CHAPTER N0.

35

Challenges In Aquaculture with Special Emphasis on Gill Health of Salmon

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Summary: Aquaculture is an increasingly important method for sustainable fish production, with salmon farming representing a major sector within industry. However, salmon aquaculture faces a range of challenges, including limited water resources, environmental constraints, parasite infestations, disease outbreaks, and fish welfare concerns. Among these, gill abnormalities have emerged as a critical health issue affecting fish performance and survival. The gills play vital roles in osmoregulation, gas exchange, pH regulation, hormone production, and excretion of nitrogenous compounds, making their integrity essential for successful salmon farming. This chapter focuses on the key factors influencing gill health in different aquaculture production systems, particularly in recirculating aquaculture systems (RAS). Several environmental and husbandry-related parameters, including water quality, pathogen load, feed composition, fish density, water exchange rate, and salinity, are known to impact gill conditions. Variations in dissolved oxygen, temperature, pH, carbon dioxide concentration, nitrogenous compounds, and particulate matter can lead to physiological stress and gill dysfunction. Moreover, the presence of toxic substances and infectious agents further exacerbates gill health deterioration. Assessment of gill health can be performed using a range of methods, such as visual gill scoring (typically on a 1–5 scale based on severity), histopathological evaluation, and molecular analyses. Gene expression profiling, particularly of structural and inflammatory markers, provides a sensitive approach for detecting early-stage gill impairments. Despite ongoing research, the relationship between water quality parameters and gene expression profiles related to gill health in RAS remains insufficiently explored. This chapter highlights current knowledge gaps and emphasizes the need for further studies to better understand and manage gill health in modern salmon aquaculture systems.
Keywords: Aquaculture, fish welfare, osmoregulation

INTRODUCTION

Is the food on Earth enough to meet the need for an ever-increasing population? Currently, between 691 million to 783 million people are facing hunger worldwide. There is a need to produce more food to fulfill the needs of the ever-increasing population. Salmon is one of those aquatic species that contribute effectively by providing food to many people. The salmon life cycle is comprised of two phases, Fresh water phase and sea water phase. The freshwater phase is considered an important phase of salmon life. Smolt is produced in the freshwater stage. It is produced by either using the flow-through system (FTS) and recirculation aquaculture system (RAS). In the FTS, new water is supplied to the farm every time and wastewater is discarded. A large quantity of water is required in this system (Fornshell & Hinshaw, 2008). This system is associated with negative environmental effects, habitat destruction, water pollution, greenhouse gas emissions, and disease transmission (De Silva et al., 2009). In RAS, outlet water from farms is used again after proper treatment of the water. Water dependence in RAS is 93% lower than FTS (Ebeling & Timmons, 2012). So, it ensures efficient utilization of the water. RAS is comprised of particle filter, biological filter, oxygen cones, CO_2 aerator, PH maintenance, chemical heaters/coolers and water disinfection units. The Provision of necessary parameters, such as proper feed, temperature, proper density, and water quality parameters are considered vital for the development and growth of all organs of the fish. Among these organs, Gills are considered important organs for osmoregulation, ion regulation, excretion, hormone production and respiratory functions (Foyle et al., 2020). Many factors, such as water quality parameters, gill diseases, and feed load, can affect the gill performance of the fish. Among these parameters, water quality parameters are an important parameter. All water quality parameters, such as particles, CO_2, nitrogenous compounds, pathogens, O_2, CO_2 and temperature in the water can affect and alter the performance of the gills. However, high particle levels, pathogens in the water, and nitrogenous compounds in the water are considered main parameters that have an impact on the gill health. Smolt production systems, such as the choice of use of FTS and RAS, can also impact the performance of the gills.

AQUACULTURE AND ITS CHALLENGES

Aquaculture

The people around 673 million are facing hunger worldwide. The reports also find that 41-42 percent of the population, more than 3.1 billion, cannot afford a healthy diet. The healthy food will increase in the future. To tackle this issue, demand for sustainable food is increasing with each passing day. According to the Food and Agriculture Organization of the United Nations, Aquatic food demand is also increasing at a rapid pace with each passing day. Aquatic food demand has increased 3.1% per year from 1990 to 2018

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(Ahmed & Turchini, 2021). It not only provides a high-quality protein source but also provides important trace minerals that are necessary for the health of the fish. Traditional fisheries and aquaculture are the main sectors that produce aquatic food. It's difficult to enhance traditional fisheries beyond a certain level. It has become stagnant. According to the Food and Agriculture Organization of the United Nations, the production of aquatic species is expected to rise to 202 million tons in 2030. This increase in production will be mainly due to aquaculture. This aquaculture production can be increased further by using advanced technology. Aquaculture is famous for providing high-quality food for humankind, increasing employment, and promoting social and economic development. Blue transformation of aquaculture to promote sustainable aquaculture is proposed by food and agricultural organizations. Salmon is also an Important specie for aquaculture.

Salmon Farming

It is an anadromous species consisting of two life cycle stages, i.e., freshwater stage and seawater stage. The juvenile stage is considered an important stage of the salmon life cycle. Eggs are produced in hatcheries from where they are shifted to the smolt production farm. The production of smolt occurs in freshwater on land, from where it is shifted to sea cages for the production of mature fish. More technical instruments are used for the production of smolt during the freshwater stage by using RAS. The smolt can be produced by using the FTS or RAS. In 2009, 90% of production was done by using the FTS (Bergheim et al., 2009). But RAS is becoming more common with the passage of time. The life cycle of smolt comprises four stages: Hatching, Start feeding, Fry, Par, and Smoltification. In the seawater phases, mature salmon are usually produced by using sea cages. Despite advancements in technology for production of both phases of salmon, Norwegian aquaculture is facing certain challenges.

Challenges in Salmon Farming

Salmon farming is facing certain challenges. Reduced availability of water, lack of availability of suitable production locations, escapes from salmon, parasite infestations, genetic interactions, environmental regulations, and increased outbreak of aquatic disease in intensive fish production systems are the main challenges of the growing stage of salmon in modern-day aquaculture. Firstly, farmed salmon are produced in an open net pen system that can spread sea lice and diseases to the free migrating wild salmon. Sea lice are a common problem in Salmon farming. The cost of sea lice is in the range of 2.27% to 13.10% of the total yearly revenue of a farm (Abolofia et al., 2017). Various methods can be used to combat sea lice. Mechanical delousing, thermal delousing and biological methods are the most used methods for delousing. The escape from salmon farming was also a major issue. Damage to net pens by storms, seals and otters was the main cause of this problem. It was a big problem in the 1990s. It has improved now due to investment in sea ranches. Nevertheless, salmon escapees have still been noticed in Norway. It was observed by the survey that actual escapes were 2 to 4 times higher than the reported number during the period from 2005 to 2011. Smolt farming is also facing certain challenges. Pathogen outbreaks, high particle load, lack of availability of fresh water and health-related challenges are the main challenges of the freshwater phase of smolt. Health and welfare-related challenges are the most important challenge among all these challenges.

Health and Welfare Challenges

Welfare Health and welfare challenges of smolts are common in highly intensive environments. Due to the sensitivity of the fish during early age, it is mandatory to provide good quality feed and an environment. It is also of paramount importance to provide good quality water to the smolt for better health and welfare of the smolt. Water can be provided to the smolt farms either by the FTS or RAS. Some producers are using the FTS while others are using the RAS. Some are using a mixture of FTS and RAS in the whole production cycle of a smolt.

Flow through system: In the fts, there is a continuous flow of water in and out of the farm. Every time new water is supplied to the farm. A large quantity of water is required in this system. In the fts, there are chances of the entrance of microorganisms and parasites into the farms with every new entering water. This system's effect depends on the quality of water and the water exchange rate. The water is usually taken from springs, streams, and groundwater (Ahmed & Turchini, 2021). This system is associated with negative environmental effects, water pollution, parasites, and disease transmission (Ahmed et al., 2019). Exotic species invasion, along with new entering water, also has negative effects on biodiversity and the ecosystem. To compensate for this, there is a need to replace it with a system that is more sustainable and environmentally friendly.

Recirculating aquaculture system: RAS is the best replacement for FTS. It ensures better utilization of water and minimum environmental effects. RAS has overall better control over the system. The stocking density range for various species varies from 70kg/m^3 to 120kg/$m^{3,}$ with fcr of 0.8 to 1, which is quite higher than the density in FTS (Ahmed & Turchini, 2021). RAS farming is also called super-intensive farming due to the high density of smolts in the tanks. RAS ensures better utilization of water. So, only a small quantity of water is required in ras. Water dependence in ras is 93% lower than fts and 90% lower than conventional aquaculture (Ebeling & Timmons, 2012). RAS also has a better waste management system. Waste produced in RAS is highly concentrated and can easily be managed. This waste can be used as a fertilizer. The eutrophication possibility is 40% lower than the net pen system (Ayer & Tyedmers, 2009) and 26 to 38% lower than FTS (Ahmed & Turchini, 2021). Although it needs modifications, it is considered a better system for fish farming.

Gill as an Organ

Gills are considered an important organ for osmoregulation, hormone production, PH regulation, excretion of nitrogenous compounds, and Na+/NH4+ and CL-/HCO3- coupled gaseous exchange (Evans et al., 2005). Gills are sensitive organs with respect to their surroundings due to one cell thick epithelial layer and a large surface area.

Therefore, Gills are good candidate to detect the poor water quality parameters that may affect the gill health (Dang et al., 2020).

Anatomical Structure of Gills

A gill is comprised of gill arches, which form the side of the larynx. Each gill arch comprises two filaments that are the basic unit of the gills. On each side of the gill filament, gill lamellae are present, which are responsible for the gaseous exchange. They have a high surface area and a thin epithelium that is surrounded by many capillaries (Wilson & Laurent, 2002). Epithelium separates the intracellular space from the extracellular fluid. Water passes through gills lamellae during its flow from the buccal cavity to the opercular cavity due to the pressure difference (Wegner & Graham, 2010). Gaseous exchange in the gills occurs at the gill lamellae level (Park et al., 2014). Lamellae have pillar cells and collagen fibers that maintain the shape of the lamellae.

Physiological Function of the Gills

Blood and water flow in opposite direction that allows 80% gaseous exchange between them. Due to counter-current exchange, more oxygen enters the blood, where it binds with the hemoglobin after entering the cells that supply it to all tissues of the body. The amount of oxygen transferred via blood depends upon the number of red blood cells, the amount of hemoglobin within red blood cells and the physiological state of the fish (Perry & Wood, 1989). Oxygen is transferred to the tissue from the red blood cells due to a pressure difference. Although O_2 uptake occurs through the gills, the main task of the gills is to remove CO_2 and nitrogenous products (Pelster et al., 2020). Metabolically produced CO_2 is converted to carbonic acid, which is converted to bicarbonate and hydrogen ions later. Carbonic anhydrases catalyze the dissociation of CO_2 to their respective ions. CO_2 transfers from the tissue to the interstitial fluid and red blood cells occur, respectively, by diffusion. HCO_3 ˜present in the blood is removed at the gill level by the Cl^- and HCO3˜ exchange. HCO_3^- is changed with the chloride ions inside RBCS, and HCO3˜ is dehydrated to CO_2. So, gills are important in maintaining base balance in the fish by adjusting plasma HCO_3^- concentration (Perry & Gilmour, 2006). Gills also remove the ammonia from the blood, which is produced by the amino acid mechanism. Gills remove ammonia from the fish through passive diffusion. Gills also maintain salt concentration at a proper level. Noticing the change in the O_2, CO_2, and NH_4, and adjusting cardiorespiratory rhythm accordingly is also the responsibility of the gills. These functions are mandatory for the survival and welfare of the fish. So, it is vital to maintain proper gill health of Atlantic salmon throughout all production phases. Different factors can affect the gill health.

Factors Affecting the Gill Health

The gill health is affected by several factors. Physical water quality parameters, pathogens, parasites, algae, zooplankton, chemicals, improper light period, and management practices are prime factors that have an impact on the fish health, including the gill health of the Salmon. A lot of gill health-determining factors, such as water quality parameters, pathogens, parasites, algae, zooplankton, and light period, are dependent on the production systems that are used for the production of salmon. FTS and RAS are the most commonly used aquaculture systems. These factors in relation to production systems' effect on the gill health of salmon have been discussed in the upcoming paragraphs.

Water quality parameters in FTS and RAS

Physical water quality parameters include particles, oxygen, temperature, ammonia andnitrite levels, PH level, pathogens, and toxic substances. All these parameters have a great impact on the gill health of Atlantic salmon. Oxygen is mandatory for fish survival. In both RAS and FTS, Oxygen is required due to the high intensity of fry/smolt in the fish tanks. In the FTS, the required amount of oxygen is added by oxygen cones every time before the entrance of water in the production systems. In RAS, it is usually the last step among all water treatment processes before water enters the tank. Fish use passive diffusion to transport oxygen in both systems from the water into the gills. Any technical error in the oxygenation unit or unavailability of oxygen cones leads to low oxygen levels in the water. In low oxygen levels, fish gills must work hard to extract oxygen from the water, which causes damage to the gills of the fish. It led to reduced growth, feed efficiency, and altered swimming ability (Jones, 1971). These effects can also create stress for the fish that may cause opportunistic pathogens to attack the gills. It can lead to complex gill disease in the fish.

The temperature of the water is also an important water quality parameter. Oxygen solubility in the water also depends upon the temperature of the water. Oxygen solubility in the water decreases with the increase in temperature. Metabolism, respiration, and oxygen demand increase with the increase in temperature (Yavuzcan Yildiz et al., 2017). Less oxygen solubility with the increase of temperature leads the gills to work harder to extract oxygen from the water. It can cause gill disease in the fish due to the attack of opportunistic pathogens on the gills. To maintain temperature both in FTS and RAS, heaters and coolers are installed. In FTS, the required temperature is provided to the water every time before its entrance into the production system. In RAS, it is comparatively easy to maintain proper temperature due to the recirculation of the same water. High levels of nitrite and nitrate are dangerous for fish. In the FTS, water is used only once. Water from outlets that contain ammonia is discarded. So, ammonia level control is not a problem. But recirculation of water in RAS may lead to fish welfare problems if its level is not controlled properly. In RAS, Ammonia is converted to nitrite and nitrate in the presence of ammonia and nitrite-oxidizing bacteria.

The nitrogen cycle begins with the ingestion and metabolism of protein from the fish feed. Ammonia is produced by the metabolism of fish and uneaten feed. High ammonia interferes with physiological processes that lead to the death of brain cells. It decreases growth and causes physiological dysfunction in the body. High ammonia levels also led to the release of the cortisol hormone that causes stress

in the fish (Yavuzcan Yildiz et al., 2017). Opportunistic pathogens can attack the gills in stress and cause disease. High nitrite levels can cause damage to the genetic structure of the Gills. Genetic material, located within the cytoplasm of the gill cells control formation of the extracellular matrix. Nitrite destroys this genetic material. As a result, extra cellular matrix is not produced. So, the extracellular matrix disappears with the passage of time. Due to stress caused by the nitrogenous compounds, there is also chances of attack by various kinds of pathogens that can cause diseases in the fish, especially the gill disease due to the opportunistic nature of bacteria.

PH maintenance is necessary for the health of the fish. Fluctuation in PH affects the ion balance in the gills, which can compromise the respiratory function of the gills. Between 6.5 and 8.5 is considered an ideal PH for aquaculture. Gill ATPase activity increased in the short term in response to the change in PH and adapted accordingly. Na.k. ATPase activity increase is due to a change in the ion permeability or activity of the ion transporter in the epithelium of the gills (Chen et al., 2023). Low PH causes bending of the lamellae, swelling of the epithelium and mucus appearance on the gills. The epithelial cells are hypertrophic and proliferate, resulting in mutual fusion between the lamellae. As PH decreases, these effects become more pronounced (Wang et al., 2013). So, it is vital to maintain proper pH.

CO_2 is the main product produced by fish. It's difficult to remove CO_2 from the body in high CO_2 concentrations in the water. High amounts of CO_2 are called hypercarbia. Hypercarbia decreases the PH of blood that leads to acidosis. Due to a decrease in blood PH along with acidosis in hypercarbia, oxygen carrying capacity of the blood decreases. Gapping mouth and bright red gills are typical signs of the high CO_2 in the blood (Yavuzcan Yildiz et al., 2017). Acidic conditions due to CO_2 can also irritate the epithelium of the gills that may impair the function of the gills. This irritation on the gills may make them hypertrophic and proliferative, resulting in mutual fusion between the gill filaments. As PH decreases, these effects become more pronounced.

Particles in water also affect the quality of the water, which can cause fish welfare problems, especially gill health problems for the fish. Particles in water can originate from the intake water or from uneaten feed, feces and sloughed microbial substances(Chen et al., 1993). Particles diameter in the hatchery vary from 1um to 100um. Particles are categorized into various kinds, depending on their size and composition. Large particles, fine particles, and colloid particles are the three main categories of particles. Large particles have diameters larger than 20 um; small particles have diameters smaller than 20um and colloid particles have diameters smaller than 10 um. It is mandatory to control particles in the water, as their decomposition can affect the water quality, which affects the health of the fish, especially gill health and other water treatment processes in RAS (Chen et al., 1993). The number of heterotrophic bacteria will increase in water due to high levels of particles in the water. It leads to a decrease in oxygen level in the water, an increase in ammonia and carbon dioxide. H_2S can be produced in water by microorganism at anaerobic spaces left by areas where accumulation of particles in the water with low circulation occurs. The particles in the water can also cause physical damage and irritation to the gills (Chapman et al., 1987). It causes opportunistic pathogens to attack the gills and cause gill disease (Bullock et al., 1994). It was recommended to keep the particle level below 15 mg/L in the water.

There are many kinds of toxic substances in the water, such as metals, antibiotic residue, industrial matter, and organic matter, that are dangerous for the fish. These substances also have an impact on the gill health of fish. Mercury, copper, cadmium, and lead are the main metals that have the potential to affect the fish's health. Metals can also affect the gill health of fish. Slight concentrations of metals can have ion regulatory effect on the gills of the fish, while high concentrations of metals cause gill damage and mucus accumulation that affects the respiratory function of the gills. The effect of metals is enhanced in poorly buffered acidic water, where metals are toxic to the fish (Spry & Wiener, 1991). Metals' effect on the gills can be reduced by inorganic ligands such as hydrogen oxide and inorganic carbon, that reduced the binding of metals to the gill surface (Playle, 1998). Pesticides and herbicides can also leach into the water that can affect the gills of the fish. Industrial chemicals can also cause damage to the gills of the fish.

Various parasites, pathogens, and algae can also affect the quality of the water and the gill health of the fish. Viruses and bacteria are the main pathogens that affect the quality of the water. *Salmon gill Pox virus and Atlantic salmon paramyxovirus* are two major kinds of viruses that can also affect the gill health of Atlantic salmon (Boerlage et al., 2020). The major bacteria include 1) *Candidatus Piscichlamydia Salmonis,* 2) *Chlavochlamydia Salmonicola,* 3) *Ca. Branchiminonas Cisticola* and *Ca. Sygnamidia Salmonis*. Parasites can also cause gill disease in the fish. Amoebic gill disease in Atlantic salmon is very common (Boerlage et al., 2020). Salmon Gill Pox virus and Atlantic salmon Paramyxovirus are major viruses that are affecting the gills of the fish. Salmon Gill Pox virus can cause high mortality in the freshwater phase of the Atlantic salmon, especially in the smoltification phase (Gjessing et al., 2017) and in the fry phase of Atlantic salmon. Gill epithelial cell apoptosis is a major histological sign of this disease. It breaks the epithelium and can cause disease. It also compromises the immunity of the fish that leads to opportunistic pathogens attacking the gills of the fish. It usually presents along with other agents that are causing the gill disease. So, it forms part of complex gill disease in the fish. The chances of AGD development can also be enhanced in the presence of this virus (Gjessing et al., 2017). Atlantic salmon paramyxovirus can also attack the gills of the fish. It is usually present along with proliferative gill inflammation (PGI) disease. The long duration of PGI is mostly due to the slow in vitro replication rate of the Atlantic salmon pox virus (Kvellestad et al., 2005). It was detected along with PGI cases (Steinum et al., 2010) and epitheliolytic cases. These viruses' detection can be confirmed by using molecular tests. Numerous bacteria can also contribute to causing the gill disease. Most of these pathogens are opportunistic in nature and present in complex gill disease in the fish. Epitheliolytic and Tenacibacculosis are two major bacterial gill diseases. Epitheliocyst is a condition in which cytoplasmic membrane-bound vesicles can be seen on the

epithelial gill surface. Sometimes bacteria in these cysts can also be easily seen. There are four kinds of bacteria t1) *Candidatus Piscichlamydia Salmonis* (Draghi et al., 2004), 2*) Chlavochlamydia Salmonicola* (Karlsen et al., 2008), 3) *Ca. Branchimonas Cysticola* (Toenshoff et al., 2012) and 4*) Ca. Sygnamedia Salmonis* (Nylund et al., 2015) that are causing this epitheliocyst. It is affecting almost 50 species of fish in freshwater and salt water. Atlantic Salmon Paramyxovirus can also be seen along with this epitheliocyst. But no direct connection can be seen between these two pathogens. Tenacibaculosis has been given different names, such as salt water culmunaris disease or gliding bacterial disease of seafish. Gram-negative bacteria associated with this disease are *Tenacibuculum maritimum.* It also affects other organs. Fish infected by this disease may be anorexic, lethargic and have increasing respiration rate. Erosions and hemorrhages within the oral cavity and around the oral cavity, scale loss, ulcerative skin lesions, and tail rot may occur in the fish. A typical yellow margin might be present around these lesions. This lesion has a focal area of necrosis and erosion in the connective tissues. Mucus and inflammation on the gills can also be seen (Boerlage et al., 2020)

There are numerous parasites that are affecting the gill health of the fish and causing various diseases in the fish. *Paramoeba Perurans* and *Desmozoon Lepeophtherae* are major parasites that are affecting the health of the fish. *Paramoeba Perurans* is a parasite that causes amoebic gill disease in fish. Amoebic gill disease is very common in Norwegian Aquaculture. It causes high morbidity and high mortality in the fish (Steinum et al., 2008). It creates disturbance in gaseous exchange and ion regulation through the gills. It can also affect appetite, growth and overall survival of the fish (Hvas et al., 2017). Lethargy, appetite, altered swimming behavior, fish swimming near the surface, and respiratory problems are typical signs of amoebic gill disease. Multifocal pale lesions on the gill surface or raised mucoid spots are typical lesions during this disease. Different scientists use different systems to classify lesions on the gill surface. These lesions may be clear, faint spots, or patches. A scoring system of 0-5 based on white patches and percentage gill coverage are commonly used system. Fresh water bathing is considered a main treatment to alleviate Amoebic Gill Disease, not eliminate Amoebic Gill Disease (Parsons et al., 2001). So, Continuous freshwater bathing is necessary to eliminate this disease. The second major parasite is *Desmozoon lepeophtherae*. Most probably, gills are the first organ infected by this parasite, and then it progresses to other organs of the body. It is present both in healthy fish and diseased fish. But it is much more numerous in diseased fish than in healthy fish. Severe gill disease, poor growth and mortality have been noticed in certain D. Lepeophtherae infections but the true association with this parasite has not been established until now (Boerlage et al., 2020). Temperature is also considered one of the main factors that affect its propagation for production and spreading of spores for establishing systemic infection (Sveen et al., 2012). A temperature of around 10° c or more than 10°c is necessary for its propagation. It is considered seasonal and more prevalent in western Norway than in northern Norway due to the effect of the Temperature (Boerlage et al., 2020). It is necessary to maintain water quality parameters and control pathogens in the production system to maintain the proper health of the gills. Maintaining good health of gills in all phases of production of salmon in different production systems is necessary for better growth and welfare of the fish.

Water Exchange Effects on Gill Health

Maintaining a proper water exchange rate is essential for the health and welfare of the fish. An increase in transcription of mucin-like 2 and 5ac, inducible nitric oxide synthetase, Lysozyme, cathepsin B and L has been noticed in the water exchange lower water than normal water flow (Sveen et al., 2016). The increment in stress level for fish also has a huge impact on the gill health of the fish. This increase in transcription of genes indicates stress on the fish. Proper water exchange is necessary for sufficient water flow across the gill surface, removal of waste, and facilitation of gaseous exchange across the gills. Water exchange rate can have an influence on maintaining oxygen level, toxic substance dilution, disease prevention, and alkaline stability. Low water exchange rate led to a decline in oxygen levels in the water. Smolts must work hard to extract oxygen from the water. In low oxygen level, the gills of the fish have also been compromised due to the stress of extracting oxygen from the water. It causes damage to the gills, reduced growth, feed efficiency, and swimming ability (Jones, 1971). Poor water exchange rate can also lead to the accumulation of nitrate, nitrite, particles, and other harmful substances in the water. These particles accommodation, along with the high retention time of water cause deterioration of water quality in the water. These particles and other substances in water can either damage the gills directly or indirectly. It can promote the growth of pathogens, bacteria, viruses, and parasites in the water that may cause various bacterial, viral, and parasitic diseases in the fish. There is a need for proper water exchange to ensure the proper health of the gills. Low pH causes bending of lamellae, swelling of epithelium and mucus appearance on the gills (Wang et al., 2013). Poor water exchange can also cause fluctuation in PH and alkalinity. This fluctuation in PH and alkalinity can damage the gills' structure and impair their ability to exchange gases. To avoid these circumstances, it is mandatory to keep a proper exchange rate for the better well-being of the fish, especially the gill health of the smolt.

Feed load and Its Composition Effects on Gill Health

The feed is considered one of the major parameters that impact the gill health of the fish. Different feed parameters, such as Feed load, feed composition, and feed processing parameters, have an impact on the welfare of the fish, especially the gill health of the fish. The higher feed load in the water can also impact the gill health indirectly. High feed load led to a high amount of undigested feed and a high amount of feces in the water. This weight decomposition in the water leads to the production of high amounts of nitrogen-containing compounds, such as ammonia and nitrate, in the water, which affects the health and welfare, including gill health, of the fish (Davidson et al., 2017).

Feed composition is a second important factor that can affect the health of the fish. Improper feed composition or

improper feed balance may lack essential nutrients that are mandatory for the health of the fish, especially for the gill health of the salmon. With the passage of time, the use of fish meal and fish oil is decreasing. It is necessary to replace it with some ingredient that not only provide required nutrients but also provide health related benefits and enhances the chances of survival of the smolt. Antarctic krill meat is one of those ingredients that provides beneficial health effects as well. It can improve the growth, survival, and health of smolt (Betancor et al., 2012; Nicol & Foster, 2016). It also reduces fat accumulation in the hepatocytes (Saleh et al., 2018). Krill meal also provides amino acids, water-soluble nitrogenous compounds, astaxanthin and omega-3 fatty acids (Kaur et al., 2022). The diet with relatively high krill meal causes relatively high expression of Caderien 13 gene in fish. High expression of caderien 13 has high anti-inflammatory effects and plays a role in metabolism regulation (Kvingedal et al., 2023). The fish that are provided with krill meal has very mild gill lamellae inflammation and hyperplasia than other groups. 12% Krill meal provides more astaxanthin than soy lecithin and marine PL, a natural antioxidant with potential anti-inflammatory properties (Kvingedal et al., 2023). In addition to it, proper processing of the feed is also essential for ensuring good growth and welfare of the fish. Poorly processed feed may contain anti-nutritional factors which can irritate the gills and impair the gill's function. The positive effects of diets including arginine, micro additives, vitamin C, and E have also been noticed on the gill health of the fish. Arginine has special effects on the prevention of pathogens related to gill disease, including amoebic gill disease (Mullins et al., 2020).

Vaccination Effects on Gill Health

Salmon can be affected by different diseases at different stages of life. IPN, Sapro, PD, HSMI, Yersiniosis and sea lice infestations are the main diseases that are affecting the fish. These diseases attack fish make the immune system weak. It can also affect the gill health of the fish. It is considered vital to provide vaccination against this disease. Vaccines have positive effects on the gill health of the fish. It stimulates the immune system of the fish which prevents the gills from being attacked by the disease. The presence of vaccine induced antibodies in the mucus layer can neutralize the pathogens upon exposure of disease. Thus, prevent the gill's structure and its function. It is highly adopted in Norway. These vaccines are given by different routes. Injection, dip, bath and oral are the most used vaccines. Salmon is usually vaccinated at the parr stage, or at the end of the smoltification phase, against several different diseases. Before vaccination, it is vital to ensure that the vaccine is not injected into the gills or near the gills to avoid damage to the gills. The injection of the vaccine near the gill may lead to inflammation of the gills and impair the function.

Fish Density in Tanks

Fish density in the smolt farm is considered an important factor for the welfare of the fish. The normal range of fish density varies from 30k to 60kg/m^3. Fish density less than this range is not considered economically viable. High fish density can impact the health and welfare of the fish. High fish density in the tanks causes stress and epithelial damage to the skin and gills. It is depicted by a high level of certain genes, indicators, and hormone levels. The transcription of mucus like genes 2 is enhanced in high-density environments. The enhancement of transcription of matrix metalloproteinase 9 and claudin 10 has also been observed in high-density environments. The increment in cortisol hormones has also been noticed in the high-density tanks of the fish. There are numerous factors responsible for stress on fish, including the gill health of the smolt.

The main factors responsible for stress on the fish in the high-density environments are epithelial damage of skin and gills, low oxygen available, high ammonia levels, aggressive behavior, and close contact between the smolts. High fish density in the tanks creates competition for dissolved oxygen in the water. Oxygen consumption by a large number of the fishes lead to a decrease in oxygen level in the tank. It causes stress for the fish, especially on the gills. Gill must work hard to extract oxygen from water that leads to stress on the gills. It is vital to provide a high amount of feed for the high number of fish in the tank. It creates high chances of feed wastage. High biomass also excretes a high number of faeces. A large quantity of ammonia is produced because of a high amount of wasted feed and faeces. This high ammonia is difficult to eradicate. High nitrogenous compounds in the water lead to stress for the fish. It creates stress for the fish in crowded conditions. This high nitrogenous compound can also affect the gill health of the fish. It leads to respiratory distress and decreased oxygen uptake. Fish may also exhibit aggressive behavior in crowding, such as finning and aggression in high condense environment. This aggressive behavior causes wounds on the skin surface and gills of the smolt. These wounds are one of the main welfare issues of the fish that lead to an increase in stress manifolds. Smolts remain close to each other in a highly dense environment. Pathogens and parasites in water can easily spread from one fish to another. High levels of morbidity have been noticed in the high-density environment once this disease entered the farm. There are multiple factors responsible for it. High fish density has effects on hydrodynamics within fish tanks, especially as it increases the mixing rate of water. Horizontal transmission is also more common in high density due to the proximity of the infected with uninfected fish.

Salinity Effect on Gill Health

The change in salinity enhances the stress level for the fish. The immune system is weakened by the high stress levels in the smolt. Numerous opportunistic pathogens can attack the gills. The probability of fish getting gill disease is increased. High levels of salinity can also cause damage to the delicate tissues of the gills. Dehydration is created by high levels of salinity that lead to disturbance in structural and functional changes in gills. The performance of the gills is affected by these changes. It decreases the efficiency of gaseous exchange through the gills. The CO_2 in the blood may lead to hypercarbia. O_2 level in the blood also decreases, which can cause hypoxia. Hypercarbia and hypoxia cause stress for the fish. It also enhances stress on the gills of the fish. Furthermore, it can also compromise the ability of the gills to transport ions. So, it disrupts the osmoregulatory function of the gills. Knowing about the gill health status is vital for

ensuring the good health and welfare of fish. Different methods are used for the evaluation of gill health.

Gill Health and Its Evaluation Methods

Maintaining gill health is necessary for the welfare of the fish. Various methods can be used to predict the gill health of Atlantic salmon. Gill Necropsy method, Histological method, and molecular methods are the main methods that can be used for evaluating the health of the gills. Scoring systems based on the number of lesions on the gill surface are commonly used methods for evaluating the gill health of fish. Histological methods include the preparation of slides to detect inflammation in gills. Molecular methods include the relative expression of those genes that are related to the immune system and inflammation.

Gross Lesions and Histological Methods

Gross lesions and histological methods can tells us about the presence of gill disease. Gross lesions are used mainly for making a presumptive diagnosis of gill disease. The Gill scoring system was developed to provide information about the presence and severity of gill disease. One to five scores are given to the gills based on the severity of the disease, as explained in the table 1. This is an effective way to tells us about the status of the gill health and the extent of the gill disease.

It is a rapid method to provide information about the gill status of the fish. The Gill scoring system is being used on most farms to predict the health status of the fish. Histological analysis of gill tissue is used to detect hyperplasia, inflammation, lamellar fusion, and damage to the gills. Some parasites can also be detected by histological methods. It is also used to detect complex gill disease, amoebic gill disease and other parasitic diseases. Histological analysis includes various steps: 1) Fixation: fixed in formalin to preserve the cellular structure of the tissue. 2) After fixation, water is removed from the tissue by using alcohol to maintain the gill structure of the tissue. 3)Staining is done to observe the specific slide under the microscope. The histological and gill lesions usually appear later. To detect disease at an earlier stage, there is a need to perform molecular analysis to detect specific genes of interest.

Molecular Analysis

Molecular analysis of tissues is done to detect specific genes of interest in the gills. There are certain genes whose expressions tells us about the health status of specific organs. These genes are divided into the following categories.

Table 1. Different gill scores based on the lesions in the gills

Gill status	Gill Score	Explanation
Clear	0	No sign of infection and healthy red color
Very light	1	1 white spot, light scaring and undefined necrotic streaking
Light	2	2-3 spots/small mucus patch
Moderate	3	Established thickened mucus patches and
Advanced	4	Established lesions covering upto 50% area
Heavy	5	Extensive lesions covering most of gill

Genes related to cell stress, apoptosis and proliferation: It includes Cyp-1 alpha, NRF2 and P-38

Genes related to inflammation and proliferation: It includes Alox 5, Interleukin 1, TNF-1 alpha, interleukin-8 and interleukin-10. Genes related to mucus production include Mucin 2, Mucin 5 and Mucin 18. Heat shock protein genes include Heat shock protein 70, and Heat shock protein 90.

These are important categories of genes, but among the above-mentioned categories, the following genes are more common. These genes, along with their primers, are mentioned below. The expression level can be checked by performing qrt-PCR. To perform PCR, there is a need to collect the gill tissues. Smolts are euthanized by mixing anesthesia in the water. Second gill lamellae are removed by using the scalpel and forceps. It is placed in the RNA later to preserve the RNA structure of the tissue. It is kept overnight at 4°c before placing it at -20°c. After this, it is kept at -20°c until the extraction of RNA. After this, RNA is extracted from the pooled RNA later by using the RNA extraction kit, denaturing homogenizer and QIA shredders (Bridle et al., 2006). Spectrophotometry was used to detect the RNA and check its integrity. It is run on 1% agarose gel and stained with ethidium bromide (Bridle et al., 2006). Analysis of RNA was done using densitometry software. Then,5µm RNA was reverse transcribed into cDNA by using the reverse transcriptase. RNAase-digested was used to remove the remaining part of the RNA template from the cDNA. PCR is performed on the cDNA to create multiple copies of the gene of interest's manifolds (Bridle et al., 2006).

PCR is a special procedure that is used to create multiple copies of the DNA of the material. It is a combination of nucleic acid hybridization and nucleic acid reactions. It results in exponential production of specific DNA/RNA. PCR comprises three steps: 1) Denaturation, 2) Annealing of the primers, and 3) primer extension. In denaturation, the DNA strand is divided into two categories. After this, a primer is attached to the specific combination on the DNA strand. Multiple copies of DNA are produced in this way. The Taq polymerase enzyme is responsible for the extension of the strand. A primer is a short segment of a DNA strand that is complementary to the DNA or RNA of the strand. The Polymerase is separated from Thermus aquaticus and is responsible for the creation of prime extension of the DNA strand. To run a polymerase chain reaction, the following components are required: DNA template, PCR buffer, deoxyribonucleoside triphosphates, primers, and Taq polymerase that generate multiple copies of DNA (Acharya Tankeshwar, 2023). PCR machines increase or decrease the temperature of the machine in a programmed way that generates copies of the DNA sequence. Extracted samples containing DNA are added to the tube containing primers, free nucleotides, and Taq polymerase. This tube is run on a PCR machine that increases or decreases the temperature of the machine automatically.

In the denaturation, separation of two hydrogen-bonded complementary chains of DNA into a pair of single-stranded molecules by heating at a temperature of 94°c to 96°c. The temperature is lowered to 45-60 °c for the attachment of primer

to the single-stranded DNA strand. Temperature is increased to 72 °C for the extension of the DNA strand (A. Once the process is completed, it is repeated for the next cycle. That procedure is repeated multiple times and creates many copies of that specific sequence of the DNA. The product of PCR is detected by different kinds of probs (Tankeshwar,2023). The probe creates radioactive, calorimetric, and fluorometric signals. Probe base amplicon serves two purposes; it allows visualization of PCR products. Amplicon is the target sequence of interest and not the results of non-specific amplification. Sometimes, gel electrophoresis is enough to detect the presence of specific amplicons. Different kinds of PCR are available on the market. Real-time PCR, Reverse transcriptase PCR (RT-PCR), Multiplex PCR, Nested PCR, High-fidelity PCR and Fast PCR are the main kinds of PCR used for the analysis of gill tissues. Among all these types of PCR, qRT-PCR is used commonly used to detect the expression level of different immune, inflammatory, and stress genes. Although gene expression analysis using qRT-PCR can tell about the disease in early stages, it has not fully explored in RAS. The exploration of gene expression analysis of important genes in relation to water quality parameters will help us to detect the gill and other diseases at an early stage.

CONCLUSION

Gill health is vital for the successful production of Salmon. It is involved in gaseous exchange. Ammonia is removed from the blood due to this gaseous exchange. It also maintains proper salt concentration in the blood. Its direct connection with water makes it an excellent organ for identifying suboptimal water quality. It is also a vital organ to assess the fish's health. Gill health can be affected by a lot of parameters. Physical water quality parameters, pathogens, parasites, zooplanktons, improper light period and chemicals are the main parameters that can impact the fish health. Among these parameters, water quality parameters are the main parameters that can affect the gill health. It can impact the gill health both in FTS and RAS. Dissolved oxygen, temperature, nitrogenous compounds, PH level, CO2 level and particles in water are the main water quality parameters that can impact the gill health. It is essential to keep these parameters within normal range to keep the gills completely active. The change in the surrounding environment that can impact fish health can be easily detected by gill health. Sub-optimal gill health is an early indication that the surrounding environment is not optimal for fish health. Different methods are used to detect gill health. Gill scoring methods are widely used to assess gill health. In addition to it, histological and molecular analysis methods can also be used to detect the gill disease in an early stage. Gene expression analysis can also be used to detect sub-clinical or early stages of gill diseases. However, gene expression analysis in relation to water quality parameters in RAS is not fully understood. There is a need to conduct further studies on gene expression analysis in relation to water quality parameters. It will help to optimize fish welfare in RAS.

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