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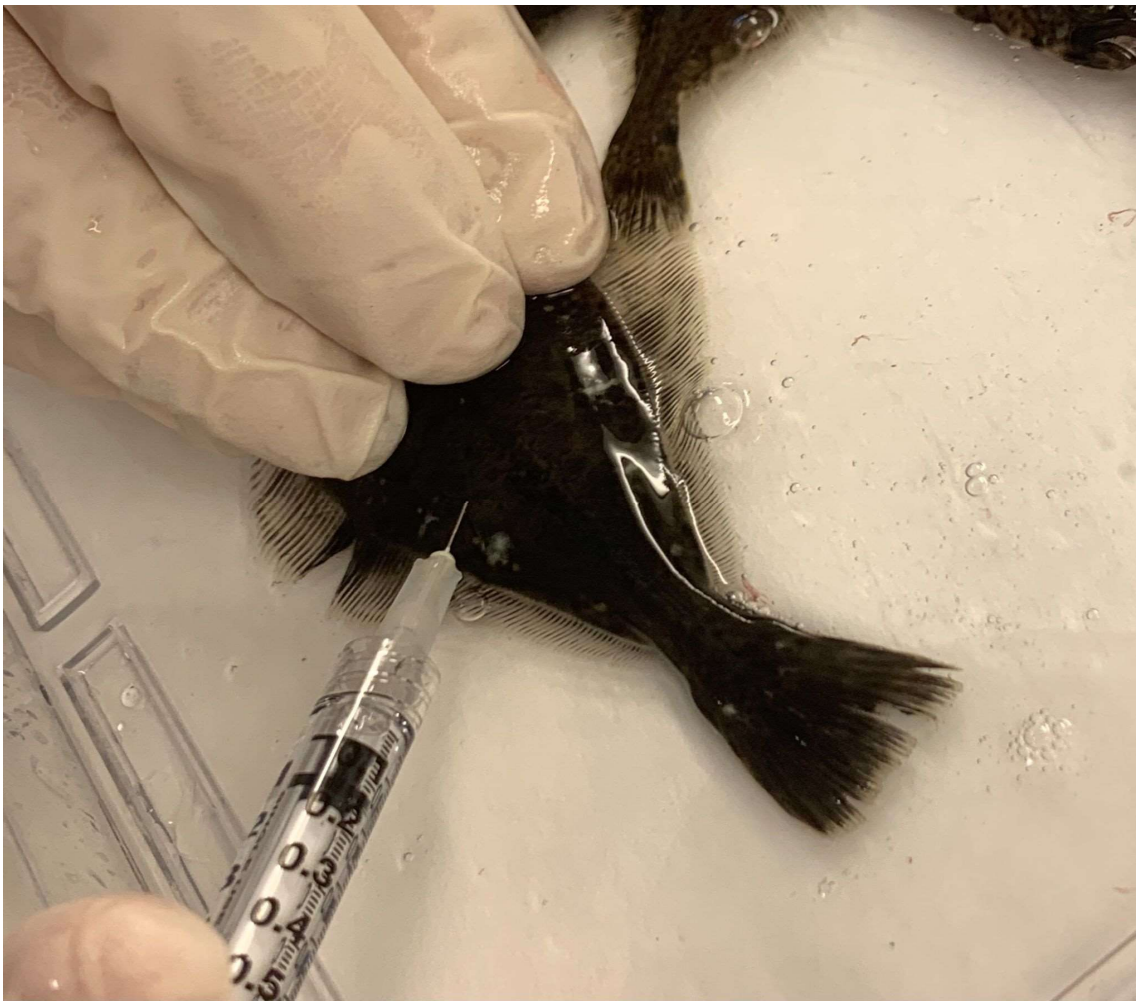
Atlantic halibut (*Hippoglossus hippoglossus* L.) fry mortality after transportation with focus on biological and chemical aspects



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Abstract

This thesis is a project finalizing Bachelor of Science degrees in the fields of biological chemistry and chemical- & environmental engineering. As the literature database for Atlantic halibut fish farming is scarce, the need for research is evident and potentially innovative. The intention for this thesis was to show initiative contacting a company within the common field of interest that is fish farming and establish a mutually positive project. As a response to inquiry, Sterling White Halibut (SWH) presented a current problem that was very intriguing and relevant in an educative regard. The thesis project was designed in collaboration with SWH. The focus areas for monitoring were biological stress and water chemistry.

Hippoglossus hippoglossus fry (6.70 ± 2.35 g) was transported approximately one thousand kilometers from Rørvik to Imsland. This thesis concerns one batch of 94 186 Atlantic halibut fry, with arrival at Imsland in March 2021. Measurements were made upon arrival and for one month after delivery. SWH reports that a late onset mortality occurs after transportation of small fry sent from the hatchery to the rearing facilities. Some fry batches have endured substantial mortality which is considered caused by the transportation and transfer of fry. The complex phenomenon of late onset mortality after transportation required investigation.

Water sampling and chemical analysis were performed in two stages. First stage was at arrival. Analysis of transportation water compared to receiving water presented a relatively large difference in water quality. The second stage was comprised of daily analysis of the water quality to monitor changes during the expected period of mortality. These differences were relatively minor. Biological parameters were measured at arrival and monitored afterwards on each of the three field excursions to complement the water quality data. These datasets served as the basis for the graphical- and statistical analysis. Mortality was considered the prime biological parameter of which the other data were evaluated against. Correlation ($r = -0.44$) between pH and mortality was discovered and 19 % of the variation observed is explained by the regression model of pH and mortality.

Abrupt environmental differences in transportation water compared to receiving water was revealed, which contributes to additional stress after transportation. Stress levels were indicated by elevated blood glucose concentration and increased red blood cell (RBC) count. Late onset mortality is suggested as a consequence of stress during and after transportation, including an unsuccessful adaptation to a new environment at the rearing facilities.

Terms and abbreviations

BMI = Body mass index

BWE = Backward elimination

DO% = Dissolved oxygen percentage

MPS = Multiparameter sonde

NORCE = Norwegian Research Centre (governmental research institute)

PSU = Practical salinity unit

RAS = Recirculating aquaculture system

RBC = Red blood cell

SPC = Specific conductance

SWH = Sterling White Halibut

TAN = Total ammonia nitrogen

UiS = University of Stavanger

YSI = Yellow Springs Instruments (US equipment production company)

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1. Introduction

1.1. Atlantic halibut farming

Norway is the biggest producer of farmed salmon in the world (Taranger et al., 2014). A variety of fish farming companies have tried to expand the horizon on aquaculture to include large-scale farming of marine species. Atlantic halibut (*Hippoglossus hippoglossus*) have long been attractive for Norwegian fishermen due to its high market price (Godø & Haug, 1988).

Production of halibut was first initiated in Norway in the mid-1980s and commercial production arose late in the 1990s (Glover, Svåsand, Olesen, & Rye, 2006). The production of Atlantic halibut has increased since the 1990s. In 2019 Norway produced 1525 tons of Atlantic halibut, which only represents 0.1 % of Norway's total aquafarming for 2019. As a comparison 1364 megatons were produced of farmed salmon the same year (SSB, 2020). Sterling White Halibut (SWH) was created to farm halibut in 2001(SWH, n.d). It was created by the world's largest farmed salmon producer, namely Mowi (Vormedal, 2017). SWH was Global Gap-certified as the first commercial halibut farming company in the world (SWH, n.d)

There are several reasons why it is not easy and straightforward to farm halibut. One bottleneck factor is the critical growing and feeding of hatched fry in the early stages of the fry phase (Bergheim, 2006). This is a vital phase of the farming process, as young and small fry are vulnerable to stressors and influencing external factors. Because of the relative to weight rapid growth, the halibut needs different environmental considerations at the different stages. To provide an optimal setting for growth, the fry needs to be transported between different facilities or areas that are specialized in the different stages of farming. This poses a major stress momentum for the growing fry, which in the case with SWH can lead to high mortality.

1.2. Sterling White Halibut

SWH aquaculture facilities for growth of young halibut fry are situated at Imsland in Ryfylke, Norway. The most critical point before rearing is transportation. The transportation distance is approximately thousand kilometers and have a duration of twenty hours. The halibut fry is transported in a truck with an accompanying trailer. They are divided into twelve separate tanks, five of them installed in the truck compartment and seven installed in the connected trailer. Five tanks in the truck have internal recirculating water flow and the same applies to the seven trailer tanks. The system used for transportation may resemble a recirculating aquaculture system (RAS), however without any water treatment units.

Biomass status before transportation is important. If the fry is too large it becomes cumbersome and costly to transport. If the fry is too small, they are considered more vulnerable with lower survival. The fry will in general experience higher levels of stress in transportation tanks compared to regular stationary fish tanks. Physical damage like blunt force trauma initiated by the tanks moving irregularly or by other individuals induces stress. This may be explained by the elevated density of fry, since the transportation tanks are relatively small as there are dimensional limitations for transportation. The water quality is also difficult to manage, and essentially a shorter drive is deemed more suitable in the case for SWH. There are no stops on the way to manage water quality, however monitoring is performed by sensors installed in the transportation tanks. The most critical parameters are recorded every fifteen minutes. These data can be valuable for SWH in terms of observing historical change, and review for possible inclusion of new measures in the process.

During the transfer from transportation tanks to the stationary destination tank a water separation slide was used. This method may inflict damage to the fry, as there is a relatively sharp slide angle with a moderately large drop to the water surface. Especially considering some of the smaller fry. Even though the chemical parameters that determine the water quality stay within the provisional accepted boundaries, they change significantly over a short amount of time. This change contributes to stress. The water quality is not only represented as direct values presenting a snapshot image of the water quality situation.

Initially two points of interest were established for the thesis investigation. Firstly, what would be the most optimal transportation size of the fry. Secondly, what environmental factors are the most crucial for survival.

1.3. Aim of the study

Because of time restrictions determined by the University of Stavanger (UiS), and practical delays during the Covid-19 pandemic, this thesis concerns the second initial point of interest. It includes biological and chemical monitoring of one halibut fry batch after transportation. The halibut fry studies are split into two sections. First section of analysis focuses on biological factors. These factors may indicate fry stress levels and help determine survivability after transportation. Second section of analysis covers monitoring of the chemical quality of water used in the farming process. The value range and fluctuations of these internal biological and external chemical factors are reviewed with theoretical knowledge and relevant literature. Possible links between environmental factors and late mortality are also proposed.

The core reason for this bachelor thesis project is to produce new knowledge about the effects of moving fry. For many years SWH has experienced elevated mortality in the fry phase, which has occurred several weeks after transportation. Some possible theories are conjectured and discussed as the analysis results drive further progress in theoretical explanations to this phenomenon.

The most substantial mortality has previously been around 40% for one delivery batch at this phase in the fish farming process. Mortality is logged after sorting out dead fry together with troubled- and emaciated fry. The latter contributes the most to mortality numbers according to SWH. Troubled fry swim around in the tank instead of lying on the bottom and emaciated fry are abnormally thin from insufficient feeding. Fry that lie on the bottom of the tank are considered healthy, as this is a sign of well-being for this type of flounder (Nelson, Grande, & Wilson, 2016). The reason for why the fry is troubled and not eating properly is not known, and the factors determining these outcomes are not monitored enough to give a complete overview.

The goal of this thesis was to give some explanation to the observed mortality by using analysis of selected water chemistry parameters together with observations from data of biological parameters. To process the data, different methods were used. Based on graphical- and statistical analysis the most important parameters for monitoring mortality are proposed.

1.4. Biology and morphology

The Atlantic halibut (*Hippoglossus hippoglossus*) is the largest of all flatfish (Orlov, Kuznetsova, & Mukhametov, 2011). It is most widespread in the northern parts of the Atlantic Ocean (Glover, 2006). Halibut is a right-side flatfish with darker skin on the eyed side, while the blind side is white (Haug, 1990). It belongs to the family Pleuronectidae and classified as a member of the order Pleuronectiformes. classified by Linnaeaus in 1758 (Roje, 2010).

Atlantic halibut live to be relatively old and can exceed 300 kg in nature. Male halibut rarely grow beyond 50 kg as growth stagnates after it has become sexually mature (Mangor-Jensen & Holm, 2004). Halibut can reach very low depths in the sea and spawning occurs at depths of 300-700 meters. Juvenile halibut are often localized at sandy bottoms at depths of 20-60 meters. Halibut spend most of their time on the sea floor as they lack a swim bladder. They therefore need to invest energy to adjust their buoyancy (Nelson et al., 2016).

1.5. Stress indicators

The physiological response to stress in fish is necessary to overcome challenges and maintain homeostasis. The response includes biochemical mechanisms in the fish biology that reflects in magnitude the challenge imposed on it. Thereby compensating for the stressor in an appropriate way, to ensure survival and maintain homeostasis (Iwama, 2006). This thesis defines stressors as either acute or chronic. Acute and chronic stress are dependent on the context and therefore not so clearly characterized. The definition used in the thesis is based on the consideration of consequence duration, not the duration of its stressor (Boonstra, 2013).

The mortality observed at SWH is acute and present in a relatively short time even though there is a late onset. There are limitations in terms of time in this project, so no long duration monitoring for prolonged consequence duration was possible. Most parameters in the field work are considered to track the acute stress and some track chronic stressor indication. The acute stress indicators are complemented by the blood cell counting data and therefore gives a more complete profile of the stress status (Marcel, Martinez & Rogelio., 2009).

The general stress response can be divided into three phases. Primary, secondary, and tertiary stress phase of response. In the primary phase of a stress reaction the production of corticosteroids and catecholamines occurs. They then initiate the secondary phase by signaling distribution of necessary resources to vital areas in the fish body. Measured as an increase in blood glucose levels. These hormones have a positive survival effect by making resources available for the fish to use when exposed to a stressor. The tertiary phase affects the whole organism as a response to long time stress. These changes can be disease resistance, growth, behavior and reproductive changes (Schreck & Tort, 2016).

Despite recovering from primary or secondary stress factors the fish may never fully recover if the stress is severe enough. A delayed mortality can occur weeks later even if clinical indicators suggests recovery (Schreck & Tort, 2016).

1.5.1. Blood glucose

When exposed to stress, the endocrine organs release stress hormones such as catecholamines and corticosteroids as part of the primary response. This is categorized as an endocrine system response, as the endocrine system controls the release of signal hormones. Then the secondary response occurs as the resulting biochemical mechanism provides glucose to the bloodstream to be readily available. This metabolic pathway is produced to prepare for an emergency (Marcel et al., 2009). Elevation in blood glucose concentration may also be explained by post prandial plasma glucose increase, which occurs after feeding. Because there is rapid insulin response this should be a short and small increase, but it is difficult to ascertain the magnitude of influence compared to the stress response. This is because of the dynamic nature of this particular response, as it should cater to a variety of stressors with different severity.

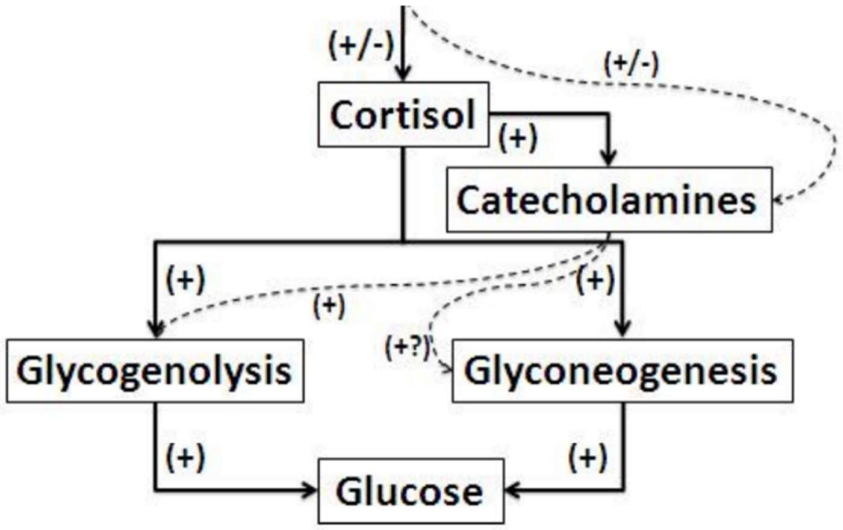


Figure 1. An overview on the complex physiological cortisol pathway. Positive and negative feedback are indicated by plus- and minus signs. "Environmental factors. physiological status. rearing history…". 2009. Martinez-Porchas. (https://panamjas.org/pdf_artigos/PANAMJAS_4(2)_158-178.pdf).

1.5.2. RBC count

Measurements of hematological parameters in fish can indicate their physiological status. In this thesis the determination of red blood cell count contributes to assessing the halibut fry health changes. As well as evaluate if any environmental stress factors monitored have an impact on their hematological values.

If the fish demands high energy output or if the water environment contains a low concentration of oxygen the fish can respond by increasing the concentration of red blood cells to raise the oxygen carrying capacity (Svobodová, 1993). This mechanism also applies by reducing the concentration of red blood cells in the fish if it is metabolically inactive and has high input of oxygen. An elevated concentration of red blood cells will normally be expected for fish exposed to stress (Fazio et al., 2015). Lower blood cell count may describe an anemic condition, which may be caused by infection. blood loss or nutrient deficiency (Editorial, 2016).

1.5.3. Density in cages

Overactive swimming halibut individuals changing depth levels often are considered stressed (Conte, 2004). Healthy unstressed halibut are generally located on the floor area and tank density in the farming tanks are therefore expressed as the tank floor area coverage in percent. This parameter serves as a biological health indicator. The density can vary from 100-200 %, without the growth being significantly affected. The fry should not be confined to live too close to each other and density coverage beyond 100 % is not recommended (Pittman et al., 1994).

In nature halibut live in the deep where there are relatively weak water currents. In aquaculture it is observed that the halibut prefer to stay in places with low water flow, and generally avoid water inlets and outlets if there are strong water flows there. If different flow conditions are created in the farming cells, and the fish can migrate between the cells, the cell with the weakest current is preferred by most fish (Pittman et al., 1994).

1.6. Water Chemistry

Water chemistry analysis is a way of determining the composition and concentration of different chemicals present in water samples. As the seawater used for pisciculture has a known general composition, a quantitative perspective on water chemistry is more suitable for the thesis purpose.

"Unfavorable water quality is often mentioned as a limiting factor and a source of suboptimal operation" (Åtland, 2015). This is the starting point for why the environmental monitoring of water quality in aquaculture is important. Theoretical evaluations on which substances posed the higher risk in the fish tank setting helped limit the investigation scope (Åtland, 2015).

It is difficult to establish and uphold conditions to simulate natural aquatic environment. As a result of suboptimal conditions the fish may become stressed (Conte, 2004). A source of environmental change is due to transportation. When moving fish. the receiving fish tanks' environmental parameters should be matched to the source water. Rapid changes in water quality stress the fish considerably, even if the values are within the tolerance range for the specie being transported (Sopinka, Donaldson, O'Connor, Suski, & Cooke, 2016). To avoid abrupt changes in water quality the fish can be acclimated to the receiving water. Acclimation not done properly contribute to stressing the fish, and may lead to delayed mortality (Harmon, 2009).

Relevant aspects of the water flow concern three stages at Imsland. First stage is the water inlet phase. This water may include microorganisms, different types of particles and seawater contaminants. It is important to have in mind that even though some of these factors are considered unwanted, some will also contribute to better fish welfare. “Bacterial diversity is favorable” (Åtland, 2015). Second stage is water treatment and encompass aeration, UV-dosing, and oxygenation. Third stage is pumping water flow to the inland fish tanks.

Dissolved oxygen is a chemical parameter of interest when monitoring the fry population after transportation. Air pumps may cause oxygen gradients in the tanks if the outlets are not evenly distributed. Temperature changes will affect the percentage of dissolved oxygen in the tanks. If a pressure gradient is present, it may result in difficulties regulating the oxygen levels.

1.7.Water quality parameters

Industrial farming of halibut on land is impossible if manipulation of parameters summarized in table 1 is not achievable. The main goal for a breeding company will always be to maximize biological production with respect to producing a healthy and strong fish with focus on minimizing consequential damage (Rosten, Kristensen, Rosseland & Braathen, 2004).

Table 1. Parameters that can be manipulated in an aquaculture fish tank.

	Controllable	Partially controllable
Temperature	X	
Salinity	X	
Alkalinity	X	
TAN		X
pH		X
Carbon dioxide		X
Oxygen	X	
Raw water quality		X

The aquatic environment is crucial for fish well-being and health. Fish have evolved specific requirements for the environmental chemical factors in terms of temperature and content of compounds such as ammonia, oxygen, carbon dioxide, pH, and various metals. For the various parameters there are specific tolerance ranges for the different fish species (Rosten et al., 2004).

Halibut has emissions that influence the water chemical processes in a water tank. Emissions of carbon dioxide will have a direct influence on the pH, which in turn affects the form of ammonia. A simple pathway on how the halibut influence the water chemistry is visualized in Figure 2.

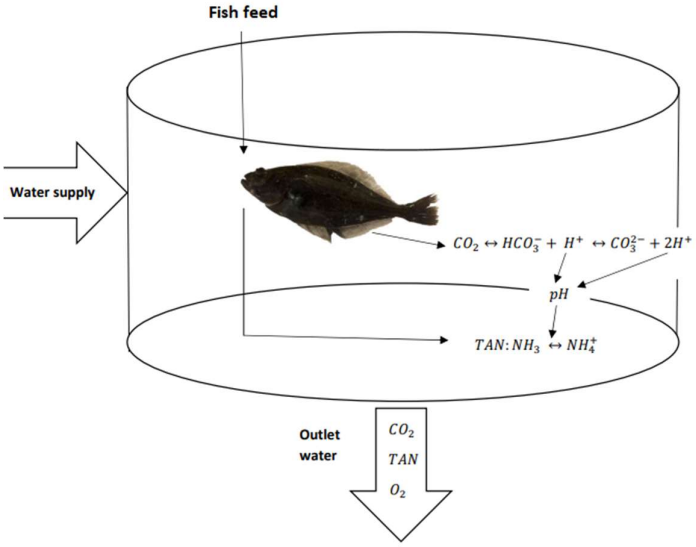


Figure 2. An illustration showing a simple cylinder shape representing the aquafarming tanks used at Imsland. Spesific influencing pathways from the fish metabolism to water quality parameters are visualized. "Illustrasjon av noen av de viktigste prosessene", 2004, Mattilsynet. (https://www.mattilsynet.no/fisk_og_akvakultur/fiskevelferd/mattilsynet__rapport_om_vannkvalitet_og_fiskevelferd_2004.5943/binary/Mattilsynet%20-%20Rapport%20om%20vannkvalitet%20og%20fiskevelferd%20(2004)).

All the parameters discussed in the next sections will focus on halibuts' tolerance and action limits to the various parameters. There is little available literature when it comes to halibut tolerance for each specific parameter and therefore some of the parameters will be based on general tolerance limits for other common farmed fish species. Interactions between different parameters can cause one parameter to act either synergistically or antagonistic to another (Rosten et al., 2004). This will also be discussed for the parameters concerned measured.

1.7.1. Dissolved oxygen (DO)

Dissolved oxygen is a vital and one of the most important parameters to be controlled when it comes to RAS (Ta & Wei, 2018). Supersaturation can lead to gas bubbles in the fish. Excess gas forms tissue emphysema and blood emboli, also called gas bubble disease (Bouck, 1980). Especially for halibut it is reported harmful bubble formation in and around the eyes due to elevated oxygen saturation. Therefore, over 100% saturation is not recommended and exceeding this value can be harmful for the fish (Åtland, 2015). On the lower limits the halibut can withstand oxygen saturation down to 84%, but a recommended oxygen saturation should be around 100% (Rosten, 2009). It is important to maintain an even DO level during transport and a saturation near 100% is recommended throughout transport (Harmon, 2009).

1.7.2. Alkalinity and pH

"Alkalinity is explained by waters capacity to neutralize a strong acid to a determined pH-value" (FHI, 2021). In short it is the buffer capacity of water, which helps keep the pH stabile. Three main negative ions are considered to contribute the most to this effect, the carbonate system, and hydroxides. As acid rain lowers the pH of waters, the buffer capacity declines and resistance to pH change diminishes. A monitoring of both alkalinity and pH is therefore adequate when describing water chemistry status, pH has in turn great influence on the water quality due to its synergistic and antagonistic properties towards other water parameters. The pH value is often decisive for whether a number of substances are present in a toxic or non-toxic form (Gebauer, Eggen, Hansen, & Eikebrokk, 1992). Especially the chemical speciation of metals is pH-dependent, of which aluminum is most important concerning fish toxicology. It is difficult to state an optimal pH range for Atlantic halibut since there is limited literature concerning this.

1.7.3. Temperature

Monitoring temperature in the aquaculture system is important. SWH has also determined likewise as they have installed temperature sensors in the transportation tanks. As one of only three monitored parameters during this crucial process. Temperature is an important parameter, as fish are poikilothermic animals. This means that the ambient temperature in the fish tanks control their body temperature (Svobodová, 1993). In turn this modulates metabolism and thereby influences the water quality. If the fish has optimal access to feed

and environmental conditions are optimal, the metabolism will increase with rising temperatures. This trend stops when the temperature reaches the start of an upper lethal limit.

Fish metabolism produces biochemical energy from feed. The amount of energy that channels directly into growth depends on how much energy the fish spends on other activities (Gebauer et al., 1992). As temperatures fluctuates the energy distribution changes. An optimal temperature for halibut farming would be approximately 5-9 °C (Brown, 2010). This is strongly dependent on the water quality condition as environmental parameters may act synergistic or antagonistic shifting the optimal temperature. The best growth conditions is generally observed in a narrower range between 13-16 °C for juvenile halibuts (Mangor-Jensen & Holm, 2004).

Vital parameters that change with temperature fluctuations is oxygen-, carbon dioxide- and ammonia concentrations. The solubility of oxygen and carbon dioxide decreases with increasing temperature and increases with decreasing temperature. Ammonia toxicity increases with decreasing temperatures, conversely the amount of ammonia increases with increasing temperature (Rosten et al., 2004). The pH is also a vital parameter, as one of the three parameters including dissolved oxygen, that is monitored by SWH during transportation.

To explain the temperature dependency of pH it is important to understand the underlying theory. A basic concept within chemistry theory is the temperature dependency of molecular dissolution. The nature of the chemical compound will determine the direction of temperature effects. If a chemical dissociation reaction needs energy to proceed the dissociation will increase with increasing temperature. If it produces energy the dissociation will decrease with increasing temperature. As in the case of water, which is the chemical compound in question regarding monitoring, the reaction of dissolution is endothermic. Since H_2O consist of bonds between hydrogen and oxygen the reaction to break them needs energy, so that they may dissociate to the smaller constituents. Increasing the temperature in a water solution the reaction will favor the direction which counteracts this change, as understood by Le Châtelier's principle. Since the reaction is endothermic, the direction preferred when adding heat is towards producing ions. It is therefore the case that an increase in temperature will lead to a higher concentration of hydrogen ions which in turn lowers the pH.

The pH-scale is based on pure water as a reference as it is defined as pH-neutral with standard conditions. As water exhibit the property of self-ionization, this must be considered

and is used as the exact reference number for neutral pH. Ion-product constant (K_w) describes this property. As one mole of water molecule produces one mole of both ions a pH of 7 is stated as neutral.

$$pH = -log[H^+].\ pOH = -log\,[OH^-] \quad \text{(Ia)}$$

$$K_w = [H^+][OH^-] = 10^{-14} \quad \text{(Ib)}$$

$$pH + pOH = -log\,(K_w) = 14 \quad \text{(Ic)}$$

1.7.4. Ammonia

Ammonia is a nitrogenous compound excreted by animals and a metabolic waste product from protein catabolism (Kir & Öz, 2015). It can also be generated from decomposition of organic materials. In seawater ammonia occurs in two different forms, the ionized form (NH_4^+) and the unionized form (NH_3). Total ammonia nitrogen (TAN) is composed of these two forms of ammonia. The ratio of ammonia and ammonium is strongly dependent on the pH value. In water solution, ammonia (NH_3) is present in equilibrium with ammonium (NH_4^+) in the given equilibrium equation (Gebauer et al., 1992).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad \text{(II)}$$

Temperature and pH influence the equilibrium. At pH values above 9.75 NH_3 is the predominant form, and pH values below 8.75 NH_4^+ is the predominant form (Li, Xu, Li, Wang, & Wang, 2020). Temperatures affect this equilibrium as well as higher temperature gives elevated amounts of unionized ammonia (Kir & Öz, 2015).

First signs of ammonia toxicity in fish are restlessness and increased respiration. In the case of halibut they congregate to the water surface (Svobodová, 1993). Ammonia can easily diffuse across the fish tissue when a concentration gradient exists. Ammonia is therefore potentially toxic for the fish, dependent on the concentration. There is disagreement as to whether only the deionized form (NH_3) makes up all the toxic effects or if the ionized form (NH_4^+) also contribute some impact on the toxicity. If the ionized form should in the future show to have toxic effects this would destabilize the understanding of ammonia toxicity within the fish farming industry. This is because ammonium presents much higher concentrations then ammonia at the pH values and temperatures that are most common for fish farming (Gebauer et al., 1992). Which gives the possibility for a small toxicity effect to give great impact on the total toxicity.

Besides pH and temperature there are other factors that influence the toxicity of ammonia. Low concentration of oxygen in water also makes ammonia more toxic (Svobodová, 1993). Salinity also influences ammonia, explained further in the next section. The specific response of Atlantic halibut to periodic ammonia exposure is currently unknown.

1.7.5. Salinity and conductivity

Salinity is one of the parameters in table 1 that can be manipulated. Lab experiments have found that the salinity levels for halibuts between 25-35 ppt are preferred (Brown, 2010). For adults the preferred salinity levels is found to be around 33 ppt (Cargnelli, Griesbach, & Morse, 1999). In contrast to pH and temperature, the relationship between salinity and ammonia works inverse. Observations done on shrimps showed that the toxicity of ammonia increased with reduced salinity levels (Kir & Kumlu, 2006; Kir & Öz, 2015).

Conductivity is highly dependent on the amount of dissolved solids in the water (Rusydi, 2018). The major ionic constituents of seawater are NaCl as it contributes approximately 91 % relative moles (Mackenzie, 2020). This intuitively indicates the relationship between salinity and conductivity. Considering ionic strength, ionic character and temperature is important when adjusting conductivity for a specific environment. To perform this type of specific adjustment a detailed seawater speciation is needed together with an empirical statistical model for the exact environmental conditions. This was considered not possible because of time limitations, also there were no apparent need for such high precision values as the salinity range is quite wide.

1.7.6. Aluminum

Aluminum poisoning can lead to acute mortality and the toxicity effect depends on the size of the fish and the concentration of aluminum. Aluminum is acutely toxic to fish in acid water, even at low concentrations. Fish gill is the target organ for waterborne aluminum and mortality occurs due to a respiratory dysfunction (Exley, Chappell, & Birchall, 1991). The accumulation of aluminum in organs is slow and does not have a toxic effect for the fish (Wilson, 2011). Aluminum is an important parameter in aquafarming due to its acute toxicity, where time limitations give little time for problem management. Problems with aluminum poisoning occur especially in the south/west of Norway due to acid rain leading to an increased release of aluminum from the soil (Gebauer et al., 1992).

2. Materials and methods

2.1. Water chemistry measurements

Only the chemical aspects of the water environment in the rearing facilities are included in the field work, as analysis before arrival was deemed impossible due to the pandemic situation and economic limitations. Water quality can be described by many parameters, however in this thesis project the extent of field measurements was limited by the available instruments.

Measurements were carried out differently at fry batch arrival compared to the stages of monitoring afterwards. MPS measurements were done in each of the sampling buckets from each of the transportation tanks, as it represents the water quality at the departure station. In the three field dates for monitoring the water quality the sonde was used as before with nine data points measured. Since all the fry from delivery were situated in one fish cage after arrival there was only need for nine data points in total to the contrary of the fry batch arrival day. At arrival, water was sampled immediately from the transportation tanks and stored for later analysis. Two parallel sample sets were obtained, where 12 of the samples included acidification as conservation using two drops of 5 M sulfuric acid. The other 12 were sampled under water to avoid later pH fluctuations by air pollution.

In subsequent weeks after arrival the technical personnel at Imsland sampled two parallel water bottles from the fish cage number four each day. From the start date of field work to the end date of field work there were 30 days in total excluding the day of arrival and including the third field day of monitoring. From all water sampling days there was a total of 29 water samples, because it was forgotten to sample the fish cage water on the 25th of March.

The reason for taking two parallel water samples each sampling day was due to the different sampling methods. These sampling methods were designed to fit each section of water analysis in the after period of field excursions. Ammonia in water is highly elusive, and to preserve the amount of interest at the sampling day some conservation was needed. Storage of acidified samples at standard superficial freezer, and the other samples at refrigerator temperature. Samples stored at freezer were later used for NH_3 analysis (Riemann & Schierup, 1978). Acidification of the water samples converted the elusive ammonia to the non-elusive ammonium, which was in analysis converted back using basification.

2.2. Materials and equipment

Four main equipment were used for measurements and analysis of biological and chemical parameters. A MPS were used to measure water chemistry parameters in real time at Imsland. An ammonia selective electrode and a pH electrode for alkalinity were utilized for the secondary analysis work on the water samples. By using correct sampling and conservation methods, water samples were used to determine additional parameters to complement MPS data. Conductivity was analyzed independently by Espen Enge in his laboratory as a supplement to the conductivity data collected with the MPS. In the results section the MPS conductivity data and the conductivity data from Espen Enge is compared.

Biological measurements and analysis were performed in separate parts. Blood extraction was utilized to provide blood tissue for analysis. The fish after analysis were placed in a container with water and a lethal dose of Aquacalm (Metomidate hydrochloride). For the halibut body measurements simple measuring equipment were used. Such as a low accuracy scale, a plastic caliper measuring tool and pictures taken of the fry postmortem.

2.3. Field measurement procedures

2.3.1. Solution preparation

A solution of heparin was made containing 5000 IU / ml. The solution was made by adding 10 ml distilled water to 0.5 g solid heparin sodium salt from porcine intestinal mucosa (see Appendix E). Solution for red blood cell counting were made in advance. Physiological saline, 995 µl, were pipetted in three identical sterile Eppendorf's and closed. Whole blood, 5 µl, were later added after blood extraction making the relative dilution ratio 1:200.

2.3.2. Blood glucose analysis

The fish was caught using twelve separate and identical sampling buckets in the shipping tanks. Between three and eight random fish individuals were gathered from each tank and stored in the sampling buckets with oxygen pumps connected. Two pumps were used in total, in a sequential manner, and the sampling buckets were aerated two and two with a set time interval of 10 minutes. After gathering the fish individuals from each transportation tank, the buckets were covered with a tarpaulin to avoid direct sunlight. This way the actual external environment the fry experience in the stationary fish cages is simulated. The fish stayed in the buckets for approximately two hours before analysis started. This was determined

observationally to be the optimal time for the fish to calm down after the stress of transportation. This time interval was replicated throughout the field work before extraction of blood to uphold experimental continuity.

To obtain blood from the fish the caudal vein was punctured. and the blood extracted using heparinized syringes. Glucose was analyzed immediately after blood extraction using a commercial glucometer, Contour Next. The strips were inserted in the Contour Next glucometer, and approximately 4 µl blood were used for each strip. The Contour Next glucometer has a detection range from 0.6-33.3 mM glucose. The reason why human glucometer is used in this thesis is because it is easily accessible and within the finical framework for this thesis. Using a human glucometer to measure fish glucose levels is also a reliably method (Bartoňková, Hyrsl, & Vojtek, 2017).

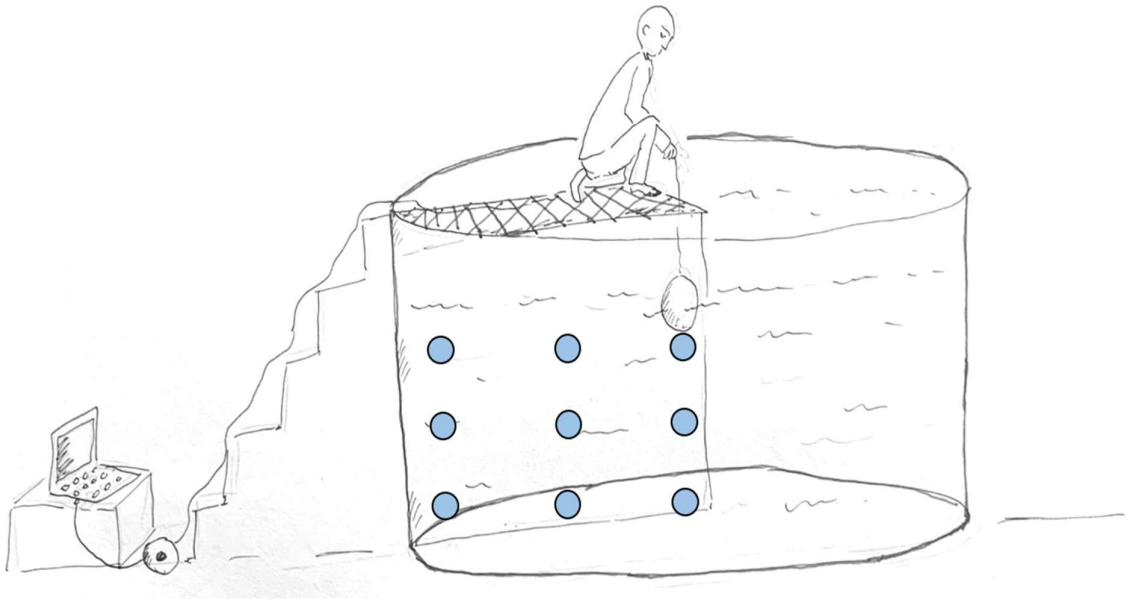
2.3.3. RBC analysis

After the glucose measurements solutions for RBC counting were mixed with whole blood. Since blood tissue has a limited time of viability ex vivo swiftness was needed. The solution was mixed using a pipette. The chamber and glass cover were cleaned using 70 % EtOH. Approximately 20 µl of the cell mixture was pipetted out and put at the edge of the cover glass. The cell mixture was then analyzed with a microscope using 40x objective to count each central grid. The microscope was connected to a VisiCam 5 Plus camera. and pictures of the blood samples were saved for later counting. All the grids in the center of the Thoma chamber was counted. If cells are touching the upper and left limits it is counted, and cells touching lower and right limits are not counted. This procedure is performed on all squares counted. The formula used to calculate RBC is given in equation II (Giri, 2021).

$$Particles\ per\ \mu l = \frac{cells\ counted * dillution\ factor}{area\ counted\ (mm^2) * depth(mm)} \qquad \text{(III)}$$

2.3.4. MPS measurements

The MPS was used in the field to measure several parameters parallelly in real time at Imsland. Measurements were done at nine different depths and locations in the tank. How the measurements were carried out is visualized in figure 3. The MPS was connected to a laptop computer during the measurements and software named KorEXO was used to establish a connection between them.



Fry batch arrival date measurements served as datasets reflecting both the environment at arrival in Imsland, but also described the water quality from departure at Rørvik. Therefore, more water samples and fry individuals were required for analysis to provide a sound data basis for later evaluation and comparison.

Table 2. Overview of MPS data points, water sampling material and fry individuals used for blood analysis.

Progress	Batch arrival	Monitoring 1	Monitoring 2	Monitoring 3
Field dates	18.03.2021	26.03.2021	10.04.2021	17.04.2021
Water chemistry:				
1. Probe data points	30	9	9	9
2. Water samples (frozen/cool)*	14/14	7/7	16/16	6/6
Blood extraction:				
Total individuals	43	9	9	9
1. Individuals for blood glucose	18	3	3	3
2. Individuals for morphology data	43	9	9	9
3. Individuals for RBC counting	0	3	3	3

*Water sampling consisted of two parallel batches. One batch with conservation stored in freezer. and one batch sampled under water to minimize CO_2 influence. 5 M sulfuric acid was used for conservation by acidification. using 2 drops by Pasteur pipette.

2.3.5. Alkalinity

An empty beaker was put on the scale and tared. Approximately 30 ml of the sample was added to the beaker. The exact volume was recorded, and a magnet was added for stirring. A pH electrode was inserted, and the value was recorded. Stirring was stopped each time the pH value recording was made. The sample was titrated using a 0.5 ml pipette with 0.02 N H_2SO_4. The pH value was logged for every 0.5 ml titrated. Titration was done until three values pH < 4.5 was recorded. The beaker was rinsed in distilled water between measurements and pH electrode temporarily stored in distilled water. The values for alkalinity were calculated (See Appendix D), using a sophisticated method modified with gran-plots according to Henriksen formula (Henriksen, 1982).

2.3.6. Ammonia

Ammonia-selective electrode method was utilized for this analysis. Selective electrode for ammonia was prepared with a standard inner solution and hydrated in distilled water for approximately 30 min. An empty beaker of cylindrical shape was used for determining the ammonia concentration. The beaker was tared on a scale with one decimal point to the exact mass of 50 g, and then 2 ml of 5 N NaOH was added. After addition, an alarm was set for 10 min, which was the determined optimal time for diffusion through the selective membrane. This determination was done based on previous testing of the electrode with different concentrations at different time intervals. Voltage potential was logged after set time interval,

and the measurement was repeated on each sample. A minimum of three calibration readings were performed every analysis day at the laboratory (See Appendix B for calibration curve and calculation).

To adjust ammonia values corresponding to environmental conditions at origin of sampling it is important to include the influencers of largest magnitude. Considering the available datasets collected of other parameters that may influence ammonia concentration, two main parameters were considered. Temperature and pH are adjusted according to Appendix B (Emerson, Russo, Lund & Thurston, 2011). The table is constructed empirically and includes adjustments for both pH and temperature. Temperature adjustment is performed by assigning a mean temperature obtained from MPS data to each monitoring phase. Ammonia percentage is also adjusted with precise pH data acquired from water chemistry analysis results for each sample. The joined parameters give a specific percentage factor from the table stated above, which in turn gives the actual percentage of ammonia in the origin environment.

2.4. Equipment overview

Equipment used for this thesis are partly provided by University of Stavanger (UiS) and Måltidets hus. Approximate uncertainties are included in table 3. The possible errors involved in the methods used are mentioned in the discussion section to determine if the optimal methods were used.

Table 3. Equipment for water chemistry analysis is presented first. then the MPS are presented with uncertainties for the different parameters measured. Lastly the equipment used for biological analysis are listed except for a standard plastic caliper measuring tool used to log halibut body measurements.

Equipment	Unit	Uncertainty
Radiometer pH-electrode	-log[H+]	±0.1
Metrohm ammonia electrode	µg/L NH3	±5-10%
Cyber Scan conductivity electrode	µS/cm	±1-2%
A&D Scale	g	±0.2
YSI Multiparameter sonde (EXO1)		
- Temperature	-5-+50 °C	±0.01
- Dissolved Oxygen	0-200%	±1
- Conductivity	0-100 mS/cm	±0.5%
- pH	0-14 pH	±0.2
Thermo Fischer pipette	0.2-2.0 µl	±2.5-12%
Thermo Fischer pipette	10.0-100 µl	±1-3%
Ohaus CS-200 Scale	G	±0.1
Contour Next glucometer	0.6-33.3 mM glucose	±10%
Thoma hemocytometer	RBC's/µL	Highly variable

Thoma and other hemocytometers have large margins of error, both systematical and human error. An investigation showed up to 55 % inaccuracy due to pipetting and sampling errors (Freund & Carol, 1964). Some of the uncertainties here will be used later in discussion.

2.5. Statistical methods

The field outline included an array of different parameters selected based on knowledge from SWH, relevant literature and personal experience from previous laboratory work. The selected parameters were expected to describe the aquaculture system at Imsland to some degree. “Chemists often collect experimental observations on a large number of variables when it is unknown which specific ones are significant for prediction of future samples” (Sutter & Kalivas, 1993). To process data from the monitoring phase a multiple regression analysis was utilized, using the backward elimination method (BWE). A correlation test was performed to validate independent variables. Parameters are successively eliminated based on the highest p-values obtained from the regression model. Significance level is fixed at 0.05, and parameters which do not describe the system under investigation are omitted in the final analysis result (Sutter & Kalivas, 1993). The parameter of significance is subsequently validated for the assumptions for linear regression, to confirm that the relationship is true.

3. Results

3.1. Water quality data

3.1.1. Monitoring phase

Ammonia values from the monitoring phase shows an average of 0.37 µg/L NH_3 over the 30 days of measuring (Table 4). This is a relatively minor concentration and should under normal operation not be a limiting factor for production. Salmonids have a standard water quality level of 21 µg/L NH_3, which is considered applicable for most marine fish species. This is because salmonids are established as one of the most sensitive species to ammonia (Eddy, 2005). The standard deviation of the ammonia measurements performed at Imsland during monitoring is 0.12 µg/L NH_3. In relative terms this deviation is large, however considering the units it is still in the micro-range. It is important not to understate this statistical observation. The deviation for NH_3-concentration measurements is 32% in relation to the average mean. Where the other parameter deviations lie in the range of 0-2%.

Mortality is logged by SWH as sorted out individuals every day after delivery. Counting is delayed by one day if detection limit is not reached when sorting out fry. Detection limit for death count at SWH is ten individuals. The statistical representation for zero is five individuals, even though the data array is too small to define an exact mean average of five for non-detection days. Total mortality for the monitoring phase is 0.4%. This is not the 40% that has been logged for previous fry batches at Imsland. The general trends and discoveries may have an importance on describing the aquaculture system at Imsland regardless of the low mortality.

Table 4. Parameters measured during field work monitoring phase. Dates 25.03 and 29.03 are omitted as they have incomplete analysis results. Original zero loggings of mortality are demarcated with underscores.

Dates	Days after delivery	Mortality	NH3 (µg/L)	ALKe (µM)	pH	Cond (mS/cm)
19.03.2021	1	28	0.70	2317	7.09	50.87
20.03.2021	2	6	0.51	2302	7.49	50.67
21.03.2021	3	19	0.53	2303	7.33	50.77
22.03.2021	4	3	0.48	2304	7.38	51.29
23.03.2021	5	21	0.47	2315	7.28	50.87
24.03.2021	6	2	0.45	2309	7.33	50.77
26.03.2021	8	5	0.39	2304	7.40	50.25
27.03.2021	9	14	0.36	2216	7.27	50.25
28.03.2021	10	5	0.42	2205	7.27	49.94
30.03.2021	12	5	0.31	2204	7.38	49.73
31.03.2021	13	10	0.14	2166	7.57	49.52
01.04.2021	14	5	0.41	2274	7.35	50.35
02.04.2021	15	22	0.35	2278	7.14	50.77
03.04.2021	16	5	0.22	2318	7.54	50.87
04.04.2021	17	4	0.15	2332	7.57	50.98
05.04.2021	18	20	0.36	2326	7.47	50.77
06.04.2021	19	34	0.40	2324	7.26	50.98
07.04.2021	20	12	0.32	2341	7.32	51.08
08.04.2021	21	13	0.30	2335	7.40	51.08
09.04.2021	22	11	0.31	2328	7.22	50.67
10.04.2021	23	6	0.22	2332	7.47	50.98
11.04.2021	24	13	0.30	2321	7.28	50.87
12.04.2021	25	5	0.36	2304	7.36	50.35
13.04.2021	26	24	0.35	2326	7.33	50.67
14.04.2021	27	5	0.30	2287	7.48	50.98
15.04.2021	28	18	0.34	2324	7.43	50.77
16.04.2021	29	5	0.39	2335	7.50	50.77
17.04.2021	30	39	0.39	2341	7.40	50.87

It is important to evaluate the dataset independency before regression analysis. Regression allows estimations by using the relation of parameters, but it does not provide information about the strength of relation. By performing a correlation test these relationship strengths are revealed for evaluation (Table 5). Values below 0.1 reveal negligible relationship, while above 0.9 indicate a significant relationship (Schober, Boer, & Schwarte, 2018). An association between parameters do not imply causation, however a large correlation coefficient indicates a large variance. A good regression model will have small variance, as it indicates close prediction of datapoints. By the conventional approach to correlation coefficient interpretation the alkalinity- and conductivity parameters possess a very strong correlation (Schober et al., 2018). According to literature on the subject this is a sound statistical observation and has previously been reported with a correlation coefficient of $r = +0.72$ with a significant probability of $p = < 0.01$ (Sechriest, 1960).

Table 5. Correlation test between assumed independent variables. Colored value in bold present a correlation coefficient r ≥ 0.9, when including standard number rounding rules to obtain value with one decimal point.

	Days after delivery	NH3 (µg/L)	ALKe (µM)	pH	Cond (µS/cm)
Days after delivery	1				
NH3 (µg/L)	-0.55	1			
ALKe (µM)	0.35	0.14	1		
pH	0.26	-0.58	0.00	1	
Cond (µS/cm)	0.18	0.17	**0.86**	-0.07	1

Before performing the multiple regression (BWE) analysis alkalinity- or conductivity parameters must be removed according to the correlation test results (Table 5). After inspection of the other correlation test results, alkalinity present the highest correlation coefficients with the other parameters and are therefore omitted.

For the statistical multiple regression (BWE) analysis, a significance level of $p < 0.05$ were chosen. All p-values above this level are removed successively from highest to lowest, to provide the best model considering the dependent variable of mortality. Analysis present pH as a parameter of significance and are therefore evaluated further to verify statistical validity (Table 6).

Table 6. Multiple regression analysis with BWE. Eliminations (E1, E2 and E3) observed with red color to indicate removal. The final p-value are marked in green color and bold text presenting significance.

	P-values E1	P-values E2	P-values E3	P-value End
pH	0.091	0.091	0.007	**0.019**
Days after delivery	0.088	0.045	0.115	
NH3 (µg/L)	0.284	0.191		
Cond (µS/cm)	0.613			

After performing the multiple regression (BWE) analysis, all basic assumptions of linear regression were verified for the significant parameter. Linearity, independence, normality, and equality of variance (Kim, 2019). To verify linear relation a fitted line from the plot of mortality and pH are observed side by side with the corresponding residuals plot (Figure 4). This is to check that the scatter does not have a trend, which is presented as random scatter points (Kim, 2019).

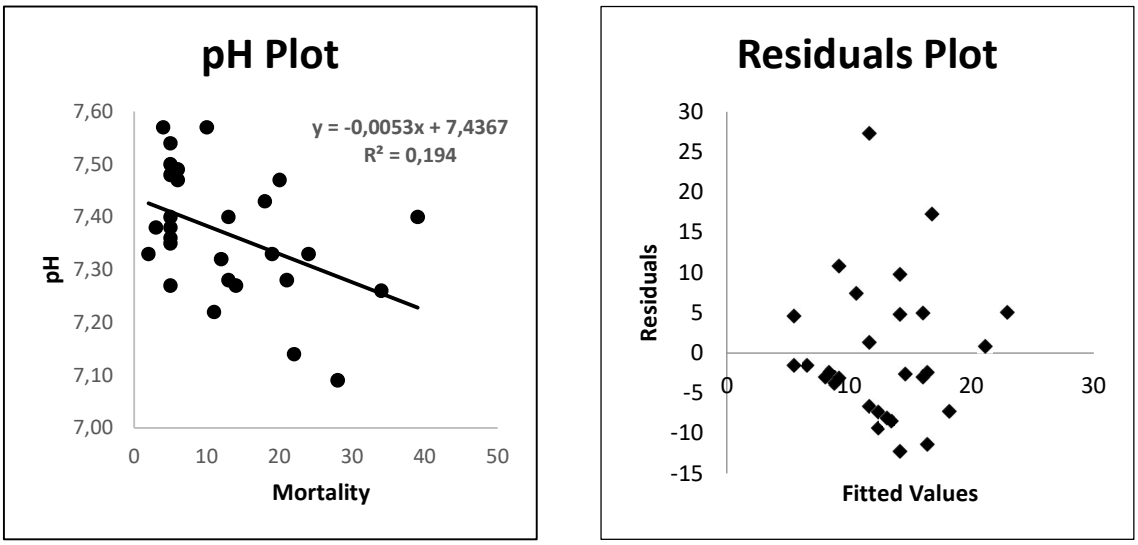


Figure 4. A pH-plot and a residuals-plot are presented juxtaposed and presents the pH and mortality relation. The pH-plot are located to the left and the residuals plot to the right. Included in the pH-plot are the equation for fitted line and R-squared value.

By observing the residuals plot (Figure 4) it is visually determined that the residuals do not possess an apparent trend. The error is not explained by the fitted line can therefore be characterized as sufficiently random (Kim, 2019). It is correct to determine the pH and mortality relationship as linear. A visual analysis for homoscedasticity is also performed on the residuals plot to see if there are same variance of data. As the scatter plot presents a tilted square around zero the variance may be skewed, however not significantly as no clear trend are revealed in right or left direction (Kim, 2019).

To verify whether the dataset present dependence through autocorrelation the pH residuals are plotted against time. Dependence are most often observed in experiments where measurements are performed from the same origin over time (Kim, 2019). Which is why this is important to verify for the data measured in the stationary tank at Imsland over time.

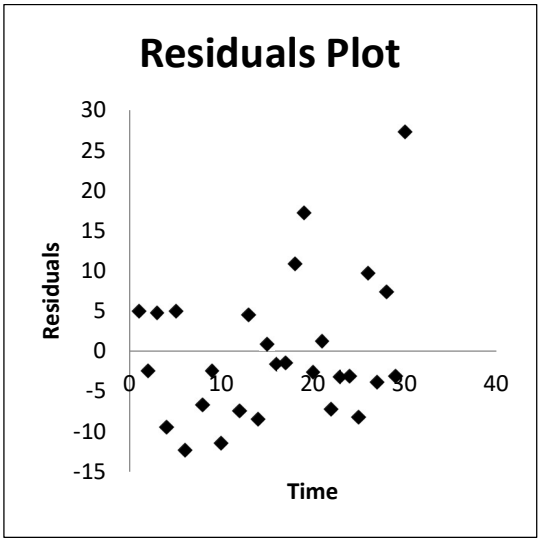


Figure 5. Residuals plotted with time as the independent variable. No apparent trend is visible. Even distribution around zero axis, with possible outlier in the upper right corner.

The error plots in longitudinal data (Figure 5) do not display autocorrelation as there are no cyclic trend between negative and positive error values (Kim, 2019). To verify the assumption of normality a quantile-quantile plot for pH and mortality is observed and evaluated (Figure 6). Theoretical normality reference of the distribution is represented by the approximate 45° linear trendline. The pH presents a normal distribution with minor variation. Mortality presents some skew in the left tail and can be explained by the sorting method and detection limit utilized at Imsland.

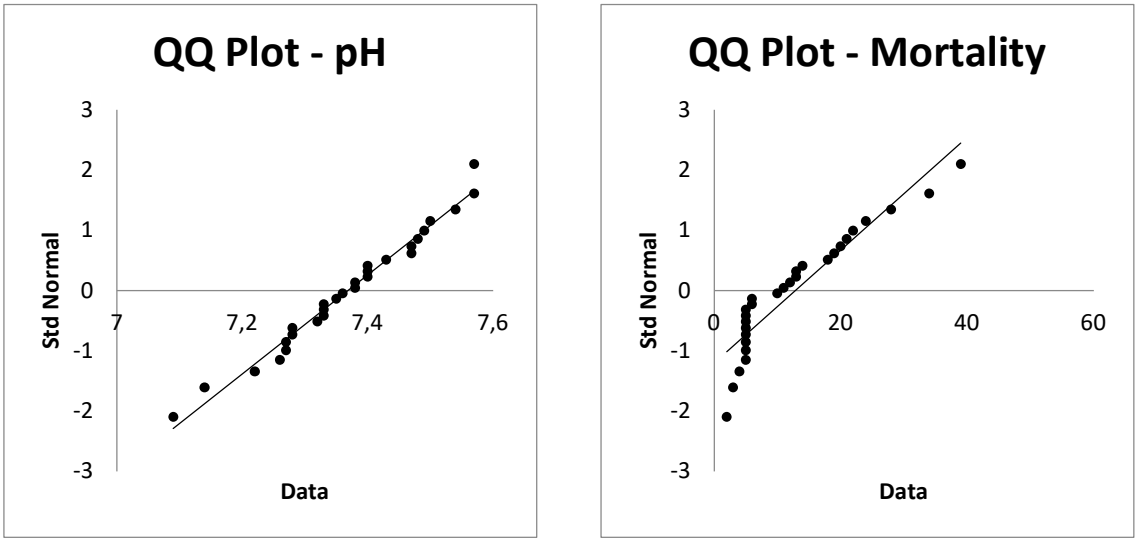


Figure 6. QQ-plots are viewed for pH to the left and mortality to the right. Standardized original data are plotted against the standard normal approximation for the corresponding datapoints. No visible trend of variation for pH, however some trend for mortality data. The graphical skew is visible at the x-axis area of data loggings for five individuals.

As the assumptions for regression analysis are sufficiently evaluated and verified, the R-squared number were used for further explanation of the pH and mortality relation. The regression analysis yield $R^2 = 0.19$ (Figure 4) for the linear model of pH and mortality with corresponding significant p-value = 0.02 (Table 6). The R-squared value is not adjusted for the number of predicators in the linear regression model. Within the significance level of 0.05 a conclusive result of statistical analysis presents pH relation to mortality. By the R-squared value, 19% of the variation that is observed can be explained by the model of pH and mortality. When checking the two arrays of pH and mortality for correlation the correlation coefficient of r = -0.44 describes a negative relation. When observing the pH plot (Figure 4) this relation is visible, and for a decreasing pH is an increasing mortality. To explain this relationship in more detail a future study on the effect of pH on all substance species encountered in seawater may be interesting and reveal mechanisms of pH effects.

3.1.2. Transportation tanks

Comparing the stationary tank water and water from the transportation tanks, some significant differences were discovered. This concerned the parameter measurement data of temperature, NH_3-levels, and dissolved oxygen (Table 7). Average water temperature for the transportation tanks were 5.2℃, which is 3.6℃ lower than the water they transferred into. After six hours of calming after transfer the temperature elevated to approximately the same temperature as before transfer. Ammonia concentrations in the water during transportation were in average 2**.**1 µg/L compared to 0.8 µg/L in the receiving tank. NH_3-levels in the stationary tank remained low when measuring after transfer and calming. Mean DO-percentage during transportation was 107%, somewhat alike the receiving tank at 101%, however drastically changed to 77% after six hours of calming.

When evaluating the pH-data, the apparent measurement values do not seem significantly changed (Table 7). It is important to have in mind the logarithmic nature of pH. Transportation water pH was 7.2 in average compared to receiving tank with pH 7.6. After transfer, a low pH was measured as well. Lower temperatures during transportation did not affect the receiving water, which makes sense as the fish thermal status are controlled by the environment. NH_3-levels were not affected long term, and after transfer and calming it was measured again as a low concentration. The DO% was however severely decreased, even though there were no drop in percentage neither at the receiving tank nor the transportation tanks. The parameter with a lingering effect was pH, as the low pH from transportation persisted after transfer. As pH and mortality are presenting a negative correlation relationship, a persisting decreased pH indicates higher mortality after transportation.

Table 7. Transportation water measurement and analysis data. *Measurements performed at nine different spatial locations where the average is presented in the table. Before transfer indicates measurements performed before the fish arrived at the tank. After transfer indicates measurements performed six hours after the fry was transferred into the tank and calmed.

	ALKe (µM)	NH3 (µg/L)	Temp (°C)	DO%	SPC	pH
Tank 4 – before transfer	2338	0.8	8.73*	100.9*	51237*	7.60*
Transportation tank 1	2101	2.4	5.44	105.0	50206	7.12
Transportation tank 2	2116	1.8	5.31	110.1	50289	7.20
Transportation tank 3	2124	2.4	5.23	109.3	50311	7.20
Transportation tank 4	2120	2.1	5.18	108.1	50293	7.20
Transportation tank 5	2115	2.1	5.22	107.6	50289	7.19
Transportation tank 6	2073	2.7	5.13	107.4	50301	7.20
Transportation tank 7	2091	1.9	5.13	105.7	50345	7.22
Transportation tank 8	2186	2.1	5.03	104.5	50434	7.23
Transportation tank 9	2184	1.2	5.00	102.5	50347	7.21
Transportation tank 10	2247	1.3	5.01	110.3	50352	7.22
Transportation tank 11	2196	2.6	5.25	106.9	50247	7.19
Transportation tank 12	2238	2.2	4.94	103.8	50400	7.14
Tank 4 – after transfer	2340	0.6	8.83*	76.8*	51282*	7.23*

3.2. Biological stress data

Measurement data and mortality numbers from Imsland rearing facilities concern stationary tank number four. Mortality was logged every day and is represented as an average of each monitoring step (Table 8). On the 18th of March, the mortality was 141 individuals which is considered directly due to the event of transportation and transfer. The mortality numbers in the monitoring phase are substantially lower and maintained stable throughout monitoring. The biological morphology data consist of body measurement values over time. The average weight had increased with 42% from delivery to end of monitoring. Length was also observed with a minor increase of 17% during the month after delivery. BMI distribution curve for the fry before transfer present a peak at approximately 0.92 (Figure 7). In comparison, peaks for the monitoring phase are situated in a right shift direction towards higher BMI (Figure 8). The right shift describes a BMI trend that is clearly increasing during the monitoring phase, and the distribution is observed to change as well. There is a visible bell-shaped curve indicating normal distribution of fry BMI before transfer (Figure 7) and for monitoring 1 (Figure 8). This distribution changes over time in the monitoring phase. The moving average curve for BMI distribution widens and lowers with time (Figure 8). The moving average curve for monitoring 3 may describe a distribution of fry in the stationary tank with a higher variability of size and weight.

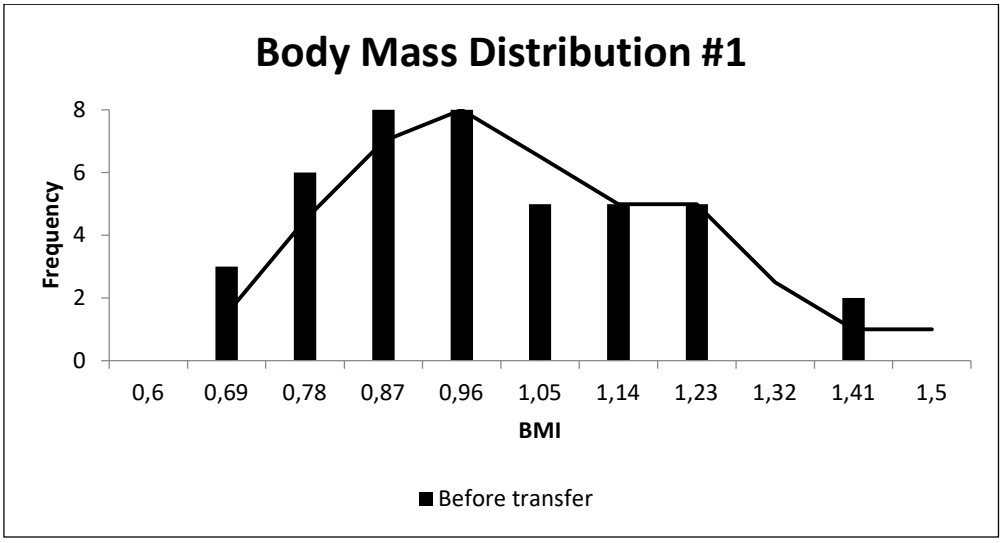


Figure 7. BMI distribution for datapoints obtained from 40 fry before transfer to the stationary tank. A trendline of moving average is also included and present a symmetric unimodal normal distribution. Slight tendency for bimodality around BMI 1.23.

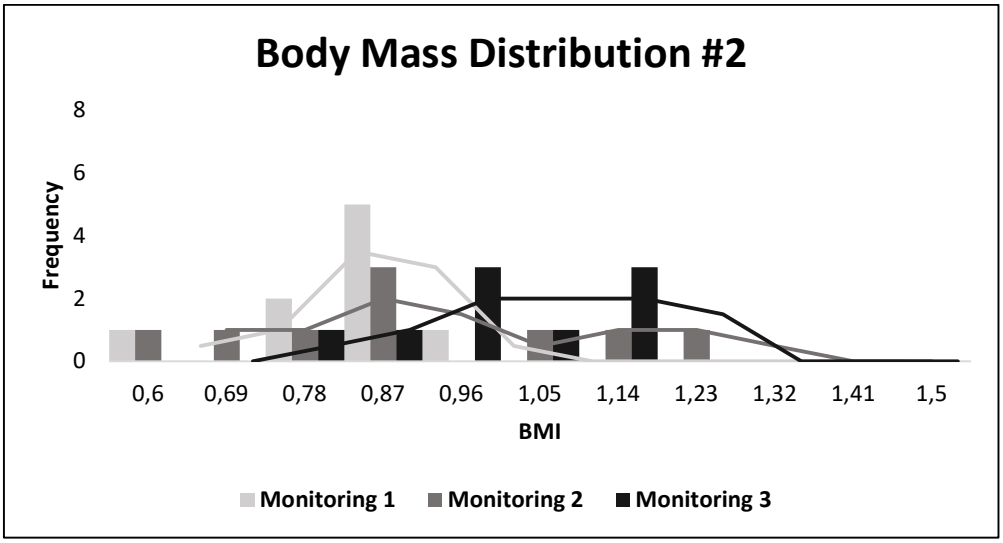


Figure 8. BMI distribution for datapoints obtained at the three different monitoring field dates. Trendlines of moving average are included, and presents unimodality for M. 1, bimodality for M. 2, and again unimodality for M. 3. A right shift and a flattening of the curve is observed along the monitoring phase.

Biological analysis of extracted blood during the monitoring phase indicates that the fry is stressed after transportation. The RBC count decreased with 63% and the blood glucose levels were steady over the monitoring phase with an average of 1.62 mM (Table 8). The most significant changes in blood glucose concentration were between transportation tank fry and from fry after calming six hours in the stationary tank. A 69% increase in blood glucose occurred after transfer and calming of the fry, which in the start of the monitoring phase showed a decrease of 31% indicating a betterment in the environmental status.

Dissolved oxygen levels in the stationary tank water presents as variable. The most significant change is observed on the batch arrival date where the DO% had decreased after the six-hour calming period (Table 8). The salinity levels were steady with an average of 33.66 psu during the monitoring phase.

Table 8. Biological and chemical measurement data from the stationary fish tank at Imsland. The column with values for "after transfer and calming" indicates measurements performed six hours after the previous measurement.

	Before transfer & calming	**After transfer & calming**	**Monitoring 1**	**Monitoring 2**	**Monitoring 3**
Field dates	**18.03.2021**	**18.03.2021**	**26.03.2021**	**10.04.2021**	**17.04.2021**
DO% (% O_2 sat)	100.97	76.83	90.64	91.24	84.98
pH	7.60	7.23	7.97	7.67	7.90
Temp (°C)	8.73	8.83	8.42	8.28	8.45
Sal (psu)			33.24	33.73	34.02
Mortality (individuals)		141	12.5	11.2	15.6
Weight (g)	6.66		5.33	7.00	9.44
Length (cm)	8.36		8.26	8.78	9.79
Glucose (mM)	1.06	2.85	1.97	1.27	1.63
RBC (cells/µL)			912 000	695 333	577 333

3.2.1. Other biological observations

A general morphological assessment of the fry used for analysis were noted. No gas bubbles were observed during the field work, which means no visible indicator for high DO%. Eye metamorphosis abnormalities were not observed in the individuals used for analysis. Some variation in general health indicators were noted on each field day, such as degree of pigmentation. It is worth noting that two individuals of the total 27 from the monitoring period had pigmentation alteration showing partial or total absence of pigments.

When considering additional morphology data logged during the monitoring phase, as fry width and depth. There is observed an increase in both parameters, however the increase in width at 11% is far smaller than for depth at 30%. Even though the Atlantic halibut is a flounder fish, it has at this stage more girth growth than bilateral growth. This indicates a body mass distribution with more central mass than peripheral mass. The average deviation is approximately twice the magnitude for depth as for width.

SWH reports the floor coverage for the stationary tank at approximately 195%, which is beyond what is recommended for fry halibut. The internal limit used by SWH at Imsland for fry tank floor coverage is fixed to a maximum of 300%, which is exceeding the recommendations from relevant literature (Pittman et al., 1994). In the early stage of rearing at Imsland there are no farming cells for the halibut to migrate between, forcing them to stay at the same level. An exploration at the Imsland facilities revealed farming cells at later stages of rearing. This would be an interesting point of interest to investigate in a future project.

4. Discussion

4.1. Factors influencing mortality

Water quality and general wellbeing of fry regarding optimal rearing tank environment and feeding are factors of influence considering mortality. In this study the stress of fry after transportation has been monitored as stress are suspected to cause late onset mortality. The fry is starved before transportation to lower metabolism and may serve as a basis for higher vulnerability to stress. Insufficient feeding and the possibility of an aggressive population may be caused by stress and therefore contribute to mortality. Transportation without water treatment give very different water quality status compared to the open system used at Imsland. There is consequently a change of water environment after transportation. Since the fry are separated from source water and transferred directly into receiving water a relative change in water quality seem to induce stress according to the elevated blood glucose concentration. Handling and movement may also contribute to stress in this stage. The stress duration may be considerably longer than the water quality indicates in the monitoring phase, as RBC count decreases over time throughout the phase indicating decreasing stress levels. By multiple regression method of water quality parameters eliminating insignificant contributors to the model a single parameter showed relation. The pH explains some of the mortality, as a decreasing pH give higher mortality.

4.2. Evaluation of results

4.2.1. Water quality data

The tank water showed less differences during the monitoring stage compared to data from the fry delivery. Not surprising that it is therefore found a maximum analysis result for undissociated ammonia of 2.7 µg/L in sampling bucket number six originating from transportation tank six. Minimum value of 0.1 µg/L is found at two analysis points within the dataset from monitoring stage two. As a reference SWH has collaborated with NIVA and Skretting in a report concerning water quality for marine fish farming in years 2011-2014, and here the maximal value of ammonia was recorded as 8 µg/L (Åtland, 2015). The report also states that these values are not significant in terms of negative health aspects, which can be explained by the high flow rate of seawater which entails a high level of water replacement. Minimum flow is 0.5 L min^{-1} kg^{-1}. The ammonia values obtained during this thesis field work is seemingly insignificant judging by the ammonia values presented in the

report. Contribution of ammonia to the water are from two main sources, fry excretion and dissolving excess fish feed. Some amounts of uneaten feed were observed in the stationary fish tank at Imsland on several occasions. This is difficult to assess since excess feed is not easy to measure. Since the water analysis present such low values of ammonia, this should not be a limiting factor in regards of fish growth and survival. Since Imsland has an open system with moderate to high water exchange rates, this should not pose as a major contributor to ammonia in the tank.

Another water quality parameter commonly known to be toxic for fish life is aluminum. The Vindafjord, where Imsland takes the production water from, is affected by influx of fresh water. The independent analysis results from the upstream origin of the freshwater waterfall located near the inlet pipes at Imsland showed 12 µg/L labile aluminum. NIVA has reported a limit of 10 µg/L labile aluminum in the pH range of 6.0-6.4 according to water regulations when talking about salmon wellbeing in limed waters. Since labile form of aluminum is highly dependent on pH the difference in pH-status between freshwater and seawater is important (NIVA, 2015). The pH is generally considered to be lower in freshwater, which can indicate that this concentration might be lower in the tanks with seawater.

This analysis was done just as a preliminary investigation, that could have led this parameter to be included in the field work. By observation, the fraction of mixing at the waterfall seawater interface was deemed not significant, also the inlet pipes for the fish tanks were located well below the interface water level. The shallowest inlet pipe is situated 13 m below water surface. All these accounts concluded in no further investigation. Upon the discovery of pH relation to mortality this parameter became more relevant, and further studies on aluminum would be interesting in the future. Temperature loggings would also complement these data, which could be useful evaluating other parameters. Possible methods of regulation of pH may be increase of water flow or introducing a new inlet pipe below the deepest one at 50 m to control water temperature which affects pH.

MPS measurement data indicates an average on 88.95 DO% with standard deviation of 4.54 in the stationary tank (see Appendix C). It has also been observed a direct pipe for oxygenation in the tank. Since there are no dampener mechanic for this flow, a lack of fry distribution is observed at this area (See Appendix F). Average DO% for measurement from location nine is 95.77 %, compared to the measurements done at location one is 84.23 %. The significant difference on 11.54 % here is explained as location nine is nearest the inlet gas

pipe. The measured values give backing to the observed conditions. An interesting parameter for future research would be water flow rates, as it was observed during the field work that fry was carried by the water flow in the stationary tank. This was especially the case for the smaller fry considering empirical notes.

4.2.2. Delivery measurements

The rapid change in 3.57℃ from transportation tanks to stationary fish tank contributes to stress for the halibut (Badr & Alfons, 2019). Both transportation- and tank water temperature are within the range of tolerance for halibut, but the sudden change contributes to stress.

The dissolved oxygen levels in the stationary tank water decreased with 24.14% after the halibut were transferred into it. This may indicate the halibut being stressed as they consume more oxygen during a stress response (Portz, Woodley, & Cech, 2006).

4.2.3. Biological parameters

Body measurement data of the fry in the monitoring phase presented a right shift and flattening of the distribution. This entails a fry population with a larger variety of length and weight values. General growth is somewhat impaired if there is a wide range in size of fry in the aquaculture fish tank. Especially for halibut this may result in aggressive behavior which is not optimal in terms of growth conditions (Pittman et al., 1994). This fact gives a data driven explanation for why sorting fry by size after delivery to rearing facilities provides better conditions for growth. When consulting SWH they confirmed that sorting of fry is common procedure usually commencing a short period after delivery at Imsland.

The average glucose levels measured in fry from transportation tanks was 1.06 mM. The measurement that was performed six hours later resulted in an average of 2.85 mM. The halibut were fed approximately three hours after they were transferred to the stationary tank and therefore the blood glucose elevation may encompass feeding also. The reason why the halibut had a relatively low glucose concentration at arrival is because they were starved three days prior to the delivery. Starving fry before transportation is normal procedure due to a decrease in the metabolism (Amend, Croy, Goven, Johnson, & McCarthy, 1982). During the three-field excursion the glucose levels kept stable with an average of 1.62 mM. Comparing glucose levels before and after they were transferred to stationary tank indicates

that the halibut were exposed to stressors during that process, or that the process itself was the stressor. The average glucose concentration after transportation was 1.23 mM higher than the calculated average of the three field excursions. Because the elevation in blood glucose concentration were substantial it is likely not only contributed by feeding effects.

The total RBC count decreased during the month of monitoring and there may be several reasons explaining this (table 8). The dissolved oxygen concentration was low on fry batch arrival day, and this may have contributed to the fish being stressed. Both low concentration of dissolved oxygen and stress will increase the amount of red blood cells. The highest value of RBC count is observed where the corresponding DO% values are lowest. Which entails a parameter relation between them (Caldwell & Hinshaw, 1994). The method of blood cell counting by manual counting chamber is prone to human error. This includes incorrect dilution, miscalculation, and procedural inaccuracy. Systematic error may also occur due to material variation in coverslip, pipettes, grids, and depth in the counting chamber. Error due to instrumentation and materials is found to be varying from 4.26-9.46 % (Biggs & Macmillan, 1948; Berkson, Magath & Hurn, 1939). A study shows that human error attributed to 55% uncertainties in sampling and pipetting, and 45% uncertainties in chamber and counting (Freund & Carol, 1964). It may be interesting in the future to use an automatic hemocytometer for RBC counting, which also clears time to use on more extensive sampling and analysis. Monitoring RBC count over time would also indicate chronic stressor existence.

Where there is substantial absence of pigmentation, it is presumed to be a disadvantage for survival (Mangor-Jensen & Holm, 2004). It has however been empirically reported by technical personnel at Imsland that the individuals with less pigmentation are not necessarily sorted out and may in fact thrive. This would be an interesting phenomenon to explore experimentally in a future project.

4.3. Limitations and sources of error

Time and funding are two big limitations for the thesis study design. In practical regard, this caused narrowing of the initial scope. If one were to continue this research or plan a similar type of investigation there are many improvement possibilities. Especially if the capacity of time and funding were expanded.

Concerning the materials section, the sampling flasks used for water samples were not optimal. Because there was a need to use conservation partly in form as freezing before ammonia analysis, it was decided to do parallel sampling. Each sampling point needed two separate samples. One for freezing and one for cooling, HDPE plastic bottles were used for freezing samples, and standard DURAN® laboratory bottles were used for samples stored in refrigerator.

If there would have been no limitation on lab resources and time, one could have performed an experiment on the different sampling flasks logging changes of standard solutions over time. Personal experience with DURAN® Protect laboratory bottles have revealed their capacity for temperature changes and pressure containing capability. This would have been more suitable to utilize for the set of samples used for alkalinity- and conductivity analysis. As alkalinity is dependent on the water pH, a sampling bottle with less permeability for gas exchange is optimal. With less exchange, and careful sampling with underwater method one would eliminate CO_2 gas contamination by air pockets and cork leakage. The Protect bottles have waterproof sealing caps, which is not present in the ordinary "blue" bottles that has been used in this thesis. This would prevent leakage in a higher degree. A minor source of error is the usage of different sampling bottles for analysis, as the same type is preferred to eliminate differences in external chemical influence on the water samples.

Concerning the MPS a calibration by the producers' standard solutions would be optimal, however these revealed quite expensive to purchase. A test of fresh solutions made at UiS were performed but deemed inaccurate as values varied a lot compared to factory reset values. It was therefore decided to keep the MPS at factory settings for all monitoring field excursions. Because of this decision validation of the data seemed appropriate. Which is why pH and conductivity were measured at the water chemistry analysis lab at Rennesøy, as an independent analysis. A comparison of these datasets provides a general overview on the validity of the MPS measurement data.

For the biological analysis, an automation of blood analysis would save a lot of time. More effective handling of the fry and less time used for analysis diminishes margins of error. This improved during the field work as mass training made for more time effective procedures. As small improvements were observed to work, they were implemented along the way of the thesis progress. Some parameters were also added after getting recommendations from a MOWI company employee, after establishing contact through the external supervisor at SWH.

If an automated analysis would be possible regarding blood, a wider variety of substances may be measured. It would be specially interesting in future studies to include cortisol as the major stress indicator, as it is the physiological stress hormone. Possible analysis methods for cortisol were evaluated. and the conclusion was that an ELISA kit would suffice for the thesis purposes. The funds were unfortunately not sufficient to explore these blood analysis options. but it would be an excellent addition to the other analysis points in a future project.

5. Conclusions

Moving fry entails unfavorable conditions to some degree. It is nevertheless a necessary process in the fish farming industry. The major negative effect of transportation is death of fry. An explanation to the observed delayed mortality occurring in the fry phase at Imsland rearing facilities are proposed. Chemical analysis of selected water parameters combined with biological indicators of stress presented a better picture of the fry environment and health status.

A major challenge when transporting fry is minimizing the amount of stress inflicted. The recorded high RBC count at the start of monitoring and the blood glucose level elevation after transportation indicated a period of stress. Mortality is the most important biological parameter. It was analyzed in relation to the chemical water parameters. A multiple regression analysis with backward elimination presented a significant negative correlation ($r = -0.44$) between pH and mortality. The statistical evaluation performed on a generated pH plot with mortality described that 19% of the variation observable is explained by the regression model of pH and mortality. Management of pH should be implemented, as suboptimal water quality contributes to mortality at Imsland. Mechanisms of pH influence on water quality should be investigated empirically in future projects to fully understand the relation to mortality.

Unsuccessful adaptation to a new environment is a consequence of differences in water quality between transportation water and receiving water. Acclimation procedures after transportation is not performed by SWH and explains the water quality differences. The most significant differences discovered concerned NH_3-concentrations and pH. Abrupt changes in these parameters cause stress and may contribute to delayed mortality.

Mimicking the water quality across environments in the transportation stage are suggested to reduce stress. Together with introducing proper pH regulation this thesis has provided new knowledge for optimizing the fry phase for Atlantic halibut. These findings will hopefully serve fish farmers together with new scientific additions to the field of marine aquaculture.

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Endnote and citation style APA 6th was used to produce the reference list.

List of Appendices

Appendix A

Overview of parameters monitored by SWH:

Table A1. Parameters recorded before. during and after transportation. Data from before is obtained by technical personnel at Rørvik station. during transportation through h installed sensors. and after by technical personnel at Imsland station.

Before delivery (general data for the batch to be delivered)	**During transportation (data tracking every 15 minutes to record live conditions)**	**After arrival (environmental readings of fish cages and seawater inlet)**
Oxygen %	Oxygen %	Oxygen %
Temperature (°C)	Temperature (°C)	Temperature (°C)
Feed type used	pH	pH
Average weight		Average weight
Biomass		Biomass
Quantity		Quantity
Mortality %		Salinity (PPT)
Growth % (month before delivery)		Redox
Starvation % (month before delivery)		CO_2 (mg/L)
Average °C (month before delivery)		TAN (mg/L)
Reference normal O_2 %		TGP (%)
Last sorting date		RES - N_2 (%)
Density fry / tank (normal: 6 kg/m^2)		N content
Formalin treatment date		NH3 content
Start starvation date		$NH4^+$ content
Spawn group number (generation code) / spawn date		
Start date live feed		
Start date dry feed		

Appendix B

Adjustment table and calibration curve for ammonia analysis:

Table A2. Percentage NH_3. in aqueous ammonia solutions for 0-30 C and pH 6-10.

	pH								
Temp (C)	**6. 0**	**6. 5**	**7. 0**	**7. 5**	**8**	**8.5**	**9. 0**	**9. 5**	**10**
0	0.00827	0.0261	0.0826	0.261	0.82	2.55	7.64	20.7	45.3
1	0.00899	0.0284	0.0898	0.284	0.891	2.77	8.25	22.1	47.3
2	0.00977	0.0309	0.0977	0.308	0.968	3	8.9	23.6	49.4
3	0.0106	0.0336	0.106	0.335	1.05	3.25	9.6	25.1	51.5
4	0.0115	0.0364	0.115	0.363	1.14	3.52	10.3	26.7	53.5
5	0.0125	0.0395	0.125	0.394	1.23	3.8	11.1	28.3	55.6
6	0.0136	0.0429	0.135	. 427	1.34	4.11	11.9	30	57.6
7	0.0147	0.0464	0.147	. 462	1.45	4.44	12.8	31.7	59.5
8	0.0159	0.0503	0.159	. 501	1.57	4.79	13.7	33.5	61.4
9	0.0172	0.0544	0.172	. 542	1.69	5.16	14.7	35.3	63.3
10	0.0186	0.0589	0.186	. 586	1.83	5.56	15.7	37.1	65.1
11	0.0201	0.0637	0.201	. 633	1.97	5.99	16.8	38.9	66.8
12	0.0218	0.0688	0.217	. 684	2.13	6.44	17.9	40.8	68.5
13	0.0235	0.0743	0.235	. 738	2.3	6.92	19	42.6	70.2
14	0.0254	0.0802	0.253	. 796	2.48	7.43	20.2	44.5	71.7
15	0.0274	0.0865	0.273	. 859	2.67	7.97	21.5	46.4	73.3
16	. 0295	. 0933	. 294	. 925	2. 87	8.54	22.8	48.3	74.7
17	. 0318	. 101	. 317	. 996	3. 08	9.14	24.1	50.2	76.1
18	. 0343	. 108	. 342	1.07	3. 31	9.78	25.5	52	77.4
19	. 0369	. 117	. 368	1.15	3. 56	10.5	27	53.9	78.7
20	. 0397	. 125	. 396	1.24	3. 82	11.2	28.4	55.7	79.9
21	0.0427	0.135	0.425	1.33	4.1	11.9	29.9	57.5	81
22	0.0459	0.145	0.457	1.43	4. 39	12.7	31.5	59.2	82.1
23	0.0493	0.156	0.491	1.54	4. 70	13.5	33	60.9	83.2
24	0.053	0.167	0.527	1.65	5. 03	14.4	34.6	62.6	84.1
25	0.0569	0.18	0.566	1.77	5. 38	15.3	36.3	64.3	85.1
26	0.061	0.193	0.607	1.89	5.75	16.2	37.9	65.9	85.9
27	0.0654	0.207	0.651	2.03	6.15	17.2	39.6	67.4	86.8
28	0.0701	0.221	0.697	2.17	6.56	18.2	41.2	68.9	87.5
29	0.0752	0.237	0.747	2.32	7	19.2	42.9	70.4	88.3
30	.080J	0.254	0.799	2.48	7.46	20.3	44.6	71.8	89

Table A2.2. Calibration values obtained with standard solutions of ammonia. Two clear outliers are removed.

NH_3-concentration	Voltage output potential	Logarithmic concentration
mg/L	**mV**	**log [mg/L]**
4	-51.6	0.60206
8	-68.9	0.90309
12	-80.3	1.079181
20	-92.9	1.30103
1	-9.6	0
2	-27.8	0.30103
4	-48.1	0.60206
2	-31.4	0.30103
1	-13.3	0
2	-32.7	0.30103
4	-50.4	0.60206
1	-14.7	0
2	-32.3	0.30103
0.2	26.4	-0.69897
2	-32.7	0.30103
1	-17.0	0
0.5	0.5	-0.30103
1	-16.0	0
0.2	25.6	-0.69897
0.5	-1.1	-0.30103
1	-8.1	0
0.5	10.6	-0.30103
0.2	33.5	-0.69897
0.20	33.8	-0.69884
0.20	37.8	-0.69897
0.20	38.3	-0.69897
0.50	13.2	-0.30103
1.00	-5.5	0
1.00	-8.3	0
0.5	6.4	-0.30103
0.2	30.1	-0.69897
1	-12.4	0
0.2	29	-0.69897
0.5	4	-0.30103

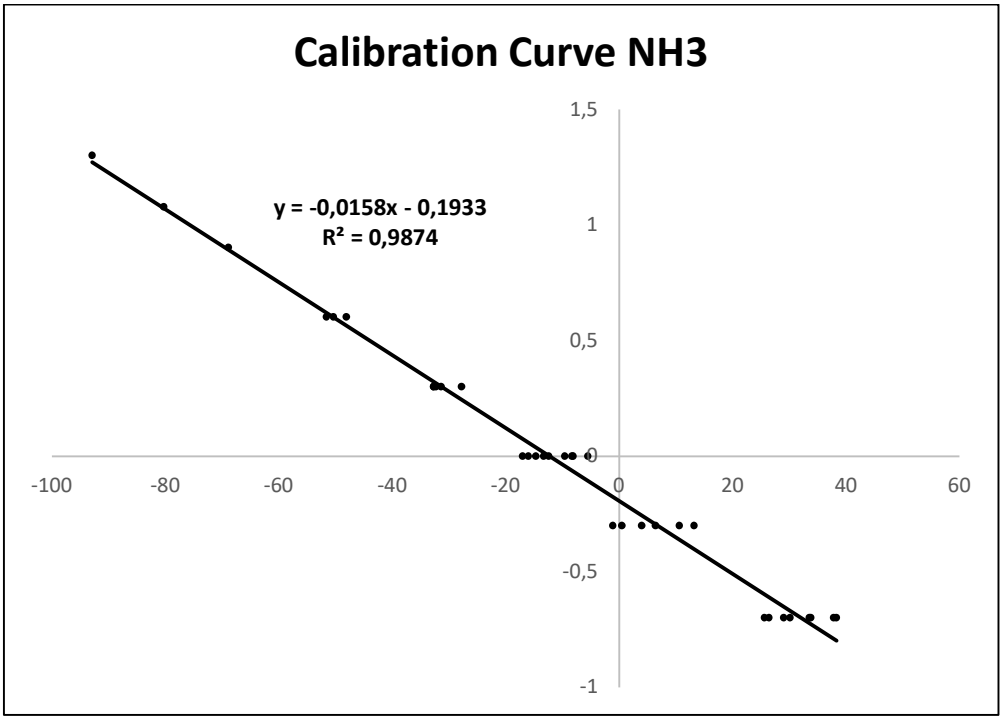

Figure A1. Total calibration curve including all calibration measurements from table A 3.2.obtained during water chemistry analysis of NH_3. Equation for fitted line together with R-squared value are included in the graph area.

Appendix C

Measurements done with MPS in tank four, and dissolved oxygen was measured different places in the tank:

Table A3. MPS measurements in tank four. Dissolved oxygen was measured.

	26.mar	**10.apr**	**17.apr**
Innermost in tank:	**OD % sat**	**OD % sat**	**OD % sat**
Location 1	83.7	86.9	82.1
Location 2	88.2	88.5	82.5
Location 3	91.5	89.8	82.4
Middle of the tank:			
Location 4	86.7	88.4	83.1
Location 5	89	90.1	85.2
Location 6	89.7	90.8	85.8
Outermost in tank:			
Location 7	91.8	93.7	86.4
Location 8	96.4	94.1	87.7
Location 9	98.8	98.9	89.6
Std	4.43		
Average location 9	95.77		
Average location 1	84.23		
Avg outermost - avg innermost	11.53		
Total average	88.95		

Appendix D

Example of how alkalinity was calculated, the experimental data pH is converted to [H+] and later used in Gran plot:

Table A4. Titration with 0.02N H_2SO_4.The first three points after pH 4.5 was used later in Gran plot.

Added **(0.02N H2SO4)**	**START:**	**0.5 ml**	**1 ml**	**1.5 ml**	**2.0 ml**	**2.5 ml**	**3.0 ml**	**3.5 ml**	**4.0 ml**	**4.5 ml**	**5.0 ml**
Weigth**:**	23.6										
pH:	7.23	6.53	6.16	5.83	5.41	4.36	3.5	3.22	3.05		
[H+] µM	0.06	0.30	0.69	1.48	3.89	43.65	316.23	602.56	891.25	######	######
Temp °C	22.5										

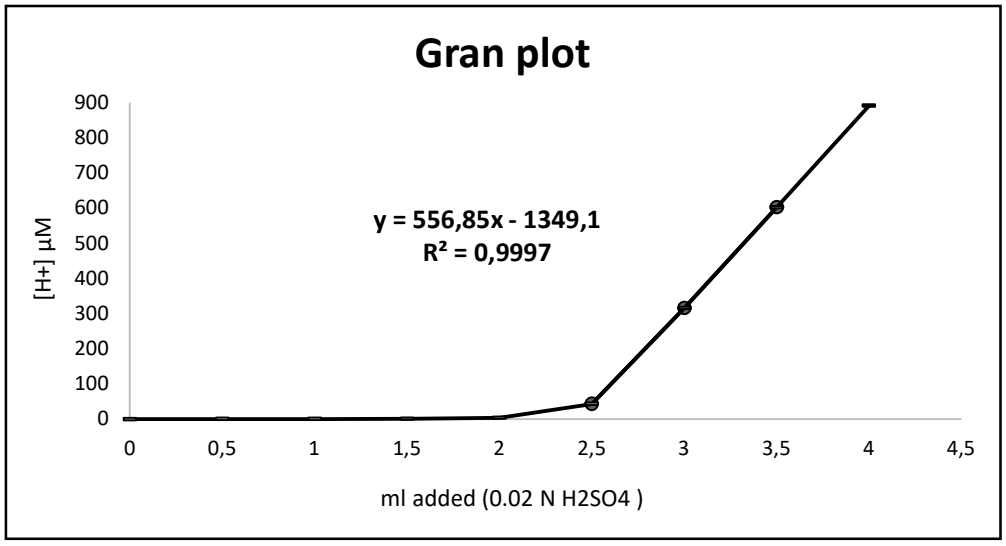

Figure A2. Showing how a Gran plot is made. Equation made from three points after equivalence.

Y=$[H+]\ at\ pH\ 4.5 = 31.62\ \mu M$

$Y = 556.85x - 1349.1$

$V_{pH\ 4.5} = 2.479\ ml$

$C_{sample} * V_{Sample} = C_{titr.} * V_{titr.}$

ALK_E=Y-32+0,646 * $\sqrt{Y - 32}$

$\boldsymbol{ALK_E} = 2116.76\ \mu M$

Appendix E

Recipe calculation for heparin solution used when extracting blood:

Table A5. Original conversion factor from international units to metric units stated on the chemical bottle are included in table. Also included are the desired solution for appropriate anti-coagulation effect of substance.and the conversion calculation used for the heparin solution preparation.

Hep-Na (s)		**Desired 10 mL solution**	
100	IU / mg	5000	IU / mL
Calculated amount			
5000	IU = 50 mg		
50	mg / mL		
= 500	mg / 10 mL		
= 0.5	g / 10 mL		

Appendix F

Collection of observational photos obtained during the field work at Imsland:

Figure A3. Surface skimmer for low density material is observed up and to the right of the image center.with accumulated mass visible. Also visible is the oxygen-rich flow from the black pipes and to the right. Less distribution of fish on the tank floor around this area of flow.

Figure A4. Four halibut fry individuals are visible. The individual at the top exhibit lack of pigmentation which is a clear contrast to the regular pigmentation of halibut fry in comparison below.