# Putative effects of QTL on the development of cardiomyopathy syndrome (CMS) in natural infected Atlantic salmon (*Salmo salar)* in marine production

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# Abstract

Cardiomyopathy Syndrome (CMS), caused by Piscine Myocarditis Virus (PMCV), is a viral disease in marine aquaculture, particularly affecting Atlantic salmon (*Salmo salar*), resulting in significant economic losses and compromised fish welfare. This study's aim was to explore the putative effects of Quantitative Trait Loci (QTL) in disease resistance and progression of CMS in naturally PMCV-infected salmon. The investigation was undertaken considering the observed variance in individual susceptibility to CMS. Through systematic observation and analysis of the effects of a particular QTL on the health and survival rates of PMCV-infected Atlantic salmon, the study unveiled the following findings. The QTL did not provide complete resistance to PMCV infection but was associated with diminished heart tissue pathology, lower viral load, and a trend with enhanced survival rates at site G after the detection of PMCV. These findings indicate that the role of this QTL may not only lie in direct disease resistance but also in mitigating the pathological impact of PMCV infection. With the continuous work on improving genetic resistance in CMS, it is a promising tool for reducing the severity of the disease. The role of QTL in Atlantic salmon's health and disease resistance appears to be more multifaceted, potentially exerting pleiotropic and polygenic influences. Indeed, these findings spur further research for validation, which may provide insights beneficial for selective breeding programs in aquaculture. Fundamentally, the results from this study highlight the importance of gaining a comprehensive understanding of genetic traits and their interplay with disease resistance, which is essential in enhancing fish health management practices.

## Abbreviations

| | |
|---|---|
| µL | Microliter |
| Atlantic Salmon | *Salmo salar* |
| BGN | Benchmark Genetics |
| *chd1* | Epithelial cadherin |
| Chr | Chromosome |
| CMS | Cardiomyopathy syndrome |
| Ct-value | Cycle threshold value |
| E | Efficacy |
| EF1A | Assay for Atlantic salmon elongation factor |
| F-primer | Forward primer |
| FDRG | Fish Disease Research Group |
| HE | Hematoxylin and eosin |
| HSMI | Heart and skeletal muscle inflammation |
| IPNV | Infectious pancreatic necrosis virus |
| ISA | Infectious salmon anemia |
| ISAV | Infectious salmon anemia virus |
| L | Liter |
| mL | Milliliter |
| N | Number of individuals |
| NE | Normalized expression |
| NTC | Non-template control |
| $O^2$ | Oxygen |
| PD | Pancreas disease |
| PMCV | Piscine myocarditis virus |
| PRV1 | Piscine orthoreovirus 1 |
| qPCR | quantitative Polymerase Chain Reaction |
| *QQ* | Positive QTL-PMCV |
| *qq* | Non-carrier QTL-PMCV |
| QTL | Quantitative trait loci |
| R-primer | Reversed primer |

| | |
|---|---|
| Rainbow trout | *Oncorhynchus mykiss* |
| Real time RT-PCR | Real time reverse transcriptase polymerase chain reaction |
| RGC | Rauma Strong, Site G |
| RGD | Rauma Weak, Site G |
| RNA | Ribonucleic acid |
| RTA | Rauma no-QTL, Site T |
| RTB | Rauma CMS, Site T |
| RTC | Rauma CMS+Robust, Site T |
| RTD | Rauma Robust, Site T |
| SAV | Salmonid Alphavirus |
| SGA | SalmoBreed – Test group, Site G |
| SGB | SalmoBreed – Control group, Site G |
| sp. | Species (unknown) within the genera |
| spp. | Species within the genera |
| Ssa12 | Chromosome 12 |
| Ssa27 | Chromosome 27 |
| STE | SalmoBreed – Test group, Site T |
| STF | SalmoBreed – Control group, Site T |
| UoB | University of Bergen |

# Table of content

# 1. Introduction

## 1.1 Norwegian aquaculture

The aquaculture industry has experienced significant growth in recent decades and is now recognized as the world's fastest-growing sector for animal-origin food production (Kibenge, 2019). Norway has emerged as a major global producer of salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) since the beginning of its aquaculture sector in the mid-1970s (Ashe et al., 2011). In 2022, Norway produced 1.54 million tons of salmon and 77,000 tons of rainbow trout, slightly lower than the previous year's figures of 1.58 million tons and 85,000 tons respectively. However, the industry achieved record-breaking revenues of NOK 105.8 billion from salmon exports and NOK 8 billion from trout exports, making it Norway's second-largest export industry and a crucial contributor to the nation's economy (SSB, 2023). The Norwegian government has implemented extensive regulations to ensure the sustainability and quality of fish produced, including stringent environmental and health rules. However, disease outbreaks have been persistent challenges in the aquaculture industry, leading to decreased fish welfare and significant financial losses. Pancreas disease (PD, Salmonid alphavirus), heart and skeletal muscle inflammation (HSMI, Piscine orthoreovirus), and cardiomyopathy syndrome (CMS, Piscine myocarditis virus) are among the top ten problems affecting Norway's salmon production in recent years (Sommerset et al., 2023). The increase in production and global trade of live aquatic animals and their products has also contributed to the prevalence of viral diseases affecting both farmed and wild fish (FAO, 2022). Addressing and resolving these disease-related challenges are critical for Norway's ability to sustainably expand its aquaculture production and industry.

## 1.2 Virus diseases in aquaculture

Similar to terrestrial animal agriculture, the concentration of a large number of animals in aquaculture can cause significant stress to the animals, leading to increased virus multiplication and clinical disease (Kibenge, 2016). However, aquaculture presents different challenges when compared to other intensive animal production methods. In aquaculture, both farmed and wild aquatic animals coexist in the same water column, and the environmental parameters cannot be as closely controlled as in captive livestock agriculture, such as poultry and swine industries (Kibenge, 2019). Viruses from wild aquatic animals, which may not be present in adequate numbers to maintain a natural transmission cycle, can quickly spread in aquaculture due to the

high density of host organisms. The sector's constant diversification and expansion, as well as farming intensification to offer high-quality animal protein for human consumption, have led to the emergence and spread of virus-borne diseases (Valero & Cuesta, 2023). Viral diseases pose a substantial threat to the sustainability and profitability of the aquaculture industry (Kitamura et al., 2007), making it crucial to understand the biological factors influencing disease outbreaks and control (Ashe et al., 2011; Robertsen et al., 2017).

For an infectious disease to occur, the causative agent must be introduced. Aquaculture, with its high population of susceptible hosts, provides favourable conditions for viruses to efficiently transmit, replicate rapidly, and shed effectively, which are correlated with their disease-causing ability (Rimstad, 2011). An infectious disease may require additional factors to be present. Variants of agents can differ in their levels of virulence, which is the capacity to cause disease. Virulence and factors such as the infectious dose and transmission routes can play a crucial role in determining whether a disease will develop or remain sporadic. Host factors, including species, age, nutritional status, stress, hormonal imbalances, pre-existing infections and disorders, genetic determinants, and other aspects, can also affect the outcome of viral infection. Besides the aforementioned factors, the aquatic environment and farming practices can exert additional influence on the prevalence and propagation of viral diseases in aquaculture. The quality of water, encompassing aspects such as temperature, salinity, levels of dissolved oxygen, and the presence of pollutants or toxins, can significantly affect the well-being of aquatic creatures and their vulnerability to viral infections (Kibenge, 2019).

In addition to understanding the factors influencing disease outbreaks, developing, and implementing effective strategies to control viral diseases in aquaculture is crucial. Common strategies to disease control include vaccination (Ma et al., 2019), biosecurity measures, the use of antiviral compounds or immune modulators, and selective breeding for disease resistance (Kjøglum et al., 2008). Vaccination has proven to be a successful strategy for some viral diseases, but it is not universally effective, as the efficacy depends on the specific virus and the immune response of the host (Burnell & Allan, 2009; Gudding et al., 1999). Biosecurity measures, such as controlling water quality, reducing stress, and preventing the introduction of pathogens through the movement of fish, equipment, or personnel, are essential for minimizing disease risk (Lotz, 1997). Antiviral compounds and immune modulators may be effective in controlling viral replication and enhancing host immunity, but their use must be carefully considered to avoid potential adverse effects on fish health, the environment, and human

consumers (Kibenge et al., 2012). Selective breeding for disease resistance can lead to improved host resilience against viral infections, but this approach requires a thorough understanding of the genetic basis of resistance and the specific pathogen involved (Doeschl-Wilson et al., 2012; Råberg et al., 2007). A comprehensive understanding of the biology of viral pathogens, their interactions with the host, and the factors influencing disease susceptibility are fundamental for developing targeted and effective disease control strategies.

## 1.3 Viruses associated with circulatory problems

Several viruses have been linked to circulatory problems in Atlantic salmon, including infectious salmon anemia virus (ISAV), piscine orthoreovirus (PRV), salmonid alphavirus (SAV), and piscine myocarditis virus (PMCV), each causing distinct diseases with varying effects on fish health and the aquaculture industry. Infectious salmon anemia (ISA) is a highly contagious viral disease caused by ISAV, an orthomyxovirus affecting Atlantic salmon. ISA is characterized by severe anemia, hemorrhaging, and circulatory disturbances, leading to high mortality rates in infected fish populations (Thorud, 1991). Piscine orthoreovirus (PRV) is a double-stranded RNA virus that infects various salmonid species, including Atlantic salmon. PRV is associated with heart and skeletal muscle inflammation (HSMI) (Markussen et al., 2013; Mikalsen et al., 2012; Palacios et al., 2010) a disease characterized by inflammation in the heart and skeletal muscles, leading to impaired swimming, reduced growth, and increased mortality in affected fish. Salmonid alphavirus (SAV) is a single-stranded RNA virus that causes pancreas disease (PD) in Atlantic salmon (Hodneland et al., 2005). PD is characterized by damage to the exocrine pancreas, heart, and skeletal muscle, resulting in reduced growth, increased mortality, and decreased product quality.

As we transition from discussing these viruses to piscine myocarditis virus (PMCV), it is important to note that each of these pathogens presents unique challenges for disease management in aquaculture. Understanding the specific biological characteristics and environmental factors influencing the prevalence and spread of these viruses is essential for developing targeted and effective strategies to prevent and control their associated diseases.

### 1.3.1 Piscine myocarditis virus

Piscine myocarditis virus (PMCV) is a double-stranded RNA virus belonging to the Totiviridae family (Wiik-Nielsen et al., 2013) and is the causative agent of cardiomyopathy syndrome (CMS), a disease affecting Atlantic salmon (*Salmo salar*) in aquaculture (Haugland et al., 2011). Primarily targeting Atlantic salmon in aquaculture, CMS is regarded as one of the most devastating diseases afflicting the Norwegian aquaculture sector, resulting in reduced welfare, elevated mortality rates, and considerable financial consequences annually (Brun et al., 2003). CMS generally affects larger, high-quality salmon late in the production cycle, causing mortality when most of the costs have already been incurred. As a result, even CMS outbreaks with low mortality rates can result in significant economic losses. CMS is among the older diseases affecting Norwegian salmon farming. Signs of the disease caused by PMCV were observed as early as the 1970s, but it wasn't formally described until the 1980s in Norway (Amin & Trasti, 1988; Ferguson et al., 1990), Scotland (Rodger & Turnbull, 2000), Ireland (Rodger et al., 2014), and Faroe Islands (Poppe & Sande, 1994) with an unknown etiology at the time. PMCV was first identified as the causative agent for CMS in 2010 (Haugland et al., 2011). In recent years, the aquaculture industry has faced a growing challenge with the increasing prevalence of PMCV. As depicted in Figure 1.1, there has been a notable rise in cases during the past decade, with 2020 and 2021 witnessing over 150 aquaculture sites testing positive PMCV resulting in CMS (Sommerset et al., 2023). It should be noted that CMS is not a notifiable disease in Norway, so the actual number of cases may be higher than what is reported.

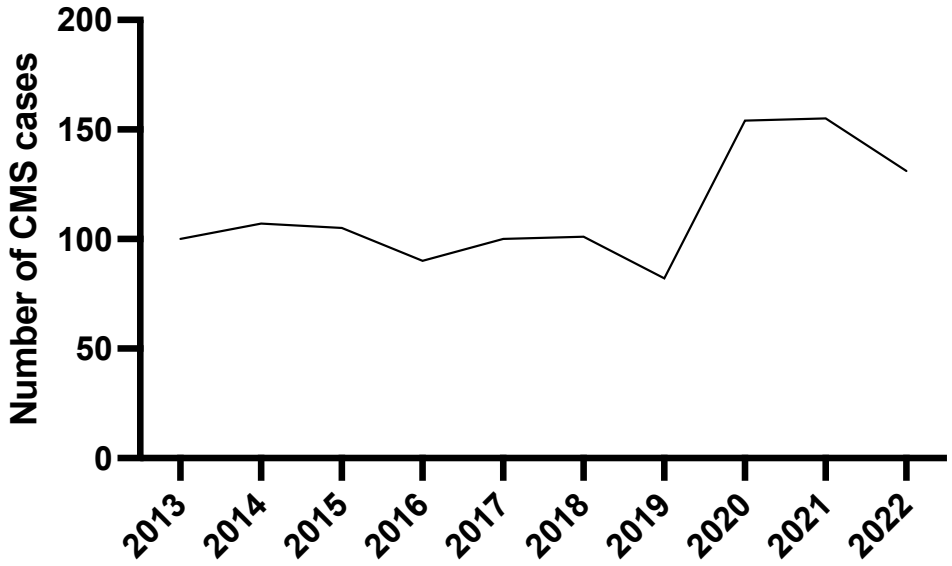


***Figure 1.1**. Number of localities with documented CMS cases in salmonid fish from 2013 to 2022. The data illustrates the fluctuation in the number of affected localities over the ten-year period, with a noticeable increase in cases during 2020-2021.*

### 1.3.2 Disease and pathology

CMS in fish may cause sudden death without any noticeable symptoms of the disease. The symptoms, if present, can be vague, such as abnormal swimming behaviour (Amin & Trasti, 1988; Ferguson et al., 1990). In diseased fish, circulatory disruptions develop gradually before any clinical symptoms become evident. Nevertheless, the cardiac alterations may lead to reduced cardiac function, and eventually rupture of the atrial wall or sinus venosus, which may result in heart failure and mortality (Bruno & Poppe, 1996; Ferguson et al., 1990). The histopathological changes observed in the heart initially manifest in the atrium and subsequently in the ventricle. These alterations are characterized by the infiltration of mononuclear cells into the subendocardium of the spongy regions, accompanied by the degeneration and necrosis of the spongy myocardial tissue (Bruno et al., 2013).

### 1.3.3 Diagnostics and detection

The process of diagnosing cardiomyopathy syndrome (CMS) is relatively straightforward and depends on the identification of clinical observations consistent with the disease. When CMS is detected, it is highly likely that the affected fish have a considerable viral load of piscine myocarditis virus (PMCV). Studies employing real-time RT-PCR analysis have elucidated a strong correlation between the amount of virus present in the heart and the severity of cardiac lesions, in addition to the prevalence of CMS-related lesions (Haugland et al., 2011; Løvoll et al., 2010; Timmerhaus et al., 2011). The diagnostic approach for CMS necessitates the evaluation of cardiac tissue through a histomorphological examination using light microscopy, which is further substantiated by molecular detection techniques such as real-time RT-PCR for the identification of PMCV genetic material (Haugland et al., 2011).

### 1.3.4 Reservoir

The reservoir for piscine myocarditis virus (PMCV) and the natural pathways of viral transmission remain incompletely understood. Although the potential role of wild Atlantic Salmon as a reservoir for PMCV has been proposed, a study conducted in 2012 suggests that this is improbable, as only a minor percentage of the tested wild salmon were found to be positive for the virus (Garseth et al., 2012). It is essential to consider that CMS is a severe disease, which can have substantial implications for the survival and behaviour of afflicted fish. Fish suffering from CMS may not only face higher mortality rates but also reduced cardiovascular capacity. This reduced fitness can limit their ability to return to the rivers where

sampling for Garseth's study took place. Moreover, even if these affected fish manage to survive and reach the rivers, the debilitating effects of CMS may still prevent them from having the strength necessary to swim upstream against the river. As such, these fish would not be included in the sampling, further skewing the observed distribution of CMS in the population. This means that the observed prevalence of PMCV in wild brood fish could potentially underestimate the actual incidence of the virus in natural settings (Garseth et al., 2012). Both field studies and experimental research have provided evidence for the horizontal transmission of viruses and the ensuing development of diseases (Fritsvold et al., 2009; Haugland et al., 2011; Jensen et al., 2013). Vertical transmission has been hypothesized but has not yet been definitively proven or identified (Bang Jensen et al., 2019; Mikalsen et al., 2020). Given the current limited understanding of PMCV reservoirs and transmission pathways, alternative approaches for mitigating the impact of this virus on the aquaculture industry are essential. One such approach could be through selective breeding developing genetic improved salmon hosts through identification of quantitative trait loci (QTL) that may increase the resistance to PMCV preventing development of CMS.

## 1.4 Use of quantitative trait loci (QTL) in breeding of farmed salmon

Selective breeding, which originated in the early 20th century following the rediscovery of Mendel's ground-breaking work, initially found its application in plant production and subsequently, within a span of 15 years, in livestock farming (Gjedrem & Robinson, 2014). Despite these advancements, aquaculture has not kept pace with terrestrial farming in terms of genetic improvement. The inception of selection experiments in aquaculture dates to the 1920s, with the Brook trout (*Salvelinus fontinalis*) selected for lowered mortality due to furunculosis (Embody & Hayford, 1925). Thereafter, the estimation of genetic and phenotypic parameters, which is fundamental for effective breeding programs, became achievable for aquaculture species.

With the continuous development within the field, selective breeding aimed at disease resistance became an indispensable strategy to elevate the health and productivity of aquaculture species (Norris, 2017). This strategy is now well-integrated, enhancing the health and productivity of aquaculture species by integrating genetic resistance to specific pathogens into breeding programs (Houston, 2017). The prediction of genetic risk factors and traits such as growth rate demands an in-depth understanding of the specific loci contributing to a

phenotype as well as the genetic architecture of the trait (Geldermann, 1975; Naish & Hard, 2008). This knowledge serves as the foundation for interpreting phenotypic differences, which are subsequently linked to the causative loci via various mapping techniques, including quantitative trait locus (QTL) mapping (Korte & Farlow, 2013).

QTL, representing genomic regions housing alleles influencing the expression of specific traits like viral infection resistance, can be identified to enable the selection of individuals exhibiting desirable traits, thereby enhancing genetic resistance to the targeted disease in the progeny (Norris, 2017). Historically, selective breeding has proven its potential in enhancing disease resistance in salmonids. For instance, Houston et al. found a single QTL explaining 83 % of the genetic variation in resistance to infectious pancreatic necrosis (IPN) in Atlantic salmon (Houston et al., 2008). In a parallel study, Moen et al. (2009) documented that after two generations of selective breeding against IPN mortality, based on challenge tests, there was an observed increase in the allele frequency associated with IPN resistance in the target population, from 0.27 to 0.44 (Moen et al., 2009). Furthermore, a QTL for PD (Pancreas disease) was found, accounting for a significant portion of the variation in this disease (Gonen et al., 2015). These findings highlight the potential for enhanced resistance targeted disease through selective breeding.

Timmerhaus et al. (2012) offered the initial evidence of potential genetic differences in CMS resistance, demonstrated through observed variations in histopathological scores of affected tissues and expression profiles of select immune-related genes. Further transcriptomic analysis revealed an upregulation in genes related to adaptive immunity, particularly in less-resistant animals. These discoveries highlighted a strong genetic underpinning for CMS resistance, suggesting the utility of selective breeding in controlling and mitigating viral outbreaks (Timmerhaus et al., 2012). Two recent studies have examined the genetic components and genomic landscape of Piscine myocarditis virus (PMCV) resistance in Atlantic salmon, using data from various populations and phenotypes. Findings indicated that additive genetic variance significantly contributes to the phenotypic variation in the host's response to this disease, with estimated heritability ranging from 0.12 to 0.51. The studies identified loci associated with increased resistance to PMCV infection, with genetic markers on chromosome 27 (Ssa27) showing a strong correlation to the resistance status, irrespective of the phenotype measured. Suggestive quantitative trait loci (QTL) were also reported on Ssa12, explaining a substantial proportion of the additive genetic variation (Boison et al., 2019; Hillestad & Moghadam, 2019).

In the case of CMS in Atlantic salmon, investigating the role of QTL in disease development will provide valuable insights for improving disease prevention and management in the aquaculture industry.

## 1.5 Aim of study

The primary aim of this study was to map the putative effects of QTL on the development of cardiomyopathy syndrome (CMS) in naturally PMCV infected Atlantic salmon (*Salmo salar*) in marine production.

*Hypothesis* $H_0$: The chosen QTL in salmon brood fish will not affect the resistance against infection with PMCV and the development of CMS.

The effect of the QTL will be monitored through observations of disease development, mortality, and the presence of PMCV in QTL salmon and salmon lacking these traits.

## 2. Material and methods

The material in this study is part of a larger project titled "Practical Effects of Selection for Increased Resistance/Tolerance for PMCV, which Causes Cardiomyopathy Syndrome in Atlantic Salmon" financed by Benchmark Genetics Norway AS. The project operates under a research permit also held by Benchmark Genetics, which is a time-limited authorization reserved for experiments concerning the breeding and genetics of salmonids in aquaculture to enhance disease resistance and fish health. The research permits connect to this study aimed to document the efficacy of selecting for increased PMCV resistance/tolerance in salmon, based on available quantitative trait loci (QTL). As it is anticipated that the response to CMS may vary depending on the salmon stock, year class within the stock, age at release (0+ or 1+), and the environment in which the salmon is farmed (particularly concerning fish handling), a series of field trials will be carried out to document the potential effects of using QTLs on the salmon's robustness against Piscine myocarditis virus (PMCV).

Over a six-year period (2019-2024), the project, “Practical Effects of Selection for Increased Resistance/Tolerance for PMCV, which Causes Cardiomyopathy Syndrome in Atlantic Salmon”, intends to conduct four field experiments (Appendix 7.7). These will evaluate four distinct year classes of SalmoBreed and several year classes of other salmon strains available in Norway (StofnFiskur and Rauma). Each field experiment will involve evaluating distinct genetic groups as both 0+ and 1+ smolts across two aquaculture regions, specifically at SalMar's sites in Møre og Romsdal, and Sinkaberg-Hansen's sites in Trøndelag, where the test groups will be examined in two commercial fish cages. Test groups produced by carriers (CMS-strong) and non-carriers (CMS-weak) of the positive QTL-PMCV (*Q*) variant will represent each salmon strain/year class. Additionally, the Rauma strain will be represented by test groups produced by carriers/non-carriers of alternative QTLs associated with enhanced survival and robustness.

Regarding the production of test groups, parents were genotyped and selected based on the QTL-PMCV variant (*QQ* or *qq*). Parents of the Rauma strain was also selected according to the positive/negative variant of the alternative QTL for increased robustness. The exact number of males and females used in the trial varied on the test site. The test groups in the study were introduced as eggs the year before the experiment started (i.e., at the conclusion of the years 2018-2021) at SalmoBreed (Lønningdal) and SalMar (Eik), respectively. The test groups was

maintained separately until tagging but synchronized to ensure a consistent time point for transportation to the test locations.

Subsequently, the material in this study was analysed at the Fish Disease Research Group (FDRG) laboratories, at the University of Bergen (UoB).

## 2.1 Study sampling

Salmon tissues were sampled from two different production sites in the sea: site T and site G (Figure 2.1). The heart tissue was fixed in formalin (Buffered 10 %) or frozen (- 40 ° C) for histology and qPCR respectively.

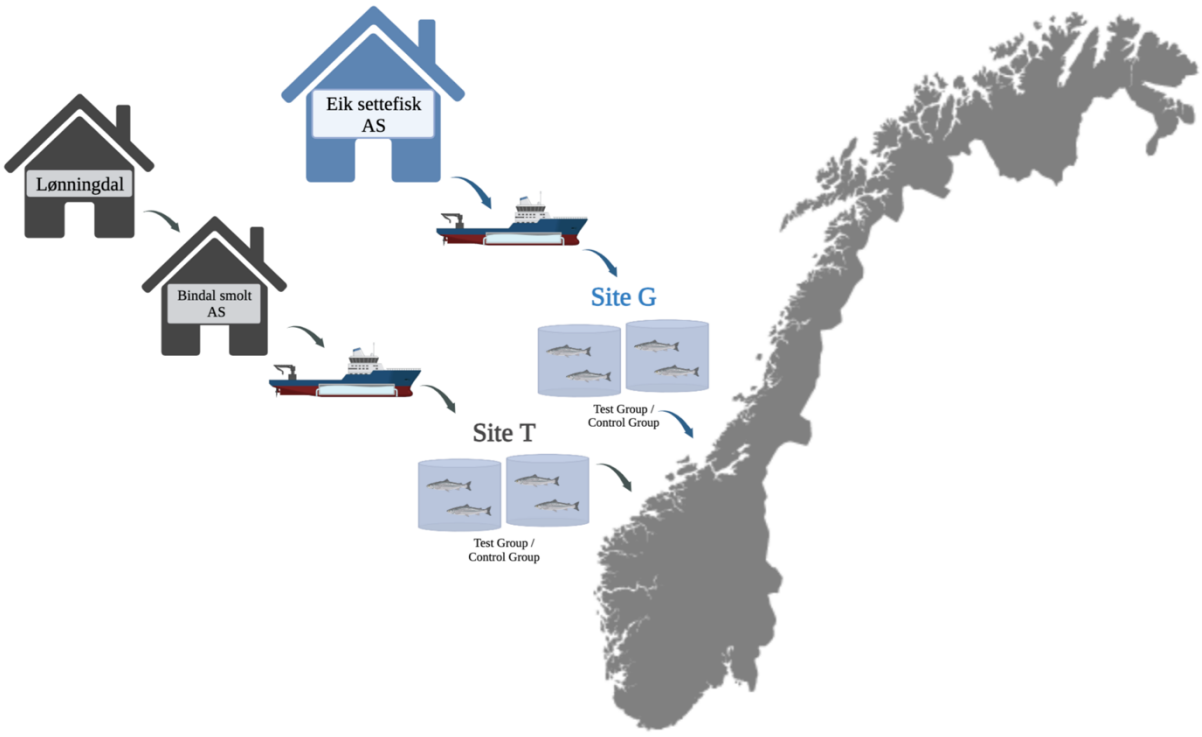


**Figure 2.1**. Map showing the two locations "Site G" located outside Molde, and "Site T" located outside Trondheim. Illustration made with www.biorender.com.

### 2.1.1 Sampling one, Site T

The project at site T was divided into two subparts. The first part focused on fish with SalmoBreed lineage, where Benchmark Genetics (BGN) selected 2 males and 2 females for Test Group, and 4 males and 5 females for Control Group (Test Group, N = 500, Control Group, N = 500). The second part involved fish with SalMar genetics, consisting of four groups, with different QTL combinations (Rauma). The eggs for the SalmoBreed part were introduced at Bindalsmolt. On October 30, 2020, they were PIT-tagged by VESO-Vikan personnel using a

Biomark needle pistol. The weight of the fish was also recorded, with the Test Group having a mean weight of 62.6 g and the Control Group 68.64 g. They were vaccinated with Clynav (Elanco) on November 10 and Alpha Ject Micro 6 on November 11, 2020. Finally, they were released at site T location on May 12, 2021, with both the Test and Control Groups exhibiting a mean weight of 179 g. The eggs with SalMar genetics were introduced at Eik and PIT-tagged on December 9, 2020, with these fish mixed with the remaining fish that constituted the experimental cage at Eik and released to sea on May 24, 2021 twelve days after the Bindalen fish. The number of fish tagged is provided in Table 2.1. On June 15, 2022 the fish were allocated across two pens. Throughout the sea production phase, the systematic of all mortalities within the experimental fish pens was carried out by scanning all dead PIT-tagged fish.

During a clinical outbreak of Pancreas Disease (PD) at site T, Atlantic Salmon (*Salmo salar*) was gathered, and the diagnosis was verified through histopathological analysis conducted by certified fish health personnel on September 27, 2022. These fish were then transported from the site to Vikenco AS slaughterhouse for additional processing and registration, during the period from October 1 to 12, 2022. Following Vikenco AS protocols, the fish were stunned, gill-cut/bled, and placed in a bleed tank. Those with PIT tags were manually chosen for further examination and sampling. A total of 6 different groups of fish had been pit-tagged at the time of release and consisted of 500 individuals each from various genetic backgrounds (Table 2.1). Among these groups, the present study only focused on SalmoBreed Test Group and SalmoBreed Control Group. PIT-tagged fish was scanned (STE, N = 242, STF, N = 260), and weight (g), length (cm), and maturation were recorded. Subsequently, samples were taken from 60 fish (30 from SalmoBreed Test Group, and 30 from SalmoBreed Control Group) for histology of gills and heart (illustrated in Figure 2.2), as well as 60 samples for real-time RT-PCR of gills, kidneys, and heart. It is important to note that no samples were collected from the other fish groups in this study, limiting the analysis to the identified and selected Test Groups and Control Groups populations.

Pictures of the fish's exterior and interior were taken to ensure comprehensive observations, along with any relevant notes.

**Table 2.1**. Fish groups of separate strains including QTL properties from site T explained. The fish groups were distributed in two separate pens.

| Strain | Group | Group Letter | Number marked | Explanation |
|---|---|---|---|---|
| SalmoBreed | Test Group | **STE** | 500 | Selected for increased resistance to CMS |
| SalmoBreed | Control Group | **STF** | 500 | Not selected for increased resistance to CMS |
| Rauma | No-QTL | **RTA** | 500 | Male and female not selected based on QTL |
| Rauma | CMS | **RTB** | 500 | Testing top marker on chr. 27 (Based on SalmoBreed) |
| Rauma | CMS+ Robust | **RTC** | 500 | Male and female have top marker on chr. 23 and 27 |
| Rauma | Robust | **RTD** | 500 | Male and female have top marker on chr. 23 |

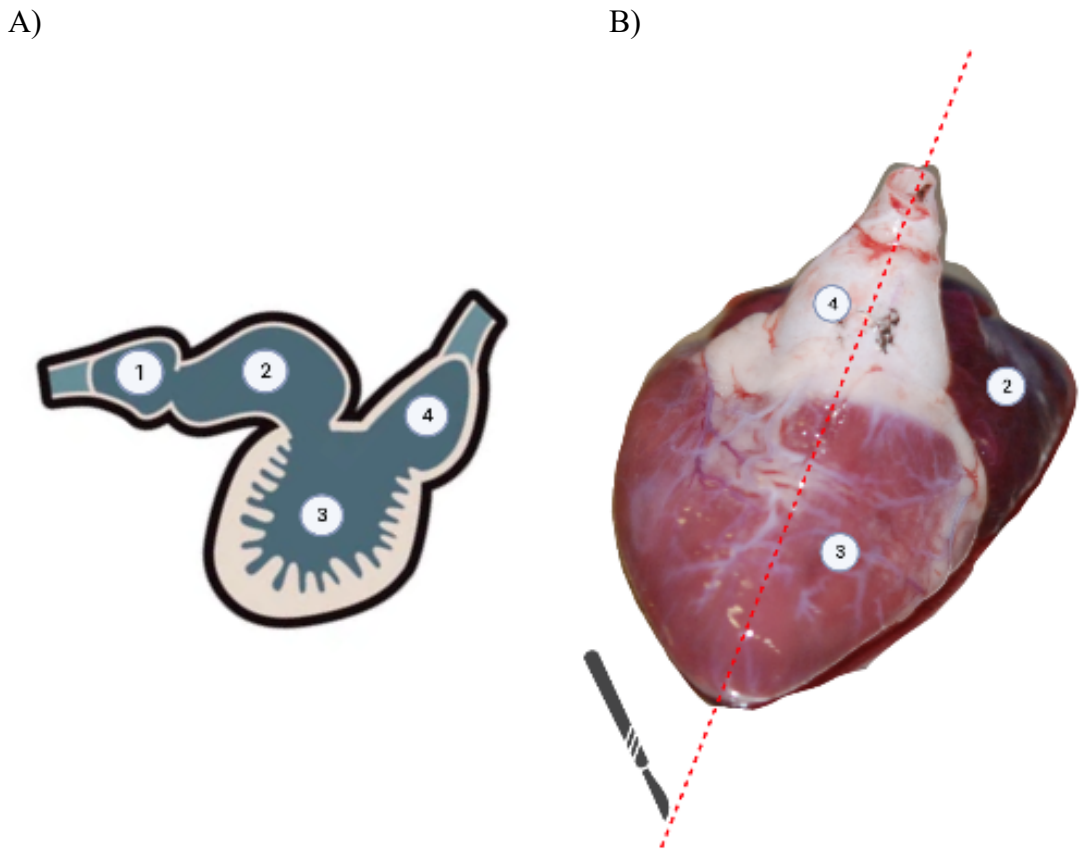


**Figure 2.2**. Illustration of sampling of the separate heart components 1; sinus venosus, 2; atrium, 3; ventricle, 4; bulbous arteriosus at site G and T. **A:** Schematic figure of separate heart components (1, 2, 3 and 4). **B:** Photo illustrating heart components 2, 3 and 4. Scalpel annotation and stapled line illustrates sectioning of the sagittal plane for sampling. Illustration made with www.biorender.com

### 2.1.2 Sampling two, Site G

The project at site G involved four groups at the Eik hatchery facility. Egg introduction took place on January 22, 2021, for Rauma strong and weak groups, as well as Benchmark test and control groups, which were selected based on genetic markers for increased resistance to CMS using one male and one female for both the test and control group. On September 6, 2021, they were vaccinated with Alpha Ject Micro 7 ILA (Pharmaq), Clynav (Elanco), and Autogen Biv.ERM (Vaxxinova). The groups were kept in separate tanks until PIT-tagging was carried out on September 13, 2021, using a Biomark needle pistol. Mean weight of the test group was 93.45 g, and 93.13 g in the control group. The number of fish tagged is provided in Table 2.2. The fish were transported by car and further carried by boat (Vikabas) to site G, cage 12, and mixed with the other fish groups September 24, 2021. At the time of release, both groups exhibited a mean weight of 104 g. In total, four groups of fish were PIT-tagged and documented as released, as illustrated in Table 2.2. Throughout the sea production phase, the systematic of all mortalities within the experimental fish pens was carried out by scanning all dead PIT-tagged fish.

During a clinical outbreak of CMS at site G, the diagnosis was confirmed by certified fish health personnel through histopathological analysis on October 31, 2022. The health situation, compounded by increased CMS mortality and sea lice levels, deemed it unsafe to proceed with sea lice treatment due to potential additional health implications. From November 22 to 24, 2022, the fish were transported from site G to InnovaMar (SalMar AS) slaughterhouse for additional processing and registration. Also at this site, the present study only focused on the SalmoBreed Test Group and SalmoBreed Control Group. Following SalMar AS protocols, the fish were stunned, gill-cut/bled, and placed in a bleed tank. At the facility, the PIT-tagged fish were automatically identified and selected using an integrated scanner on the slaughter line (SGA, N = 363, SGB, N = 402). Following this, the same sampling procedure employed at site T was carried out.

**Table 2.2**. Fish groups of separate strains including QTL properties explained from site G. The fish groups were distributed in one pen.

| Strain | Group | Group Letter | Number marked | Explanation |
|---|---|---|---|---|
| SalmoBreed | Test-group | **SGA** | 502 | Selected for increased resistance to CMS |
| SalmoBreed | Control-group | **SGB** | 504 | Not selected for increased resistance to CMS |
| Rauma | Strong | **RGC** | 485 | Selected families from Elite Robust strong chr. 23 |
| Rauma | Weak | **RGD** | 504 | Selected families from Elite lice weak from chr. 23 |

## 2.2 Histology

Formalin-fixed material (both ventricle and atrium) were sent to the Norwegian Veterinary Institute (VI) in Bergen for paraffin embedding, sectioning, and hematoxylin and eosin (HE)-staining. By studying every single histological section "blinded", both from the group with QTL and the group without the QTL, possible changes in the ventricle and atrium could be identified without knowing the genetics of the fish. The histological sections were scored based on a system of identifying infiltration of immune cells and degeneration (Timmerhaus et al., 2011). The two different tissues, ventricle and atrium, were sectioned through the endocardium, myocardium, and epicardium. In the ventricle tissue, the myocardium also was divided into the spongiosum and compactum layers. All the tissue were scored at each section, and the scoring system addressed differences in the heart tissue with a score range = 0-3 (Figure 4, atrium and Figure 5, ventricle). The different scores were given based on the percentage of affected tissue. Score 0-3; score 0 indicates no infiltration/degeneration (no changes) in the tissue, score 1 is given with less than 10-20 % of infiltration/degeneration (mild changes), score 2 indicates between 20-50 % infiltration/degeneration (moderate changes), and score 3 is given if more than 50 % of the tissue is affected (extensive changes). The heart scoring was done using a light microscope (Leica DM500 light microscope and Zeiss ® Axio Scope. A1 with Axiocam 105 colour camera, and the pictures were taken and processed in ZEN lite 2012 v.1.1.2.0.

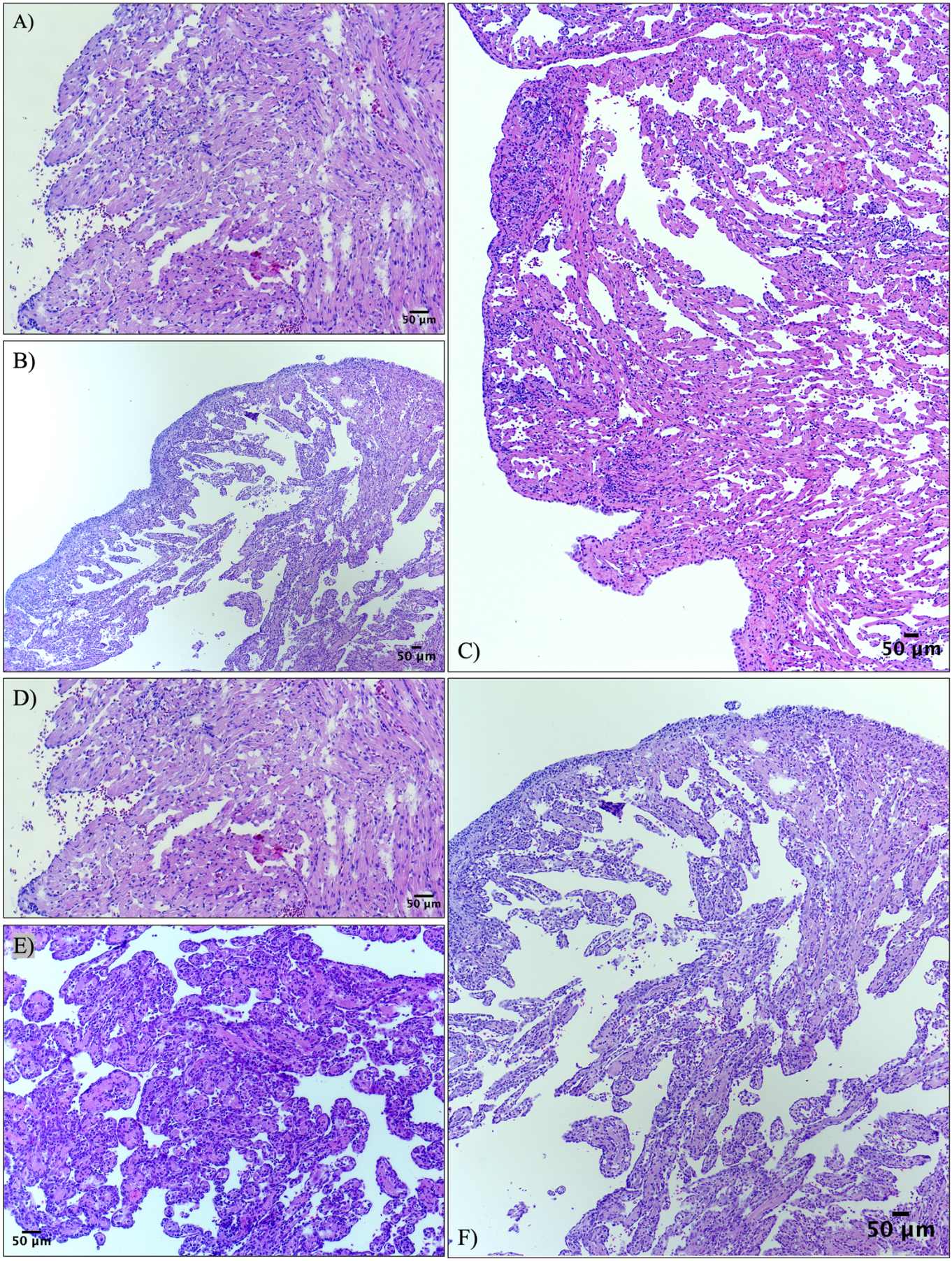


**Figure 2.3**. Atrium histopathological assessments using a defined scoring system (Scores 0-3). This figure provides six representative images of atrial tissues, reflecting varying degrees of histopathological changes in Atlantic salmon. The images were evaluated and scored in a blinded manner, based on the extent of immune cell infiltration and tissue degeneration. **A)** and **D)** Exhibit atrial tissues with mild changes (Score 1) where less than 10-20% of the tissue is affected. **B)** and **F)** Display atrial tissues demonstrating extensive changes (Score 3), with more than 50% of the tissue affected by immune cell infiltration and degeneration. **C)** Depicts an atrial tissue showing moderate changes (Score 2), with 20-50% of the tissue affected. **E)** Illustrates a severe case (Score 3) where both the Myocardium and Endocardium are heavily infiltrated by immune cells.

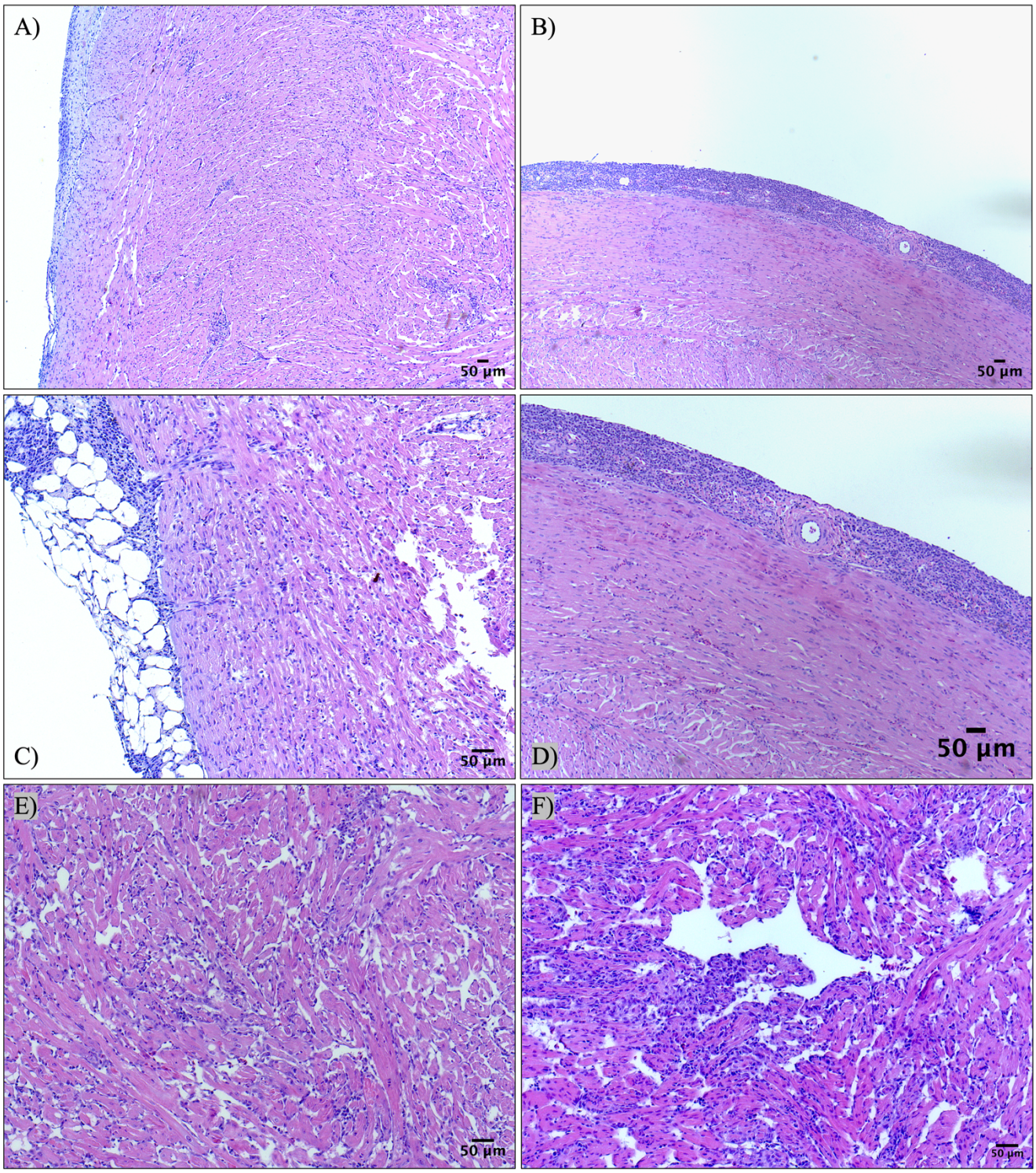


**Figure 2.4**. Ventricle histopathological assessments using a defined scoring system (Scores 0-3). This figure comprises six distinct images depicting varying levels of histopathological alterations in ventricular tissues of Atlantic salmon. Each tissue section, assessed blind to the fish's genetic status, was scored based on infiltration of immune cells and degeneration. **A)** Illustrates an Epicardium and Compact Myocardium both presenting with less than 10-20% affected tissue, indicative of a mild change (Score 1). **B)** Exhibits an Epicardium with extensive changes, where over 50% of the tissue is affected (Score 3). **C)** Epicardium with moderate changes (Score 2), and a Compact Myocardium showing mild to moderate alterations (Scores 1-2). **D)** Emphasizes a severely affected Epicardium, replicating the scenario in **B)** with Score 3. **E)** Spongious Myocardium with moderate infiltration/degeneration (Score 2). **F)** Spongious Myocardium evidencing extensive changes (Score 3), contrasted against an Endocardium with only mild alterations (Score 1).

## 2.3 Detection of pathogens

### 2.3.1 RNA extraction

RNA was extracted with TRIzol® Reagent (Life Technologies) after the manufacturer's protocol, with a few modifications. 1.0 ml of Trizol was added to the heart tissue samples before homogenizing in a TissueLyser II (Qiagen) for 5 minutes at a speed of 30 oscillations per second. To permit the dissociation of nucleoprotein complexes, it was necessary to incubate the homogenized samples for 5 minutes at room temperature. After the incubation, 200 µl chloroform was added and heavily shaken for 15-30 seconds. Further, the samples were incubated for 5 minutes at room temperature, and then centrifuged for 15 minutes at 12 000 x g and 4°C (Thermo Scientific™ Heraeus Fresco™ 21). This step will separate the mixture into a lower red phenol-chloroform phase, an interphase, and a colourless upper aqueous phase (which contains the RNA). 350 µl of the aqueous phase was pipetted into an Axygen Microtube 1.5 ml, containing 500 µl of isopropanol. After 10 minutes of incubation at room temperature, the samples were again centrifuged for 15 minutes at 12 000 x g and 4 °C. This step ensures the separation of the RNA pellet from the rest of the mixture. To guarantee the quality of the RNA extraction, the pellet was washed twice with both 1.0 ml 75 % ethanol and 1.0 ml 100 % ethanol. After adding the ethanol, the pellet was rinsed by vortex for a couple of seconds followed by centrifugation for 5 minutes at 12 000 x g and 4 ºC. The same step was repeated for 100 % ethanol. The ethanol was then removed, and the pellet dried for about 10 minutes until the alcohol had evaporated. Lastly, 50-200 µl (depending on the size of the pellet) of RNAase-free water (Sigma-Aldrich) with a temperature of about 70 °C was added to the sample. One RNA extraction control was added for every tenth tissue sample to identify possible contaminations. The samples were then stored at -25 °C until further analysis. After elution, the RNA concentration (ng/µl) and purity of the samples were measured by use of a NanoDrop ND-1000 spectrophotometer (NanoDrop™ 1000, Thermo Scientific). Both concentration and purity were measured for every sample, including the RNA extraction controllers.

### 2.3.2 One-step real-time RT-PCR

With the extracted RNA qPCR was carried out using the AgPath-ID™ One-Step RT-PCR Kit (ThermoFisher Scientific) and Applied Biosystems® QuantStudio™ Real-Time PCR System (ThermoFisher Scientific). The AgPath-ID™ One-Step RT-PCR Reagents are designed for sensitive, robust amplification of RNA targets using a single-tube TaqMan® real-time reverse transcription PCR (RT-PCR) strategy. Combining the master mix (containing specific primers and probes) and the template in a reaction plate with 96 wells (Applied Biosystems® MicroAmp® Optical 96-Well Reaction Plate), the Real-Time RT-PCR will give an amplification curve that indicates how much of the template is present in the sample.

**Table 2.3** Components of the MasterMix (MM)

| Component | General |
|---|---|
| 2X RT-PCR Buffer | 6.25 |
| Forward Primer | 1.00 |
| Reverse Primer | 1.00 |
| Probe | 0.22 |
| 25X RT-PCR Enzyme mix | 0.25 |
| RNase-free water | 1.78 |
| RNA Template | 2.0 |
| Total volume | 12.5 |

The master mix was prepared according to *Table 2.3*. 10.5 µl of the master mix and 2.0 µl of template were added to every well on the reaction plate. For each essay (in addition to the templates) one non-template control (NTC) and one negative control (RK, from the RNA extraction) were analysed.

The qPCR was performed using the Applied Biosystems® QuantStudio™ Real-Time PCR System, with the following cycle parameters: 10 minutes at 45 °C (reverse transcription), 10 minutes at 95 °C (reverse transcriptase inactivation and PCR polymerase activation), 45 cycles of 95 °C/15s (DNA dissociation) and 45s at 60 °C (annealing and elongation). QuantStudio Design™ & Analysis Software (v1.5.1) was used for showing and analysing the amplification curves. For every analysis, the threshold line was set manually to 0.1.

Analysis was performed on 120 heart tissue samples, with 60 obtained from site G and 60 from site T, to detect the presence of RNA from Piscine myocarditis virus (assay - PMCV), Piscine orthoreovirus (assay - PRV1-M2), and Salmonid alphavirus (assay - nsp1). Additionally, 60 samples from site G were tested for Infectious salmon anemia virus (ISAV), *Paranucleospora theridion* (assay – NUC) and Infectious pancreatic necrosis virus (assay – IPNV). The assay for elongation factor (assay - Elf -alpha) was used for reference genes.

**Table 2.4**.Primers and probes for assays used in Real-time RT-PCR analysis. Efficacy is given in the references.

| Assay | Primer | Sequence | Reference |
|---|---|---|---|
| Salmonid alphavirus (nsP1) | Probe | CTG GCC ACC ACT TCG A | (Hodneland & Endresen, 2006) |
| | Forward | CCG GCC CTG AAC CAG TT | |
| | Reverse | GTA GCC AAG TGG GAG AAA GCT | |
| Infectious salmon anemia virus (Segment 7) | Probe | CAC ATG ACC CCT CGT C | (Plarre et al., 2005) |
| | Forward | TGG GAT CAT GTG TTT CCT GCT A | |
| | Reverse | GAA AAT CCA TGT TCT CAG ATG CAA | |
| *Piscine orthoreovirus* 1 (PRV1-M2) | Probe | CTG GCT CAA CTC TC | (Nylund et al., 2018) |
| | Forward | CAA TCG CAA GGT CTG ATG CA | |
| | Reverse | GGG TTC TGT GCT GGA GAT GAG | |
| Piscine myocarditis virus (PMCV) | Probe | TGG TGG AGC GTT CAA | (Nylund et al., 2018) |
| | Forward | AGG GAA CAG GAG GAA GCA GAA | |
| | Reverse | CGT AAT CCG ACA TCA TTT TGT GA | |
| Infectious pancreatic necrosis virus (IPNV) | Probe | TCT TGG CCC CGT TCA TT | (Watanabe et al., 2006) |
| | Forward | ACC CCA GGG TCT CCA GTC | |
| | Reverse | GGA TGG GAG GTC GAT CTC GTA | |
| *Paranucleospora theridion* (Nuc) | Probe | TTG GCG AAG AAT GAA A | (Nylund et al., 2010) |
| | Forward | CGG ACA GGG AGC ATG GTA TAG | |
| | Revers | GGT CCA GGT TGG GTC TTG AG | |

### 2.3.3 Efficacy test of real-time RT-PCR assay

Prior efficacy testing and optimization have been performed on each assay (Table 2.4). This test involves assessing a 1:10 dilution series ($10^0$-$10^8$) in triplicate using a template with a known concentration. The test's slope and regression number (R2) are then calculated using the Ct values for each triplicate, which are subsequently shown in a standard curve. Additionally, the formula (1) is used to obtain the efficacy value (E):

(1) $E = 10^{(-1)/slope}$ (Pfaffl, 2004)

## 2.4 The density of pathogens

The degree of an individual pathogen infection in each fish is indicated by its RNA density. In this study, density is utilized to show how much of a particular pathogen's RNA is present in the sample (volume) that is being analysed. Reversed Ct-values and normalized expression (NE) are two ways to visualize the density.

### 2.4.1 Normalization of expression values

Real-time RT-PCR analysis data has been normalized against the EL1A reference gene. This is done to make up for any discrepancies that may have existed in the tissue sampling's quantity. Formula (2) was used to calculate the normalized expression values (NE):

(2) $NE_{Heart\ tissue} = \frac{(E_{ref})^{Ct\ referance\ gene}}{(E_{target})^{Ct\ target\ gene}}$

To further highlight the amount of pathogen RNA at each sampling, the normalized expression values were transformed into NE-fold and the data was Log2 transformed (Andersen et al., 2010). To do this, the NE-values were divided by the lowest NE-value, according to the following formula (3):

(3) $NE_{fold} = \frac{NE}{NE_{min}}$

### 2.4.2 Reversed Ct-values

An improved way to see the Ct-values from the analysis is using reversed Ct-values. This method's high values (low Ct-values) indicate high density, whereas low values (high Ct-values) indicate low density. The following formula (4) is used to determine reversed Ct-values:

(4) $Density = 40 - Ct\ value$

## 2.5 Prevalence

Prevalence is the proportion of a population, in percentage, that has a specified trait in a given time period. The percentage of individuals infected with a certain pathogen will be the specific trait in this study. The presence of the microparasite RNA in the population under study will be indicated. A study population of 30 fish analysed will detect a prevalence of 10%, with a 95% confidence interval. Using formula (5), the prevalence is determined:

(5) $Prevalence = \frac{Number\ of\ positive\ samples}{Total\ number\ of\ samples}\ x\ 100$

## 2.6 Statistics

The changes in pathogen density across different groups were evaluated using NE-fold values derived from positive individuals as the basis for statistical analysis. Given the non-normal distribution of these NE-fold values, the nonparametric Mann-Whitney test was the statistical test of choice for comparing the median ranks of the two groups. For the examination of histological scores within the different groups, the Mann-Whitney test was also selected due to the non-normal distribution of the scores among the various groups and heart layers. To compare the histological scores with reversed Ct-values, the Spearman correlation test was chosen for analysis, considering the non-normal distribution of these values. The same method was used for the weight, length, and condition factor statistics.

The statistical significance for all tests was set at a $p$-value less than 0.05. Different levels of significance are represented in the results as follows: * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$, and **** for $p \leq 0.0001$. The appendix contains the corresponding adjusted P-values.

# 3. Results

## 3.1 Study sampling

### 3.1.1 Recapture rate, clinical signs, and diagnosis of salmon at site T

Throughout the production cycle in the experimental cages, the total fish population at site T experienced a consistent mortality rate, culminating at 6.9 % from the point of release to slaughter. The predominant cause of mortality, quantified by the number of fish, was attributed to complications arising from sea lice treatments. From the initial population of 500 individuals in each of the two experimental groups, a total of 32 fish were registered as dead in both Test and Control Groups under production in the sea phase as illustrated in Figure 3.1 and Table 3.1, with a clear mortality top in relation to the release of the two fish groups on May 12, 2021. Due to issues with registration during the production, caused by malfunctioning PIT readers, not all PIT-tagged morts were recorded. Pancreas disease (caused by Salmonid Alphavirus, SAV) was detected and diagnosed on September 27, 2022. Viral detection and histological findings consistent with the disease were observed, but the site showed no signs of lethargy or poor appetite. Prior to slaughter, 19 control samples were also collected to assess the presence of Piscine myocarditis virus (PMCV), with no positive results. When harvesting 242 PIT-tagged fish were recorded in the Test Group and 260 in the Control Group.

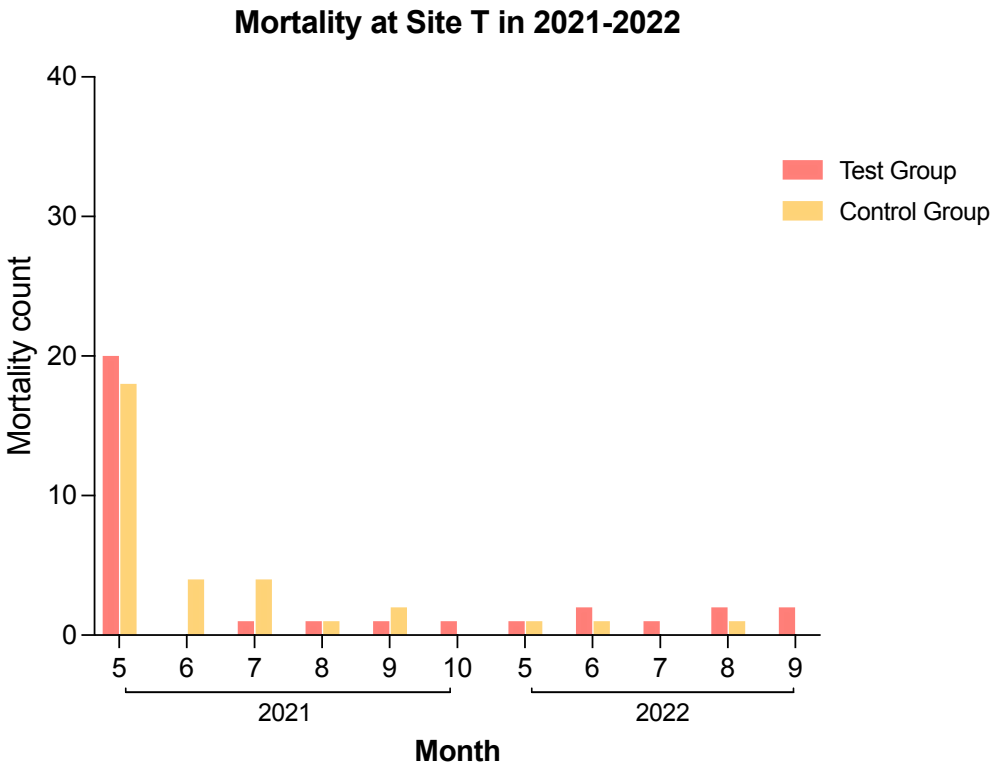


**Figure 3.1**. The registered mortality rate at site T during the sea phase production from May, 2021 to October, 2022. The red line represents the mortality rates for the test group, and the yellow line represents the mortality rates for the control group.

**Table 3.1**. Distribution of Atlantic Salmon in Test Group (STE) and Control Group (STF) at Site T. The table shows the numbers for each group at different points in the study: PIT-tagged fish, fish released to the sea, fish that died and were recorded at the sea production phase, fish recorded at the harvest line, and fish with unknown status.

| Group | PIT-tagged | Fish released | Dead at sea | Harvesting | Unknown |
|---|---|---|---|---|---|
| Test Group, STE | 500 | 500 | 32 | 242 | 226 |
| Control Group, STF | 500 | 500 | 32 | 260 | 208 |

Among the 60 fish examined and sampled at site T, 30 from Test Group (STE) and 30 from Control Group (STF), 28 demonstrated clear clinical signs of circulatory problems, specifically bloody ascites and blood in the pericardial cavity, or a combination of both. A detailed group-wise distribution revealed that bloody ascites was observed in 2 fish from each of the STE and STF groups. Blood in the pericardial cavity was noted in 6 fish from group STE and 13 from group STF. A combination of bloody ascites and blood in the pericardial cavity was seen in 4 fish from group STE and 1 fish from group STF (Figure 3.2).

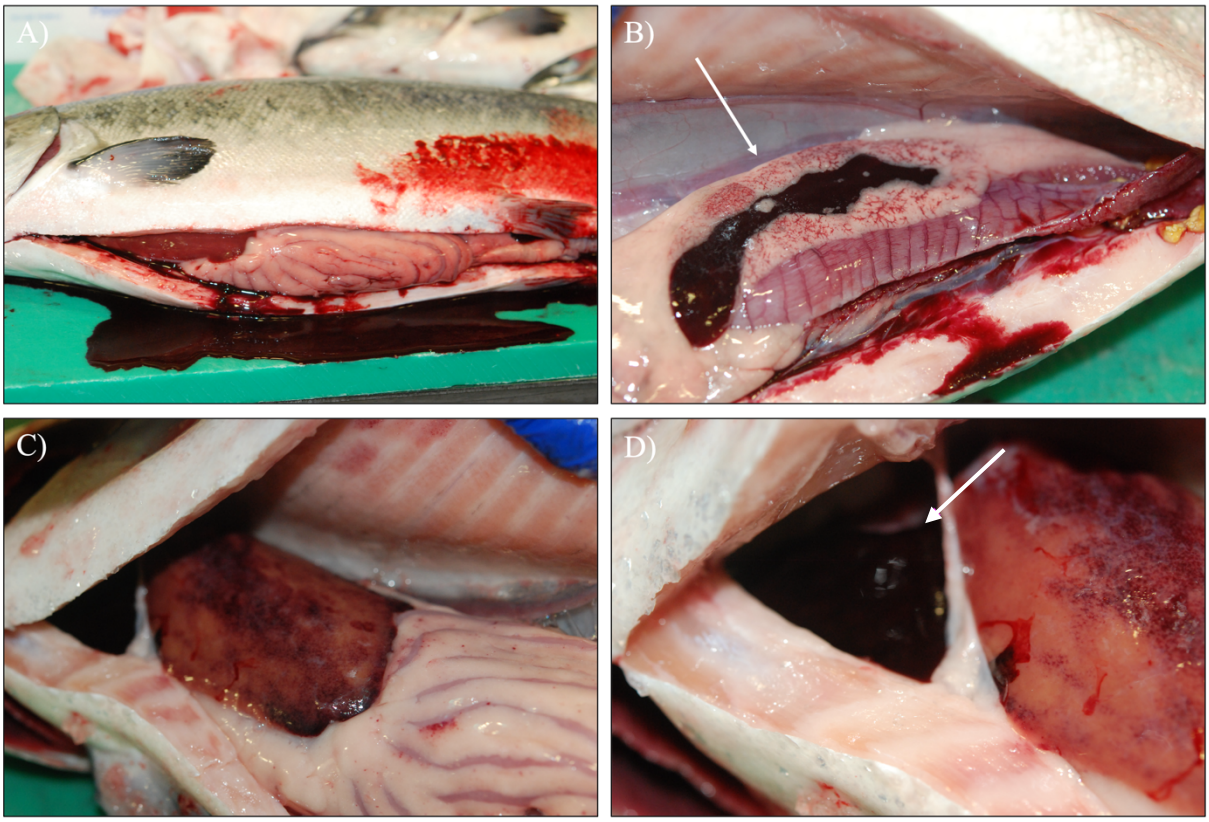


**Figure 3.2**. **A)** Recently opened fish with clear signs of circulatory disorders, showing blood and bloody ascites flowing out. Punctate/ petechial hemorrhages are also visible in the pylorus region. **B)** Punctate/petechial hemorrhages in the mesenteric fat are indicated by the white arrow. **C)** and **D)** depict fish with CMS symptoms, showing a marbled liver with fibrin layer formation and a bloody pericardial cavity indicated by a white arrow. All pictures are taken form fish within the Test Group.

Furthermore, an examination of pigmentation patterns in both groups revealed notable differences. In Group STE, 25 fish displayed significant pigmentation in the pyloric caeca and mesenteric fat. Conversely, in Group STF, 18 fish exhibited similar pigmentation traits. This distinction underscores the variability in pigmentation patterns across the two investigated groups (Figure 3.3).

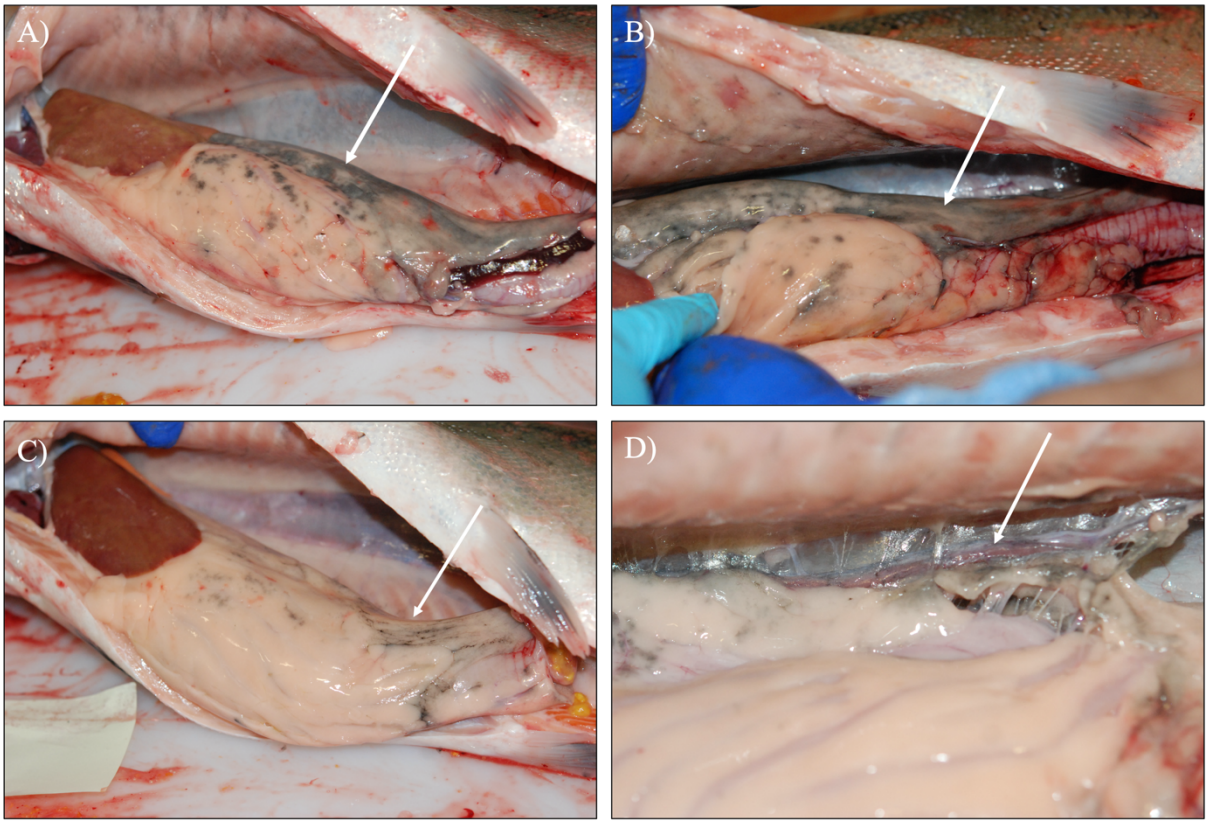


**Figure 3.3**. **A)** and **B)** show two different fish with a strong degree of pigmentation where most of the pyloric caeca and mesenteric fat are affected. The affected area is indicated with a white arrow. Both fishes from group STE. **C)** Organs are affected to a moderate degree, with a pigmented area indicated by the white arrow. **D)** The white arrow shows connective tissue with an associated degree of pigmentation, probably a granulomatous inflammation. Fish in both **C)** and **D)** are from group STF.

Upon macroscopic examination of the 60 fish, clear indications of circulatory problems in the liver were relatively infrequent, with only 9 instances of noticeable changes, such as a bloody liver or marbled/spotted liver. Specifically, 3 fish from the STE group presented with livers exhibiting a distinct bloody or red coloration (Figure 3.4, D). In the STF group, 6 fish demonstrated similar bloody or red-coloured livers, with some additionally manifesting marbled (Figure 3.4, B) or punctate haemorrhages (Figure 3.4, D).

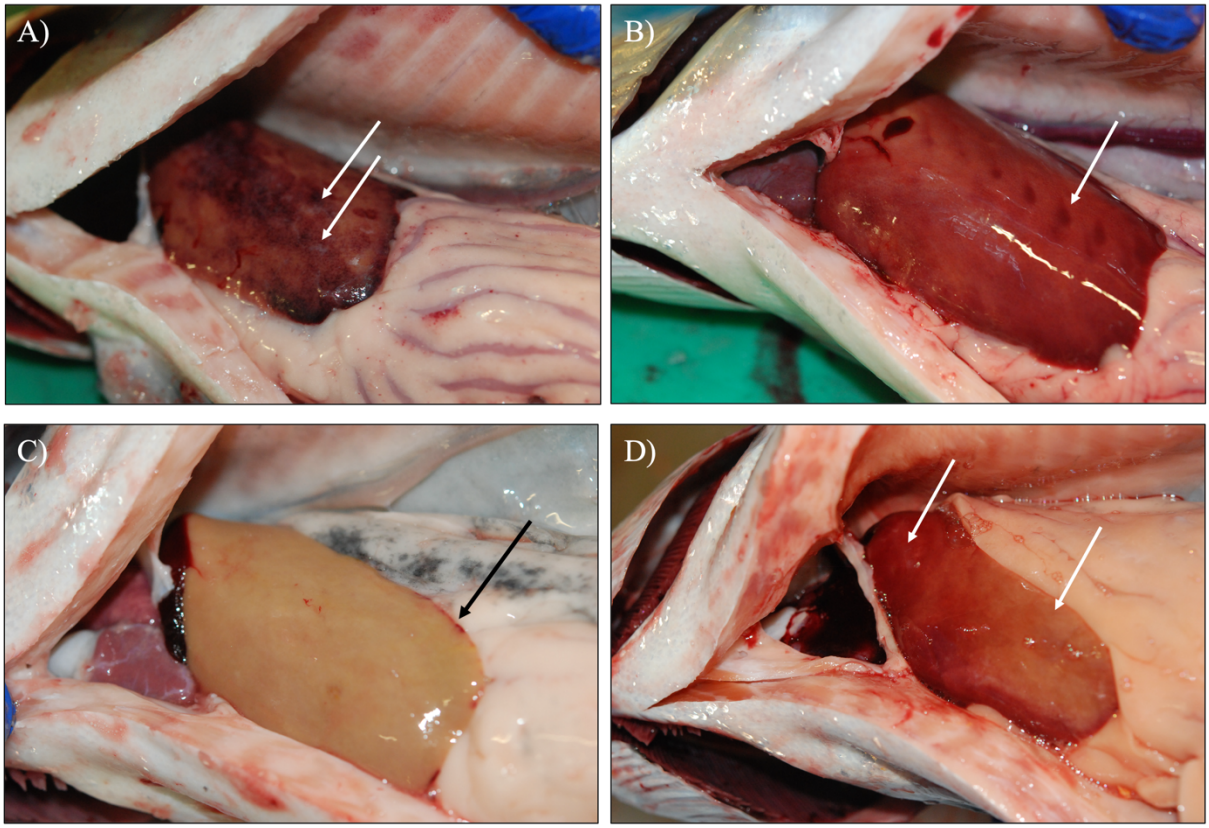


**Figure 3.4**. Overview of different clinically observed types of the liver. **A)** haemorrhagic and speckled liver with signs of fibrin layer formation (indicated by white arrows), fish from SGB group, **B)** bloody liver with probable post-mortem artifacts (seen at white arrow), fish from SGB group, **C)** pale liver with bleeding in the outer parts (indicated by black arrow), and a hint of punctate haemorrhages, fish from group STE, **D)** a bloody liver with colour changes within the organ, as indicated by the presence of white arrows, fish from STF group.

Sexual maturation was assessed for all PIT-tagged fish obtained at the harvest (STE, N = 242. STF, N = 260), which included both sampled and non-sampled individuals. A degree of maturation was recorded, ranging from 1, indicating early maturation, to 2, indicative of advanced maturation or completion of the maturation process. Among the 60 fish sampled, six demonstrated signs of maturation: three were assessed as degree 1 and three as degree 2, all of which were part of the STE group. In an assessment of the entire fish population within the STE and STF groups (STE, N = 242. STF, N = 260), a total of 27 fish exhibited signs of maturation. The distribution of maturation stages was as follows: within the STE group, 7 fish were at maturation degree 1, and 12 were at degree 2, whereas in the STF group, 4 fish were each at maturation degrees 1 and 2. Notably, all instances of recorded maturation were exclusively observed in male fish. Illustrative examples of maturation stages are presented in Figure 3.5

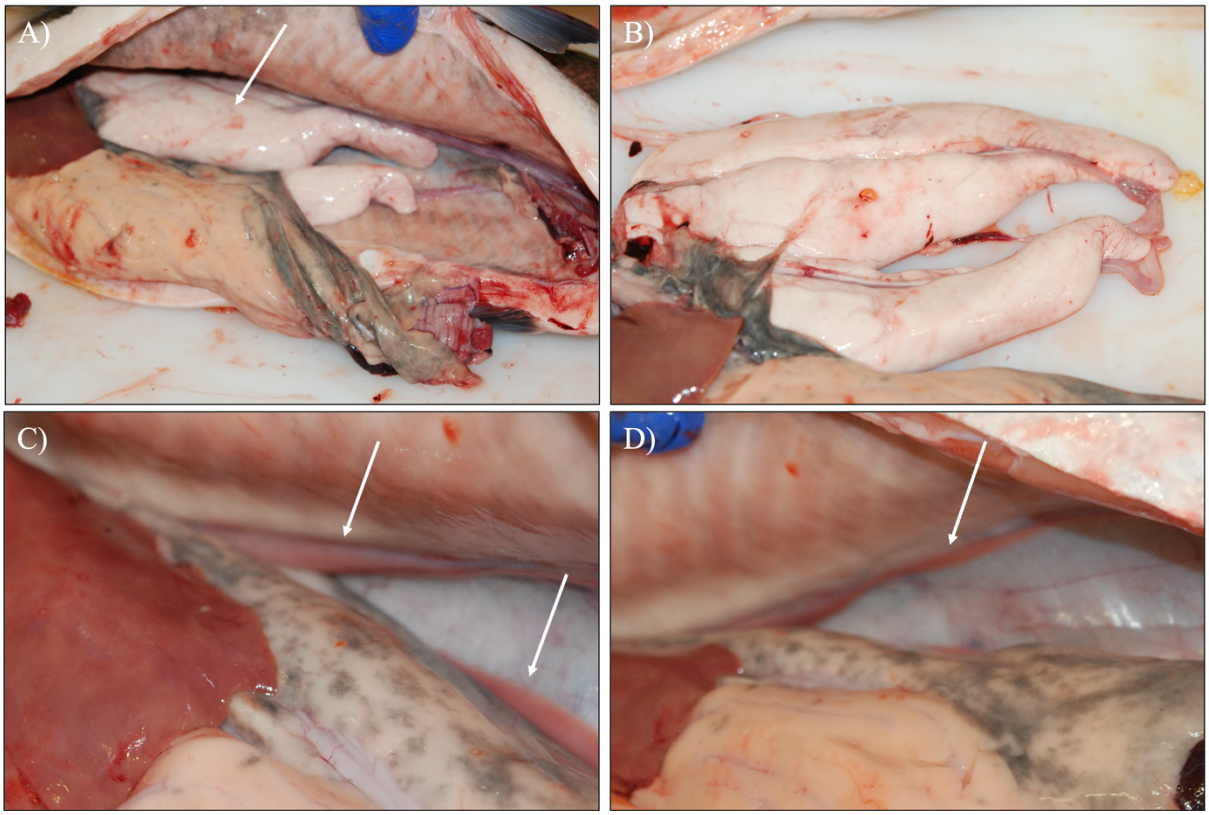


**Figure 3.5**. Comparative visualization of different stages of testicular maturation in sampled fish. Panels **A)** and **B)** depict significant testis enlargement, indicative of a maturation score of 2. Conversely, panels **C)** and **D)** depict a modest enlargement of the testis, corresponding to a maturation score of 1. In all panels, the white arrows denote the location of the testis. Both fish illustrated are from group STE.

### 3.1.2 Recapture rate, clinical signs, and diagnosis of salmon at site G

The initial population were 502 individuals PIT-tagged in the Test Group, and 504 in the Control Group. Of these, 496 from the Test Group and 498 from the Control Group were released to sea. During the sea phase, a total of 78 fish from the Test Group and 37 from the Control Group were registered as dead, as illustrated in Figure 3.6 and Table 3.2, with a peak in mortality for the Test Group in April 2022 (caused by an external factor). Throughout the production cycle in the experimental cages, CMS-related mortality was observed during the final month before slaughter. Due to worsening health conditions and increasing lice infestations, an early harvest was required, resulting in low fish weights. Mortality numbers from this critical period, revealed a higher survival rate in the Test Group (SGA) compared to the Control Group (SGB), as illustrated in Figure 3.6. When harvesting, 363 PIT-tagged fish were recorded in the Test Group, and 402 in the Control Group (Table 3.2).


**Figure 3.6**. Mortality rates at site G during the sea phase production from January, 2022 to November, 2022. The red line represents the mortality rates for the test group, and the yellow line represent the mortality rates for the control group.

**Table 3.2**. Distribution of Atlantic Salmon in Test Group (SGA) and Control Group (SGB) at Site G. The table shows the numbers for each group at different points in the study: PIT-tagged fish, fish released to the sea, fish that died and were recorded at the sea production phase, fish recorded at the harvest line, and fish with unknown status.

| Group | PIT-tagged | Fish released | Dead at sea | Harvesting | Unknown |
|---|---|---|---|---|---|
| Test Group, SGA | 502 | 496 | 78 | 363 | 55 |
| Control Group, SGB | 504 | 498 | 37 | 402 | 59 |

Among the 60 fish examined and sampled at site G, comprising 30 from Test Group (SGA) and 30 from Control Group (SGB), clinic compatible with circulatory disturbances were apparent in 23 individuals. These presentations included a fibrinous coat on the liver, petechial haemorrhages, ascites, bloody ascites, and blood in the pericardial cavity, observed individually or in conjunction. An analysis of the symptomatology across the two groups revealed that a single case of ascites was observed in group SGB, while none was found in group SGA. Neither group showed instances of bloody ascites. The presence of blood in the pericardial cavity was detected in 3 fish from group SGA and 9 fish from group SGB. Bloody ascites, in conjunction with blood in the pericardial cavity, were noted in 1 fish from group SGA and 8 fish from group SGB. Additionally, one fish from group SGB exhibited a fibrinous layer on the liver. Visual exemplars of these circulatory disturbances are provided in Figure 3.2.

Within the sample of 60 fish, a degree of variation in liver appearances was expected, given that liver morphology can exhibit substantial differences among individual fish, even in the absence of explicit disease symptoms. Macroscopic examination revealed a relatively high prevalence of circulatory problems in the liver, with 31 fish presenting with discernible changes suggestive of such issues. Specifically, in the Test Group, 2 fish presented with bloody liver, 5 with pale liver, and 4 with a marbled or spotted liver appearance. Conversely, in the Control Group, 6 fish showed a bloody liver, with one of these additionally exhibiting a fibrinous layer (Figure 3.4, A), 8 fish presented with a pale liver (Figure 3.4, C), while 5 demonstrated a marbled or spotted liver appearance (Figure 3.4, D).

Lesions such as wounds likely caused by mechanical means (Figure 3.7, B) and spine deformity (Figure 3.7, A) not thought to be related to PMCV infections were observed on some fish. Unlike the previous sampling event, no evidence of pigmentation abnormalities was detected at site G.

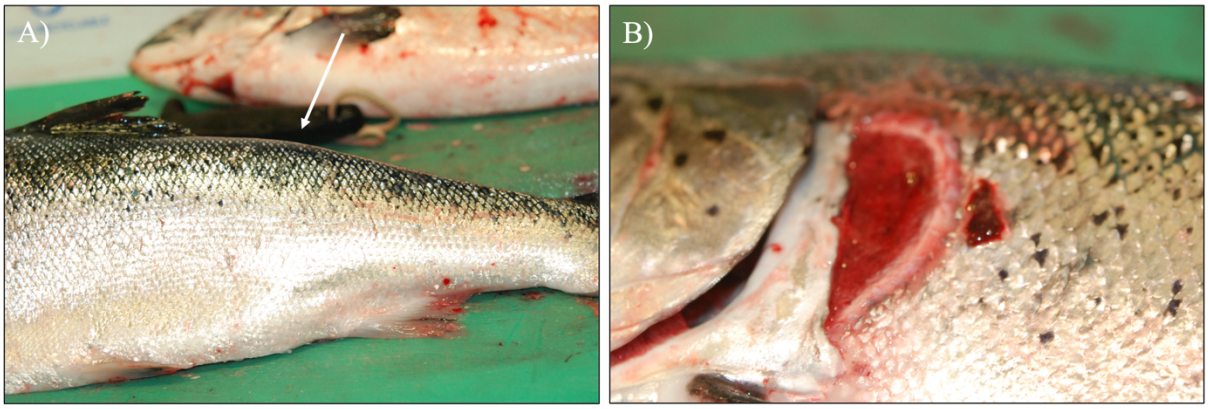


**Figure 3.7**. **A)** Fish with spinal deformity indicated by the white arrow, from group SGB. **B)** A significant wound in the area surrounding the gill cover is likely the result of mechanical damage, from group SGA.

### 3.1.3 Weight and length

Weight, length, and condition factor measurements were conducted to contrast the experimental groups located at sites T and G.

#### *3.1.3.1 Sampling one, site T*

At site T, the mean weight of the sampled fish from the Test Group was found to be 6.399 kg, slightly higher than the mean weight of the Control Group, which was 6.307 kg (Table 3.3). An unpaired t-test revealed that this difference was not statistically significant ($p = 0.7641$). When the weights of all identified PIT-tagged fish were considered (Test Group, N = 242; Control Group, N = 260), the median weight was lower in the Test Group (6.280 kg) compared to the Control Group (6.580 kg). This difference was found to be statistically significant * ($p = 0.0411$, Mann-Whitney U = 28143) (Figure 3.8).

In terms of fish length, the mean length of the sampled fish from the Test Group was 79.00 cm, slightly less than the Control Groups mean length of 80.07 cm. The unpaired t-test showed no statistically significant difference between these lengths ($p = 0.3272$). However, when considering the lengths of all PIT-tagged fish identified, the Control Groups median length (80.00 cm) was significantly greater than that of the Test Group (79.00 cm) *** ($p = 0.0005$, Mann-Whitney U = 25783) (Figure 3.8).

The condition factor, reflecting physical and biological circumstances (Baxter, 1998) showed a statistically significant difference * ($p = 0.0100$, Mann-Whitney U = 27277) between the two groups. The median condition factor for the Test Group was 1.297, higher than the Control Groups median condition factor of 1.262 (Figure 3.8). *p* values are given in the appendix.

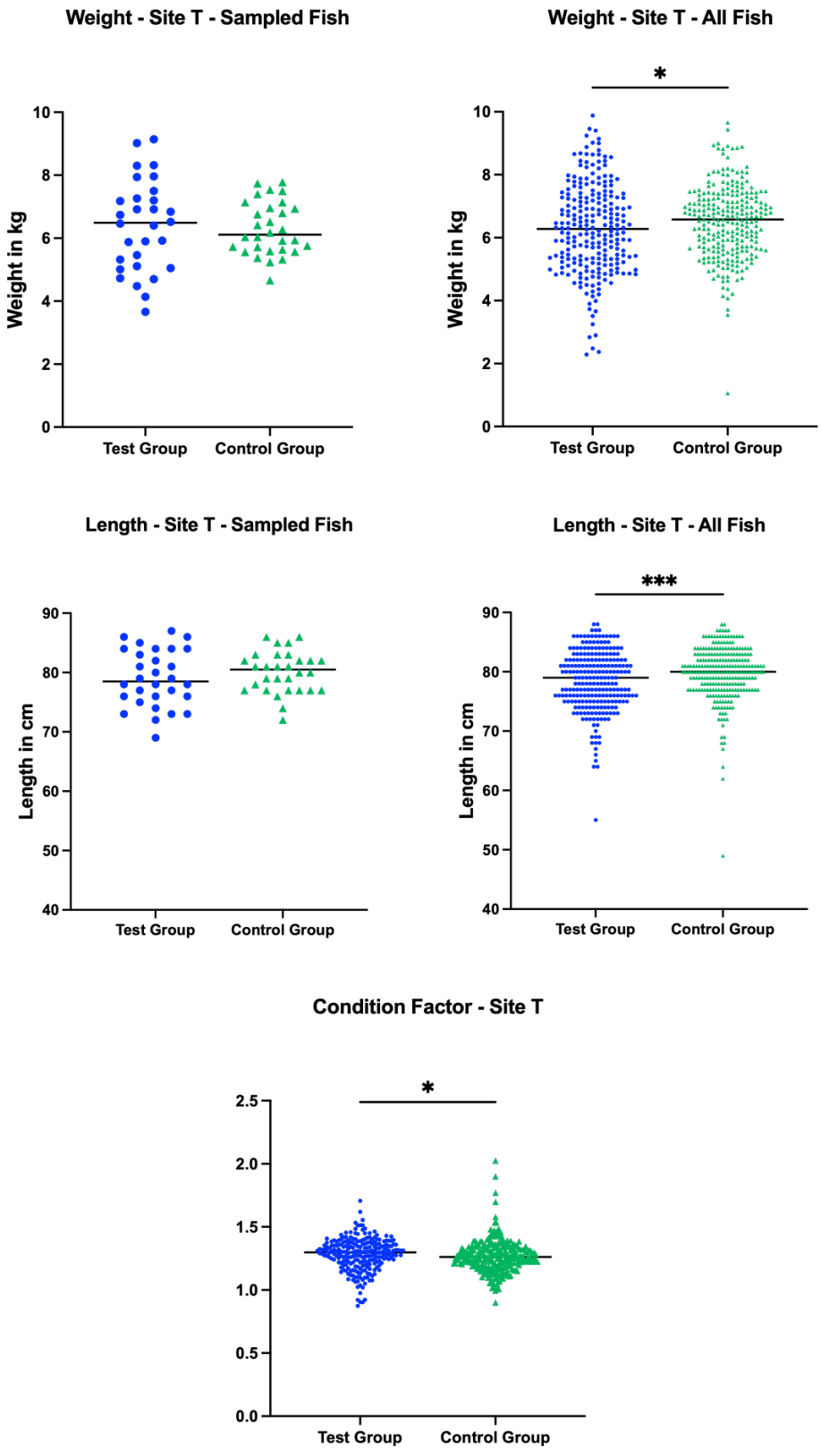


**Figure 3.8**. Analysis of weight and length across experimental groups at site T. The figure consists of five illustrations representing individual weight and length for sampled fish (Test Group, N = 30; Control Group, N = 30) and all registered fish (Test Group, N = 242; Control Group, N = 260) in the Test and Control groups. Statistically significant differences were observed in the weight (*) and length (***) of all registered fish at site T. The fifth illustration shows the condition factor, with a statistically significant difference observed between the Test and Control groups (*). Unless otherwise specified, group comparisons were not statistically significant (ns). Weight and length of sampled fish were analysed using a two-tailed t-test, while other variables were analysed using a Mann-Whitney test.

#### *3.1.3.2 Sampling two, site G*

At site G, the average weight of the sampled fish from the Test Group was 3.690 kg, marginally higher than the Control Group's mean weight of 3.567 kg (Table 3.3). However, the unpaired t-test revealed that this difference was not statistically significant ($p$ = 0.4437). For all PIT-tagged fish identified (Test Group, N = 363; Control Group, N = 402), the difference between the Test Groups mean weight of 3.659 kg and the Control Group mean weight of 3.596 kg was statistically different *** ( $p$ = 0.0002) (Figure 3.9).

Looking at the length of the fish, the sampled Test Group fish had a median length of 68.00 cm, which was higher than the Control Group's median length of 66.50 cm. This difference, however, was not statistically significant ($p$ = 0.2276, Mann-Whitney U = 368.5). When considering all PIT-tagged fish identified, the median length of the Test Group (68.00 cm) was significantly longer than the median length of the Control Group (67.00 cm), as shown by a significant p-value **** ($p < 0.0001$, Mann-Whitney U = 59776) (Figure 3.9).

In terms of condition factor at site G, there was a significant difference **** ($p < 0.0001$, Mann-Whitney U = 55308) between the two groups. The Control Group had a higher median condition factor of 1.186 compared to the Test Groups median condition factor of 1.151 (Figure 3.9). $p$ values are given in the appendix.

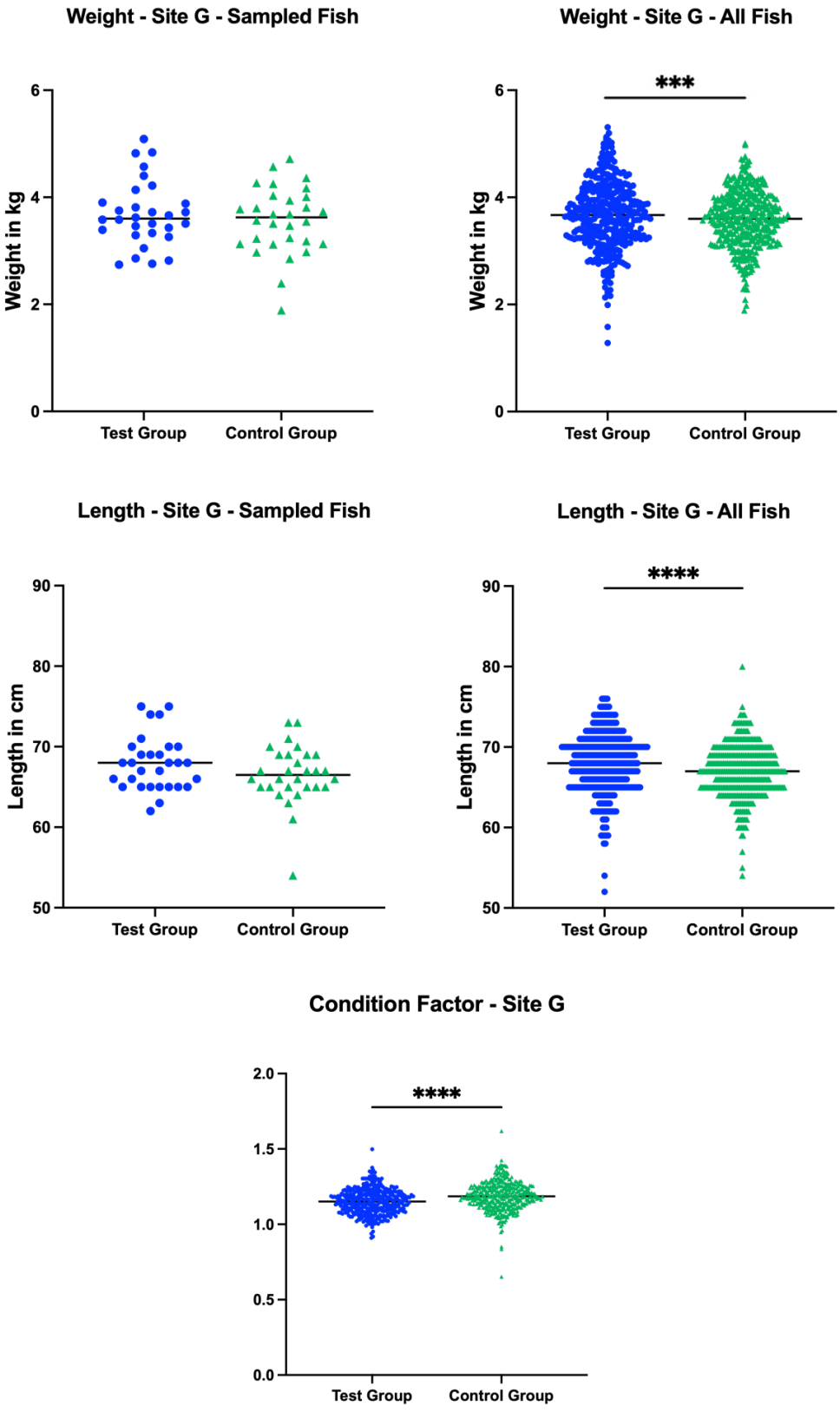


**Figure 3.9**. Analysis of weight and length across experimental groups at site G. The figure consists of five illustrations representing individual weight and length for sampled fish (Test Group, N = 30; Control Group, N = 30) and all registered fish (Test Group, N = 363; Control Group 402) in the Test and Control groups. Statistically significant differences were observed in the length (****) of all registered fish at site G. The fifth illustration shows the condition factor, with a statistically significant difference observed between the Test and Control groups (****). Unless otherwise specified, group comparisons were not statistically significant (ns). The weight of sampled fish and the weight of all fish were analysed using a two-tailed t-test, while other variables were analysed using a Mann-Whitney test.

**Table 3.3**. Overview of mean weight and length across both sampled fish groups, the total slaughter, from sites T and G, and the distribution of males and females within these groups. The test groups are in blue, while the control groups are in green.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1st sampling – Site T | Numbers form the 60 fishes with both tissue samples and histology | | | | | | | |
| | Mean weight – Test Group | Mean weight – Control Group | Mean length – Test Group | Mean length – Control Group | Number of females – Test Group | Number of females – Control Group | Number of males – Test Group | Number of males – Control Group |
| | 6.399 kg | 6.307 kg | 79.00 cm | 80.06 cm | 13 | 20 | 17 | 10 |
| | Numbers from the total slaughter | | | | | | | |
| | 6.353 kg | 6.354 kg | 78.328 cm | 78.332 cm | 131 | 144 | 109 | 116 |
| 2nd sampling – Site G | Numbers form the 60 fishes with both tissue samples and histology | | | | | | | |
| | Mean weight – Test Group | Mean weight – Control Group | Mean length – Test Group | Mean length – Control Group | Number of females – Test Group | Number of females – Control Group | Number of males – Test Group | Number of males – Control Group |
| | 3.690 kg | 3.567 kg | 67.93 cm | 66.53 cm | 15 | 15 | 16 | 14 |
| | Numbers from the total slaughter | | | | | | | |
| | 3.659 kg | 3.596 kg | 68.03 cm | 67.06 cm | 159 | 211 | 204 | 191 |

## 3.2 Real time RT-PCR

Real-time RT-PCR assays were performed to evaluate pathogen prevalence within 120 samples from heart tissue across the four sample groups SGA (Test Group), SGB (Control Group), STE (Test Group), and STF (Control Group) from the two different sampling sites, site T (STE and STF) and site G (SGA and SGB) (Table 3.4 and/or 3.5). The Ct-values, which are inversely proportional to the amount of target nucleic acid in the sample, provide valuable insights into the viral load across the samples.

Piscine orthoreovirus (PRV1) was detected across all sample groups, indicating a 100% prevalence (Table 3.4). The Ct-values for the STE and STF groups ranged from 19.0 to 31.9 and 21.5 to 29.1, respectively. The SGA and SGB groups exhibited a wider range of Ct-values, from 14.2 to 23.3 for SGA and 16.7 to 24.0 for SGB (Table 3.3). For PRV1, the density analysis showed statistically significant differences between the STE and STF groups from site T. However, no significant difference was found between the SGA and SGB groups from site G. The Mann-Whitney test confirmed these findings, revealing a statistically significant difference between the STE and STF groups with a *p*-value of 0.0076 ** (Figure 3.10), but no significant difference between the SGA and SGB groups with a *p*-value of 0.2601 (Figure 3.11).

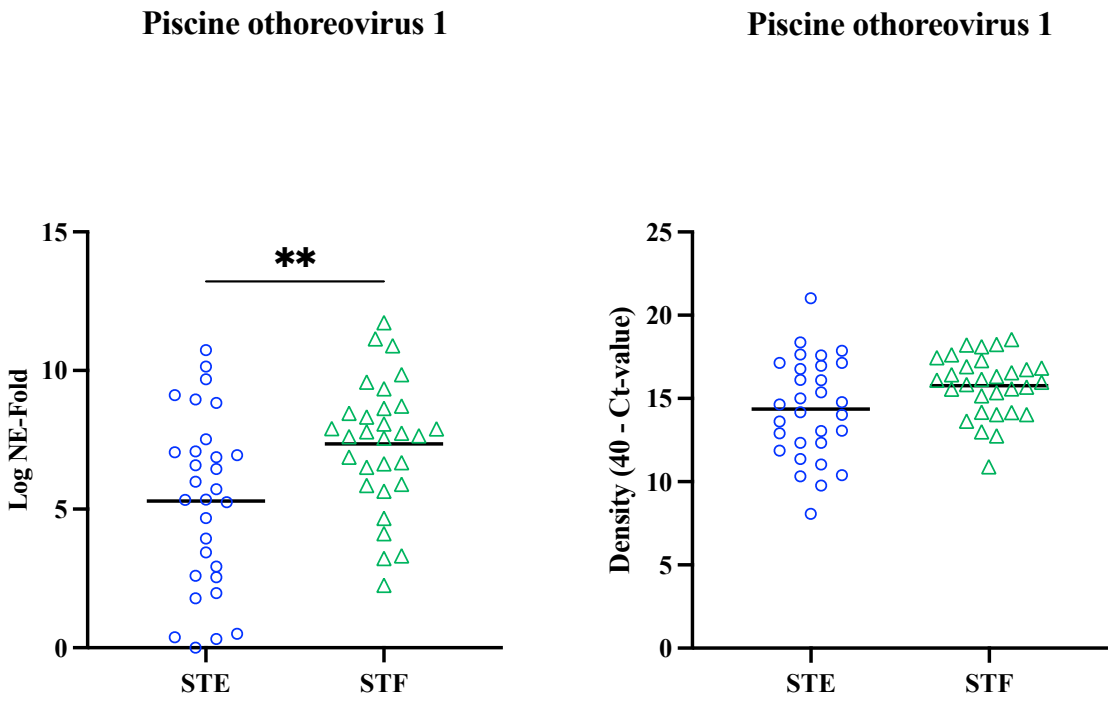


**Figure 3.10**. Density of Piscine orthoreovirus 1 from heart tissue for STE and STE groups from site T. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for PRV1 across STE and STF from Site T. In both groups N = 30. As depicted in the left panel, the Log2-transformed NE-fold for the STE and STF groups are statistically significant differences with p-value = 0.0076 **. The right panel displays the reversed Ct-values (40-Ct-value).

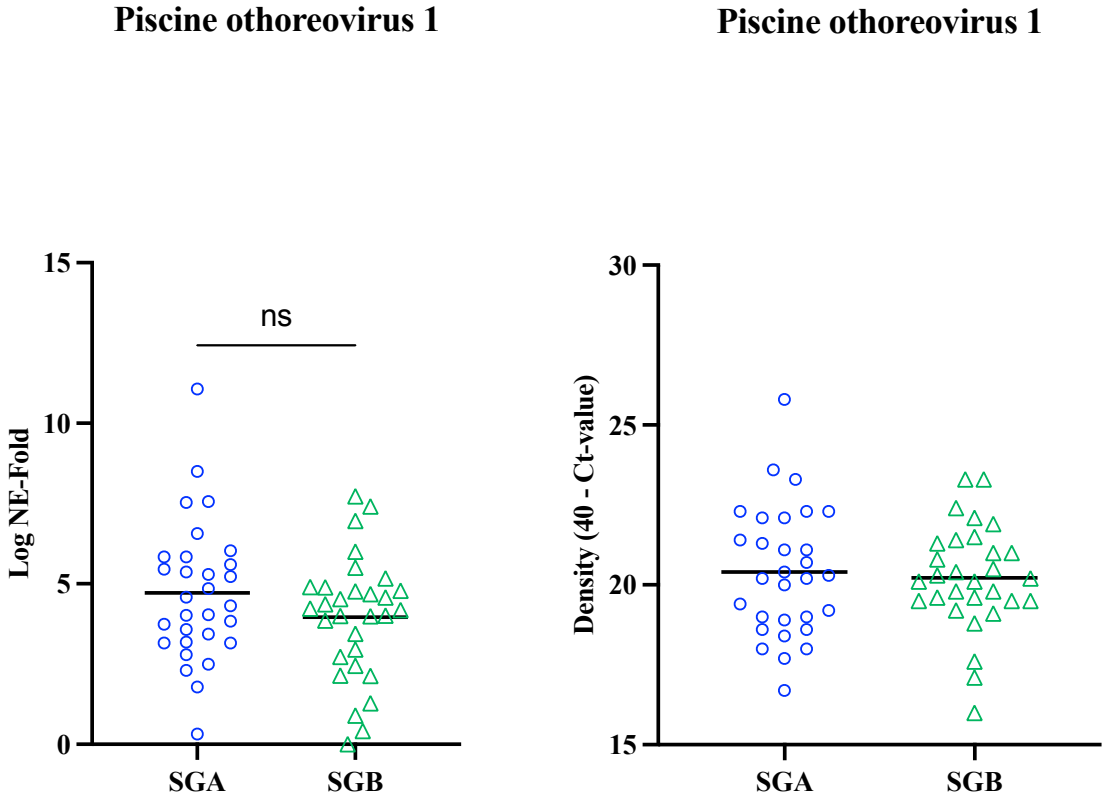


**Figure 3.11**. Density of Piscine orthoreovirus 1 from heart tissue for SGA and SGB groups from site G. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for PRV1 across SGA and SGB from Site G. In both groups N = 30. As depicted in the left panel, the Log2-transformed NE-fold shows no significance for the SGA and SGB groups with a p = 0.9737. The right panel displays the reversed Ct-values (40-Ct-value).

Salmonid alphavirus (SAV) was detected across all sample groups (Table 3.4). In the STE and STF groups, the Ct-values ranged from 23.6 to 36.9 (STE) and 27.9 to 36.4 (STF), respectively. The SGA and SGB groups showed a more extensive range of Ct-values, ranging from 19.7 to 35.5 (SGA) and 23.6 to 36.1 (SGB), respectively (Table 3.3).

The density of SAV for the analysed heart tissue within both groups at both sites showed no statistical difference between either groups (*p* values are given in the appendix). The Mann-Whitney test confirmed these findings, revealing no statistical difference between the STE and STF groups ($p = 0.1113$) (Figure 3.12), or SGA and SGB groups ($p = 0.4223$) (Figure 3.13).

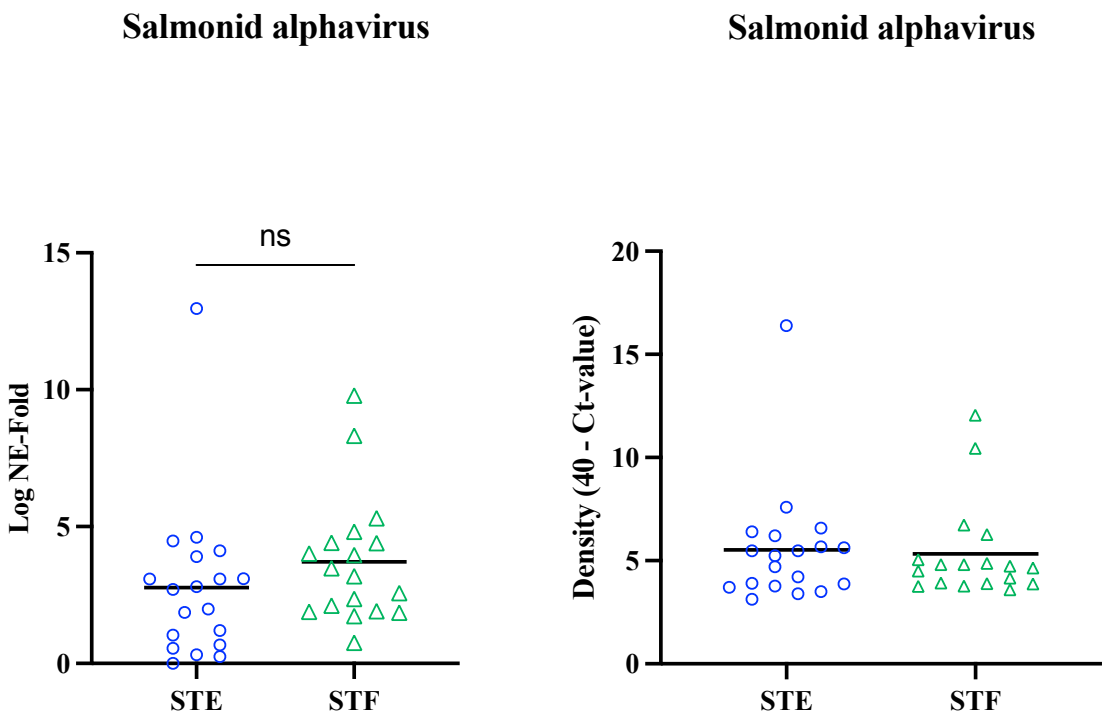


**Figure 3.12**. Density of Salmonid alphavirus from heart tissue for STE and STE groups from site T. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for SAV across STE and STF from Site T. In group STE, N = 19, in group SGB, N = 18. As depicted in the left panel, the Log2-transformed NE-fold for the STE and STF groups are statistically significant differences with p-value = 0.1113. The right panel displays the reversed Ct-values (40-Ct-value).

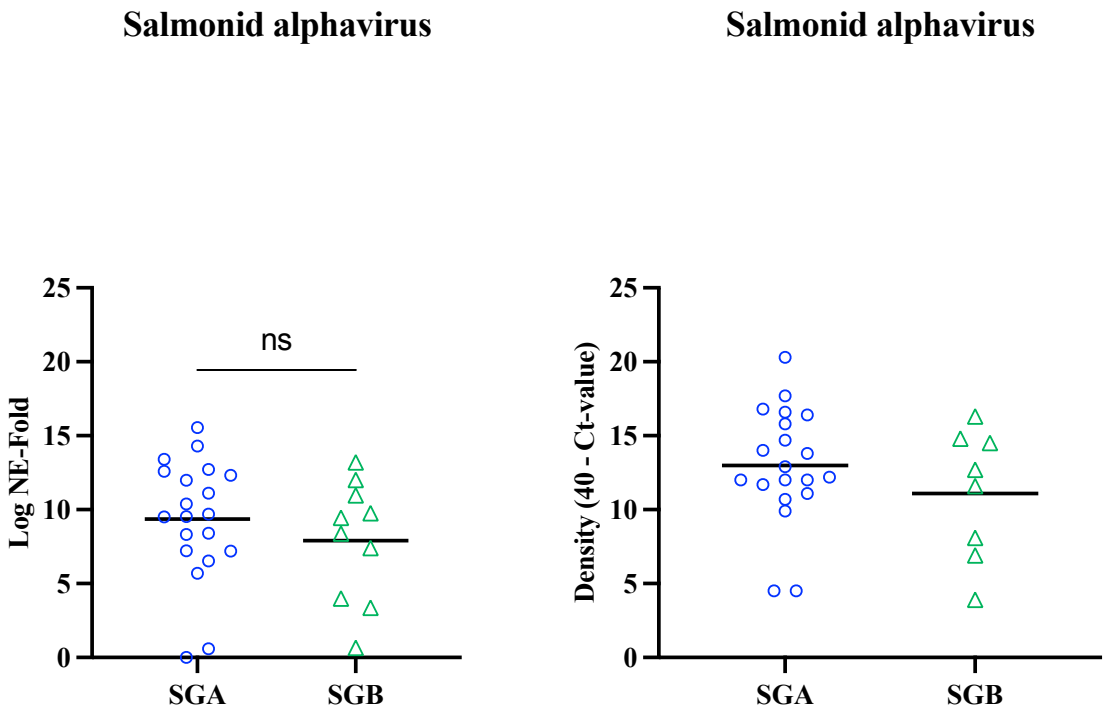


**Figure 3.13**. Density of Salmonid alphavirus from heart tissue for SGA and SGB groups from site G. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for SAV across SGA and SGB from Site G. In group SGA, N = 20, in group SGB, N = 10. As depicted in the left panel, the Log2-transformed NE-fold shows no significance for the SGA and SGB groups with a p = 0.4223. The right panel displays the reversed Ct-values (40-Ct-value).

Piscine myocarditis virus (PMCV) was not detected in the STE and STF groups (Table 3.4). However, PMCV was present in both the SGA and SGB groups, given the prevalence of 100 % within these two groups. The SGA group showed Ct-values ranging from 11.4 to 26.5, while the SGB group had Ct-values between 10.7 and 19.9 (Table 3.5). The density analysis of PMCV for the analysed heart tissue in revealed significant statistical differences between SGA and SGB ($p < 0.0001$) (Figure 3.14).

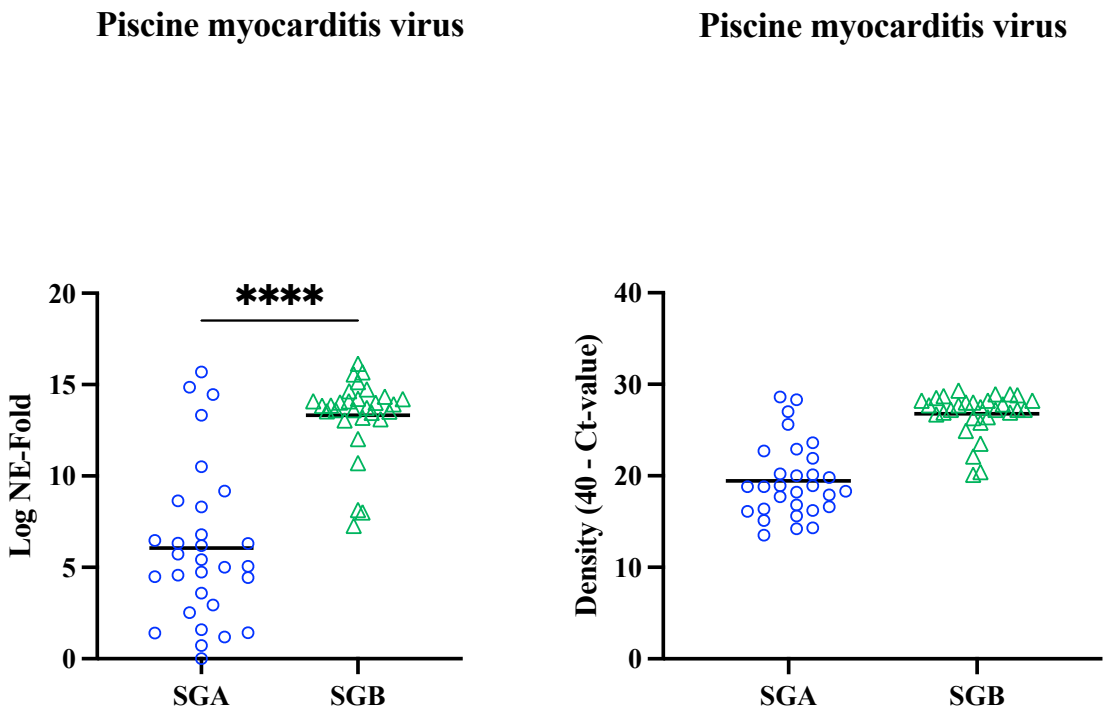


**Figure 3.14**. Density of Piscine myocarditis virus from heart tissue for SGA and SGB groups from site G. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for SAV across SGA and SGB from Site G. In both groups N = 30. As depicted in the left panel, the Log2-transformed NE-fold shows statistically significant differences between the SGA and SGB groups with a $p < 0.0001$. The right panel displays the reversed Ct-values (40-Ct-value).

The prevalence of *Paranucleospora theridion* (NUC) was 100 % for both SGA and SGB groups (Table 3.5). The density of the parasite in the heart tissue was significant between SGA and SGB. The Mann-Whitney test confirmed these findings, revealing a statistically significant difference between the SGA and SGB groups ($p$ = 0.0006) (Figure 3.15).

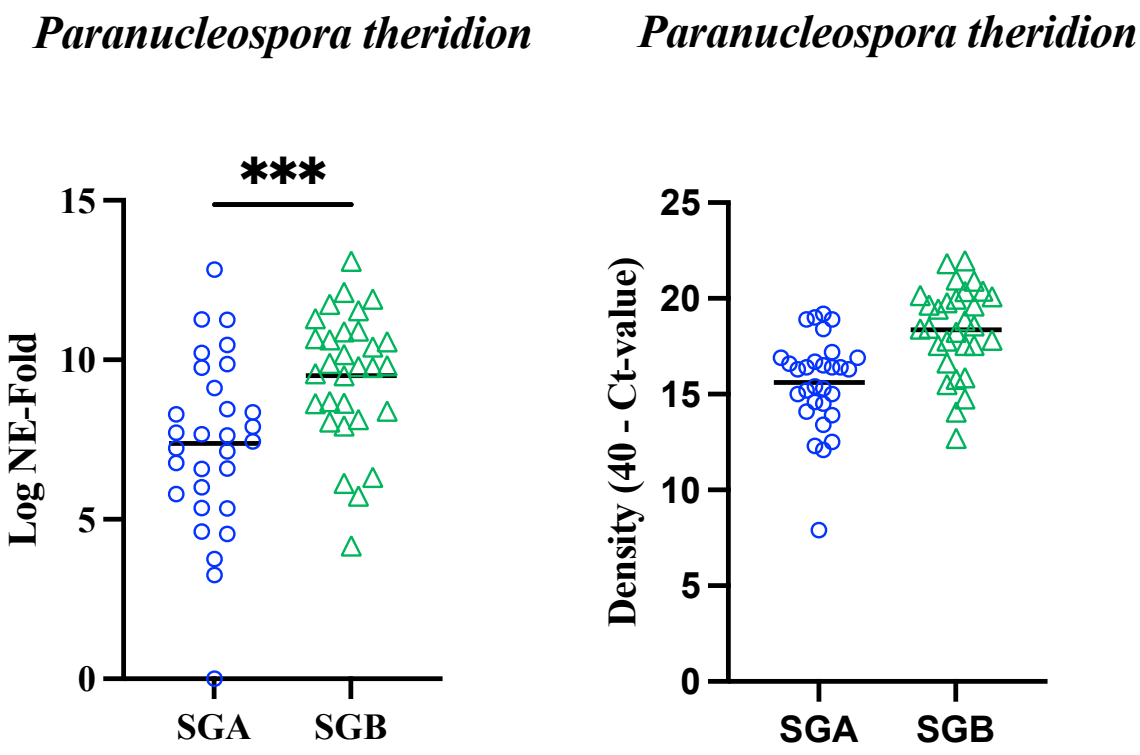


**Figure 3.15**. Density of Paranucleospora theridion from heart tissue for SGA and SGB groups from site G. Each point represents an individual fish, with the black line indicating the average Ct-value within each group. The data is presented as Log2-transformed NE-fold and reversed Ct-values (40 – Ct-value) for SAV across SGA and SGB from Site G. In both groups N = 30. As depicted in the left panel, the Log2-transformed NE-fold shows statistically significant differences between the SGA and SGB groups with a p = 0.0006. The right panel displays the reversed Ct-values (40-Ct-value).

No detection of Infectious salmon anemia virus (ISAV) and Infectious pancreatic necrosis virus (IPNV) was observed in any of the samples from the SGA and SGB groups (Table 3.5). These pathogens, therefore, had a prevalence of 0 % (Table 3.4). All *p* values are given in the appendix.

**Table 3.4**. Prevalence in percent of the different pathogens in the heart tissue that were analysed within the four groups.

| | Site G (2nd sampling) | | | | Site T (1st sampling) | | | |
|---|---|---|---|---|---|---|---|---|
| | SGA | | SGB | | STE | | STF | |
| | Number of positive individuals | Prevalence (%) | Number of positive individuals | Prevalence (%) | Number of positive individuals | Prevalence (%) | Number of positive individuals | Prevalence (%) |
| PMCV | 30 of 30 | 100.0 | 30 of 30 | 100.0 | 0 of 30 | 0.0 | 0 of 30 | 0.0 |
| PRV | 30 of 30 | 100.0 | 30 of 30 | 100.0 | 30 of 30 | 100.0 | 30 of 30 | 100.0 |
| SAV | 20 of 30 | 66.6 | 10 of 30 | 33.3 | 19 of 30 | 63.3 | 18 of 30 | 60.0 |
| ISAV | 0 of 30 | 0.0 | 0 of 30 | 0.0 | - | - | - | - |
| *P. theridion* | 30 of 30 | 100.0 | 30 of 30 | 100.0 | - | - | - | - |
| IPNV | 0 of 30 | 0.0 | 0 of 30 | 0.0 | - | - | - | - |

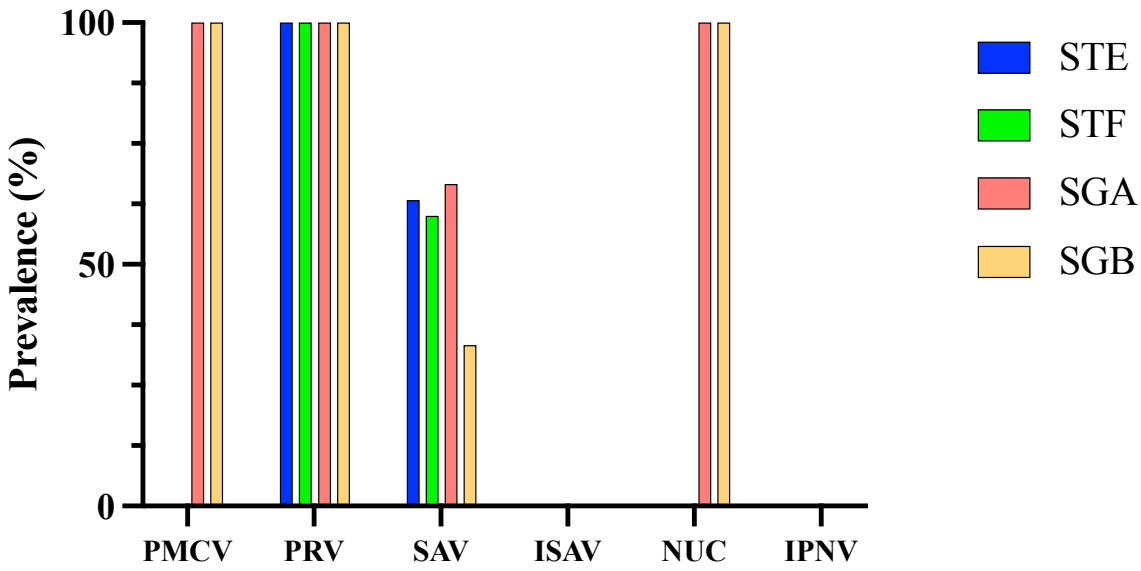


**Figure 3.16**. Prevalence of pathogens in heart tissue from the different groups. **STE group**: PRV was detected in all individuals (30 of 30), SAV was detected in 19 of 30 individuals, and PMCV was not detected (0 of 30). **STF group**: PRV was detected in all individuals (30 of 30), SAV was detected in 18 of 30 individuals, and PMCV was not detected (0 of 30). **SGA group**: PRV, PMCV, and *P. theridion* were detected in all individuals (30 of 30), SAV was detected in 20 of 30 individuals, Ca. B. cysticola EPIT was detected in 3 of 30 individuals, ISAV and IPNV were not detected (0 of 30). **SGB group**: PRV, PMCV, and *P. theridion* were detected in all individuals (30 of 30), SAV was detected in 10 of 30 individuals, ISAV and IPNV were not detected (0 of 30). PRV = Piscine orthoreovirus, SAV = Salmonid alphavirus, PMCV = Piscine myocarditis virus, ISAV = Infectious salmon anemia virus, NUC = Paranucleospora theridion, IPNV = Infectious pancreatic necrosis virus.

**Table 3.5**. The average and range of Ct-values and prevalence in percent of the different assays at each sampling at sites G and T.

| Assay | Site G (2nd sampling) | | | | | | Site T (1st sampling) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SGA | | | SGB | | | STE | | | STF | | |
| | Ct-value | | % | Ct-value | | % | Ct-value | | % | Ct-value | | % |
| | Average | Range | Prevalence | Average | Range | Prevalence | Average | Range | Prevalence | Average | Range | Prevalence |
| Piscine myocarditis virus | 20,568 | 11,4 - 26,5 | 100.0 | 13,230 | 10,7 - 19,9 | 100.0 | Neg | Neg | Neg | Neg | Neg | Neg |
| Piscine orthoreovirus 1 | 19,602 | 14,2 - 23,3 | 100.0 | 19,8 | 16,7 - 24,0 | 100.0 | 25,6 | 19,0 - 31,9 | 100.0 | 24,2 | 21,5 - 29,1 | 100.0 |
| Salmonid alphavirus | 26,888 | 19,7 - 35,5 | 66.6 | 27,927 | 23,6 - 36,1 | 33.3 | 34,5 | 23,6 - 36,9 | 63.3 | 34,7 | 27,9 - 36,4 | 60.0 |
| Infectious salmon anemia virus | Neg | Neg | Neg | Neg | Neg | Neg | - | - | - | - | - | - |
| Infectious pancreatic necrosis virus | Neg | Neg | Neg | Neg | Neg | Neg | - | - | - | - | - | - |
| *Paranucleospora theridion* | 24,4 | 20,8 - 30,1 | 100.0 | 21,6 | 18,0 - 27,3 | 100.0 | - | - | - | - | - | - |

## 3.3 Histological scores

Histological examinations were performed on various sections of heart tissue, including the endocardium, myocardium, and epicardium of both the atrium and ventricle. The myocardium of the ventricle was further divided into the spongiosum and compactum layers for a more detailed evaluation (explained in 2.2, Histology). Notably, these histopathological scores were exclusively from fish collected at site G. An initial assessment of 20 histology slides from site T unveiled no notable findings, leading to a decision not to forego further investigation of the remaining 40 slides from this site. Comparative analysis of average histological scores was carried out between two fish groups from site G: SGA (Test Group) and SGB (Control Group). The distribution of fish within the various histological scores, stratified by the two groups, is depicted in Figure 3.17.

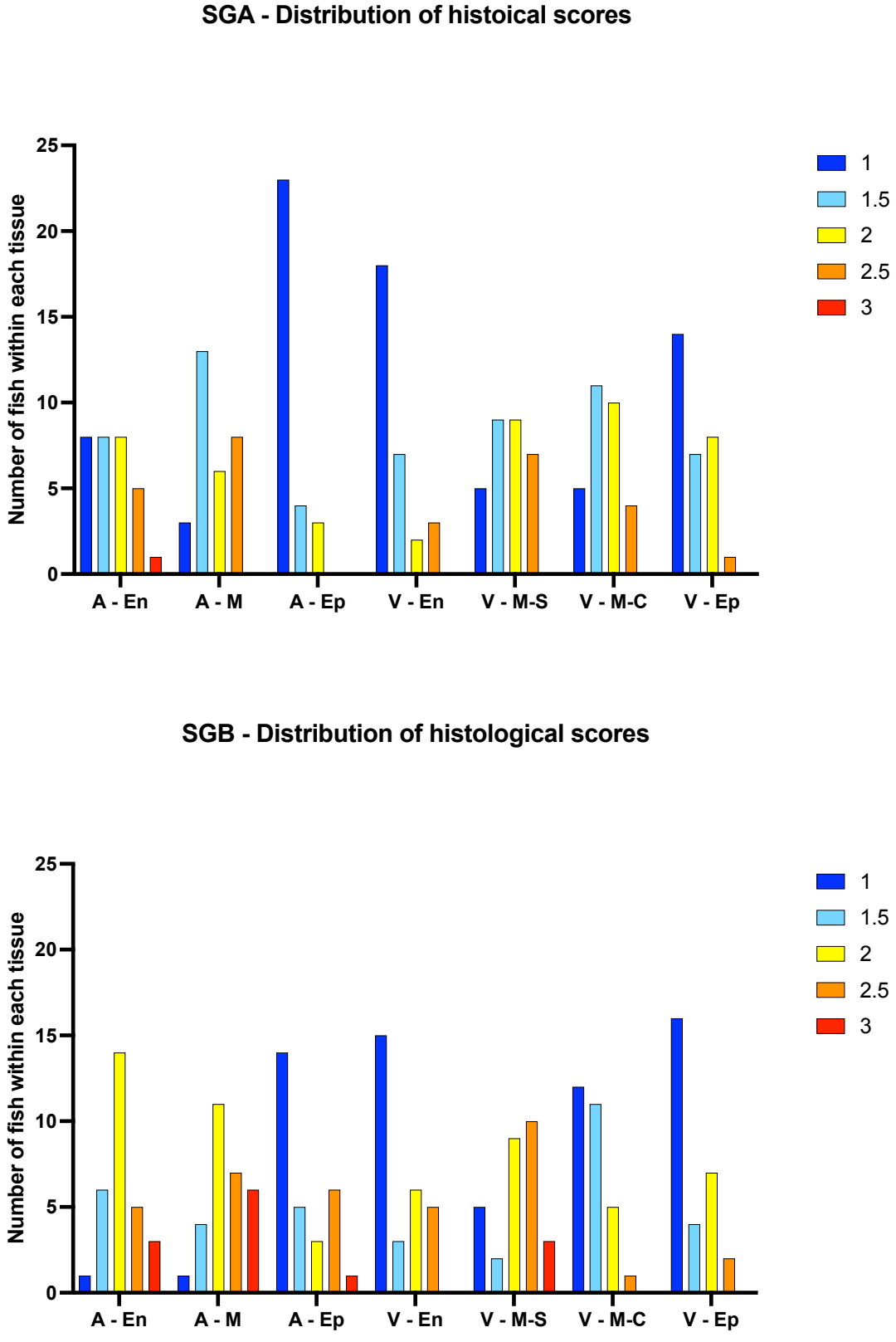


**Figure 3.17**. Distribution of fish within distinct histological scores for Test Group (SGA) and Control Group (SGB). The two diagrams present a representation of fish corresponding to diverse histological scores for groups SGA (first diagram) and SGB (second diagram). The classifications are color-coded as follows: Score 1 (depicted in dark blue), Score 1.5, a score between 1 and 2 (depicted in light blue), Score 2 (depicted in yellow), Score 2.5, a score between 2 and 3 (depicted in orange) and Score 3 (depicted in red). **A-En**: Atrium – Endocard, **A-M**: Atrium - Myocard, **A-Ep**: Atrium – Epicard, **V-En**: Ventricle – Endocard, **V-M-C**: Ventricle – Myocard-Spongiosum, **V-M-C**: Ventricle – Myocard - Compactum and **V-Ep**: Ventricle – Epicard.

Analysis of the histological scores using a Mann-Whitney test exhibited variations across the heart tissues in both groups, reflecting differing degrees of changes.

In the endocard of the atrium, there was a significant difference between the SGA and SGB groups, as evidenced by a p-value of 0.0175 (*) (Figure 3.18). A similar pattern was observed in the myocardium of the atrium, where the difference between the SGA and SGB groups was also significant, evidenced by a p-value of 0.0037 (**) (Figure 3.18). The trend continued in the atrium epicard, with a statistically significant difference between the SGA and SGB groups * ($p$ = 0.0141) (Figure 3.18).

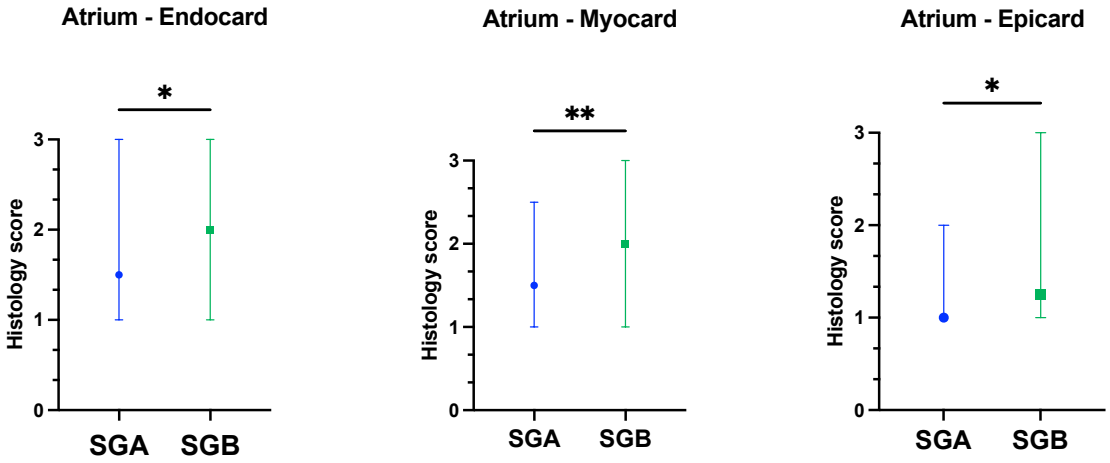


**Figure 3.18**. Comparative analysis of histological scores across heart tissues in SGA and SGB groups. This figure illustrates the median histological scores and their statistical significance across various heart tissue sections in both SGA (test group) and SGB (control group). The heart tissue sections include the atrial endocardium, myocardium, and epicardium. The * and ** annotations denote statistically significant differences at the 0.05 and 0.01 levels respectively, as determined by the Mann-Whitney U test. 'ns' denotes non-significant differences. The lines in the bars represent the range of scores in each group.

Contrastingly, in the endocardium of the ventricle, no statistically significant difference was observed between the SGA and SGB groups, as indicated by a $p$-value of 0.3510 (ns) (Figure 3.19). However, the spongiosum layer of the ventricular myocardium showed a significant difference between the SGA and SGB groups *($p$ = 0.0478) (Figure 3.19). The compactum layer of the ventricular myocardium also revealed a significant divergence between the SGA and SGB groups, with a p-value of 0.0089 (**) (Figure 3.19). In the epicardium of the ventricle, no significant difference was found between the SGA and SGB groups ($p$ = 0.6968) (Figure 3.19).

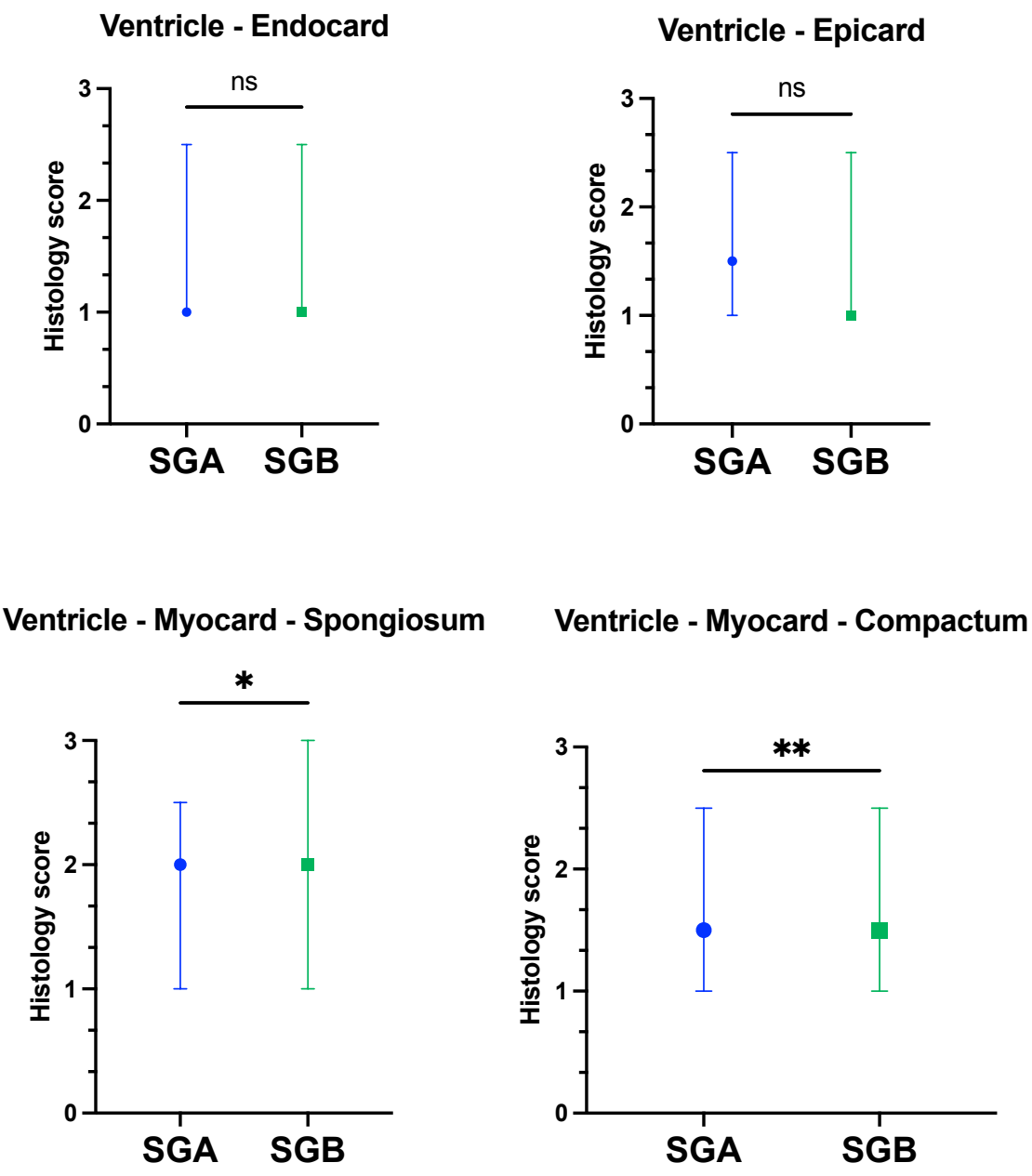


**Figure 3.19**. Comparative analysis of histological scores across heart tissues in SGA and SGB groups. This figure depicts the median histological scores and their statistical significance across various heart tissue sections in both SGA (test group) and SGB (control group). The heart tissue sections include the ventricular endocardium, myocard spongiosum, myocard compactum, and epicardium. Statistically significant differences are denoted by * for $p < 0.05$ and ** for $p < 0.01$, as determined by the Mann-Whitney U test. 'ns' denotes non-significant differences. The lines in the bars represent the range of scores for each group.

In summary, the Mann-Whitney U test disclosed statistically significant differences between the SGA and SGB groups in several heart tissues, specifically in the atrial endocardium, myocardium, atrium epicard, the spongiosum layer of the ventricular myocardium and the compactum layer of the ventricular myocardium (Table 3.6). These findings, illustrated in Figure 3.18 and Figure 3.19, use the * and ** annotations to denote significant correlations at the 0.05 and 0.01 levels (two-tailed), respectively, while 'ns' signifies non-significant differences. *p* values are given in the appendix.

**Table 3.6**. Comparative analysis of histological scores across heart tissues in SGA and SGB groups. This table presents the median histological scores, range of scores, Mann-Whitney U values, and their corresponding p-values for various heart tissue sections in both SGA (Test Group) and SGB (Control Group). The heart tissue sections include the atrial endocardium, myocardium, and epicardium, as well as the endocardium, myocardium (spongiosum and compactum), and epicardium of the ventricle. The * and ** annotations denote statistically significant differences at the 0.05 and 0.01 levels respectively, as determined by the Mann-Whitney U test. 'ns' denotes non-significant differences.

| | SGA – Test group | | SGB – Control Group | | | |
|---|---|---|---|---|---|---|
| | Median | Range (min-max) | Median | Range (min-max) | Mann-Whitney U | *p* value |
| Atrium – Endocard | 1.500 | 1.00-2.00 | 2.000 | 1.00-3.00 | 296.2 | 0.0175 * |
| Atrium – Myocard | 1.500 | 1.00-2.00 | 2.000 | 1.00-3.00 | 263.5 | 0.0037 ** |
| Atrium – Epicard | 1.000 | 1.00-1.50 | 1.250 | 1.00-1.50 | 307 | 0.0141* |
| Ventricle – Endocard | 1.000 | 1.00-1.50 | 1.000 | 1.00-1.50 | 393 | 0.3510 |
| Ventricle – Myocard – Spongiosum | 2.000 | 1.00-3.00 | 2.000 | 1.00-3.00 | 320.5 | 0.0478 * |
| Ventricle – Myocard – Compactum | 1.500 | 1.00-2.00 | 1.5000 | 1.00-2.00 | 281 | 0.0089 ** |
| Ventricle – Epicard | 1.500 | 1.00-2.00 | 1.000 | 1.00-2.00 | 423 | 0.6968 |

## 3.4 Correlations between Ct-values and histological scores

In the present study, the relationship between reversed CT-values (40 - Ct-value) and histological scores for different layers of heart tissue in Atlantic salmon was explored.

Spearman's rank correlation was applied to ascertain these relationships. In the SGA group, PMCV displayed significant correlations in the Atrium - Myocard (rho = 0.3902, $p$ = 0.0330), Ventricle – Myocard – Spongiosum (rho = 0.5056, $p$ = 0.0044), and Ventricle - Epicard (rho = -0.4318, $p$ = 0.0172) regions ($r$ and $p$ values are given in the appendix). In contrast, no significant correlations were observed for PMCV in the SGB group. For PRV, no significant correlations were found in either the SGA or SGB groups. In the context of SAV, no significant correlations were discerned in either the SGA or SGB groups. Similarly, NUC displayed no significant correlations in either group.

**Table 3.7**. Spearman's correlation values (rho) and corresponding p-values for each examined heart tissue layer in both the SGA and SGB groups. The reported values detail the correlations between reversed Ct-values (40 – C-value) and histological scores for the presence of four different pathogens (PMCV, PRV, SAV, and NUC). For each tissue layer and pathogen, rho values are provided to show the strength and direction of these correlations, while the p-values indicate their statistical significance. Values highlighted with asterisks represent significant correlations (*p < 0.05, **p < 0.01).

| | | Atrium – Endocard | Atrium – Myocard | Atrium – Epicard | Ventricle – Endocard | Ventricle – Myocard – Spongiosum | Ventricle – Myocard – Compactum | Ventricle – Epicard |
|---|---|---|---|---|---|---|---|---|
| PMCV - SGA | Sprearman´s rho | 0,2016 | 0,3902 | -0,02152 | 0,1326 | 0,5056 | -0,1797 | -0,4318 |
| | *p* value | 0,2855 | 0,0330 * | 0,9101 | 0,4850 | 0,0044 ** | 0,3419 | 0,0172 * |
| PMCV - SGB | Sprearman´s rho | -0,03750 | -0,08890 | 0,1015 | -0,3173 | 0,2765 | -0,2425 | 0,3389 |
| | *p* value | 0,8440 | 0,6404 | 0,5934 | 0,0876 | 0,1391 | 0,1967 | 0,0669 |
| PRV - SGA | Sprearman´s rho | -0,3331 | -0,9151 | -0,3339 | 0,1147 | 0,04897 | 0,1546 | 0,008966 |
| | *p* value | 0,0721 | 0,6305 | 0,0713 | 0,5463 | 0,7972 | 0,4145 | 0,9625 |
| PRV - SGB | Sprearman´s rho | -0,3523 | -0,03072 | -0,1734 | 0,08600 | 0,1377 | -0,1352 | 0,1945 |
| | *p* value | 0,0562 | 0,8720 | 0,3595 | 0,6514 | 0,4682 | 0,4761 | 0,3030 |
| SAV - SGA | Sprearman´s rho | -0,08841 | -0,05997 | 0,01913 | 0,07192 | 0,05337 | 0,1634 | 0,1991 |
| | *p* value | 0,7109 | 0,8017 | 0,9362 | 0,7632 | 0,8219 | 0,4913 | 0,4000 |
| SAV - SGB | Sprearman´s rho | -0,1091 | -0,02471 | 0,3712 | -0,1826 | -0,1386 | -0,2199 | -0,01277 |
| | *p* value | 0,8214 | 0,9655 | 0,3810 | 0,6571 | 0,7500 | 0,5952 | >0,9999 |
| NUC - SGA | Sprearman´s rho | -0,05307 | -0,09432 | -0,06219 | 0,1889 | -0,08337 | -0,008414 | -0,009323 |
| | *p* value | 0,7806 | 0,6200 | 0,7441 | 0,3173 | 0,6614 | 0,9648 | 9610 |
| NUC - SGB | Sprearman´s rho | -0,1478 | 0,07256 | 0,2635 | -0,2302 | 0,1555 | -0,08304 | 0,3236 |
| | *p* value | 0,4358 | 0,7032 | 0,1594 | 0,2208 | 0,4119 | 0,6627 | 0,0811 |

## 3.5 Histology

The histological scores within both the Test Group (SGA) and Control Group (SGB) varied, as evidenced by the results presented in the appendix. As depicted in Figure 3.17, 3.18 and 3.19, there is an overall higher histopathological score in the control group (SGB) versus the test group (SGA). Figures 3.20-3.24 represent the most severe cases within each group. The layers with the highest score of pathological changes were predominantly found in the atrium and the spongiosum layers of the ventricle.

Overall, both the atrium and ventricle displayed some level of pathological changes in all individuals examined. No fish was given the score = 0, meaning all tissue exhibited at least some degree of change (Figure 3.17). The observed pathological changes were compatible with CMS lesions, characterized by the infiltration of inflammatory cells infiltrating the subendocardium of the spongy regions, concomitant with degeneration and necrosis of the spongy myocardial tissue. In individuals with severe infection, the atrium displayed the highest degree of inflammation and cell infiltration (Figure 3.23), although significant degeneration was also observed in the ventricle (Figure 3.24).

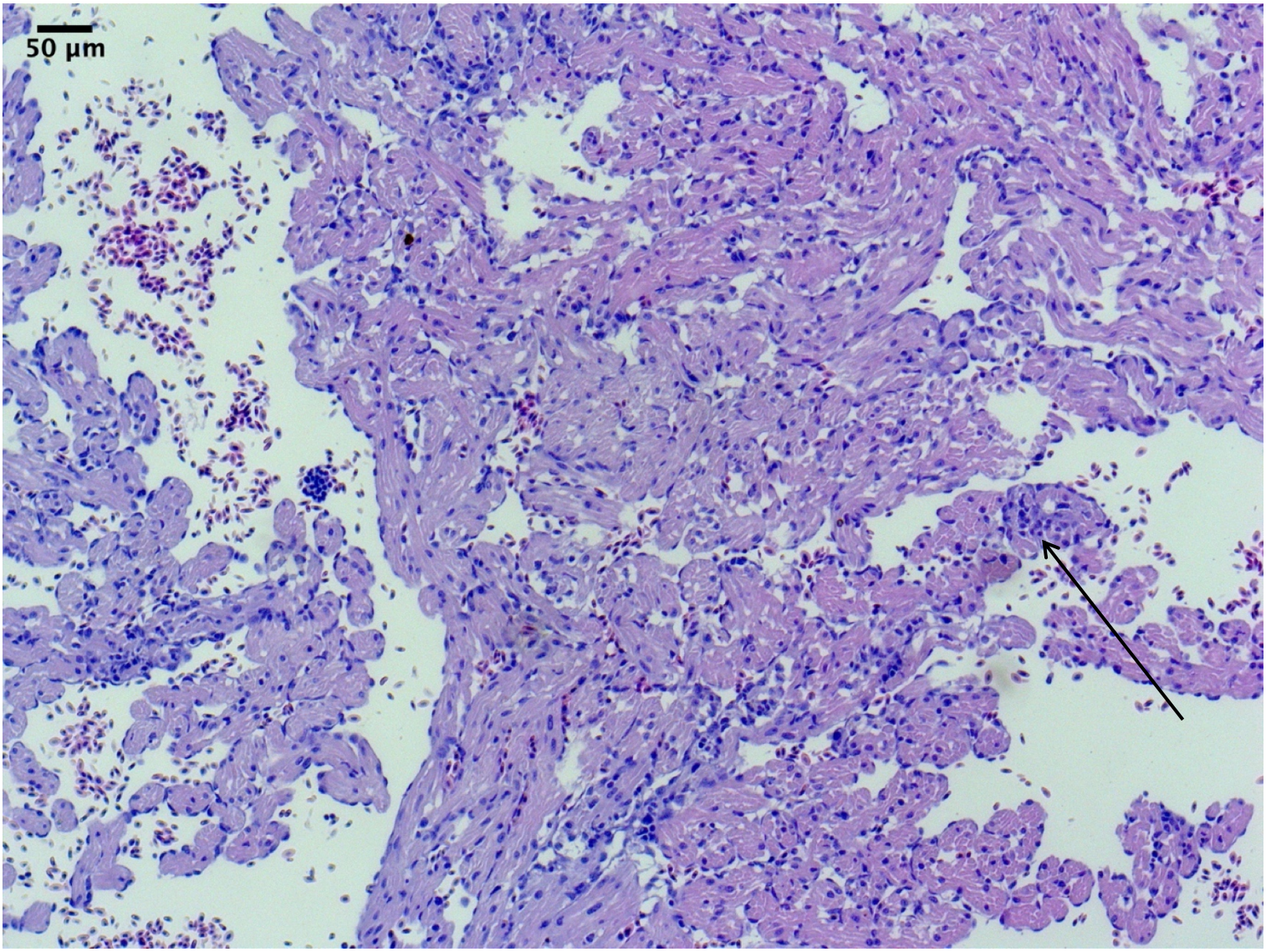


**Figure 3.20**. Histological section of fish nr. 42 from **test group**, with mild pathological changes. A mildly infiltration of inflammatory cells was observed in the myocardium. Scores within the different atrium layers were given; endocard = 1, myocard = 1-2, epicard = 1. Scale: rod = 50µm.

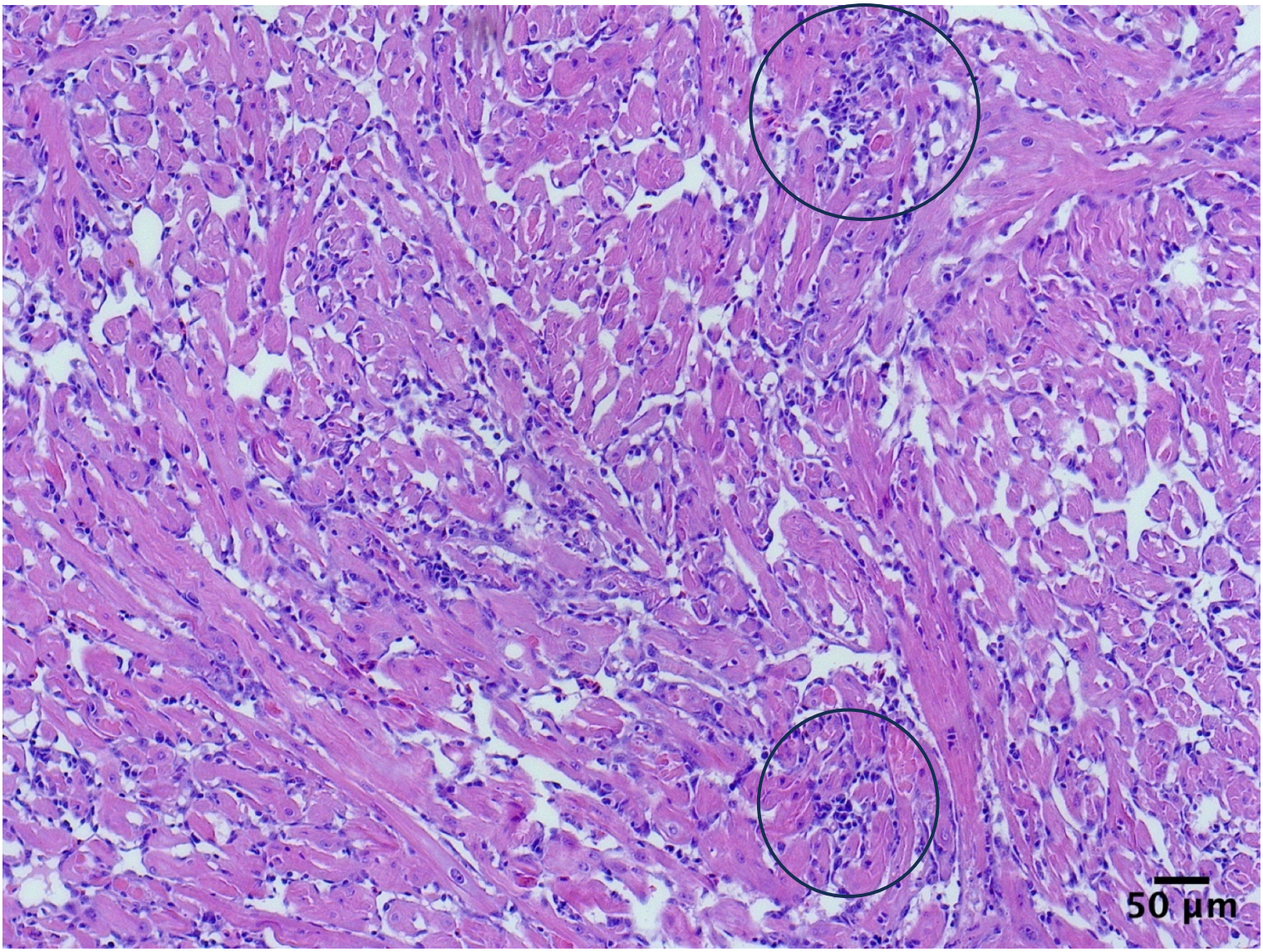


**Figure 3.21**. Histological section of fish nr. 11 from **test group**, with mild pathological changes in the ventricle. The spongiosum and compactum layers of the ventricle show mild infiltration of inflammatory cells. A mild infiltration of inflammatory cells was observed in the myocardium. Scores within the different ventricle layers were given; endocard = 1, myocard – spongiosum = 2, myocard – compactum = 1-2, epicard = 2.
Scale: rod = 50µm.

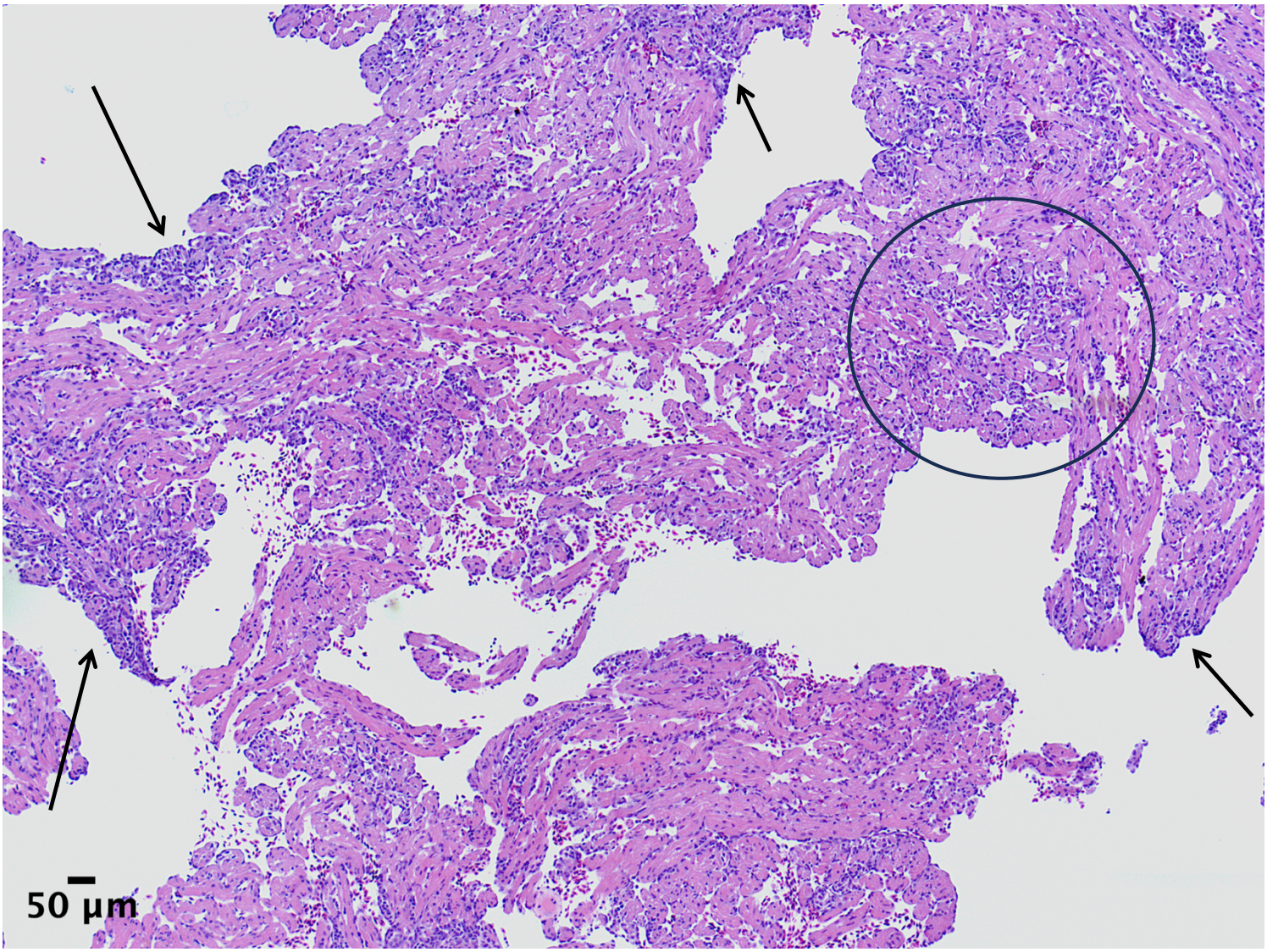


**Figure 3.22**. Histological section of fish nr. 35 from **test group**, with mild to severe pathological changes. Mainly the myocardium and endocard of the atrium are affected, with mild changes in the epicard. These observations representing the most severe case in the test group. Scores within the different atrium layers were given; endocard = 2, myocard = 2-3, epicard = 1. Scale: rod = 50µm.

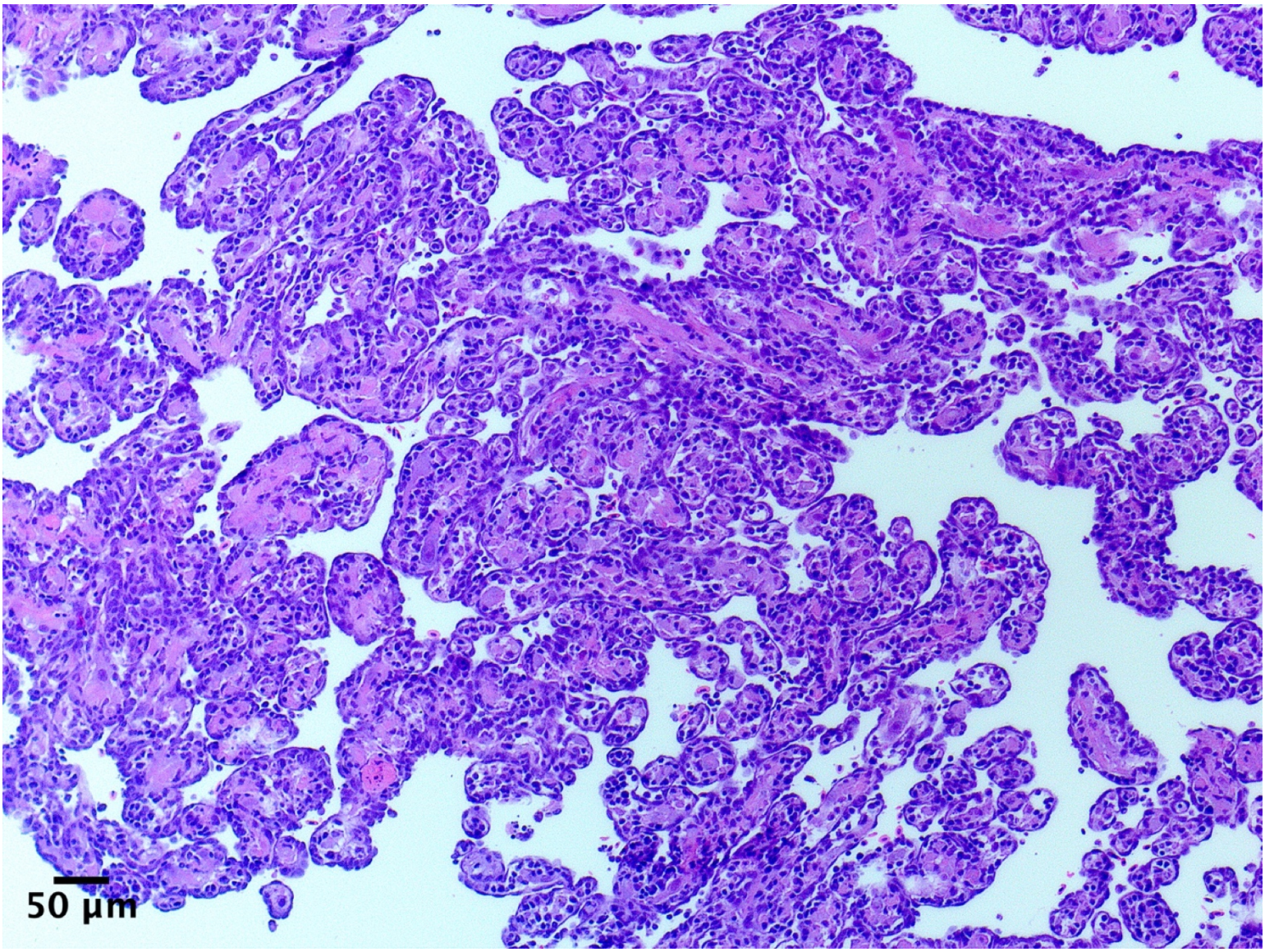


**Figure 3.23**. Histological section of fish nr. 57 from **control group**, with severe pathological changes. All layers in the atrium affected given the heart setion designation pancarditt. These observations representing the most severe case in the control group. Scores within the different atrium layers were given; endocard = 3, myocard = 3, epicard = 3. Scale: rod = 50µm.

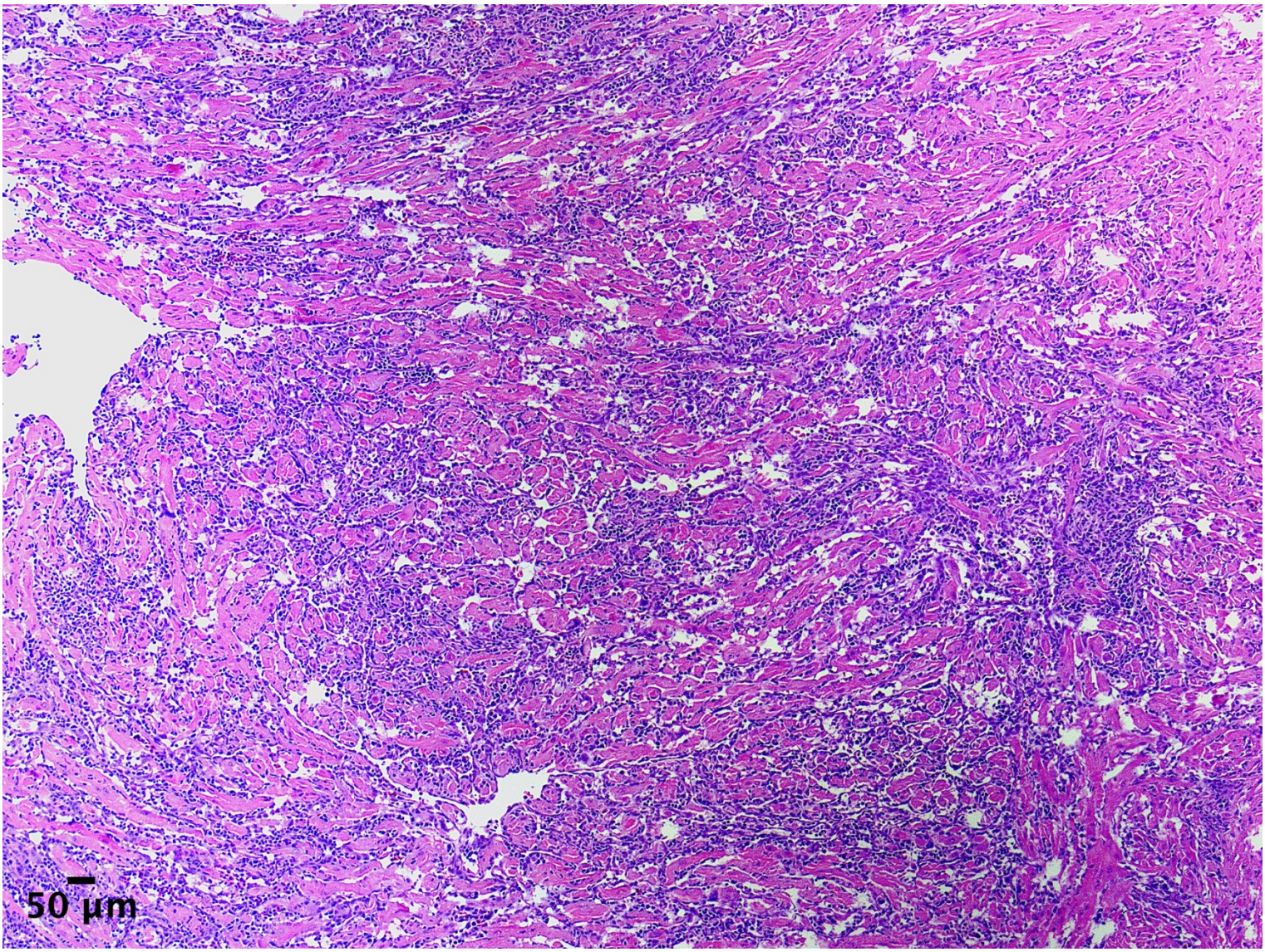


**Figure 3.24**. Histological section of fish nr. 57 from the **control group**, with severe pathological changes in the ventricle. The spongiosum layer and endocard of the ventricle show heavy infiltration of inflammatory cells. Scores within the different ventricle layers were given; endocard = 2-3, myocard – spongiosum = 2-3, myocard – compactum = 2, epicard = 2. Scale: rod = 50µm.

# 4. Discussion

This is a study of the putative effects of Quantitative Trait Loci (QTL) on the development of cardiomyopathy syndrome (CMS) in Atlantic salmon (*Salmo salar*) naturally infected with Piscine Myocarditis Virus (PMCV), in marine production. The primary aim was to examine whether the chosen QTL in salmon brood fish affects the resistance against infection with PMCV and the subsequent development of CMS. The QTL impact was assessed by tracking recapture rate, growth parameters such as weight, length, and condition factor, disease development as evidenced by histopathological changes, and assessing the presence of PMCV and other pathogens in salmon with and without the QTL.

## 4.1 The role of QTL on salmon health

The findings from this study provide a complex and multifaceted picture of the role of PMCV-QTL in salmon health and survival. It was observed that at site G, all fish, irrespective of the presence of the QTL, were infected with PMCV. These data suggest that the prevalence of infection does not differ between the groups (Figure 3.16). Yet, when considering the heightened mortality in the control group at site G post-CMS detection (Figure 3.6) combined with the data from histology and viral qPCR (viral load), a distinct pattern appears. These factors collectively reveal a positive trend of the PMCV-QTL, as the test group with CMS showed lower mortality rates, reduced viral loads, and less heart pathology compared to the control group.

It is crucial to acknowledge potential factors that could influence the accuracy of these findings. This especially applies to the recapture rate and registration of mortalities within the test and control groups. The methodology used PIT registration to identify individual fish and their genetics during the sea phase and at harvest. Throughout the production there were reported problems with the PIT readers, especially at site T. This probably resulted in instances where the deaths of PIT-tagged fish were not registered, thus affecting the reliability of the data. Also, the fish were manually selected from the harvest line at site T. Given the challenge of manually identifying whether the fat fin has been cut off or not (indicating PIT-tagging), there is a chance that not all PIT-tagged fish were identified (Table 3.1). There is also a chance that a PIT tag could become dislodged from the fish or stop functioning during production, causing it to go undetected through registration. It is also a fact that not all PIT-tag fish at site G was identified as dead or recorded at harvest (Table 3.2). Therefore, while the observed positive mortality

trend concerning the CMS test group at site G after the CMS detection is intriguing, uncertainties surrounding the registration of deceased fish throughout the production process renders its significance questionable.

Traits of major economic relevance, including growth rate, meat quality, and persistence of diseases are influenced by various genes, environmental conditions, and the interactions between them. The objective of QTL mapping is to comprehend the influence of genes responsible for a particular trait, facilitating selective breeding programs aimed at expediting the enhancement of key traits (Naish & Hard, 2008). Understanding the degree of associated responses is crucial in making informed decisions about suitable QTL alleles responsible for resistance and averting any unwanted correlated selection response. In genetics, it is common to find that a single gene can impact multiple characteristics, a concept known as pleiotropy. On the other hand, it is also usual for several genes to work together to determine a single characteristic, a phenomenon known as polygenicity (Falconer, 1996). Despite evidence from previous research suggesting that IPNV-resistance QTL does not exhibit notable effects on crucial production parameters, including weight, length, condition factor, and fillet quality (Gheyas et al., 2010), it remains possible that different outcomes may be observed in the context of PMCV-resistance QTL.

Previous research on QTL related to Infectious pancreas necrosis virus (IPNV) resistance in Atlantic salmon has identified a major QTL in salmon that is responsible for a considerable portion of genetics variations in IPNV susceptibility (Houston et al., 2008; Moen et al., 2009). The differences in IPN mortality between fish with homozygous resistant (*QQ*-QTL) and homozygous susceptible (*qq*-QTL) were marked (Houston et al., 2008; Moen et al., 2009). The gene linked to IPN resistance, *cdh1*, located in the cell membrane, binds to IPNV, *in vitro*. It co-locates with IPNV within the liver cells of individuals with *qq*-QTL but is absent from the hepatocytes of *QQ*-QTL individuals, suggesting that cdh1 may be part of the cellular machinery that IPNV exploits for infection, acting as a co-receptor for the virus (Moen et al., 2015). In the context of fish carrying the resistant genotype, this could mean that IPNV cannot effectively attach or enter the host cell. However, it has been confirmed that while susceptible fish show significant mortality, both resistant and susceptible Atlantic salmon fry can be infected with IPNV (Reyes-López et al., 2015; Robledo et al., 2016). This indicates that resistance may not totally rely on the inability of IPNV to enter and replicate within the host cells. Instead, it appears that superior regulation of the immune response could help protect resistant fish against

the virus, as indicated by the comparison of gene expression profiles between the resistant and susceptible fish challenged with IPNV (Reyes-López et al., 2015; Robledo et al., 2016). Similarly, Timmerhaus et al. documented an elevation in the expression of genes linked to adaptive immunity among fish that were less resistant, indicating a potential genetic factor in CMS resistance. These genes are instrumental in facilitating T and B cell responses, the presentation of major histocompatibility complex (MHC) antigens, and apoptosis processes (Timmerhaus et al., 2011; Timmerhaus et al., 2012).

Beyond the discussion of the QTL's direct effect on PMCV infection and survival, it is also important to consider the influence of the QTL on other significant factors in salmon such as weight and length. The observations of divergent patterns in weight and length seen in this study, as depicted in Figures 3.8 and 3.9, indicate that the presence of QTL may be associated with variations in physical attributes, which in turn, could potentially influence the health and disease resistance of the Atlantic salmon. Body weight has been found to be correlated with immune functions in some studies (Falconer, 1996; Scotland et al., 1990), which supports the idea that body weight should be considered when looking at disease resistance in QTL analyses. Taking this into account the condition factor, a measure reflecting both physical and biological circumstances (Baxter, 1998), should be considered. The results of the present study were divergent across sites. At site T, where no PMCV was detected, the Test Group exhibited a significantly superior condition factor. However, this trend was reversed at site G, where the Control Group had a significantly higher condition factor suggesting that other factors than QTL may have influenced the condition factor.

One factor that could have influenced the results is the presence of Pancreas Disease (PD), which was detected at site T prior to harvest. PD, caused by Salmonid Alphavirus (SAV), typically infects salmon during the smolt stage in their first year at sea, often resulting in reduced growth (Taksdal et al., 2007). The presence of PD at Site T suggests the possibility that the QTL linked to PMCV resistance may also have influenced the establishment of PD in the salmon at this site. Another explanation can be the detection of both PRV1 and SAV at this site. A co-infection of these pathogens is known to occur at the same locations (Lund et al., 2016). Existing studies also have demonstrated that fish groups diagnosed with viral diseases such as HSMB or PD possess a higher risk of developing CMS (Jensen et al., 2013; Jensen et al., 2020).

This aligns with the findings of Repstad in 2011. In this study, a smolt population that was previously infected with the PRV exhibited resistance to PD in their first year at sea. This group did not show increased expression of general antiviral immune response genes (genes encoding the production of interferon and Mx-proteines), compared to a smolt population with high mortality (Repstad, 2011). Repstad (2011) suggested that the group with 100% PRV infection may have stimulated a non-specific cellular immune response before seawater transfer, possibly making them more resistant to an SAV infection in the first year after seawater transfer. Supporting this, experimental findings by Lund et al. (2016), demonstrated that primary PRV infections can potentially mitigate the disease progression of subsequent SAV infections. These findings were evidenced by lower levels of SAV RNA, less severe PD pathological lesions, and higher condition factors in co-infected groups (Lund et al., 2016).

## 4.2 Histopathological correlations with QTL

A closer histological examination of the sampled fish at site T (STE and STF groups) did not yield remarkable changes warranting further study; hence, only a subset of 20 samples from this site underwent detailed histological analysis. These results are surprising given that Pancreas Disease (PD), caused by Salmonid alphavirus (SAV), was detected at the facility before slaughtering. Since PD is known to cause changes in the heart's tissue structure (McLoughlin et al., 2002), a correlation between these changes and the presence of SAV was expected.

At site G substantial differences in histopathological changes were identified in the heart tissues of the Test and Control groups. A positive trend was observed with less pathology in the heart of the SGA group with CMS QTL. These alterations were particularly evident in certain layers of the heart, notably in the atrium endocardium, myocardium, and epicardium, as well as in the ventricles spongiosum and compactum layers of the myocardium. No significant differences were detected in the endocardium or epicardium of the ventricle. These observations echo the findings of Bruno et al. (2013), who noted that cardiac histopathological alterations initially manifest in the atrium and subsequently in the ventricle. Such changes are often characterized by mononuclear cells infiltrating the subendocardium of the spongy regions, concomitant with degeneration and necrosis of the spongy myocardial tissue (Bruno et al., 2013).

The study also showed correlations between pathogen load (PMCV, PRV, SAV and *P. theridion*), as quantified by Ct-values, and histological scores across different heart tissue layers (Table 3.7) at site G. The significant positive correlations for PMCV within multiple heart compartments, Table 3.7, could imply that an increase in viral load (lower Ct-values) coincided with more severe pathological changes within these heart layers. In the Test Group, which is considered more protected against PMCV and demonstrated higher Ct-values, only one significant positive correlation with PMCV emerged in the ventricle (Table 3.7). This singular correlation is notable and may be influenced by the observed significant fat deposition within the ventricle-epicardium, a common phenomenon in farmed Atlantic salmon (Poppe & Taksdal, 2000).

The significant negative correlation with PRV within the Test Group in the Atrium – epicard and – endocard (Table 3.5), indicates that higher viral load (lower original Ct-values) was associated with less severe histopathological changes. No significant correlations were discovered in the Control Group in the case of PRV, and no significant correlations were detected for SAV or *P. theridion* in either the Test or Control groups, implying that these pathogens might not have a similar impact on the heart tissue pathology as PMCV and PRV, or their effects may not be easily detectable through the methods utilized in this study.

These observations add to the understanding of the intricate relationship between viral load and histopathological changes in Atlantic salmon, substantiating previous studies that identified a strong correlation between PMCV viral load and the severity of cardiac lesions (Haugland et al., 2011; Løvoll et al., 2010; Timmerhaus et al., 2011). However, given the variability and complexity of these correlations across different heart regions and groups, demand investigations to unravel the underpinning mechanisms and potential impacts of QTL on fish health.

## 4.3 Density of pathogens in correlation with QTL

While understanding the relationship between histological scores and Ct-values provides valuable insights into how viral load affects different layers of heart tissue, it is also critical to consider a broader view of disease occurrence and distribution. Evaluating the prevalence of pathogens within each group allows a comprehensive understanding of their health status, providing insights into their resistance or susceptibility profiles.

The comprehensive pathogen screening using real-time RT-PCR at the facilities, Site T and Site G, provides insights into the differential presence and impacts of various pathogens in Atlantic salmon. The most crucial divergence between these sites is the lack of PMCV detection at site T. Given the typical distribution of PMCV along the Norwegian coastline (Kongtorp et al., 2005), the no detection of PMCV at site T is an essential factor to consider.

The presence of PRV1 prevalence of 100 % across all samples from both sites, Table 3.4, is a common observation in salmon farms. Site G exhibits lower Ct-values than site T. Data from site T, (Table 3.5), show that the Test Group presents a higher average Ct-value than the Control Group. A pattern emerges with SAV, where the Test Group at Site T may be associated with a higher prevalence and lower average Ct-value than the Control Group, albeit these differences not achieving statistical significance (Figure 3.12). However, the presence of PMCV at Site G within both the Test and Control groups presents a key point of interest for further exploration, especially considering the selective breeding for PMCV resistance in the Test Group.

Indeed, the relationship between the two groups and the detection of other pathogens at Site G unveils a significant divergence that warrants further investigation. PRV1, for instance, implies a somewhat higher response in the Test Group (Table 3.5), suggesting a potential impact of the QTL in this context. The SAV situation furthers this narrative, as the Test Group is associated with a lower minimum and average Ct-value than the Control Group. Observations concerning *Paranucleospora theridion* detection contribute an additional layer of complexity to this analysis. A statistically significant difference exists in average viral load between the two groups, as the Test Group manifests a slightly lower average load (Table 3.5).

In the discussion about the influence of QTL on heart health and the association histopathological changes, it is crucial to address the importance of the heart-gill axis on the overall health status of the salmon. The intricate interplay between these two primary organs can significantly influence the fish's viability. Given the heart's role in pumping blood throughout the fish's body, any cardiac distress could impact the efficiency of blood oxygenation in the gills, ultimately compromising the fish's respiratory health. Vice versa the gills influence the heart function. Any disturbance in the gill function can lead to a reduction in the oxygen levels in the blood, thereby increasing cardiac stress. Fish exposed to stress is shown to increase mortality during a CMS outbreak (Skrudland et al., 2002). In this context, understanding the role of the QTL in determining the severity of histopathological changes

within the heart may also provide insight into its potential impacts on gill health. Our results indicate that the presence of the QTL in Atlantic salmon may be linked to less severe histopathological changes in the heart (Figures 3.18 and 3.19). This finding suggests that fish carrying the QTL might experience reduced cardiac stress, which could in turn promote better gill function due to the improved heart-gill axis performance.

# 5. Conclusion

This study aimed to clarify the potential role of Quantitative Trait Loci (QTL) in the development of cardiomyopathy syndrome (CMS) in Atlantic salmon (*Salmo salar*) naturally infected with Piscine Myocarditis Virus (PMCV). The QTL did not provide a complete resistance PMCV infection, but it did exhibit a several beneficial effects in regards to CMS disease severity both in mitigating viral load and heart tissue pathology. Importantly, there was also a positive trend observed in the mortality rate following the CMS detection at site G. The divergence of the observations in viral load, heart tissue pathology, and a declining trend in mortality post-CMS detection at site G points to a potential protective role of the PMCV-QTL.

The specific QTL in this study, may reduce cardiac stress and promote better gill function, potentially affecting resistance to other diseases. This further highlights the multifaceted nature of genetic and environmental interplay in disease resistance. The divergent roles of QTL in heart health and resistance to infectious agents contrast with established understandings of QTL, such as those related to Infectious Pancreas Necrosis Virus (IPNV) resistance in Atlantic salmon. This underscores the genetic complexity and the potential pleiotropic and polygenic effects in the QTLs function.

Considering the uncertainties associated with PIT-tag registration, the study cannot definitively determine the mortality rate. At Site G, even though a noticeable trend in mortality data appeared after CMS detection, the registration issues imply it can't be considered statistically significant. Not all PIT-tag fish were recorded at the harvesting, and the ability to make a more detailed conclusion is limited due to the potential sources of error. However, what we observed at Site G is important and should be looked into more.

Our findings signify the need for further research to validate these observations and explore potential applications in selective breeding programs. The complexity and influence of QTL on Atlantic salmon's overall health status, particularly concerning CMS and PMCV, demonstrate that a deeper understanding of genetic traits and their interplay with disease resistance is essential for optimal fish health management.

## 5.1 Future perspective

The nuanced impact of Quantitative Trait Loci (QTL) on the health of Atlantic salmon (*Salmo salar*), especially its apparent mitigating effect on heart tissue pathology and potential implications on susceptibility to other pathogens, offers new avenues for further research. The following recommendations aim to provide guidance for future studies.

Sampling Modifications: To thoroughly investigate the QTL's effect on gill health, immediate sample collection post-euthanasia is crucial due to the autolytic nature of gills. This involves separating QTL fish from other experimental fish before harvest. Similar protocol adjustments are required for more accurate macroscopic observations of the liver.

Benchmark Genetics Project Refinements: The current approach of statistical analysis when harvesting has limitations and could benefit from monitoring fish from the smolt stage to harvest. Screening smolts before transfer to sea can give deeper insight into possible positive correlations with co-infections. Maintaining cohort studies, wherein all fish are placed in the same pen and subjected to identical conditions, can yield more robust comparative data. Additionally, examining gene regulation, potential differences between QTL and non-QTL groups, and antibody production in response to PMCV are promising areas for future work.

Mortality Evaluation: Future research should consider analysing fish mortality throughout the production cycle by continuous register all PIT-tagged fish that die, to better understand the QTL's effect on overall survival rates.

Understanding QTL Specificity: There is a need for further investigations to ascertain whether resistance to PMCV in QTL fish is due to higher antibody production, a nonspecific immune response, or other yet-unidentified factors.

Bacterial Disease Examination: Future studies should also explore the QTL's impact on other diseases without effective vaccines, such as *Moritella viscosa*, *Tenacibaculum*, *Yersinia*, and the re-emerging *Renibacterium*. Additionally, assessing the effectiveness of current vaccines on both QTL and non-QTL groups could provide valuable insights into the broader implications of QTL on disease resistance.

Co-infection Analysis: Understanding the interaction between multiple infections is also a crucial aspect for future studies. As demonstrated in this study with PRV1 and SAV, the presence of co-infections could have significant implications for disease progression and resistance in both QTL and non-QTL groups. The findings by Repstad (2011) and the experimental results by Lund et al. (2016) suggest that past infections could play a role in shaping the future immune response of the salmon. In line with these findings, future research could aim to investigate how early PRV infections (or other pathogens) might affect subsequent susceptibility to diseases like CMS. This could potentially broaden our understanding of the role of QTL in multi-pathogen environments and their implications for the health of salmon.

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# 7. Appendix

## 7.1 Log 2 and reversed ct-values

| "Table Analyzed" | "Log2 - PRV; STE, STF" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0076 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "736 , 1094" |
| "    Mann-Whitney U" | 271 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "5.534, n=30" |
| "    Median of column B" | "7.694, n=30" |
| "    Difference: Actual" | 2,160 |
| "    Difference: Hodges-Lehmann" | 1,956 |

| "Table Analyzed" | "Log2 - SAV; SGA, SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,4223 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "329 , 136" |
| "    Mann-Whitney U" | 81 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "9.618, n=20" |
| "    Median of column B" | "8.911, n=10" |
| "    Difference: Actual" | -0,7068 |
| "    Difference: Hodges-Lehmann" | -1,366 |

| "Table Analyzed" | "Density - PRV STE/STF" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0532 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "784 , 1046" |
| "    Mann-Whitney U" | 319 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "14.41, n=30" |
| "    Median of column B" | "16.03, n=30" |
| "    Difference: Actual" | 1,628 |
| "    Difference: Hodges-Lehmann" | 1,344 |

| "Table Analyzed" | "Density - PRV SGA/SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,9737 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "917.5 , 912.5" |
| "    Mann-Whitney U" | 447,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "20.25, n=30" |
| "    Median of column B" | "20.15, n=30" |
| "    Difference: Actual" | -0,1000 |
| "    Difference: Hodges-Lehmann" | 0,000 |

| "Table Analyzed" | "Log2 - SAV; STE, STF" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,1113 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "308 , 395" |
| "    Mann-Whitney U" | 118 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "2.701, n=19" |
| "    Median of column B" | "3.335, n=18" |
| "    Difference: Actual" | 0,6336 |
| "    Difference: Hodges-Lehmann" | 1,161 |

| "Table Analyzed" | "Log2 - SAV; SGA, SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,4223 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "329 , 136" |
| "    Mann-Whitney U" | 81 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "9.618, n=20" |
| "    Median of column B" | "8.911, n=10" |
| "    Difference: Actual" | -0,7068 |
| "    Difference: Hodges-Lehmann" | -1,366 |

| "Table Analyzed" | "Density - SAV STE/STF" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,8926 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "366 , 337" |
| "    Mann-Whitney U" | 166 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "5.247, n=19" |
| "    Median of column B" | "4.693, n=18" |
| "    Difference: Actual" | -0,5544 |
| "    Difference: Hodges-Lehmann" | -0,06215 |

| "Table Analyzed" | "Density - SAV SGA/SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,3484 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "309 , 97" |
| "    Mann-Whitney U" | 61 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "12.55, n=20" |
| "    Median of column B" | "12.15, n=8" |
| "    Difference: Actual" | -0,4000 |
| "    Difference: Hodges-Lehmann" | -1,700 |

| "Table Analyzed" | "Log2 - PMCV; SGA, SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | <0.0001 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | **** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "564 , 1266" |
| "    Mann-Whitney U" | 99 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "5.246, n=30" |
| "    Median of column B" | "13.85, n=30" |
| "    Difference: Actual" | 8,609 |
| "    Difference: Hodges-Lehmann" | 8,129 |

| "Table Analyzed" | "Density - PRV SGA/SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,9737 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "917.5 , 912.5" |
| "    Mann-Whitney U" | 447,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "20.25, n=30" |
| "    Median of column B" | "20.15, n=30" |
| "    Difference: Actual" | -0,1000 |
| "    Difference: Hodges-Lehmann" | 0,000 |

| "Table Analyzed" | "Log2 - NUC; SGA, SGB" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0006 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | *** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "689 , 1141" |
| "    Mann-Whitney U" | 224 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "7.538, n=30" |
| "    Median of column B" | "9.807, n=30" |
| "    Difference: Actual" | 2,269 |
| "    Difference: Hodges-Lehmann" | 2,176 |

| "Table Analyzed" | "Density - NUC" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | <0.0001 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | **** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "639 , 1191" |
| "    Mann-Whitney U" | 174 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "16.30, n=30" |
| "    Median of column B" | "18.52, n=30" |
| "    Difference: Actual" | 2,217 |
| "    Difference: Hodges-Lehmann" | 2,898 |

## 7.2 Histological scores

| SGA | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Atrium | | | Ventrikkel | | | |
| Fish nr. | Endocard | Myocard | Epicard | Endocard | Myocard - Spongiosum | Myocard - Compactum | Epicard |
| 2 | 2,5 | 2 | 2 | 1,5 | 1,5 | 2 | 2,5 |
| 3 | 1 | 1,5 | 2 | 1 | 1,5 | 1,5 | 2 |
| 6 | 1 | 1,5 | 1 | 1 | 1,5 | 1 | 1,5 |
| 8 | 2 | 1,5 | 1,5 | 1 | 2 | 1,5 | 1 |
| 10 | 3 | 2 | 2 | 1 | 2 | 1 | 1 |
| 11 | 1 | 1 | 1 | 1 | 2 | 1,5 | 2 |
| 12 | 1,5 | 1,5 | 1 | 1 | 2 | 2 | 2 |
| 15 | 2,5 | 2,5 | 1 | 1 | 2 | 2 | 2 |
| 19 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 24 | 1,5 | 1,5 | 1 | 1 | 1,5 | 1 | 1 |
| 25 | 2,5 | 2,5 | 1 | 1 | 2,5 | 2,5 | 1 |
| 28 | 1 | 1,5 | 1 | 1 | 1 | 1 | 1 |
| 31 | 2,5 | 2,5 | 1,5 | 2,5 | 2,5 | 2 | 2 |
| 33 | 1,5 | 2 | 1 | 1,5 | 2 | 2 | 1 |
| 35 | 2 | 2,5 | 1 | 2 | 2,5 | 1,5 | 1 |
| 36 | 1 | 1,5 | 1 | 1 | 1,5 | 1,5 | 1 |
| 37 | 1,5 | 2 | 1,5 | 1 | 1,5 | 1,5 | 2 |
| 38 | 1,5 | 1,5 | 1 | 1 | 1 | 1,5 | 1 |
| 39 | 2 | 2 | 1 | 1 | 1,5 | 1,5 | 1 |
| 40 | 1 | 1 | 1 | 1 | 1,5 | 1,5 | 1,5 |
| 41 | 1,5 | 1,5 | 1 | 1 | 1 | 2 | 1,5 |
| 42 | 1 | 1,5 | 1 | 1,5 | 2 | 1,5 | 1 |
| 46 | 1,5 | 2 | 1 | 1,5 | 2,5 | 2 | 2 |
| 47 | 2 | 2,5 | 1 | 2,5 | 2 | 2 | 1,5 |
| 48 | 1,5 | 1,5 | 1 | 1 | 1 | 2,5 | 1,5 |
| 49 | 2 | 2,5 | 1 | 1,5 | 2 | 2,5 | 1,5 |
| 51 | 2 | 1,5 | 1 | 2 | 2,5 | 2,5 | 2 |
| 53 | 2 | 2,5 | 1 | 1,5 | 2,5 | 1,5 | 1,5 |
| 54 | 2 | 1,5 | 1 | 1,5 | 1,5 | 2 | 1 |
| 55 | 2,5 | 2,5 | 1,5 | 2,5 | 2,5 | 2 | 1 |

| SGB | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Atrium | | | Ventrikkel | | | |
| Fish nr. | Endocard | Myocard | Epicard | Endocard | Myocard - Spongiosum | Myocard - Compactum | Epicard |
| 1 | 2,5 | 2,5 | 1 | 1 | 2,5 | 1 | 1 |
| 4 | 2 | 3 | 1 | 1 | 3 | 1 | 2,5 |
| 5 | 2 | 1,5 | 2 | 1 | 2 | 1 | 1,5 |
| 7 | 1,5 | 1,5 | 1 | 1 | 1 | 1 | 1 |
| 9 | 2 | 2 | 1 | 1 | 1 | 2 | 1 |
| 13 | 2 | 2 | 2 | 1 | 2 | 1 | 1 |
| 14 | 2 | 2 | 1 | 1 | 2,5 | 1 | 2 |
| 16 | 2,5 | 3 | 2,5 | 1 | 2 | 1,5 | 1 |
| 17 | 1,5 | 2 | 1,5 | 1 | 2 | 1 | 2 |
| 18 | 2 | 2 | 1,5 | 1 | 2,5 | 1 | 1 |
| 20 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| 21 | 2 | 2 | 2 | 1 | 1,5 | 1 | 1 |
| 22 | 2 | 2 | 1 | 1 | 2,5 | 1,5 | 1 |
| 23 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| 26 | 1,5 | 1 | 1 | 2 | 1,5 | 1 | 1 |
| 27 | 2 | 2 | 1 | 1 | 2 | 1 | 1 |
| 29 | 3 | 2,5 | 1,5 | 2,5 | 2,5 | 2,5 | 1,5 |
| 30 | 1 | 2,5 | 2,5 | 2,5 | 2,5 | 1,5 | 1 |
| 32 | 3 | 3 | 2,5 | 2,5 | 2,5 | 1,5 | 2 |
| 34 | 1,5 | 2 | 2,5 | 1 | 1 | 1,5 | 2 |
| 43 | 2 | 2,5 | 2,5 | 2 | 3 | 1,5 | 2 |
| 44 | 2,5 | 3 | 1 | 2 | 2,5 | 1,5 | 1,5 |
| 45 | 1,5 | 1,5 | 1 | 1,5 | 2 | 1,5 | 2 |
| 50 | 2 | 3 | 2,5 | 2,5 | 3 | 2 | 2,5 |
| 52 | 2 | 2,5 | 1 | 1,5 | 2 | 1,5 | 1 |
| 56 | 2,5 | 2,5 | 1 | 2 | 2 | 2 | 1 |
| 57 | 3 | 3 | 3 | 2,5 | 2,5 | 2 | 2 |
| 58 | 1,5 | 1,5 | 1 | 1,5 | 2 | 1,5 | 1 |
| 59 | 2,5 | 2,5 | 1,5 | 2 | 2,5 | 2 | 1 |
| 60 | 2,5 | 2,5 | 1,5 | 2 | 2,5 | 1,5 | 1,5 |

## 7.3 Analysed - Histological scores

| "Table Analyzed" | "(atrium - endocard)" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0175 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | * |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "761.5 , 1069" |
| "    Mann-Whitney U" | 296,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.500, n=30" |
| "    Median of column B" | "2.000, n=30" |
| "    Difference: Actual" | 0,5000 |
| "    Difference: Hodges-Lehmann" | 0,5000 |

| "Table Analyzed" | (atrium-myocard) |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0037 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "728.5 , 1102" |
| "    Mann-Whitney U" | 263,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.500, n=30" |
| "    Median of column B" | "2.000, n=30" |
| "    Difference: Actual" | 0,5000 |
| "    Difference: Hodges-Lehmann" | 0,5000 |

| "Table Analyzed" | "(atrium - epicard)" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0141 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | * |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "772 , 1058" |
| "    Mann-Whitney U" | 307 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.000, n=30" |
| "    Median of column B" | "1.250, n=30" |
| "    Difference: Actual" | 0,2500 |
| "    Difference: Hodges-Lehmann" | 0,000 |

| "Table Analyzed" | "(ventricle - endocard)" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,3510 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "858 , 972" |
| "    Mann-Whitney U" | 393 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.000, n=30" |
| "    Median of column B" | "1.000, n=30" |
| "    Difference: Actual" | 0,000 |
| "    Difference: Hodges-Lehmann" | 0,000 |

| "Table Analyzed" | "(ventricle - myocard -spong)" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0478 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | * |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "785.5 , 1045" |
| "    Mann-Whitney U" | 320,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "2.000, n=30" |
| "    Median of column B" | "2.000, n=30" |
| "    Difference: Actual" | 0,000 |
| "    Difference: Hodges-Lehmann" | 0,5000 |

| "Table Analyzed" | "(ventricle - myocard - comp)" |
|---|---|
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0089 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "1084 , 746" |
| "    Mann-Whitney U" | 281 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.500, n=30" |
| "    Median of column B" | "1.500, n=30" |
| "    Difference: Actual" | 0,000 |
| "    Difference: Hodges-Lehmann" | -0,5000 |

| | |
|---|---|
| "Table Analyzed" | "(ventricle - epicard)" |
| | |
| "Column B" | SGB |
| vs. | vs. |
| "Column A" | SGA |
| | |
| "Mann Whitney test" | |
| "  P value" | 0,6968 |
| "  Exact or approximate P value?" | Exact |
| "  P value summary" | ns |
| "  Significantly different (P < 0.05)?" | No |
| "  One- or two-tailed P value?" | Two-tailed |
| "  Sum of  ranks in column A,B" | "942 , 888" |
| "  Mann-Whitney U" | 423 |
| | |
| "Difference between medians" | |
| "  Median of column A" | "1.500, n=30" |
| "  Median of column B" | "1.000, n=30" |
| "  Difference: Actual" | -0,5000 |
| "  Difference: Hodges-Lehmann" | 0,000 |

## 7.4 Histological scores vs. reversed Ct-values

Correlation of Histoscore - SGA - PMCV (Reverced Ct-values)

| | CT-value (Reverced_PMCV_SGA) vs. Atrium - Endocard | CT-value (Reverced_PMCV_SGA) vs. Atrium - Myocard | CT-value (Reverced_PMCV_SGA) vs. Atrium - Epicard | CT-value (Reverced_PMCV_SGA) vs. Ventricle - Endocard | CT-value (Reverced_PMCV_SGA) vs. Ventricle - Myocard - Spongiosum | CT-value (Reverced_PMCV_SGA) vs. Ventricle - Myocard - Compactum | CT-value (Reverced_PMCV_SGA) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| Spearman r | | | | | | | |
| r | 0,2016 | 0,3902 | −0,02152 | 0,1326 | 0,5056 | −0,1797 | −0,4318 |
| 95% confidence interval | -0.1819 to 0.5318 | 0.02373 to 0.6643 | -0.3884 to 0.3512 | -0.2496 to 0.4790 | 0.1669 to 0.7376 | -0.5154 to 0.2038 | -0.6913 to -0.07362 |
| P value | | | | | | | |
| P (two-tailed) | 0,2855 | 0,0330 | 0,9101 | 0,4850 | 0,0044 | 0,3419 | 0,0172 |
| P value summary | ns | * | ns | ns | ** | ns | * |
| Exact or approximate P value? | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| Significant? (alpha = 0.05) | No | Yes | No | No | Yes | No | Yes |
| Number of XY Pairs | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

Correlation of Histoscore - SGB - PMCV (Reverced Ct-values)

| | CT-value (Reverced_PMCV_SGB) vs. Atrium - Endocard | CT-value (Reverced_PMCV_SGB) vs. Atrium - Myocard | CT-value (Reverced_PMCV_SGB) vs. Atrium - Epicard | CT-value (Reverced_PMCV_SGB) vs. Ventricle - Endocard | CT-value (Reverced_PMCV_SGB) vs. Ventricle - Myocard - Spongiosum | CT-value (Reverced_PMCV_SGB) vs. Ventricle - Myocard - Compactum | CT-value (Reverced_PMCV_SGB) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| Spearman r | | | | | | | |
| r | −0,03750 | −0,08890 | 0,1015 | −0,3173 | −0,2765 | −0,2425 | 0,3389 |
| 95% confidence interval | -0.4019 to 0.3371 | -0.4442 to 0.2906 | -0.2789 to 0.4544 | -0.6150 to 0.05967 | -0.5865 to 0.1041 | -0.5620 to 0.1400 | -0.03543 to 0.6299 |
| P value | | | | | | | |
| P (two-tailed) | 0,8440 | 0,6404 | 0,5934 | 0,0876 | 0,1391 | 0,1967 | 0,0669 |
| P value summary | ns | ns | ns | ns | ns | ns | ns |
| Exact or approximate P value? | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| Significant? (alpha = 0.05) | No | No | No | No | No | No | No |
| Number of XY Pairs | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

Correlation of Histoscore - SGA - PRV(Reverced Ct-values)

| | CT-value (Reverced_PRV_SGA) vs. Atrium - Endocard | CT-value (Reverced_PRV_SGA) vs. Atrium - Myocard | CT-value (Reverced_PRV_SGA) vs. Atrium - Epicard | CT-value (Reverced_PRV_SGA) vs. Ventricle - Endocard | CT-value (Reverced_PRV_SGA) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverced_PRV_SGA) vs. Ventricle - Myocard - Compactu | CT-value (Reverced_PRV_SGA) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| Spearman r | | | | | | | |
| r | −0,3331 | −0,09151 | −0,3339 | 0,1147 | 0,04897 | 0,1546 | 0,008966 |
| 95% confidence interval | -0.6259 to 0.04205 | -0.4463 to 0.2882 | -0.6265 to 0.04108 | -0.2666 to 0.4649 | -0.3269 to 0.4114 | -0.2284 to 0.4962 | -0.3622 to 0.3776 |
| P value | | | | | | | |
| P (two-tailed) | 0,0721 | 0,6305 | 0,0713 | 0,5463 | 0,7972 | 0,4145 | 0,9625 |
| P value summary | ns | ns | ns | ns | ns | ns | ns |
| Exact or approximate P value? | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| Significant? (alpha = 0.05) | No | No | No | No | No | No | No |
| Number of XY Pairs | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

Correlation of Histoscore - SGB - PRV (Reverced Ct-values)

| | CT-value (Reverced_PRV_SGB) vs. Atrium - Endocard | CT-value (Reverced_PRV_SGB) vs. Atrium - Myocard | CT-value (Reverced_PRV_SGB) vs. Atrium - Epicard | CT-value (Reverced_PRV_SGB) vs. Ventricle - Endocard | CT-value (Reverced_PRV_SGB) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverced_PRV_SGB) vs. Ventricle - Myocard - Compactu | CT-value (Reverced_PRV_SGB) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| **Spearman r** | | | | | | | |
| **r** | −0,3523 | −0,03072 | −0,1734 | 0,08600 | 0,1377 | −0,1352 | 0,1945 |
| **95% confidence interval** | -0.6390 to 0.02028 | -0.3962 to 0.3431 | -0.5106 to 0.2100 | -0.2933 to 0.4419 | -0.2447 to 0.4830 | -0.4811 to 0.2471 | -0.1890 to 0.5266 |
| | | | | | | | |
| **P value** | | | | | | | |
| **P (two-tailed)** | 0,0562 | 0,8720 | 0,3595 | 0,6514 | 0,4682 | 0,4761 | 0,3030 |
| **P value summary** | ns | ns | ns | ns | ns | ns | ns |
| **Exact or approximate P value?** | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| **Significant? (alpha = 0.05)** | No | No | No | No | No | No | No |
| | | | | | | | |
| **Number of XY Pairs** | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

Correlation of Histoscore - SGA - SAV (Reverced Ct-values)

| | CT-value (Reverceed_SAV_SGA) vs. Atrium - Endocard | CT-value (Reverceed_SAV_SGA) vs. Atrium - Myocard | CT-value (Reverceed_SAV_SGA) vs. Atrium - Epicard | CT-value (Reverceed_SAV_SGA) vs. Ventricle - Endocard | CT-value (Reverceed_SAV_SGA) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverceed_SAV_SGA) vs. Ventricle - Myocard - Compactu | CT-value (Reverceed_SAV_SGA) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| **Spearman r** | | | | | | | |
| **r** | −0,08841 | −0,05997 | 0,01913 | 0,07192 | 0,05377 | 0,1634 | 0,1991 |
| **95% confidence interval** | -0.5213 to 0.3806 | -0.5001 to 0.4048 | -0.4384 to 0.4688 | -0.3947 to 0.5091 | -0.4100 to 0.4954 | -0.3136 to 0.5745 | -0.2799 to 0.5988 |
| | | | | | | | |
| **P value** | | | | | | | |
| **P (two-tailed)** | 0,7109 | 0,8017 | 0,9362 | 0,7632 | 0,8219 | 0,4913 | 0,4000 |
| **P value summary** | ns | ns | ns | ns | ns | ns | ns |
| **Exact or approximate P value?** | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| **Significant? (alpha = 0.05)** | No | No | No | No | No | No | No |
| | | | | | | | |
| **Number of XY Pairs** | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

Correlation of Histoscore - SGB - SAV (Reverced Ct-values)

| | CT-value (Reverced_SAV_SGB) vs. Atrium - Endocard | CT-value (Reverced_SAV_SGB) vs. Atrium - Myocard | CT-value (Reverced_SAV_SGB) vs. Atrium - Epicard | CT-value (Reverced_SAV_SGB) vs. Ventricle - Endocard | CT-value (Reverced_SAV_SGB) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverced_SAV_SGB) vs. Ventricle - Myocard - Compactu | CT-value (Reverced_SAV_SGB) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| **Spearman r** | | | | | | | |
| **r** | −0,1091 | −0,02471 | 0,3712 | −0,1826 | −0,1386 | −0,2199 | −0,01277 |
| **95% confidence interval** | | | | | | | |
| | | | | | | | |
| **P value** | | | | | | | |
| **P (two-tailed)** | 0,8214 | 0,9655 | 0,3810 | 0,6571 | 0,7500 | 0,5952 | >0.9999 |
| **P value summary** | ns | ns | ns | ns | ns | ns | ns |
| **Exact or approximate P value?** | Exact | Exact | Exact | Exact | Exact | Exact | Exact |
| **Significant? (alpha = 0.05)** | No | No | No | No | No | No | No |
| | | | | | | | |
| **Number of XY Pairs** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

Correlation of Histoscore - SGA - NUC (Reverced Ct-values)

| | CT-value (Reverced_NUC_SGA) vs. Atrium - Endocard | CT-value (Reverced_NUC_SGA) vs. Atrium - Myocard | CT-value (Reverced_NUC_SGA) vs. Atrium - Epicard | CT-value (Reverced_NUC_SGA) vs. Ventricle - Endocard | CT-value (Reverced_NUC_SGA) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverced_NUC_SGA) vs. Ventricle - Myocard - Compactu | CT-value (Reverced_NUC_SGA) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| **Spearman r** | | | | | | | |
| **r** | −0,05307 | −0,09432 | −0,06219 | 0,1889 | −0,08337 | −0,008414 | −0,009323 |
| **95% confidence interval** | -0.4149 to 0.3232 | -0.4486 to 0.2856 | -0.4224 to 0.3150 | -0.1946 to 0.5224 | -0.4397 to 0.2957 | -0.3772 to 0.3626 | -0.3780 to 0.3619 |
| | | | | | | | |
| **P value** | | | | | | | |
| **P (two-tailed)** | 0,7806 | 0,6200 | 0,7441 | 0,3173 | 0,6614 | 0,9648 | 0,9610 |
| **P value summary** | ns | ns | ns | ns | ns | ns | ns |
| **Exact or approximate P value?** | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| **Significant? (alpha = 0.05)** | No | No | No | No | No | No | No |
| | | | | | | | |
| **Number of XY Pairs** | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

Correlation of Histoscore - SGB - NUC (Reverced Ct-values)

| | CT-value (Reverced_NUC_SGB) vs. Atrium - Endocard | CT-value (Reverced_NUC_SGB) vs. Atrium - Myocard | CT-value (Reverced_NUC_SGB) vs. Atrium - Epicard | CT-value (Reverced_NUC_SGB) vs. Ventricle - Endocard | CT-value (Reverced_NUC_SGB) vs. Ventricle - Myocard - Spongiosu | CT-value (Reverced_NUC_SGB) vs. Ventricle - Myocard - Compactu | CT-value (Reverced_NUC_SGB) vs. Ventricle - Epicard |
|---|---|---|---|---|---|---|---|
| **Spearman r** | | | | | | | |
| **r** | −0,1478 | 0,07256 | 0,2635 | −0,2303 | 0,1555 | −0,08304 | 0,3236 |
| **95% confidence interval** | -0.4909 to 0.2350 | -0.3056 to 0.4309 | -0.1179 to 0.5772 | -0.5531 to 0.1526 | -0.2275 to 0.4969 | -0.4395 to 0.2960 | -0.05261 to 0.6194 |
| | | | | | | | |
| **P value** | | | | | | | |
| **P (two-tailed)** | 0,4358 | 0,7032 | 0,1594 | 0,2208 | 0,4119 | 0,6627 | 0,0811 |
| **P value summary** | ns | ns | ns | ns | ns | ns | ns |
| **Exact or approximate P value?** | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate | Approximate |
| **Significant? (alpha = 0.05)** | No | No | No | No | No | No | No |
| | | | | | | | |
| **Number of XY Pairs** | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

## 7.5 Weight, length, and condition factor

| "Table Analyzed" | "Weight – Site T - 60 fish" |
|---|---|
| "Column B" STF | |
| vs. vs. | |
| "Column A" STE | |
| "Unpaired t test" | |
| " P value" | 0,7641 |
| " P value summary" | ns |
| " Significantly different (P < 0.05)?" | No |
| " One- or two-tailed P value?" | Two-tailed |
| " t, df" | "t=0.3016, df=58" |
| | |
| "How big is the difference?" | |
| " Mean of column A" | 6,399 |
| " Mean of column B" | 6,307 |
| " Difference between means (B - A) ¬± SEM" | "-0.09200 ¬± 0.3051" |
| " 95% confidence interval" | "-0.7027 to 0.5187" |
| " R squared (eta squared)" | 0,001566 |
| | |
| "F test to compare variances" | |
| " F, DFn, Dfd" | "3.003, 29, 29" |
| " P value" | 0,0042 |
| " P value summary" | ** |
| " Significantly different (P < 0.05)?" | Yes |
| | |
| "Data analyzed" | |
| " Sample size, column A" | 30 |
| " Sample size, column B" | 30 |

| "Table Analyzed" | "Weight – Site T - all fish" |
|---|---|
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| " P value" | 0,0411 |
| " Exact or approximate P value?" | Approximate |
| " P value summary" | * |
| " Significantly different (P < 0.05)?" | Yes |
| " One- or two-tailed P value?" | Two-tailed |
| " Sum of ranks in column A,B" | "57546 , 68707" |
| " Mann-Whitney U" | 28143 |
| | |
| "Difference between medians" | |
| " Median of column A" | "6.280, n=242" |
| " Median of column B" | "6.580, n=260" |
| " Difference: Actual" | 0,3000 |
| " Difference: Hodges-Lehmann" | 0,2400 |

| | |
|---|---|
| "Table Analyzed" | "Length – Site T - 60 fishes" |
| | |
| "Column B" STF | |
| vs. vs. | |
| "Column A" STE | |
| | |
| "Unpaired t test" | |
| " P value" | 0,3272 |
| " P value summary" | ns |
| " Significantly different (P < 0.05)?" | No |
| " One- or two-tailed P value?" | Two-tailed |
| " t, df" | "t=0.9881, df=58" |
| | |
| "How big is the difference?" | |
| " Mean of column A" | 79,00 |
| " Mean of column B" | 80,07 |
| " Difference between means (B - A) ¬± SEM" | "1.067 ¬± 1.080" |
| " 95% confidence interval" | "-1.094 to 3.228" |
| " R squared (eta squared)" | 0,01655 |
| | |
| "F test to compare variances" | |
| " F, DFn, Dfd" | "1.931, 29, 29" |
| " P value" | 0,0815 |
| " P value summary" | ns |
| " Significantly different (P < 0.05)?" | No |
| | |
| "Data analyzed" | |
| " Sample size, column A" | 30 |
| " Sample size, column B" | 30 |

| "Table Analyzed" | "Length – Site T - all fishes" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0005 |
| "    Exact or approximate P value?" | Approximate |
| "    P value summary" | *** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "55186 , 71068" |
| "    Mann-Whitney U" | 25783 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "79.00, n=242" |
| "    Median of column B" | "80.00, n=260" |
| "    Difference: Actual" | 1,000 |
| "    Difference: Hodges-Lehmann" | 1,000 |

| "Table Analyzed" | "Condition Factor - Site T" |
|---|---|
| | |
| "Column B" | STF |
| vs. | vs. |
| "Column A" | STE |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,0100 |
| "    Exact or approximate P value?" | Approximate |
| "    P value summary" | * |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "65047 , 61207" |
| "    Mann-Whitney U" | 27277 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "1.297, n=242" |
| "    Median of column B" | "1.262, n=260" |
| "    Difference: Actual" | -0,03516 |
| "    Difference: Hodges-Lehmann" | -0,02620 |

| | |
|---|---|
| "Table Analyzed" | "Weight - Site G - Sampled Fish " |
| | |
| "Column B" | "Control Group" |
| vs. | vs. |
| "Column A" | "Test Group" |
| | |
| "Unpaired t test" | |
| "    P value" | 0,4437 |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    t, df" | "t=0.7712, df=58" |
| | |
| "How big is the difference?" | |
| "    Mean of column A" | 3,690 |
| "    Mean of column B" | 3,567 |
| "    Difference between means (B - A) ± SEM" | "-0.1233 ± 0.1599" |
| "    95% confidence interval" | "-0.4435 to 0.1968" |
| "    R squared (eta squared)" | 0,01015 |
| | |
| "F test to compare variances" | |
| "    F, DFn, Dfd" | "1.056, 29, 29" |
| "    P value" | 0,8849 |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| | |
| "Data analyzed" | |
| "    Sample size, column A" | 30 |
| "    Sample size, column B" | 30 |

| | |
|---|---|
| "Table Analyzed" | "Weight - Site G - All Fish " |
| | |
| "Column B" | "Control Group" |
| vs. | vs. |
| "Column A" | "Test Group" |
| | |
| "Unpaired t test" | |
| "    P value" | 0,1313 |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    t, df" | "t=1.511, df=763" |
| | |
| "How big is the difference?" | |
| "    Mean of column A" | 3,659 |
| "    Mean of column B" | 3,596 |
| "    Difference between means (B - A) ± SEM" | "-0.06295 ± 0.04167" |
| "    95% confidence interval" | "-0.1448 to 0.01886" |
| "    R squared (eta squared)" | 0,002981 |
| | |
| "F test to compare variances" | |
| "    F, DFn, Dfd" | "1.460, 362, 401" |
| "    P value" | 0,0002 |
| "    P value summary" | *** |
| "    Significantly different (P < 0.05)?" | Yes |
| | |
| "Data analyzed" | |
| "    Sample size, column A" | 363 |
| "    Sample size, column B" | 402 |

| | |
|---|---|
| "Table Analyzed" | "Length - Site G - Sampled Fish" |
| "Column B" | "Control Group" |
| vs. | vs. |
| "Column A" | "Test Group" |
| | |
| "Mann Whitney test" | |
| "    P value" | 0,2276 |
| "    Exact or approximate P value?" | Exact |
| "    P value summary" | ns |
| "    Significantly different (P < 0.05)?" | No |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "996.5 , 833.5" |
| "    Mann-Whitney U" | 368,5 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "68.00, n=30" |
| "    Median of column B" | "66.50, n=30" |
| "    Difference: Actual" | -1,500 |
| "    Difference: Hodges-Lehmann" | -1,000 |

| | |
|---|---|
| "Table Analyzed" | "Length - Site G - All Fish" |
| "Column B" | "Control Group" |
| vs. | vs. |
| "Column A" | "Test Group" |
| | |
| "Mann Whitney test" | |
| "    P value" | <0.0001 |
| "    Exact or approximate P value?" | Approximate |
| "    P value summary" | **** |
| "    Significantly different (P < 0.05)?" | Yes |
| "    One- or two-tailed P value?" | Two-tailed |
| "    Sum of  ranks in column A,B" | "152216 , 140779" |
| "    Mann-Whitney U" | 59776 |
| | |
| "Difference between medians" | |
| "    Median of column A" | "68.00, n=363" |
| "    Median of column B" | "67.00, n=402" |
| "    Difference: Actual" | -1,000 |
| "    Difference: Hodges-Lehmann" | -1,000 |

| Table Analyzed | Condition Factor - Site G |
|---|---|
| Column B | Control Group |
| vs. | vs, |
| Column A | Test Group |
| Mann Whitney test | |
| P value | <0,0001 |
| Exact or approximate P value? | Approximate |
| P value summary | **** |
| Significantly different (P < 0.05)? | Yes |
| One- or two-tailed P value? | Two-tailed |
| Sum of ranks in column A,B | 121374 , 171621 |
| Mann-Whitney U | 55308 |
| Difference between medians | |
| Median of column A | 1,151, n=363 |
| Median of column B | 1,186, n=402 |
| Difference: Actual | 0,03500 |
| Difference: Hodges-Lehmann | 0,03658 |

## 7.6 Ct-values and prevalence

| Assay | Site G (2nd sampling) | | | | | | Site T (1st sampling) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SGA | | | SGB | | | STE | | | STF | | |
| | Ct-value | | % | Ct-value | | % | Ct-value | | % | Ct-value | | % |
| | Average | Range | Prevalence | Average | Range | Prevalence | Average | Range | Prevalence | Average | Range | Prevalence |
| Piscine myocarditis virus | 20,568 | 11,4 - 26,5 | 100.0 | 13,230 | 10,7 - 19,9 | 100.0 | - | - | - | - | - | - |
| Piscine orthoreovirus 1 | 19,602 | 14,2 - 23,3 | 100.0 | 19,8 | 16,7 - 24,0 | 100.0 | 26,428 | 22,4- 31,9 | 100.0 | 23,353 | 21,5 - 27,0 | 100.0 |
| Salmonid alphavirus | 26,888 | 19,7 - 35,5 | 66.6 | 27,927 | 23,6 - 36,1 | 33.3 | 35,165 | 33,4 - 36,9 | 60.0 | 33,576 | 27,9 - 36,2 | 80.0 |
| Infectious salmon anemia virus | - | - | - | - | - | - | - | - | - | - | - | - |
| *Candidatus* Branchiomonas cysticola | 32,036 | 27,4 - 37,0 | 10.0 | 32,378 | 27,8 - 36,0 | 16.7 | - | - | - | - | - | - |

## 7.7 Project description; Benchmark Genetics

**VEDLEGG 1:**

**Praktiske effekter av seleksjon for økt resistens/toleranse for viruset (PMCV) som forårsaker CMS hos laks**

**Bakgrunn**:

Kardiomyopatisyndrom (CMS), også kalt hjertesprekk, er en alvorlig hjertelidelse som rammer oppdrettslaks i sjø. CMS antas å være forårsaket av piscint myokardittvirus (PMCV) ved vannbåren (horisontal) smitte mellom fisk. Det ser ut til å være en klar sammenheng mellom virusmengde i fisken og grad av sykdommen. PMCV har vært påvist i oppdrettsanlegg med laks lang tid forut for utbrudd. De fleste sykdomspåvisningene er gjort i Møre og Romsdal og Trøndelag. I henhold til Veterinærinstituttet (www.vetinst.no/sykdom-og-agens/kardiomyopatisyndrom-cms) er hjerte-forandringer forårsaket av PMCV ikke til noen større plage i seg selv, men gjør laksen mer sårbar og i dårlig stand til å tåle selv mindre stressituasjoner eller fysiske påkjenninger. Sortering, flytting, avlusing og annen medisinering gjør at tilsynelatende klinisk friske fisk med CMS-forandringer i hjertet utvikler akutt hjertedød. Det er hittil ikke funnet noen aktuelle virus-reservoarer utover laks, og vertikal overføring ser ikke ut til å være en viktig smittevei for viruset. Dødeligheten på CMS-lokaliteter kan variere, men er ofte lav. Den totale dødeligheten kan imidlertid være betydelig og de økonomiske tapene store siden CMS kan forårsake at stor slaktemoden fisk dør.

Kontrollerte smittetester har vist betydelig variasjon i nivå av PMCV hos laks av SalmoBreed-stammen. Påfølgende analyser avdekket en sterk QTL (*Quantitative Trait Loci*) som forklarer mye av denne variasjonen; individer som var bærere av den gunstige varianten av QTL-en (enten *QQ* eller *Qq*) hadde svært lave nivåer av PMCV og viste få tegn til hjerteskader, mens individer med den ugunstige varianten (*qq*) hadde betydelig høyere virusbeslastning og mer hjerteskader. SalmoBreed har påstartet seleksjon i sitt avlsarbeid og tilbyr også å produsere kommersielle egg basert på bærere av denne QTL-en for å øke laksens robusthet. Basert på feltdata av Rauma-stammen er det funnet en annen QTL som er assosiert til overlevelse/robusthet ved CMS-utbrudd, men på et annet kromosom enn QTL-en utviklet ved PMCV-smittetesting av Salmobreed-stammen.

Effekten av å selektere basert på QTL assosiert til egenskapen av interesse, kan variere for ulike genetiske grupper av laks. Det er derfor viktig å verifisere hvilken effekt denne seleksjonen har i feltforsøk med ulike laksestammer og årsklasser innen samme laksestamme. Observasjoner av laks i produksjon tilsier at også regime for smoltproduksjon kan ha betydning for hvor sensitiv laksen er overfor PMCV. Dokumentasjonen av seleksjon for økt PMCV-resistens/toleranse bør derfor foretas med forsøksgrupper produsert både som 0+ og 1+ smolt.

**Formål:** Formålet er å dokumentere effekten av å selektere for økt PMCV-resistens/toleranse hos laks basert på tilgjengelig QTL. Siden det forventes at responsen kan variere avhengig av både laksestamme, årsklasse innen stamme, alder ved utsett (0+ eller 1+) og hvilket miljø laksen oppdrettes i (spesielt mht. håndtering), vil det gjennomføres en serie med feltforsøk for grundig å dokumentere hvilken praktisk effekt en slik seleksjon har for å øke laksens robusthet.

**Gjennomføring:** Det vil gjennomføres fire feltforsøk i løpet av en 6-årsperiode (2019-2024) hvor fire ulike årsklasser av SalmoBreed testes sammen med ulike årsklasser av andre tilgjengelige laksestammer i Norge (StofnFiskur og Rauma). Hvert feltforsøk vil gjennomføres ved testing av ulike genetiske grupper både som 0+ og 1+ smolt i to oppdrettsregioner (SalMars lokaliteter i Møre og Romsdal, og Sinkaberg-Hansens lokaliteter i Trøndelag). Hver stamme/årsklasse av laks vil være representert med testgrupper produsert av bærere (CMS-sterk) og ikke-bærere (CMS-svak) av den positive varianten av QTL-PMCV (*Q*). Rauma-stammen vil i tillegg være representert med testgrupper produsert av bærere/ikke-bærere av alternativ QTL som er assosiert til økt overlevelse/robusthet. Testgruppene i hvert feltforsøk vil testes i to kommersielle merder ved lokaliteter i både Møre og Romsdal (operert av SalMar) og Trøndelag (operert av Sinkaberg-Hansen), og også inngå i kontrollerte smittetester hos VESO, Vikan.

**Produksjon av testgrupper:** Foreldre for å produsere testgrupper vil genotypes og selekteres basert på variant av QTL-PMCV (*QQ* eller *qq*). Foreldre av Rauma-stammen vil dessuten selekteres basert på positiv/negativ variant av alternativt QTL for økt robusthet. Minimum 5 hanfisk og 5 hunfisk vil bidra til å produsere hver testgruppe. Testgruppene i forsøket vil legges inn som rogn året før forsøksoppstart (dvs. i slutten av årene 2018-2021) hos henholdsvis SalmoBreed (Lønningdal) og SalMar (Eik). Testsgruppene vil holdes atskilt frem til merking, men synkroniseres for å sikre felles tidspunkt for transport til i testlokalitetene.

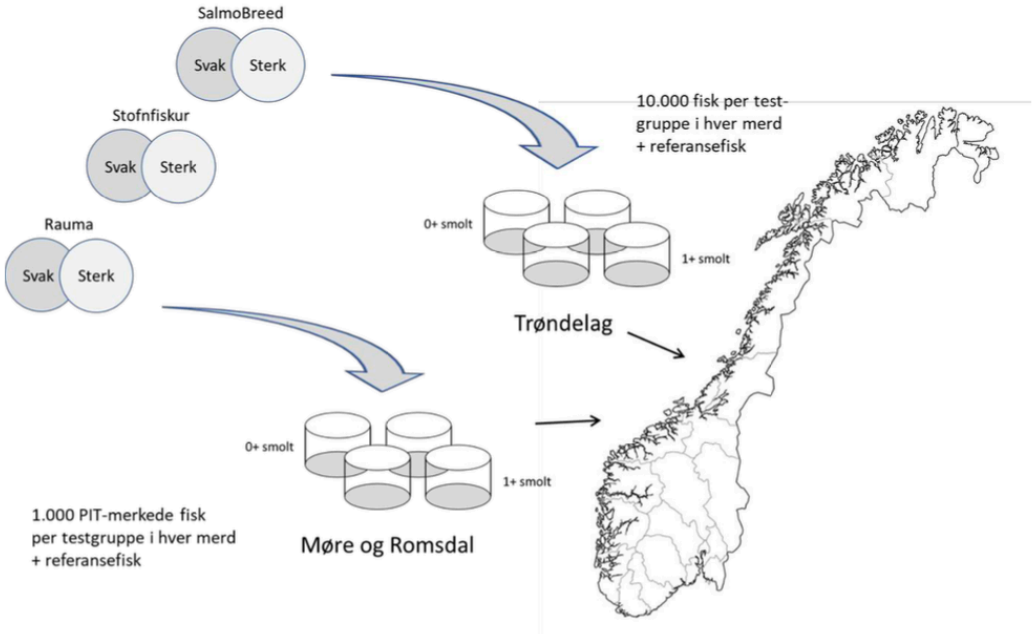
***Utsett 2019/20***: Testgrupper (CMS-sterk og CMS-svak) av SalmoBreed-stammen (årsklasse 1) vil legges inn som rogn hos SalmoBreed (Lønningdal) høsten 2018. Testgrupper av Rauma-stammen vil legges inn som rogn hos SalMar (Eik) høsten 2018. Testgruppene vil produseres både som 0+ og 1+ smolt og være klar til utsetting ved lokaliteter hos SalMar (Møre og Romsdal) og Sinkberg-Hansen (Trøndelag) høsten 2019 (0+) og våren 2020 (1+).

***Utsett 2020/21***: Testgrupper (CMS-sterk og CMS-svak) av SalmoBreed-stammen (årsklasse 2) og Stofnfiskur-stammen (årsklasse 1) vil legges inn som rogn hos SalmoBreed (Lønning-dal) høsten 2019. Testgrupper av Rauma-stammen vil legges inn som rogn hos SalMar (Eik) samme høst. Testgruppene vil produseres som 0+ og 1+ smolt og være klar til utsetting ved lokaliteter hos SalMar (Møre og Romsdal) og Sinkberg-Hansen (Trøndelag) høsten 2020 (0+) og våren 2021 (1+).

***Utsett 2021/22***: Testgrupper (CMS-sterk og CMS-svak) av SalmoBreed-stammen (årsklasse 3) og Stofnfiskur-stammen (årsklasse 2) vil legges inn som rogn hos SalmoBreed (Lønning-dal) høsten 2020. Testgrupper av Rauma-stammen vil legges inn som rogn hos SalMar (Eik) samme høst. Testgruppene vil produseres som 0+ og 1+ smolt og være klar til utsetting ved lokaliteter hos SalMar (Møre og Romsdal) og Sinkberg-Hansen (Trøndelag) høsten 2021 (0+) og våren 2022 (1+).

***Utsett 2022/23***: Testgrupper (CMS-sterk og CMS-svak) av SalmoBreed-stammen (årsklasse 4) og Stofnfiskur-stammen (årsklasse 3) vil legges inn som rogn hos SalmoBreed (Lønning-dal) høsten 2021. Testgrupper av Rauma-stammen vil legges inn som rogn hos SalMar (Eik) samme høst. Testgruppene vil produseres som 0+ og 1+ smolt og være klar til utsetting ved lokaliteter hos SalMar (Møre og Romsdal) og Sinkberg-Hansen (Trøndelag) høsten 2022 (0+) og våren 2023 (1+).

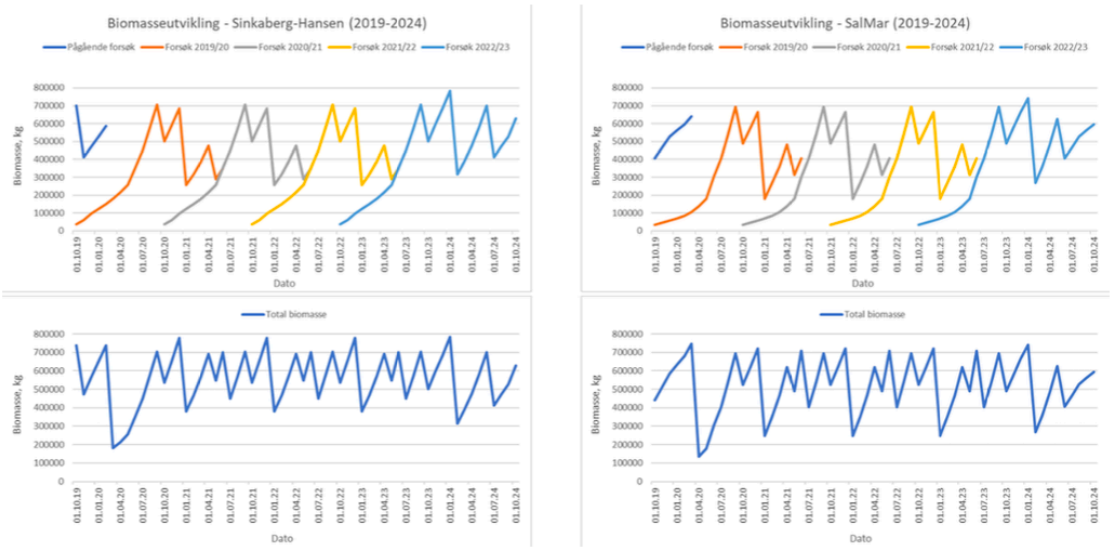
**Feltforsøk:** Testgrupper i hvert feltforsøk (*Figur 1*) vil testes sammen i totalt åtte kommersielle merder fordelt på to oppdrettsregioner (Møre og Romsdal, Trøndelag), dvs. testgrupper produsert som både 0+ og 1+ smolt vil testes i to merder i hver oppdrettsregion (2 smoltgrupper x 2 regioner x 2 merder). I Møre og Romsdal (lokaliteter operert av SalMar) vil testgruppene PIT-merkes (1,000 tilfeldige fisk per testgruppe) før testing sammen med ca. 180,000 referansefisk i hver merd. I Trøndelag (lokaliteter operert av Sinkaberg-Hansen) vil testgruppene klippemerkes (10,000 tilfeldige fisk per testgruppe) før testing sammen med ca. 140,000 referansefisk i hver merd. Forsøksfisken vil følge normale produksjonsrutiner (inkl. sorteringer, avlusninger etc.), og det er derfor svært viktig å holde et godt regnskap på antall fisk, spesielt dersom disse splittes på flere merder i løpet av produksjonstiden.




***Figur 1.*** *Forsøksdesign; hvert feltforsøk vil teste sterke og svake testgrupper av ulike stammer/ årsklasser av laks produsert som både 0+ og 1+ smolt i to ulike produksjonsregioner (Møre og Romsdal, Trøndelag) som er spesielt utsatt for CMS.*

**Biomasseutvikling:** Det er vist betydelig variasjon i dødelighet forårsaket av CMS både mellom og innen oppdrettslokaliteter. Feltforsøkene vil derfor nytte flere merder ved lokaliteter både i Møre og Romsdal og Trøndelag for å sikre god dokumentasjon. Dette medfører imidlertid at forsøkene har et potensiale for å produsere betydelig mer fisk enn hva som er tillat i våre to forsøks-konsesjoner. For likevel å kunne gjennomføre de planlagte feltforsøkene har SalMar og Sinkaberg-Hansen sagt seg villige til å overføre fisk til deres konsesjoner etterhvert som FoU-konsesjonene når maksimal biomasse (*Figur 2*). Nødvendige registreringer vil foretas også etter at de overtar forsøksfisken.

**Registreringer:** Vevsprøver fra alle testgrupper (25 tilfeldige individer/testgruppe ved merking) vil genotypes for å bekrefte at disse er bærere av riktig QTL. Antall fisk og gjennomsnittlig fiskestørrelse (basert på gjennomsnittlig vekt av 100 fisk) vil registreres for hver testgruppe ved forsøksstart (utsett i kommersielle merder) og forsøksavslutning (slakting). Ved slakting skal kjønn og status for kjønnsmodning også registreres. Disse dataene vil brukes til å beregne testgruppenes relative robusthet basert på tilvekst (SGR, vekstfaktor) og overlevelse (%, antall ved slakting – antall ved forsøksstart). Antall forsøksfisk ved slakting vil enten registreres ved automatisk oppsamling av alle PIT-merkede fisk på slaktelinja (for lokaliteter operert av SalMar) eller ved å registrere relativt antall fisk fra ulike testgrupper i flere tilfeldige utvalg av 5,000 slaktefisk (for lokaliteter operert av Sinkaberg-Hansen). Død forsøksfisk vil dessuten bli plukka opp og registrert gjennom hele produksjonssyklusen. Prøver ("organpakke"; lever, blindsekker m/pankreas, gjeller og hjerte) av stor død fisk (>2 kg, inntil 50 tilfeldige fisk per testgruppe) vil sikres (i beholder m/formalin) for senere å kunne dokumentere om CMS er dødsårsak ved histologi. Tilsvarende prøver av slaktefisk (50 tilfeldige fisk per testgruppe) vil lagres på RNALater for verifisering av CMS ved PCR. Antall prøver som analyseres vil være avhengig av om det dokumenteres et CMS-utbrudd ved oppdrettslokalitetene.




***Figur 2**. Forventet utvikling av biomasse i forsøkskonsesjone hos Sinkaberg-Hansen (Trøndelag) og SalMar (Møre og Romsdal) i løpet av 2019-2024.*