and Freshwater Research, 47*, 243-249. https://doi.org/10.1071/MF9960243.

Pittman, K., Jelmert, A., Næss, T., Harboe, T., & Watanabe, K. (1998). Plasticity of viable postmetamorphic forms of farmed Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture Research, 29*(12), 949-954. https://doi.org/10.1046/j.1365-2109.1998.29120949.x

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Björnsson, B.T., Einarsdottir, I.E., Canario, A.V.M., & Sweeney, G.E. (2001), Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 130*, 447–459. https://doi.org/10.1016/S1532-0456(01)00271-X

Perrichon, P., pers. comm. [Unpublished study]

Santos, G.A., Schrama, J.W., Mamauag, R.E.P., Rombout, J.H.W.M., & Verreth, J.A.J. (2010). Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): the combined effects of fish crowding and water quality deterioration. *Aquaculture, 299*(1-4), 73-80. https://doi.org/10.1016/j.aquaculture.2009.11.018

Schreiber, A.M. & Specker, J.L. (2000) Metamorphosis in the summer flounder, *Paralichthys dentatus*: Thyroidal status influences gill mitochondria-rich cells. *Gen. Comp. Endocrinol., 117*, 238-250. https://doi.org/10.1006/gcen.1999.7407

Schreiber, A.M., Wang, X., Tan, Y., Sievers, Q., Sievers, B., Lee, M., & Burrall, K. (2010) Thyroid hormone mediates otolith growth and development during flatfish metamorphosis. *Gen. Comp. Endocrinol. 169*, 130-137. https://doi.org/10.1016/j.ygcen.2010.08.008

Sclower, A. (1930). Die bedeutung der schilddrüse für die metamorphose des aales und der platt- fishe (The involvement of the thyroid in the metamorphosis of eels and flatfishes). *Forsch. Fortschr. Deutsch. Wiss., 6*, 435-436.

Seo, J., & Park, J. (2023). Does stocking density affect growth performance and hematological parameters of juvenile olive flounder *Paralichthys olivaceus* in a recirculating aquaculture system? *Animals, 13*(1), 44. doi: 10.3390/ani13010044

Shao, C., Bao, B., Xie, Z., et al. (2017). The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nature Genetics, 49*, 119-124. doi: 10.1038/ng.3732

Skoge, R. H., Nylund, A., Solheim, K., & Plarre, H. (2019). Complete genome of Atlantic halibut reovirus (AHRV) associated with mortality in production of Atlantic halibut (*Hippoglossus hippoglossus*) fry. *Aquaculture, 509*, 23-31. https://doi.org/10.1016/j.aquaculture.2019.04.080.

Solemdal, P., Tilseth, S., & Øiestad, V. (1974). Rearing of halibut. I. Incubation and the early larval stages. *ICES (International Council for the Exploration of the Sea) Council Meeting F: 41*, 1-12. http://hdl.handle.net/11250/101764

Statistics Norway (SSB). (2023). Fakta om Norsk nærinsliv. Available from: https://www.ssb.no/nasjonalregnskap-og-konjunkturer/faktaside/norsk-naeringsliv

Sterling White Halibut AS. (2023a). About Sterling® Halibut. Available from: https://sterlingwhitehalibut.com/en/about-sterling-halibut

Sterling White Halibut AS. (2023b). Contact. Avalable from: https://sterlingwhitehalibut.com/en/contact

Tilseth, S. (1990). New marine fish species for cold-water farming. *Aquaculture, 85*, 235-245. https://doi.org/10.1016/0044-8486(90)90023-G.

van der Meeren, T. (1995). Feed consumption and gut evacuation in Atlantic halibut (*Hippoglossus hippoglossus* L.) *larvae*.

Venizelos, A. & Benetti, D.D. (1999). Pigment abnormalities in flatfish. *Aquaculture, 176*, pp. 181–188. doi: 10.1016/S0044-8486(99)00060-5

Wang, X., Tan, Y., Sievers, Q., Sievers, B., Lee, M., Burrall, K., & Schreiber, A.M. (2011), Thyroid hormone-responsive genes mediate otolith growth and development during flatfish metamorphosis. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 158*, 163–168. https://doi.org/10.1016/j.cbpa.2010.10.014

Zhang, B., Shao, C., Chen, S., & Bao, B. (2022). Understanding asymmetrical malpigmentation in flatfishes and improvement measures for aquaculture. *Aquaculture, 14,* 1333-1344. doi: 10.1111/raq.12652

6. Appendix

6.1 Normal distribution

3.A(37) MH		*3.A(37) weight*		*3.C(38) MH*		*3.C(38) weight*	
Mean	4,97333333	Mean	0,098	Mean	7,04375	Mean	0,193125
Standard Error	0,15567569	Standard Error	0,00626403	Standard Error	0,08365044	Standard Error	0,00582514
Median	4,9	Median	0,1	Median	7,1	Median	0,19
Mode	4,9	Mode	0,08	Mode	7,1	Mode	0,2
Standard Deviation	0,60292936	Standard Deviation	0,02426049	Standard Deviation	0,33460175	Standard Deviation	0,02330057
Sample Variance	0,36352381	Sample Variance	0,00058857	Sample Variance	0,11195833	Sample Variance	0,00054292
Kurtosis	-0,4178577	Kurtosis	0,44740677	Kurtosis	0,74990441	Kurtosis	1,20066781
Skewness	0,6435466	Skewness	0,82284644	Skewness	-0,241534	Skewness	0,9766873
Range	2	Range	0,09	Range	1,4	Range	0,09
Minimum	4,2	Minimum	0,06	Minimum	6,3	Minimum	0,16
Maximum	6,2	Maximum	0,15	Maximum	7,7	Maximum	0,25
Sum	74,6	Sum	1,47	Sum	112,7	Sum	3,09
Count	15	Count	15	Count	16	Count	16

2.5.C(35) MH		*2.5.C(35) weight*		*4.A(39) MH*		*4.A(39) weight*	
Mean	5,58666667	Mean	0,10666667	Mean	6,52	Mean	0,202
Standard Error	0,10861319	Standard Error	0,00522509	Standard Error	0,0641427	Standard Error	0,00518239
Median	5,6	Median	0,1	Median	6,4	Median	0,2
Mode	5,6	Mode	0,1	Mode	6,4	Mode	0,2
Standard Deviation	0,42065708	Standard Deviation	0,02023669	Standard Deviation	0,2484236	Standard Deviation	0,0200713
Sample Variance	0,17695238	Sample Variance	0,00040952	Sample Variance	0,06171429	Sample Variance	0,00040286
Kurtosis	-0,4246126	Kurtosis	0,10396472	Kurtosis	-0,9929962	Kurtosis	-0,8373089
Skewness	0,36663365	Skewness	0,94366207	Skewness	0,67218726	Skewness	-0,0709418
Range	1,4	Range	0,07	Range	0,8	Range	0,06
Minimum	5	Minimum	0,08	Minimum	6,2	Minimum	0,17
Maximum	6,4	Maximum	0,15	Maximum	7	Maximum	0,23
Sum	83,8	Sum	1,6	Sum	97,8	Sum	3,03
Count	15	Count	15	Count	15	Count	15

4.B(43) MH		*4.B(43) weight*		*4.C(40) MH*		*4.C(40) weight*	
Mean	5,59375	Mean	0,13375	Mean	5,89375	Mean	0,14875
Standard Error	0,08778228	Standard Error	0,00712244	Standard Error	0,09935993	Standard Error	0,01244572
Median	5,7	Median	0,13	Median	5,75	Median	0,14
Mode	5,8	Mode	0,1	Mode	5,7	Mode	0,12
Standard Deviation	0,35112913	Standard Deviation	0,02848976	Standard Deviation	0,39743972	Standard Deviation	0,04978286
Sample Variance	0,12329167	Sample Variance	0,00081167	Sample Variance	0,15795833	Sample Variance	0,00247833
Kurtosis	-1,4444873	Kurtosis	-0,5242833	Kurtosis	0,60658283	Kurtosis	5,22168506
Skewness	-0,3525535	Skewness	0,61345773	Skewness	1,18138502	Skewness	1,98193786
Range	1	Range	0,09	Range	1,3	Range	0,2
Minimum	5	Minimum	0,1	Minimum	5,5	Minimum	0,1
Maximum	6	Maximum	0,19	Maximum	6,8	Maximum	0,3
Sum	89,5	Sum	2,14	Sum	94,3	Sum	2,38
Count	16	Count	16	Count	16	Count	16

Appendix 1: Calculation of normal distribution of the experimental growth data used for the statistical analyses to the start-feeding tanks. The measurements are named with the tank, age of the larvae in that tank, and if it is MH or weight growth. At least one of the kurtosis (how quickly the data tails off) or skewness (symmetry around the mid point) value must be between 2 and -2 to ensure enough homogeneity and a normal distribution of the variance within the tanks data. All tanks are within 2 and -2 for skewness and kurtosis for both MH and weight measurements, except for the weight measurements to 4.C. 4.C has a kurtosis value of 5.22. However, the skewness is 1.98, which is within the acceptable range. The 4.C weight data are therefore tailing of from the mid point at different raters but the symmetry around the middle point is still acceptable.

Low-larval density, Not approved		*Low-larval density, Approved*		*High-larval density, Not approved*		*High-larval density, Approved*	
Mean	4,66666667	Mean	108	Mean	17,6666667	Mean	119,666667
Standard Error	3,66666667	Standard Error	7,54983444	Standard Error	6,76592771	Standard Error	17,4578858
Median	1	Median	114	Median	13	Median	112
Mode	1	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	6,35085296	Standard Deviation	13,0766968	Standard Deviation	11,7189306	Standard Deviation	30,2379453
Sample Variance	40,3333333	Sample Variance	171	Sample Variance	137,333333	Sample Variance	914,333333
Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	#DIV/0!
Skewness	1,73205081	Skewness	-1,6300592	Skewness	1,50780848	Skewness	1,06760471
Range	11	Range	24	Range	22	Range	59
Minimum	1	Minimum	93	Minimum	9	Minimum	94
Maximum	12	Maximum	117	Maximum	31	Maximum	153
Sum	14	Sum	324	Sum	53	Sum	359
Count	3	Count	3	Count	3	Count	3

Uneven light spread, Not approved		*Uneven light spread, Approved*		*Even light spread, Not approved*		*Even light spread, Approved*	
Mean	58,3333333	Mean	66	Mean	11,1666667	Mean	113,833333
Standard Error	7,8683614	Standard Error	5,43445796	Standard Error	4,50493556	Standard Error	8,89725301
Median	53	Median	62,5	Median	10,5	Median	113
Mode	#N/A	Mode	#N/A	Mode	1	Mode	#N/A
Standard Deviation	19,2734705	Standard Deviation	13,311649	Standard Deviation	11,0347935	Standard Deviation	21,79373
Sample Variance	371,466667	Sample Variance	177,2	Sample Variance	121,766667	Sample Variance	474,966667
Kurtosis	-1,3204685	Kurtosis	0,20541506	Kurtosis	2,06541556	Kurtosis	2,11694183
Skewness	0,512069	Skewness	0,95920671	Skewness	1,27186485	Skewness	1,25388495
Range	50	Range	36	Range	30	Range	60
Minimum	36	Minimum	52	Minimum	1	Minimum	93
Maximum	86	Maximum	88	Maximum	31	Maximum	153
Sum	350	Sum	396	Sum	67	Sum	683
Count	6	Count	6	Count	6	Count	6

Low aeration, Not approved		*Low aeration, Approved*		*High aeration, Not approved*		*High aeration, Approved*	
Mean	47	Mean	75	Mean	69,6666667	Mean	57
Standard Error	6,65832812	Standard Error	7,5055535	Standard Error	11,6952032	Standard Error	3,21455025
Median	46	Median	75	Median	76	Median	56
Mode	#N/A	Mode	#N/A	Mode	#N/A	Mode	#N/A
Standard Deviation	11,5325626	Standard Deviation	13	Standard Deviation	20,2566861	Standard Deviation	5,56776436
Sample Variance	133	Sample Variance	169	Sample Variance	410,333333	Sample Variance	31
Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	#DIV/0!	Kurtosis	#DIV/0!
Skewness	0,38726566	Skewness	0	Skewness	-1,2694107	Skewness	0,78215212
Range	23	Range	26	Range	39	Range	11
Minimum	36	Minimum	62	Minimum	47	Minimum	52
Maximum	59	Maximum	88	Maximum	86	Maximum	63
Sum	141	Sum	225	Sum	209	Sum	171
Count	3	Count	3	Count	3	Count	3

Appendix 2: Calculation of normal distribution of the experimental eye migration data. The measurments are named with the factor influencing eye migration (density, light spread and aeration) and eye migration (not approve or approved). At least one of the kurtosis (how quickly the data tails off) or skewness (symmetry around the mid point) value must be between 2 and -2 to ensure enough homogeneity and a normal distribution of the variance within the tanks data. All factors influencing eye migration are within 2 and -2 for skewness for both not approved and approved measurements. The light distribution eye data was enough to also provide a kurtosis value. The uneven light distribution tanks have a kurtosis value within 2 and -2 (), however the even light distributed tanks are slightly above. The data to the even light distributed tanks are therefore tailing of from the mid point at different raters but the symmetry around the middle point is still acceptable.

6.2 Growth, development and culture conditions

t-Test: Two-Sample Assuming Unequal Variances		
3m MH	*3.A(37)*	*3.C(38)*
Mean	4,97333333	7,04375
Variance	0,36352381	0,11195833
Observations	15	16
Hypothesized Mean Difference	0	
df	22	
t Stat	-11,71536	
P(T<=t) one-tail	3,1476E-11	
t Critical one-tail	1,71714437	
P(T<=t) two-tail	6,2952E-11	
t Critical two-tail	2,07387307	

t-Test: Two-Sample Assuming Unequal Variances		
3m weight	*3.A(37)*	*3.C(38)*
Mean	0,098	0,193125
Variance	0,00058857	0,00054292
Observations	15	16
Hypothesized Mean Difference	0	
df	29	
t Stat	-11,120569	
P(T<=t) one-tail	2,8116E-12	
t Critical one-tail	1,69912703	
P(T<=t) two-tail	5,6231E-12	
t Critical two-tail	2,04522964	

Appendix 3: t-test results showing the p-value for the 3m MH growth(left) and the weight increase(right).

Anova: Single Factor

SUMMARY 4m MH

Groups	*Count*	*Sum*	*Average*	*Variance*
4.A(39)	15	97,8	6,52	0,06171429
4.B(43)	16	89,5	5,59375	0,12329167
4.C(40)	16	94,3	5,89375	0,15795833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6,87384574	2	3,436922872	29,7525171	6,7113E-09	3,20927802
Within Groups	5,08275	44	0,115517045			
Total	11,9565957	46				

Anova: Single Factor

SUMMARY 4m weight

Groups	*Count*	*Sum*	*Average*	*Variance*
4.A(39)	15	3,03	0,202	0,00040286
4.B(43)	16	2,14	0,13375	0,00081167
4.C(40)	16	2,38	0,14875	0,00247833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,03949085	2	0,019745426	15,799213	6,7403E-06	3,20927802
Within Groups	0,05499	44	0,001249773			
Total	0,09448085	46				

Appendix 4: ANOVA results showing the p-value for the 4m MH growth(left) and the weight increase(right).

Anova: Single Factor

SUMMARY Even spread of light, MH

Groups	*Count*	*Sum*	*Average*	*Variance*
3.A(37)	15	74,6	4,97333333	0,36352381
3.C(38)	16	112,7	7,04375	0,11195833
2.5.C(35)	15	83,8	5,58666667	0,17695238

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	35,2820018	2	17,6410009	82,0419231	2,101E-15	3,21448033
Within Groups	9,24604167	43	0,21502422			
Total	44,5280435	45				

Anova: Single Factor

SUMMARY Even spread of light, weight

Groups	*Count*	*Sum*	*Average*	*Variance*
3.A(37)	15	1,47	0,098	0,00058857
3.C(38)	16	3,09	0,193125	0,00054292
2.5.C(35)	15	1,6	0,10666667	0,00040952

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,08657857	2	0,04328928	84,1629614	1,3586E-15	3,21448033
Within Groups	0,02211708	43	0,00051435			
Total	0,10869565	45				

Appendix 5: ANOVA results showing the p-value for the even light distributed tanks, MH growth(left) and the weight increase(right).

Anova: Single Factor

SUMMARY Low aeration, MH

Groups	*Count*	*Sum*	*Average*	*Variance*
3.A(37)	15	74,6	4,973333333	0,36352381
3.C(38)	16	112,7	7,04375	0,11195833
2.5.C(35)	15	83,8	5,586666667	0,17695238
4.A(39)	15	97,8	6,52	0,06171429

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	39,7220895	3	13,24069649	74,6505034	9,8762E-20	2,76643793
Within Groups	10,1100417	57	0,177369152			
Total	49,8321311	60				

Anova: Single Factor

SUMMARY Low aeration, weight

Groups	*Count*	*Sum*	*Average*	*Variance*
3.A(37)	15	1,47	0,098	0,00058857
3.C(38)	16	3,09	0,193125	0,00054292
2.5.C(35)	15	1,6	0,106666667	0,00040952
4.A(39)	15	3,03	0,202	0,00040286

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0,13901669	3	0,046338896	95,158307	3,5725E-22	2,76643793
Within Groups	0,02775708	57	0,000486966			
Total	0,16677377	60				

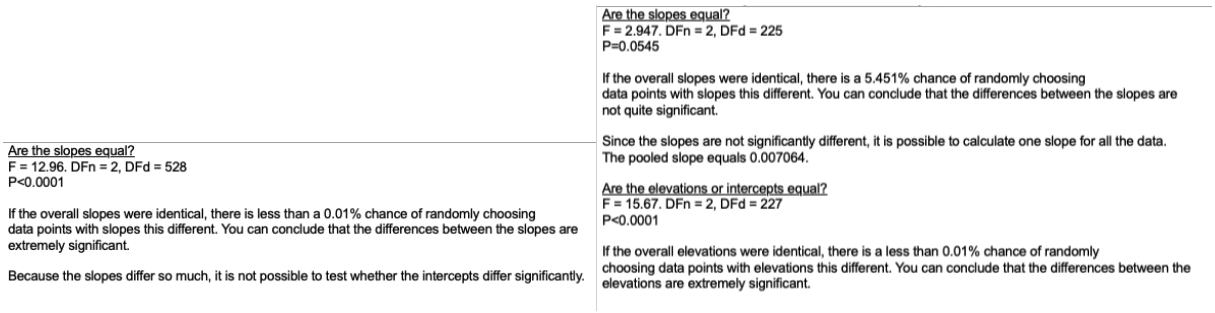
Appendix 6: ANOVA results showing the p-value for the low aeration distributed tanks, MH growth(left) and the weight increase(right).

6.3 Eye migration

Fry tank	Eye migration quality: 0	Eye migration quality: 1	Eye migration quality: 2	Eye migration quality: 3	Approved eye migration: (2+ 3)	SF Tank	Larval dencity
101	24	12	27	48	68%	4.A	Medium
102	31	15	37	51	66%	4.A	Medium
103	1	0	8	106	99%	3.C	Low
104	11	20	41	53	75%	3.A	High
109	20	27	50	13	57%	4.B	Medium
118	0	1	89	28	99%	3.C	Low
203	100	13	16	0	12%	4.C	Medium
206	83	21	8	0	7%	4.C	Medium
207	42	60	32	1	24%	4.B	Medium
216	2	7	133	20	94%	3.A	High
217	1	20	75	14	81%	3.A	High
303	71	25	38	4	30%	4.C	Medium
304	44	42	34	18	38%	4.B	Medium
311	4	30	94	1	74%	3.A	High
314	1	12	104	8	90%	3.A	High
317	6	53	60	2	51%	4.A	Medium
402	30	46	53	3	42%	4.B	Medium
416	5	37	80	20	70%	2.5.C + 4.B	Low
418	0	12	49	44	89%	2.5.C	Low

Appendix 7: The eye migration data is collected from 19 fry tanks, where 2391 halibut fry have been graded from 0-3. Approved eye migration has a grading of 2 and 3, and represents the number of fish kept in the production.

6.4 Growth and larval density



Appendix 8: Simple linear regression lines showing the p-value between the growth and the larval density. MH growth(left) and the weight increase(right).

Simple linear regression Tabular results	A	B	C
	Low	Medium	High
	Y	Y	Y
Best-fit values			
Slope	0.1767	0.1489	0.1446
Y-intercept	-0.4422	-0.3395	-0.5831
X-intercept	2.503	2.279	4.032
1/slope	5.660	6.714	6.915
Std. Error			
Slope	0.004365	0.003551	0.004907
Y-intercept	0.1083	0.1033	0.1789
95% Confidence Intervals			
Slope	0.1681 to 0.1853	0.1419 to 0.1559	0.1349 to 0.1544
Y-intercept	-0.6561 to -0.2283	-0.5429 to -0.1361	-0.9389 to -0.2274
X-intercept	1.352 to 3.558	0.9544 to 3.498	1.678 to 6.109
Goodness of Fit			
R squared	0.9100	0.8618	0.9118
Sy.x	0.5805	0.7255	0.5606
Is slope significantly non-zero?			
F	1639	1759	868.4
DFn, DFd	1, 162	1, 282	1, 84
P value	<0.0001	<0.0001	<0.0001
Deviation from zero?	Significant	Significant	Significant
Equation	Y = 0.1767*X - 0.4422	Y = 0.1489*X - 0.3395	Y = 0.1446*X - 0.5831
Data			
Number of X values	2640	3108	3204
Maximum number of Y replicates	1	1	1
Total number of values	164	284	86
Number of missing values	2476	2824	3118

Simple linear regression Tabular results	A	B	C
	Low	Medium	High
	Y	Y	Y
1/slope	106.3	145.9	170.8
Std. Error			
Slope	0.0009311	0.0008119	0.0008215
Y-intercept	0.03064	0.03126	0.03657
95% Confidence Intervals			
Slope	0.007549 to 0.01127	0.005248 to 0.008464	0.004198 to 0.007509
Y-intercept	-0.2474 to -0.1250	-0.1858 to -0.06193	-0.1815 to -0.03409
X-intercept	16.50 to 22.03	11.76 to 22.02	8.087 to 24.27
Goodness of Fit			
R squared	0.6110	0.3807	0.5358
Sy.x	0.02969	0.04154	0.03200
Is slope significantly non-zero?			
F	102.1	71.31	50.78
DFn, DFd	1, 65	1, 116	1, 44
P value	<0.0001	<0.0001	<0.0001
Deviation from zero?	Significant	Significant	Significant
Equation	Y = 0.009408*X - 0.1862	Y = 0.006856*X - 0.1238	Y = 0.005854*X - 0.1078
Data			
Number of X values	822	1302	1410
Maximum number of Y replicates	1	1	1
Total number of values	67	118	46
Number of missing values	755	1184	1364

Appendix 9: R square to the simple linear regression lines, MH growth(left) and the weight increase(right).

6.5 Growth and eye migration

```
> dataAug <- data.frame(
+   SF_tank = c("3.A", "3.C", "2.5.C", "4.A", "4.B", "4.C"),
+   Density = c("High", "Low", "Low", "Medium", "Medium", "Medium"),
+   Spread_of_light = c("Even", "Even", "Even", "Uneven", "Uneven", "Uneven"),
+   Aeration = c("Low", "Low", "Low", "Low", "High", "High"),
+   Growth_rate = c("Low", "High", "High", "High", "Low", "Low"),
+   Eye_migration = c(0.83, 0.99, 0.89, 0.61, 0.40, 0.17)
+ )
> library(ggplot2)
> ggplot(dataAug, aes(x = Density, y = Eye_migration)) + geom_boxplot() + theme_minimal()
> ggplot(dataAug, aes(x = Spread_of_light, y = Eye_migration)) + geom_boxplot() + theme_minimal()
> ggplot(dataAug, aes(x = Aeration, y = Eye_migration)) + geom_boxplot() + theme_minimal()
> ggplot(dataAug, aes(x = Growth_rate, y = Eye_migration)) + geom_boxplot() + theme_minimal()
> density_model <- aov(Eye_migration ~ Density, data = dataAug)
> summary(density_model)
            Df Sum Sq Mean Sq F value Pr(>F)
Density      2 0.3982 0.19911   5.864 0.0919 .
Residuals    3 0.1019 0.03396
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> spread_of_light_model <- aov(Eye_migration ~ Spread_of_light, data = dataAug)
> summary(spread_of_light_model)
               Df Sum Sq Mean Sq F value Pr(>F)
Spread_of_light  1 0.3902  0.3902    14.2 0.0196 *
Residuals        4 0.1099  0.0275
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> aeration_model <- aov(Eye_migration ~ Aeration, data = dataAug)
> summary(aeration_model)
            Df Sum Sq Mean Sq F value Pr(>F)
Aeration     1  0.396   0.396   15.22 0.0175 *
Residuals    4  0.104   0.026
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> growth_rate_model <- aov(Eye_migration ~ Growth_rate, data = dataAug)
> summary(growth_rate_model)
            Df Sum Sq Mean Sq F value Pr(>F)
Growth_rate  1 0.1980 0.19802   2.622  0.181
Residuals    4 0.3021 0.07552
```

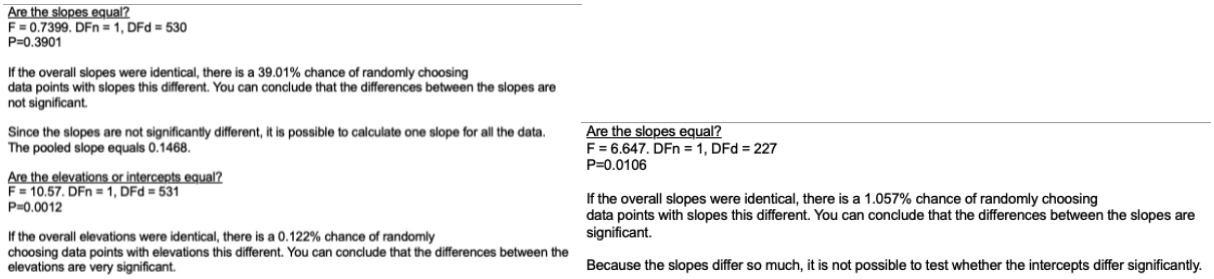
Appendix 10: ANOVA results showing the p-value for growth rate and eye migration at the bottom, p = 0.181.

6.6 Larval density and eye migration

Table Analyzed	Chi-square, Larval density and eye migration
P value and statistical significance	
Test	Chi-square test for trend
Chi-square, df	0.1703, 1
P value	0.6799
P value summary	ns
One- or two-sided	NA
Statistically significant (P < 0.05)?	No
Data analyzed	
Number of rows	6
Number of columns	2

Appendix 11: Chi-square results showing the p-value for larval density and eye migration, p = 0.6799.

6.7 Light distribution and growth



Appendix 12: Simple linear regression lines showing the p-value between the light distribution and growth. MH growth(left) and the weight increase(right).

Simple linear regression Tabular results		A Even light distribution Y	B Uneven light distribution Y
9	Y-intercept	0.1128	0.1033
10			
11	**95% Confidence Intervals**		
12	Slope	0.1369 to 0.1520	0.1419 to 0.1559
13	Y-intercept	-0.2341 to 0.2103	-0.5429 to -0.1361
14	X-intercept	-1.529 to 1.548	0.9544 to 3.498
15			
16	**Goodness of Fit**		
17	R squared	0.8506	0.8618
18	Sy.x	0.7548	0.7255
19			
20	**Is slope significantly non-zero?**		
21	F	1412	1759
22	DFn, DFd	1, 248	1, 282
23	P value	<0.0001	<0.0001
24	Deviation from zero?	Significant	Significant
25			
26	**Equation**	Y = 0.1444*X - 0.01187	Y = 0.1489*X - 0.3395
27			
28	**Data**		
29	Number of X values	3204	3108
30	Maximum number of Y replicates	1	1
31	Total number of values	250	284
32	Number of missing values	2954	2824

Simple linear regression Tabular results		A Even light distribution Y	B Uneven light distribution Y
1	**Best-fit values**		
2	Slope	0.004515	0.006856
3	Y-intercept	-0.03543	-0.1238
4	X-intercept	7.848	18.06
5	1/slope	221.5	145.9
6			
7	**Std. Error**		
8	Slope	0.0004586	0.0008119
9	Y-intercept	0.01746	0.03126
10			
11	**95% Confidence Intervals**		
12	Slope	0.003606 to 0.005424	0.005248 to 0.008464
13	Y-intercept	-0.07002 to -0.0008419	-0.1858 to -0.06193
14	X-intercept	0.2323 to 12.97	11.76 to 22.02
15			
16	**Goodness of Fit**		
17	R squared	0.4662	0.3807
18	Sy.x	0.03590	0.04154
19			
20	**Is slope significantly non-zero?**		
21	F	96.93	71.31
22	DFn, DFd	1, 111	1, 116
23	P value	<0.0001	<0.0001
24	Deviation from zero?	Significant	Significant
25			
26	**Equation**	Y = 0.004515*X - 0.03543	Y = 0.006856*X - 0.1238
27			
28	**Data**		
29	Number of X values	1410	1302
30	Maximum number of Y replicates	1	1
31	Total number of values	113	118
32	Number of missing values	1297	1184

Appendix 13: R square to the simple linear regression lines, MH growth(left) and the weight increase(right).

6.8 Light distribution and eye migration

Table Analyzed	Chi-square, Light spread and eye migration
P value and statistical significance	
Test	Chi-square test for trend
Chi-square, df	49.68, 1
P value	<0.0001
P value summary	****
One- or two-sided	NA
Statistically significant (P < 0.05)?	Yes
Data analyzed	
Number of rows	6
Number of columns	2

Appendix 14: Chi-square results showing the p-value for light spread and eye migration, $p < 0.0001$.

6.9 Aeration and growth

Are the slopes equal?
F = 4.431. DFn = 1, DFd = 530
P=0.0358

If the overall slopes were identical, there is a 3.576% chance of randomly choosing data points with slopes this different. You can conclude that the differences between the slopes are significant.

Because the slopes differ so much, it is not possible to test whether the intercepts differ significantly.

Are the slopes equal?
F = 0.09935. DFn = 1, DFd = 227
P=0.7529

If the overall slopes were identical, there is a 75.29% chance of randomly choosing data points with slopes this different. You can conclude that the differences between the slopes are not significant.

Since the slopes are not significantly different, it is possible to calculate one slope for all the data. The pooled slope equals 0.005174.

Are the elevations or intercepts equal?
F = 34.41. DFn = 1, DFd = 228
P<0.0001

If the overall elevations were identical, there is a less than 0.01% chance of randomly choosing data points with elevations this different. You can conclude that the differences between the elevations are extremely significant.

Appendix 15: Simple linear regression lines showing the p-value between low and high aeration and growth rate. MH growth(left) and the weight increase(right).

	Simple linear regression Tabular results	A Low aeration	B High aeration
		Y	Y
1	**Best-fit values**		
2	Slope	0.1488	0.1379
3	Y-intercept	-0.03661	-0.3078
4	X-intercept	0.2460	2.231
5	1/slope	6.720	7.250
6			
7	**Std. Error**		
8	Slope	0.003192	0.003809
9	Y-intercept	0.09573	0.1061
10			
11	**95% Confidence Intervals**		
12	Slope	0.1425 to 0.1551	0.1304 to 0.1454
13	Y-intercept	-0.2249 to 0.1517	-0.5169 to -0.09862
14	X-intercept	-1.060 to 1.456	0.7520 to 3.574
15			
16	**Goodness of Fit**		
17	R squared	0.8668	0.8700
18	Sy.x	0.7284	0.6336
19			
20	**Is slope significantly non-zero?**		
21	F	2174	1311
22	DFn, DFd	1, 334	1, 196
23	P value	<0.0001	<0.0001
24	Deviation from zero?	Significant	Significant
25			
26	**Equation**	Y = 0.1488*X - 0.03661	Y = 0.1379*X - 0.3078
27			
28	**Data**		
29	Number of X values	3204	2922
30	Maximum number of Y replicates	1	1
31	Total number of values	336	198
32	Number of missing values	2868	2724

	Simple linear regression Tabular results	A Low aeration	B High aeration
		Y	Y
1	**Best-fit values**		
2	Slope	0.005129	0.005512
3	Y-intercept	-0.04855	-0.09358
4	X-intercept	9.467	16.98
5	1/slope	195.0	181.4
6			
7	**Std. Error**		
8	Slope	0.0004268	0.001085
9	Y-intercept	0.01643	0.04100
10			
11	**95% Confidence Intervals**		
12	Slope	0.004286 to 0.005972	0.003349 to 0.007676
13	Y-intercept	-0.08101 to -0.01609	-0.1754 to -0.01180
14	X-intercept	3.741 to 13.62	3.511 to 22.93
15			
16	**Goodness of Fit**		
17	R squared	0.4791	0.2695
18	Sy.x	0.03758	0.03487
19			
20	**Is slope significantly non-zero?**		
21	F	144.4	25.82
22	DFn, DFd	1, 157	1, 70
23	P value	<0.0001	<0.0001
24	Deviation from zero?	Significant	Significant
25			
26	**Equation**	Y = 0.005129*X - 0.04855	Y = 0.005512*X - 0.09358
27			
28	**Data**		
29	Number of X values	1410	1110
30	Maximum number of Y replicates	1	1
31	Total number of values	159	72
32	Number of missing values	1251	1038

Appendix 16: R square to the simple linear regression lines, MH growth(left) and the weight increase(right).

6.10 Aeration and eye migration

1	**Table Analyzed**	Chi-square, Aeration and eye migration
2		
3	**P value and statistical significance**	
4	Test	Chi-square test for trend
5	Chi-square, df	30.65, 1
6	P value	<0.0001
7	P value summary	****
8	One- or two-sided	NA
9	Statistically significant (P < 0.05)?	Yes
10		
11	**Data analyzed**	
12	Number of rows	6
13	Number of columns	2

Appendix 17: Chi-square results showing the p-value for aeration and eye migration, $p < 0.0001$.