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Quantitative genetics of cardiac traits in Atlantic salmon (*Salmo salar L.*)

En kvantitativ genetisk analyse av hjerteegenskaper hos Atlantisk laks (*Salmo salar L.*)

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Abstract

Mortality of apparently healthy looking fish is frequently reported close to harvesting size of Atlantic salmon (*Salmo salar L.*), causing concerns from an economic and ethical perspective for the Aquaculture industry. Autopsy and further analyses often reveal smaller, abnormal hearts and signs of cardiac disorders. The aim of this thesis was therefore to investigate the magnitude of genetic variation in ventricle weight and heart index (ventricle weight/body weight), and the impact of the traits on body weight, survival and cardiomyopathy syndrome (CMS).

The study was based on a total of 4667 fish, the offspring of 173 sires and 341 dams (341 fullsib families) of the Mowi strain. In the sea water period the fish were naturally challenged with CMS and fish that died were classified as death due to CMS or unknown causes. At harvest size body weight and ventricle weight were recorded, and a subjective visual score was given to indicate the presence (score 1, 2, 3) or not (score 0) of CMS in different organs. A tissue sample from the ventricle tip of a small sample of 47 of the dead fish was taken for qPCR analysis for CMS.

The study showed that ventricle weight (h^2=0.34) and heart index (h^2=0.20) were heritable traits. Ventricle weight had a very high, positive genetic correlation with body weight (0.92). However, as this correlation was less than one, direct selection for increased harvest weight may over time result in a relatively smaller ventricle or heart index as also indicated by the negative correlation between body weight and heart index (-0.23).

Survival of CMS had higher heritability (h^2=0.25) than both overall survival (h^2=0.17) and the visual score for CMS recorded at harvest (h^2=0.09). Genetic correlations between overall survival on one hand, and smolt weight, harvest body weight, ventricle weight and heart index on the other, were in general low, but positive and thus favorable. The magnitude of the genetic correlation of CMS score at harvest with harvest weight (0.33), ventricle weight (0.51) and heart index (0.48) indicates an unfavorable genetic association between these traits and CMS. Consequently, direct selection also for increased resistance against CMS is needed to reduce economic losses and improve welfare in the sea phase for Atlantic salmon.

Worth a notice is that the estimated genetic correlations may be biased as the body weight and ventricle weight were missing for those fish that died prior to harvest, although including also smolt weight in the multitrait sire and dam model should to some extent account for this.

Sammendrag

Det rapporteres hyppig om dødelighet av tilsynelatende friske fisk nær slaktestørrelse av Atlantisk laks (*Salmo salar L.*), noe som fører til bekymring fra både et økonomisk og et etisk perspektiv i akvakulturindustrien. Obduksjon og videre analyser avslører ofte mindre, unormale hjerter og tegn på hjertesykdommer. Målet med denne oppgaven var derfor å undersøke omfanget av genetisk variasjon i ventrikkelvekt og hjerteindeks (ventrikkelvekt/kroppsvekt), og egenskapenes innvirkning på kroppsvekt, overlevelse og kardiomyopatisyndrom (CMS).

Studien var basert på totalt 4667 fisk, avkommene til 173 fedre og 341 mødre (341 fullsøskenfamilier) av Mowi-stammen. I sjøfaseperioden fikk populasjonen et naturlig utbrudd av CMS, og fisker som døde ble klassifisert som død på grunn av CMS eller på grunn av ukjente årsaker. Ved slakt ble kroppsvekt og ventrikkelvekt registrert, og en subjektiv visuell scoring for å indikere om fisken hadde CMS (score 1, 2, 3) eller ikke (score 0) ble utført. En vevsprøve av ventrikkelspissen på 47 av de døde fiskene ble sendt til qPCR for analyse for CMS.

Studien viste at ventrikkelvekt (h^2=0.34) og hjerteindeks (h^2=0.20) var arvelige egenskaper. Ventrikkelvekt hadde en veldig høy, positiv korrelasjon med kroppsvekt (0.92), men på grunn av at denne korrelasjonen var mindre enn 1, kan direkte seleksjon for økt kroppsvekt ved slakt føre til mindre ventrikkel eller hjerteindeks over tid, noe som også ble indikert av den negative korrelasjonen mellom kroppsvekt og hjerteindeks (-0.23).

Overlevelse av CMS hadde høyere arvelighet (h^2=0.25) enn total overlevelse (h^2=0.17) og visuell score for CMS ved slakt (h^2=0.09). Genetiske korrelasjoner for total overlevelse på en side, og smoltvekt, kroppsvekt ved slakt, ventrikkelvekt og hjerteindeks på den andre, var generelt lave, men positive og dermed gunstige. Omfanget av de genetiske korrelasjonene for visuell scoring av CMS ved slakt (0.33), ventrikkelvekt (0.51) og hjerteindeks (0.48) indikerte ugunstige genetiske assosiasjoner mellom disse egenskapene og CMS. Følgelig trengs direkte seleksjon for også økt motstand mot CMS for å redusere økonomiske tap og øke fiskevelferden i sjøfasen hos Atlantisk laks.

Verdt å merke seg er at de genetiske korrelasjonene kan være noe skjeve på grunn av at kroppsvekt og ventrikkelvekt manglet for fisk som døde før slakt, selv om å inkludere smoltvekt i multitrait mor- og far modellen til en viss grad tar hensyn til dette.

Table of contents

1 Introduction

Norway is the world's leading producer of Atlantic salmon (*Salmo salar L.*), and in 2015 a total of 1.31 tons were produced. This corresponds to a first-hand value of NOK 44.3 billion (SSB 2016). Farming of Atlantic salmon started at the end of the 1960s in Norway (Gjedrem 2005), and since then the Norwegian family-based breeding programs, first started in 1975, have developed to become among the most advanced in international aquaculture (Gjerde et al. 2007). Due to efficient breeding programs combined with improvement of environmental factors and rapid developing technology within engineering and feed, great improvements have been achieved in growth rate, feed efficiency, sexual maturation, disease resistance and meat quality (Gjedrem 2000; Gjøen & Bentsen 1997; Kolstad et al. 2004). This overall effect has led to a significantly shorter and more cost-efficient production cycle of Atlantic salmon.

As for intensive farming of livestock animals, undesirable diseases and side effects are big challenges also for the aquaculture industry. There seems to be a higher risk for behavioral, physiological and immunological problems for livestock animals that have been genetically selected for high production efficiency (Rauw et al. 1998). Possible similar responses to intensive selection in the aquacultural industry cannot be neglected.

The Norwegian Veterinary report 2016 (Hjeltnes et al. 2017) reported that approximately 20 % of the fish die or disappear in the sea phase, and the major death cause is infectious diseases. Further the report showed that beside mortality due to treatment against the salmon lice (*Lepeophtheirus salmonis*), bacterial diseases and viral diseases are the main causes for the high mortality. Infectious salmon anemia (ISA), infectious pancreatic necrosis (IPN), pancreas disease (PD), heart and skeletal muscle inflammation (HSMI), and cardiomyopathy syndrome (CMS) are the most important viral diseases. Severe pathological changes in the heart, such as myocarditis (inflammation of the heart muscle) and cardiomyocytic necrosis, are common to the three latter diseases (Finstad et al. 2012; Haugland et al. 2011; Taksdal et al. 2007). In addition to viral diseases affecting the heart, other heart disorders and abnormalities, such as small and round hearts (Poppe et al. 2003; Walde & Yousaf 2017), misaligned hearts, *situs inversus*,(Takle et al. 2005; Tørud & Hillestad 2004), absent and hypoplastic *septum transversum* (Brocklebank & Raverty 2002; Poppe & Taksdal 2000), inflammation of the epicardium (epicarditis) (Dalum et al. 2016; Shehzad 2009), thickening of the coronary arteries (coronary arteriosclerosis) (Dalum et al. 2016; Saunders & Farrell 1988), high amounts of epicardial fat (Poppe & Taksdal 2000; Shehzad 2009), myocardial

necrosis (Poppe et al. 2007), cardiac tamponade- and congestions are frequently observed. Many of the heart abnormalities have unknown and complex causes (Hjeltnes et al. 2017; Tørud & Hillestad 2004).

The heart is the pump of the circulatory system, and supplies blood to the gills for re-oxygenation before it serves the fish body with oxygenated blood, other gasses, hormones and nutrients that are important for the metabolism. Reduced heart capacity may therefore have serious consequences.

The morphology of the ventricle, the main pump of the heart, varies greatly among different fish species and reflects their behavioral habits (Agnisola & Tota 1994; Gamperl & Farrell 2004). Also differences within species are common, and the fish cardiac physiology and anatomy show a great ability for intraspecific modifications when exposed to different environmental changes and physical demands (Gamperl & Farrell 2004). Atlantic salmon, which is an active species, has a triangular shape of the heart (Farrell & Jones 1992; Poppe et al. 2003). In farmed salmon, smaller and rounder hearts have been observed compared to wild salmon (Poppe et al. 2003; Tørud & Hillestad 2004), which can be thought to reflect their less active life in cages with continuous feed supply. Any change in heart morphology may reduce the capacity of the heart, and thereby the circulation (Claireaux et al. 2005; Tørud & Hillestad 2004).

Positive correlation between ventricle weight and exercise training is observed (Castro et al. 2013). A large ventricle and a large heart index (the relationship between ventricle weight and body weight) may imply efficient heart capacity and circulation. Larger ventricles imply larger and more efficient pumps, resulting in improved cardiac output (stroke volume x heart rate) and gas- and nutrient exchange (Castro et al. 2011).

Common to many of the cardiac diseases and abnormalities, both infectious and non-infectious, is that the fish often die when exposed to stressing situations (Hjeltnes et al. 2017; Tørud & Hillestad 2004) such as crowding, pumping, transportation, suboptimal conditions, treatment and other handling. It is frequently reported by veterinarians that the fish show few clinical signs, and appear seemingly healthy. The mortality cause is often perceived as unexplainable, but during autopsy and further analyses, abnormal hearts and typical signs of viral diseases are observed (Poppe et al. 2003; Tørud & Hillestad 2004).

Mortality due to cardiac diseases or disorders is not necessarily very high, but the economic loss will be high if the mortality takes place close to harvesting size, which often occurs with cardiac diseased fish. Retarded growth and feed utilization because of chronical morbidity may give additional, indirect losses (Poppe & Seierstad 2003). In addition to the economic aspect, fish welfare is important from an ethical perspective.

The overall objective of this study is to obtain more knowledge of the genetic parameters of cardiac traits at harvest, including ventricle weight, heart index and CMS, and growth and survival until harvest in Atlantic salmon. By this, we will investigate if there is a genetic foundation for implementing cardiac traits in the breeding objective. This will be obtained by estimating the magnitude of genetic variation in the studied traits, and their genetic-, residual and phenotypic correlations. In addition the relationship between visual and histological observed CMS and qPCR CMS-viral load was studied on a limited number of dead and slaughtered fish in order to arrive at good phenotypic measures of recording CMS.

2 Theoretical Background

This chapter consists of four main sections. A general introduction about the morphology of the fish heart will be given, and differences between and within species (inter- and intraspecific differences) will be described. Thereafter, a short overview of the general heart health in fish and a description of cardiomyopathy syndrome (CMS) are presented. Last, six main possible causative factors for cardiac deviations and deformities are described and reflected upon. Based on previous studies, the overall purpose of this chapter is to present knowledge on the morphology of the fish heart, and which consequences diseases, deviations and deformities of the heart may have on the circulatory system and on fish health in general.

2.1 Morphology of the heart

2.1.1 Structure of the heart

The circulation system found in fish is single circuit and closed (Kisia 2016) (figure 1). The heart is the pressure generating pump for the circulation of blood, and pumps blood to the gills for oxygenation and for elimination of carbon dioxide. The blood then transfers oxygen and nutrients to the different organs in the body through arteries, before it returns to the heart via the vein system.

The fish heart is contained in a pericardial sac in the pericardial cavity, medially behind the gills and ventrally of the esophagus (Kryvi & Poppe 2016).The cavity is posteriorly bounded from the abdominal cavity by septum transversum (Olson 2000).

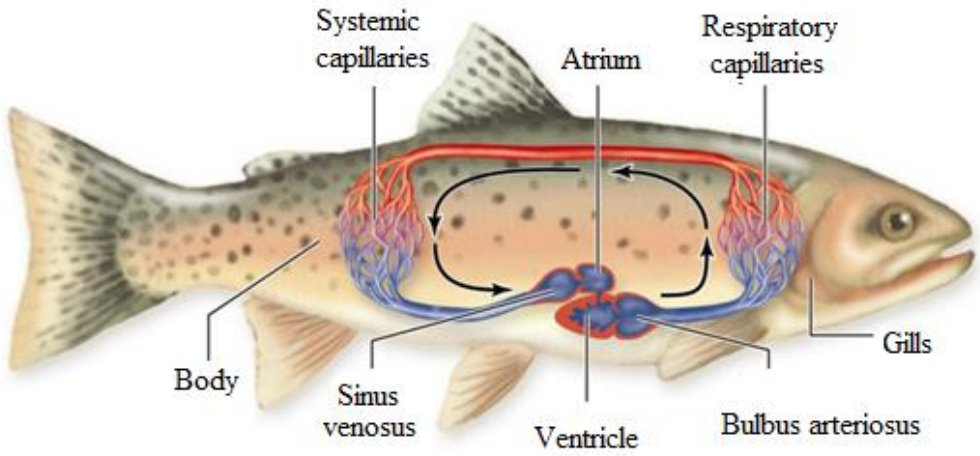

Figure 1. Simplified schematic illustration of the circulation of a typical teleost fish. Retrieved and reproduced from URL: https://www.studyblue.com/notes/note/n/ichthyology-exam-1/deck/3746583 (17.04.2017).

Fish hearts are four-chambered, and teleost hearts consist of *sinus venosus*, atrium, ventricle and *bulbus arteriosus* arranged in an S-shaped series in which the blood follows in that order (figure 2) (Farrell & Jones 1992; Pombo et al. 2012; Randall 1968; Tørud & Hillestad 2004)

Sinus venosus

Sinus venosus is a thin-walled chamber, mainly composed of connective tissue with varying degree of cardiac muscle. The chamber receives venous blood from the body, acting as venous reservoir, before the blood is pumped into the atrium (Kryvi & Poppe 2016; Santer 1985). Sinus venosus possesses specialized pacemaker cells, which makes it the important site of initiation and control of the heart beat (Farrell & Jones 1992; Santer 1985).

Atrium

The atrium is a high-volume chamber with thin trabecular (muscular bundles) walls. Filling of the ventricle occurs mainly when the atrium is contracted (Farrell & Jones 1992). Another important feature of the atrium is macrophage rich endocardium, which constitutes a significant site of the immune system in many fish (Kryvi & Poppe 2016; Roberts & Ellis 2012).

Ventricle

The ventricle is the main pump of the heart, and pumps the blood throughout the body via the bulbus arteriosus and the gills. The walls of the ventricle are thicker than the other chambers, and consist primarily of cardiomyocytes (cardiac muscle cells).When it comes to ventricular morphology there are large differences in size, shape and histology between and within species (Farrell & Jones 1992; Olson & Farrell 2005). This is further described in chapter 2.1.2 and 2.1.3.

Bulbus arteriosus

The white, pear-shaped chamber, bulbus arteriosus, is located between the ventricle and the ventral aorta (the main artery) (Farrell & Jones 1992). It is built up from elastic connective tissue with smooth muscle cells. One of its main functions is to dampen the pulsatile blood flow from the ventricle. This is to generate a more continuous circulation and to protect the fragile gill filaments where the gas exchange occurs (Farrell et al. 2010).

There are valves between the four different chambers that ensure a unidirectional blood flow (Kisia 2016; Tørud & Hillestad 2004).

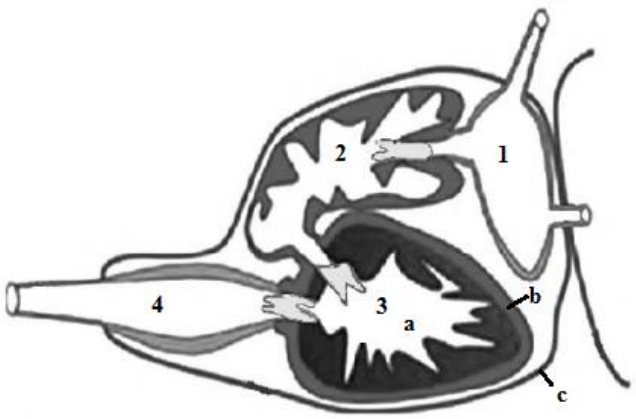

Figure 2. Anatomy of the teleost heart: (1) Sinus venosus (2) atrium (3) ventricle (4) bulbus arteriosus (a) spongy myocardium (b) compact myocardium (c) pericardial sac. The light grey areas between the different chambers picture the valves that prevent backflow of the blood. The figure is reproduced and slightly modified from Gamperl and Shiels (2013).

Histology of the heart

The pericardial sac, which surrounds the heart, constitutes a layered membrane, called the pericardium. The outer layer is tough and fibrous, whereas the inner part has two smooth and serous layers that surround the pericardial cavity. The pericardial cavity contains fluid that acts as a lubricant which prevents friction between the heart and other organs, and ensures smooth contractions of the heart (Farrell & Jones 1992; Kisia 2016). The pericardial sac also maintains the correct position of the heart and creates space for the expanding heart during diastole.

The heart wall is composed of three layers: epicardium, myocardium and endocardium (figure 3). The innermost serous layer of the pericardium is also known as the epicardium, which constitute the outermost layer of the heart wall. The myocardium is the middle layer and contains the cardiac muscle. This layer is the thickest and is responsible for the spontaneous contractions of the heart. The endocardium is a thin layer of elastic connective tissue with a simple squamous epithelium that covers the inside of the heart (Kisia 2016). The endocardium reduces the friction between the heart and the blood flow.

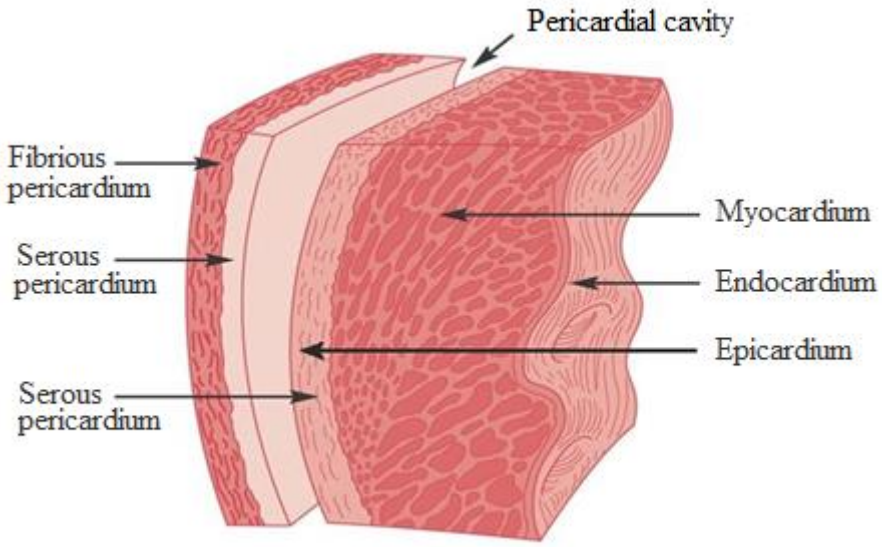

Figure 3. Layers of the heart wall. Retrieved and reproduced from URL: http://cephalicvein.com/2016/07/layers-of-the-heart/ (11.01.2017).

2.1.2 Interspecific differences

Different fish species show great variation in heart morphology, particularly the ventricle (Farrell & Jones 1992; Kryvi & Poppe 2016; Pombo et al. 2012). The shape, mass, histology, vascularization and work dynamics of the ventricle vary widely between species. Attempts of categorization omit many species because the ventricles not quite fit into any of the categories (Icardo 2012). Despite this, there are two features used to categorize the fish heart; ventricular shape (Santer 1985) and arrangement of the ventricular myocardium (Tota 1989; Tota & Gattuso 1996). These features rather reflect the ecophysiological adaptions of the different fish species than phylogenetic traits (Farrell & Jones 1992). The ventricles can be sacular, tubular or pyramidal (figure 4), seen in relation with four different classes based on the two basic myocardial arrangements; spongiosa (*stratum spongiosum*) and compacta (*stratum compactum*).

Sacular and tubular ventricles are typical in sedate fish species. These do often have type I ventricle, a ventricle which only consist of spongiosa myocardium (Farrell & Jones 1992; Pombo et al. 2012; Santer 1985), built up of a complex meshwork of trabeculae that gives a spongy appearance. Type I ventricle is supplied with venous blood from the systemic circulation only (Tota & Gattuso 1996). The sacular ventricle is the most common form, and is typical in elasmobranchs and many marine teleosts. The tubular ventricle, the least common form, is specific for fish species with elongated body shape, such as eels (Farrell & Jones 1992; Gamperl & Shiels 2013; Sanchez-Quintana et al. 1995; Santer 1985).

Ventricles with a pyramidal shape typify species with an active lifestyle, such as salmonids (Farrell & Jones 1992; Sanchez-Quintana et al. 1995; Santer 1985). Pyramidal ventricles always belong to ventricle type II, III or IV; ventricles containing a compact layer of variable thickness and a varying degree of vascularization through coronary circulation. In this way the heart also receives oxygenated blood from the gills in addition to the deoxygenated venous blood from the systemic circulation. The compacta is a layer of neatly and densely arranged trabeculae enclosing the spongiosa (Tota & Gattuso 1996), which gives the heart the potential to act as a pressure pump (Agnisola & Tota 1994). Optimum cardiac output is generated through a relatively high heart rate, high pressure and a small stroke volume (Branson & Turnbull 2008; Tota & Gattuso 1996). For species with constant swimming, and thereby a high level of energy consumption, the pyramidal ventricle is advantageous in terms of this great pumping capacity. Abnormalities in the heart shape will consequently affect the pumping capacity and reduce the cardiac output (Branson & Turnbull 2008; Brocklebank & Raverty 2002; Poppe et al. 2003).

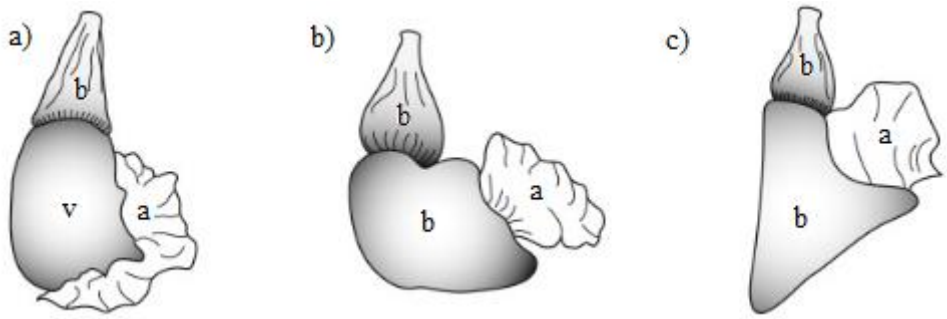

Figure 4. Ventricular shapes: a) tubular b) sacular and c) pyramidal. v=ventricle, a=atrium and b=bulbus arteriosus. The figure is reproduced and slightly modified from Farrell (2011).

2.1.3 Intraspecific differences

As for heart morphology between species there is also variation within species. Several studies have shown placidity in ventricle morphology in fish, which seem to be a response to environment and physical demands, life cycle, and domestication: In eel (*Anguilla anguilla L.*) both the compact- and spongy layers increase in relative thickness with age (Cerra et al. 2004), the same is observed for salmonids (Kryvi & Poppe 2016). Powell et al. (2002) reported that Atlantic salmon with heavy infestation of AGD (amoebic gill disease) had a significantly thicker compact layer compared to fish with only light AGD. The ventricle mass of rainbow trout (*Oncorhynchus mykiss*) increase greatly during cold-acclimation (Graham & Farrell 1989), and the ventricle shape of European seabass (*Dicentrarchus labrax*) changes from tubular, in larval stage, to sacular (Pombo et al. 2012). Poppe and Taksdal (2000)

observed that diseased smolt, sampled based on clinical appearance, had smaller and rounder ventricles than healthy smolt from the same farm. They also had conspicuous fat deposits, irregular bulbus arteriosus and considerably thinner compacta. In Atlantic salmon, significantly heavier hearts have been observed for the total male fish compared to the total female fish (Shehzad 2009). Sexually maturation increases the heart index significantly in male rainbow trout and Atlantic salmon, but not in females (Franklin & Davie 1992; Graham & Farrell 1992). Studies have also shown that the heart morphology of several domesticated fish species differ from the corresponding wild fish. Poppe et al. (2003) reported that the hearts from both farmed Atlantic salmon and rainbow trout were rounder, had a different alignment of bulbus arteriosus, and notable higher amount of epicardial fat compared to their wild counterparts. Relative ventricle mass and relative ventricle area (heart index) are reported to be lower in farmed rainbow trout and Atlantic salmon, respectively, than in wild fish of the same species (Graham & Farrell 1992; Moltumyr 2015).

2.2 Status: heart health in general

Several heart diseases and abnormalities can cause deviations from normal cardiac function. This may lead to less robust and more vulnerable fish in stressing situations, which farmed fish are exposed to, such as crowding, pumping, transportation, suboptimal conditions, treatments and other handling. Many heart conditions are described (Tørud & Hillestad 2004; Walde & Yousaf 2017), both infectious and non-infectious. The causative factors seem to be diverse and complex, and for many of them there are found no clear explanation.

Cardiomyopathy syndrome (CMS) (Ferguson et al. 1990; Haugland et al. 2011), heart and skeletal inflammation (HSMI) (Finstad et al. 2012; Kongtorp et al. 2004), and pancreas disease (PD) (Munro et al. 1984; Taksdal et al. 2007) are viral diseases that induce severe cardiac inflammation and necrosis in Atlantic salmon, and lead to large annual losses. There is a vaccine available on the market for PD, but its effect is questioned although positive results are obtained (Bang Jensen et al. 2012). Vaccines for CMS and HSMI are under development (Mikalsen et al. 2016).

Bacterial diseases such as furunculosis (Koppang et al. 2000) and cold water vibriosis (Totland et al. 1988) do not primarily affect the heart, but may induce some structural changes to it that affect the cardiac output.

In addition to the viral diseases mentioned above, the most common deviations from the classical pyramidal shaped heart are smaller and bean shaped hearts (Poppe et al. 2003; Walde

& Yousaf 2017). Misaligned hearts, *situs inversus* (Takle et al. 2005; Tørud & Hillestad 2004), absent and hypoplastic *septum transversum* (Brocklebank & Raverty 2002; Poppe & Taksdal 2000), inflammation of the epicardium (epicarditis) (Dalum et al. 2016; Shehzad 2009), thickening of the coronary arteries (coronary arteriosclerosis) (Dalum et al. 2016; Saunders & Farrell 1988), high amounts of epicardial fat (Poppe & Taksdal 2000; Shehzad 2009), myocardial necrosis (Poppe et al. 2007), cardiac tamponade- and congestions are also common deviations and abnormalities observed in Atlantic salmon. Every deviation from the normal pyramidal shape and function of the heart may give a reduction in cardiac capacity and, thereby, robustness of the fish (Poppe et al. 2003).

2.3 Cardiomyopathy Syndrome (CMS)

Cardiomyopathy syndrome (CMS) is a major cardiac disease of Atlantic salmon, first described in Norway in 1985 (Ferguson et al. 1990), and later in Faroe Island (Poppe & Sande 1994), Scotland (Rodger & Turnbull 2000), Canada (Brocklebank & Raverty 2002) and Ireland (Rodger et al. 2014). CMS has also been reported in wild Atlantic salmon (Poppe & Seierstad 2003). For many years the causative agent of the disease was unknown, but in 2011 it was identified with the double-stranded RNA virus *Piscine myocarditis virus* (PMCV) from the *Totiviridae* family (Haugland et al. 2011). CMS affects mainly large fish in their second year in the sea, and the past decade 75-85 sea farms in Norway have been diagnosed each year, resulting in annual losses up to €9 million (Martinez-Rubio et al. 2014).

Diseased fish appear apparently healthy and die often without any clinical signs. In some cases dermal hemorrhage and edema, raised scales, and marked exophthalmos (protruding eyes) have been observed (Rodger & Turnbull 2000). At autopsy, however, a variety of abnormalities are found, especially in the heart. This include rupture of the atrium or sinus venosus and thereby a cardiac tamponade in the pericardial sac, distended atria with associated hemorrhages, ascites (accumulation of fluid in the abdominal cavity) and fibrin exudates and blood clots in the heart and abdomen (Martinez-Rubio et al. 2014; Mikalsen et al. 2016; Rodger et al. 2014). Lesions in other organs, such as kidney, gills, pancreas, and liver, can also be present, likely due to failing circulation (Ferguson et al. 1990; Mikalsen et al. 2016).

Histopathological studies of fish with CMS show infiltration of inflammatory lymphocytes and macrophages, accompanied with severe inflammations and necrosis of the atrium and spongiosa part of the myocardium layer of the ventricle (Mikalsen et al. 2016). These lesions

develop into more extensive lesions at later stages of the disease, and may then also affect the compact layer of the ventricle (Fritsvold et al. 2009). Fibrous tissue is occasionally observed in late stages, and is assumed to be a reparative process to replace destroyed cardiomyocytes (Mikalsen et al. 2016). This type of compensation mechanism is also seen in arrhythmogenic right ventricular cardiomyopathy (ARVC) in humans (Bowles et al. 2002).

Real-time quantitative polymerase chain reaction (real-time qPCR) is a molecular technique used to detect pathogens with known genomic sequences. Since the genome is identified for piscine myocarditis virus (PMCV), this method can be used to detect CMS in Atlantic salmon. Haugland et al. (2011) found that there is a linear relationship between the histoscores of the spongious layer of the ventricle and the PMCV- Ct values in the heart tissue samples. The higher virus load, the larger changes in the heart morphology were observed. The study also showed that the virus was spread horizontally to cohabiting fish, which developed histopathological changes typical of CMS. The spread does most likely occur via mucosal surfaces. The source of the virus infection, however, is unidentified.

The acute mortality seen in cages with CMS is not very high, but due to the fact that the disease affects large fish, the economic consequences are significant. The morbidity is often high and long-lasting, causing a high total mortality through the lifespan of the disease (Poppe & Seierstad 2003). Changes in growth and feed utilization, as a result of the disease, may give an additional economic impact.

There are no vaccines available to protect against CMS at present time (Mikalsen et al. 2016), and more research is needed on the origin of PMCV and its replication mechanisms to develop one. However, Martinez-Rubio et al. (2014) showed that functional feeds, with reduced lipid composition and increased eicosapentaenoic acid (EPA) levels, can reduce the severity of heart lesions in Atlantic salmon infected by PMCV. The study showed significant differences in pathology and immune- and inflammatory responses in the heart tissues, especially at early and late stages after the infection. These results show that clinical nutrition may have a role in controlling the effects of infection of PMCV in Atlantic salmon.

A report from Akvaforsk (Refstie et al. 1993) showed that there was a genetic foundation for reduction of CMS through family selection, due to genetic variation in myocardial changes in atrium (h^2=0.23) and ventricle (h^2=0.27) after disease outbreak.

Today, AquaGen delivers Atlantic salmon eggs that are selected for CMS through quantitative trait loci (QTL), and are supposed to give better heart health, which again gives a more robust fish (AquaGen 2016).

2.4 Possible causative factors for cardiac deviations and deformities

Morphological deformities and variations in skeletal and soft tissues in wild and farmed fish have been observed and documented for a long time (Dawson 1964; Dawson & Heal 1976). The causative factors are often complex and multifactorial, which is also true for cardiac deformities in Atlantic salmon. The abnormalities may (partly) be a result of the aquaculture rearing conditions (Poppe & Taksdal 2000). Poppe et al. (2003) suggest that the tendency of a rounded heart in farmed salmon could be a consequence of "breeding, fast growth, sedentary lifestyle, or a combination of all these factors". A description of aquaculture factors that may contribute to heart abnormalities follows.

2.4.1 Water temperature

Water temperature is a key water quality parameter in aquaculture as it has major influence on the physiology and behavior of fish (Elliott & Elliott 2010). During incubation of salmon eggs and in early life stages the temperature is raised to intensify the production of smolt (Bjørnevik et al. 2003; Johnston & McLay 1997; Poppe & Taksdal 2000). Bjørnevik et al. (2003) saw that groups of Atlantic salmon incubated at high temperatures (6-10°C) hatched and were start fed two months earlier than the groups incubated at low temperatures (2-6°C). The pericardial cavity and the heart tube of Atlantic salmon are developed early in the embryonic ontogeny, while spontaneous cardiac contractions commence later (Gorodilov 1996). It has been demonstrated that high water temperatures during this embryonic stage can induce both spinal and soft tissue deformities in salmon (Baeverfjord & Helland 2005). Several studies have, for instance, shown a direct relation between high egg incubation temperatures and missing *septum transversum* (Takle et al. 2005; Takle et al. 2006). This might suggest that it can impact other cardiac features too. Today there is a recommended upper water temperature limit of 8°C for normal embryonic development in Atlantic salmon, while 12°C is lethal (Gunnes 1979; Takle et al. 2005).

Water temperature is also important later in the life cycle. Salmonids go through physical and enzymatic processes, called acclimatization or acclimation, as a response to the changing temperature, where the heart is important (Gamperl & Farrell 2004). Salmonids show some cardiac placidity to compensate for fluctuating temperatures (Farrell & Jones 1992; Gamperl

& Farrell 2004). Studies have shown that exposure to cold temperatures can cause hypertrophy of the heart in salmonids, where the proportion of compacta seem to be reduced, while spongiosa increases (Graham & Farrell 1989; Klaiman et al. 2011; Tervonen et al. 2001). An increase in the ventricular mass might compensate for the reduction in contractility induced by the lowered temperature, and thereby maintain the cardiac function (Gamperl & Farrell 2004; Klaiman et al. 2011). Klaiman et al. (2011) showed that acclimation to warmer temperatures had opposite effects on ventricle morphology, which is thought to increase the coronary circulation and thereby the oxygen level to the heart itself. The optimal sea water temperature for Atlantic salmon is 13-15°C, 22°C and higher is critical, while 27-28°C is lethal (Jonsson & Jonsson 2009).

2.4.2 Level of activity

Wild salmonids are active fish, and are designed for tough, long-distance movement during their spawning runs. This is reflected by their long, streamlined body shape and big tail (McCormick et al. 1998; Von Cramon-Taubadel et al. 2005). Domestication of Atlantic salmon, with a life in tanks and pens with limited water volume, space and velocities, has resulted in a less active life style than their wild counterparts.

Activity and training can alter the cardiac morphology and performance in fish (Farrell et al. 1991; Gamperl & Farrell 2004), but studies show varying results (Davison 1997; Farrell & Jones 1992). Some studies show that cardiac mass can increase with aerobic exercise training (Castro et al. 2013; Gallaugher et al. 2001; Hochachka 1961), but these changes are usually relatively small (Davison 1997). Cardiomyocyte hypertrophy and hyperplasia are also observed after some training regimes (Castro et al. 2013; Walker & Emerson 1978).What is seen in most training studies of fish is isometric cardiac growth (Farrell & Jones 1992) where heart size and shape are constant, but stroke volume, maximum oxygen consumption and maximum cardiac output are significantly increased (Castro et al. 2013; Farrell et al. 1990; Farrell et al. 1991; Gallaugher et al. 2001). Although most studies show that exercise training has positive effects on cardiac capacity, the impacts are relatively small compared to the larger increase that is seen in for example heart size during acclimation to temperature changes and sexual maturation (Davison 1997; Takle et al. 2010) According to Takle et al. (2010) the main reason for the small changes might come from lacking focus on the training amount, frequency and intensity during the experiments.

Both water temperature and activity influence the oxygen consumption of fish. The cardiac placidity observed after temperature changes and activity at different levels reveal the fishes'

ability to do compensatory changes in the heart that are necessary to maintain sufficient oxygen levels in the body (Farrell et al. 1990). Takle et al. (2010) reported that trained fish had lower oxygen consumption than untrained fish during a swimming test, and thereby learned to economize their swimming

A study showed that hearts from poor swimmers of rainbow trout had significantly rounder ventricles and lower maximum cardiac output compared to good swimmers (Claireaux et al. 2005).

2.4.3 Feed

Wild salmon live of organisms found in its natural environment, and have naturally occurring periods of starvation. Farmed salmon, on the other hand, have a continuous, external feed supply for efficient growth. As fish is the natural lipid- and protein source in wild salmon diet, this has been the preferred lipid- and protein source also for farmed salmon, derived from marine products with little human demand or marine by-products.

Due to increased prices, limited access and environmental control, the use of fish oil and fish meal in commercial feed for farmed fish will not be economically sustainable in the long run (Tacon & Metian 2008). The aquaculture industry is therefore reducing and replacing the marine lipid- and protein sources (Burr et al. 2012; Jirsa et al. 2015). Today, rapeseed oil and soy beans are the largest substitutes for fish oil and fish meal, respectively (EWOS 2017), but also corn, wheat, barley, peas, sunflower, olive, palm, and linseed are alternative substitutes (Tocher et al. 2003).

Several studies show no significant negative impact on growth, feed utilization or mortality in salmonids when fish oil is replaced with diets with high inclusion of different vegetable oils (Bell et al. 2001; Bell et al. 2004; Rosenlund et al. 2001; Torstensen et al. 2005). Likewise, diets where fish meal is substituted with alternative proteins sources with a well-balanced amino acid compositions show little negative consequences (Burr et al. 2012; Espe et al. 2006).

There is, however, some concerns regarding the replacement of marine fatty acid- and protein sources. The different fatty acid composition of the new feeds can affect the fish health (Bell et al. 1991; Rosenlund et al. 2001; Thomassen & Røsjø 1989) and fish quality (Thomassen & Røsjø 1989; Waagbø et al. 1993). Bell et al. (1991) found that Atlantic salmon post-smolt fed a diet with sunflower oil as the lipid component had an increased ratio of omega-6 to omega-3 polyunsaturated fatty acids in the phospholipids, compared to the control diet with fish oil. A

high proportion of these fish also developed cardiomyopathy, with thinning of the ventricular wall and necrosis of the muscle. Similar observations were seen in a later study (Bell et al. 1993). Unpublished results from Remø et al. and Frøyse et al. (Torstensen et al. 2013) show that there is a tendency for increased pericardial fat in fish fed a diet containing vegetable oil instead of fish oil. How this affects salmonids is unknown, but in humans it is associated with cardiovascular diseases, such as atherosclerosis (Mahabadi et al. 2009).

The fatty acid composition in feeds can also have an effect on virus diseases. Martinez-Rubio et al. (2012) and Martinez-Rubio et al. (2014) demonstrated that feeds with reduced levels of total lipid content and larger proportions of long-chain polyunsaturated fatty acids, significantly reduced viral load and gave a milder and delayed inflammatory response in fish with HSMI and CMS. These results suggest that fatty acids can serve as an important component in functional feeds.

2.4.4 Vaccination

The aquaculture industry relies on effective vaccines in controlling many severe infectious diseases, and have the past decade replaced the extensive use of antibiotics to a minimum. Today, all smolt in Norway are vaccinated before transfer to sea, most commonly intraperitoneally (injected into body cavity) with multivalent oil-adjuvanted vaccines (Sommerset et al. 2005). There are certain side effects with oil-adjuvanted vaccines, and most visible and common seem to be adhesion of internal organs, lesions at injection site, and subsequent reduced feed conversion, retarded growth and increased mortality (Berg et al. 2007; Midtlyng 1996; Poppe & Breck 1997). Fish size (Berg et al. 2007) and temperature (Grini et al. 2011) at injection time, poor handling, anesthetics, and the particular vaccine can be causative or contributing factors to the side-effects (Poppe & Koppang 2014).

Less visible and obvious side-effects of vaccination may be overseen. Effects on the fish heart are not well documented, and few publications are available. However, a study showed that vaccinated Atlantic salmon had relatively larger hearts and more triangular shaped ventricles than unvaccinated fish (Fraser et al. 2015). Both can be indications of increased cardiac capacity of vaccinated fish. Ackerman et al. (2000) and Skinner et al. (2010) both found that vaccination of rainbow trout increased the metabolic rate compared to unvaccinated fish. This may have increased the cardiac workload. An alternative, as suggested by Fraser et al. (2015), is that heart growth is stimulated due to increased cortisol levels as a result of chronic stress caused by vaccination. So far, it is not documented that vaccination has any negative impacts on the fish heart.

2.4.5 Stress

Atlantic salmon and other aquaculture species are regularly exposed to different stressors, such as bacteria, virus, parasites, crowding, pumping, transportation, suboptimal conditions, treatment and other handling. Failure of acclimation to these short-and long-term stressors may cause chronic stress, which has negative impact on the animal. Chronic stress can induce changed features related to the metabolism, osmoregulation, respiratory-, cardiovascular- and immune functions (Barton 2002). Cortisol, an important stress hormone which has various effects on different tissues, is often used as an indicator on the level of stress (Barton & Iwama 1991; Marcel et al. 2009). Johansen et al. (2011) showed that rainbow trout genetically selected for high cortisol post-stress response (HR) had an averaged heart index 34 % higher than trout selected for low cortisol response (LR). This was mainly due to hypertrophy of the compact layer of the ventricle. In a later study, Johansen et al. (2014) showed that rainbow trout fed a diet with a cortisol supplementation had a 34 % increase in heart index in 45 days. Also in this study the relative heart growth came from hypertrophy of the compactum. This was associated with an increase of molecular markers for heart diseases at an mRNA level in the heart. The hearts of the cortisol fed group had also lower pump capacity compared to the control group, with significantly lower circulation, although the heart rate was increased. Results from the above studies indicate that cortisol production due to stress can induce myocardial remodeling, and might be an important explanatory factor for cardiac abnormalities-and diseases.

Pottinger and Carrick (1999) suggested that manipulation of stress-responsiveness by selective breeding might optimize the performance of fish under stressing farming conditions. This could also possibly produce fish with a better functioning cardiovascular system in general.

2.4.6 Breeding and selection

Farmed Atlantic salmon is not only becoming more and more different from its wild origin environmentally, but also genetically (Fleming & Einum 1997; Jonsson & Jonsson 2006). The genotypic change is a long term response to the national selective breeding experiment started by Akvaforsk at the beginning of the 1970s, and also the several private breeding programs that were initiated in the 1970s and 1980s (Gjedrem 2000), such as Mowi, which Marine Harvest bases their fish on today. Selection strategy and program design have varied between the companies, but for all of them growth was the only trait in the breeding goal the first years. The complexity of the breeding programs has increased, and today early sexual

maturation, fillet fat, fillet color and other quality traits, and several disease resistance traits (Gjedrem 2000) have been included. The overall goal is to produce fish with high productivity and sustainability (Gjedrem & Baranski 2010). As a result of the intensive selection the last decades, production of salmon has become significantly more efficient, and a doubling of the growth rate of Atlantic salmon was achieved after five generations of selection in the national breeding program (today AquaGen) (Thodesen et al. 1999). Reported genetic gains for growth rate, disease resistance and sexual maturation are 17.8% and 11-19% (Gjedrem & Rye 2016) and 3-8 % units (Gjedrem 2005), respectively.

Selection for one trait may affect other genetically correlated traits. Fast growth has always been one of the major breeding goals in domesticated animals, but little is known about the mechanisms that are underlying the growth process and their potential side effects. However, Rauw et al. (1998) showed in a review that broiler chickens selected for fast growth have resulted in a high prevalence of heart failure syndrome, ascites and leg problems. As selection for fast growth is practiced in Atlantic salmon, a similar response should not be excluded for this species. Studies have shown that fish with rounded ventricles had the highest condition factor in the studied group (Claireaux et al. 2005), and that coronary artery lesions have a high genetic correlation to fish size and growth rate (Farrell 2002). It seems like Atlantic salmon selected for high body weight and fast growth have a tendency for development of cardiac lesions and abnormalities.

Arne Storset from AquaGen said in the heart-report 2004 (Tørud & Hillestad) that breeding for fast growth may have resulted in larger fillet yield at the expenses of the development of heart- and circulation system. If so, there might be a disproportionate relationship between the two traits, and an imbalance between the circulation-and respiratory systems and the oxygen demand of the fish might occur. Fish with high body mass have higher metabolic rate than smaller fish (Clarke & Johnston 1999), and are therefore dependent on well-functioning circulation-and respiratory systems. Today, the relation between heart weight and body weight (heart index) is selected for in AquaGen's breeding program (AquaGen 2017).

Refstie et al. (1993) found no significant positive or negative phenotypic correlation between growth and heart lesions associated with CMS, and concluded that there was no foundation to claim that fast growing fish were more exposed to CMS than slower growing fish. However, a negative genetic correlation between growth and CMS was observed, which indicated that families with the smallest fish had most heart lesions.

Inbreeding occurs when two individuals related to each other by ancestry are mated and produce offspring. Entirely avoidance of inbreeding in a closed breeding population, such as in salmon farming, is impossible (Gjedrem & Baranski 2010) as a relatively low number of broodstock is used to produce new generations in salmon breeding. Consequently, some inbreeding will occur (Wang et al. 2002). Nothing is known about inbreeding and its impact on cardiac health in salmon. Negative effects cannot be eliminated as a reduction in fitness and performance is a well-associated side effect of inbreeding (Fjalestad 2005; Gjerde et al. 1983; Kincaid 1983).

On the background of the former chapter, a concluding remark is that it is important to know the magnitude of genetic selection due to genetic correlations between traits and the undesirable side effects it may lead to over time.

3 Materials and Methods

3.1 Fish data

The analyzed data was obtained from Marine Harvest breeding program, based on an informant group of fish from the fourth generation since the start of the family based breeding program of the Mowi strain. The eggs were reared and hatched at the Marine Harvest hatchery Øyerhamn, Åkra, Norway. A total of 4667 fish, the offspring of 173 sires and 341 dams (341 fullsib families), were individually pit-tagged 14 months after fertilization, at an average smolt weight of 68.4 grams. In May 2015 they were transferred to two net cages in the sea at Marine Harvest Fish Feed Averøy at an average weight of 97 grams**.** Five months earlier the fish had been vaccinated with the commercially available oil adjuvanted vaccine Alpha Ject Micro 6 (PHARMAQ AS, Oslo). In November 2015 the fish were split into four net cages: C7, C8, C9 and C10.

The fish were fed commercial feed in both the freshwater- and saltwater phase. Each net cage was added 8 % lump suckers (*Cyclopterus lumpus)* as cleaner fish at arrival at sea and later increased to 20 %. Lump suckers were continuously replaced if any mortality occurred.

285 (6.1%) fish died before transfer to sea, while 905 (19.4%) were registered as dead during sea phase, adding up to a total mortality of 25.5 %. Examination of the dead fish was performed continuously, and any sign that could be a contributory cause of death was registered. In retrospect, these observations were evaluated by veterinarians, and categorized into two categories:

1: Death caused by CMS

2: Unknown cause of death

Mortality was divided into three periods, and period 3 was further divided into the two categories of death:

Period 1: Before transfer to sea

Period 2: Transfer to sea to splitting of nets

Period 3: Splitting of nets to slaughter

- death caused by CMS
- unknown cause of death

A total of 3477 fish were slaughtered between 22-25th of August 2016. The fish were anesthetized with FINQUEL® vet. before they were bled by cutting the gills. Examination took place directly after a 20 minutes bleed out of the fish. Passive Integrated Transponders (PIT) tags were read by a PIT-tag scanner to identify the ID of the fish, and automatically registered on the computer. Body weight (bleeded weight) and body length were registered. The ventricle of each fish was sampled, photographed, weighted, and preserved in formalin. Atrium and bulbus arteriosus were removed from the ventricle.

Table 1 shows descriptive statistics by trait for all fish in the dataset (n=4667). Due to considerably similar mean values between cages (see appendix), an overall table is presented. Outliers due to obvious experimental errors were excluded from the dataset.

Table 1. Descriptive statistics by studied trait.

Trait	N	Mean	S.D	Min	Max	CV
Smolt weight (g)	4667	68.4	18.2	15.9	165.4	26.6
Body Weight (kg)	3477	4.14	1.13	0.44	8.26	27.3
Body Length (cm)	3477	68.9	6.2	35.0	88.0	9.0
Ventricle Weight (g)	3423	3.442	0.967	0.574	7.928	28.1
Heart Index (%)	3423	0.084	0.012	0.040	0.168	14.3
Mortality (%)[1]	1190	25.5				
- Period 1	285	6.1				
- Period 2	164	3.5				
- Period 3	741	15.9				
- CMS	414	8.9				
- Unknown	327	7.0				

[1]Mortality is divided into three periods: before transfer to sea (period 1), transfer to sea to splitting of nets (period 2), and splitting of nets to slaughter (period 3).

Cardiomyopathy syndrome (CMS) was first detected by veterinarians in February 2016 by qPCR and histology analyses of ventricle and atrium tissues, after a routine visit at the research station. During lice picking in the end of May and beginning of June 2016 many fish died, and typical signs of CMS, as described in table 2, were observed on many fish during examination. A tissue sample from the ventricle tip of a random sample of 47 of the dead fish

were taken and sent to PHARMAQ Analytic for qPCR analysis for CMS. The disease was confirmed for all fish (Ct ≤ 25.1). At slaughter, ventricle samples were taken of 3423 fish for the same purpose. A veterinarian also scored the fish visually for CMS during post mortem examination, based on the classification described in table 3. Ventricles preserved in formalin from fish with high CMS score (CMS-score>1, n=28) and samples from fish with high (Ct≥25, n=45) and low (Ct≤21, n=43) qPCR-Ct values were sent to histology analysis at Fish Vet Group Norge.

qPCR

Quantitative polymerase chain reaction (qPCR) is a modification of PCR and can precisely quantify specific nucleic acids by accumulation of fluorescent signals of labeled PCR products over a range of cycles. Results of qPCR are presented as threshold cycle-values (Ct-values). A threshold level is set, which represents a level above background fluorescence. The Ct-value is the spot where the reaction curve crosses the threshold cycle, and is the average number of cycles it takes to detect a signal from the PCR-sample (Shipley 2007). Real time qPCR results were expressed in Ct-values, and are inversely correlated with the amount of viral nucleic acids in the PCR-sample, and thereby with viral load (Huijskens et al. 2012).

Low Ct-values indicate high viral load, while high Ct values means lower viral load. Ct-values <29 normally indicate abundant amount of nucleic acids, while Ct-values> 35-40 are regarded false/negative (Jørgensen, S.M., personal communication 14.10.2016), and may be infected or environmentally contaminated.

Visually CMS-scoring

Presence of CMS was classified into four scores, as listed in table 2. Classical signs of CMS are shown in figure 5.

Table 2. Visually scoring of CMS in different organs of Atlantic salmon.

Score	Description
0	No signs of CMS
1	Signs of edema in liver and spleen. Atrium filled with blood. Score 1 describes a congestion condition.
2	Edema, signs of fibrin exudates on liver surface, filled atrium, serous fluid in abdominal cavity.
3	Edema, fibrin exudates on liver surface, hemorrhage in pericardial cavity and/ or abdominal cavity.

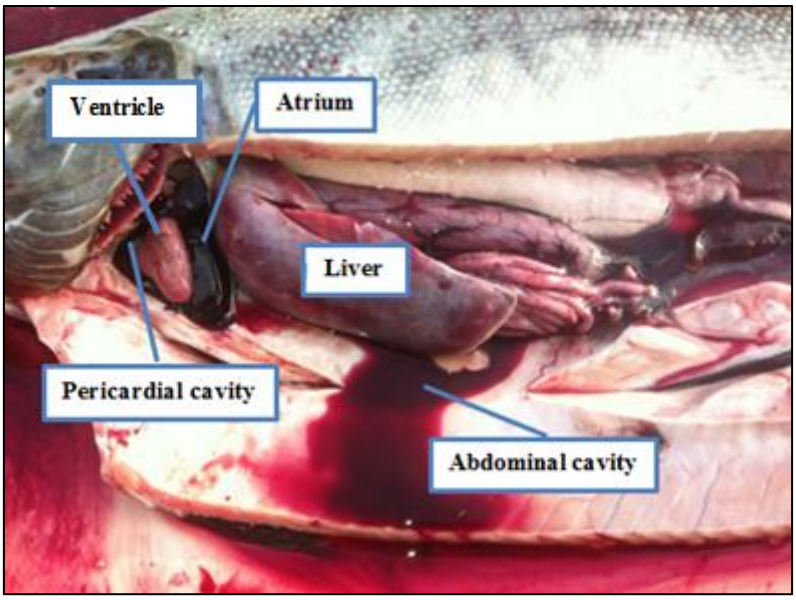

Figure 5. Classical signs of *Cardiomyopathy syndrome* (CMS), score 3. Atrium filled with blood, fluid in abdominal cavity, hemorrhage in pericardial cavity/abdominal cavity and edema on liver surface. Photo: Per Helge Bergtun.

Histology scoring

Histological changes in the ventricle tissue were classified into five scores based on the severity of the lesions, as listed in table 3. Figure 6 shows micrographs according to the classification.

Table 3. Histological scoring of lesions in endo-, epi- and/or myocardium for Atlantic salmon (Fritsvold et al. 2009).

Score	Description
0	No pathological findings, or slightly increased number of leukocytes
1	One or a few focal lesions, increased number of leukocytes
2	Several distinct lesions and small to moderate increase in number of leukocytes
3	Multifocal to confluent lesions and moderate to severe increase in number of leukocytes
4	Severe confluent lesions comprising > 75 % of the tissue and massive leukocyte infiltration

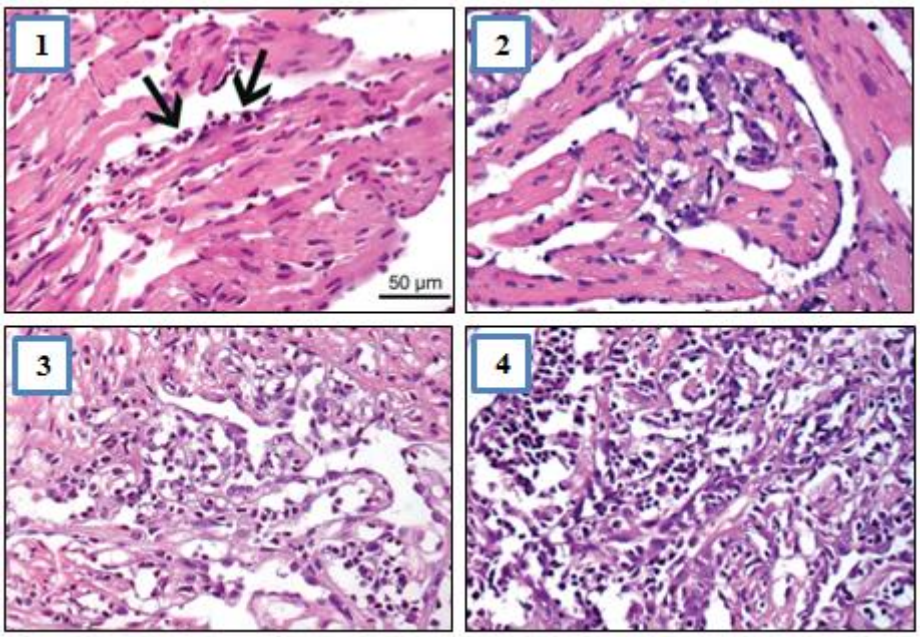

Figure 6. Micrographs representing the histological scores of lesions in the ventricle tissue of Atlantic salmon, according to the classification in table 3. Score 1: Arrows show minor inflammatory lesions with a sparse number of focal subendocardial infiltrated leukocytes. Score 2: Several inflammatory lesions and an increase of leukocytes. Score 3: Multifocal to confluent lesions and much tissue has been replaced by infiltrated leukocytes. Score 4: Severe and confluent lesions where most of the tissue has been replaced by massive leukocyte infiltration (Fritsvold et al. 2009).

Heart index

The heart index (HI) was calculated by the following formula:

$$HI = \frac{Ventricle\ weight\ (g)}{Body\ weight\ (g)} * 100$$

3.2 Data analysis

Statistical Analysis

R Studio and Microsoft Excel were used for preparation and organization of the data. In addition, calculation of descriptive statistics and non-genetic statistical analyses, such as regression, Least square means, box-plots, Pearson's correlation coefficient and tests of statistical significance were performed in the same software. The level of significance was set to 5% ($P < 0.05$).

Genetic analysis

The pedigree file for the Mowi-strain was received from Marine Harvest.

Estimates of genetic and residual variance components for the studied traits (smolt weight, body weight, ventricle weight, heart index, CMS at harvest and survival in three different periods) were obtained from a multitrait sire and dam model, using ASREML software (Gilmour et al. 2006). The variance component for sire was set equal to the variance component for dam and equal to ¼ of the additive genetic variance. In addition to the random effects of sire and dam, a combined fixed effect of cage and sex was included in the model.

When ventricle weight and heart index were included in the model, the parameters did not converge due to high genetic correlations between the traits. Consequently, these two traits were included in the model one at a time.

When CMS at harvest was analyzed, the trait was treated as a binary trait, where fish scored 0 was 0 and fish scored >1 was labeled 1.

For the survival traits a Bayesian approach with Gibbs sampling was used and a threshold sire and dam model was fitted to the survival traits. The variance components for the survival traits were obtained on the non-observable underlying liability scale, one at a time, in the multivariate analyses, as the software used does not allow obtaining variance component on the liability scale simultaneously for two such traits. Survival in period 3 was used in the genetic correlation analyses.

For all traits the following model was used:

$$y = Xb + Z_s u_s + \mathrm{Z}_d u_d + e$$

Where y = is a vector of observed trait

b = is a vector of fixed effects [combined effect of sex and cage]

u_s = is the vector of ½ the sire additive genetics values

u_d = is the vector of ½ the dam additive genetic values

e = is the vector of residual errors

X, Z_s, Z_d = incidence matrices that assign each observation to its appropriate level of the fixed and random effects

For smolt weight an additional random effect of full-sib family was included to account for a possible combined non-additive genetic and maternal environmental effect.

For all traits, except smolt weight, heritability (h^2) was calculated as follows:

$$h^2 = \frac{4\sigma_{sd}^2}{2\sigma_{sd}^2 + \sigma_e^2}$$

Where σ_{sd}^2 = additive genetic sire=dam variance

σ_e^2 = residual variance

For smolt weight, common environmental variance (σ_c^2) was added to the total phenotypic variance. For survival, the residual variance was assumed to be 1.

Genetic, residual- and phenotypic correlations were categorized into three groups based on numerical values:

Low correlation	0.000-0.333
Moderate correlation	0.334-0.666
High correlation	0.667-1.000

Heritability was categorized as follows:

Low heritability	0.000-0.199
Moderate heritability	0.200-0.399
High heritability	0.400-1.000

4 Results

4.1 Mortality

Figure 7 shows the mortality in each period in percentage of total PIT-tagged fish. Figure 8 shows the time course of the mortality in the sea phase (period 2 and 3).

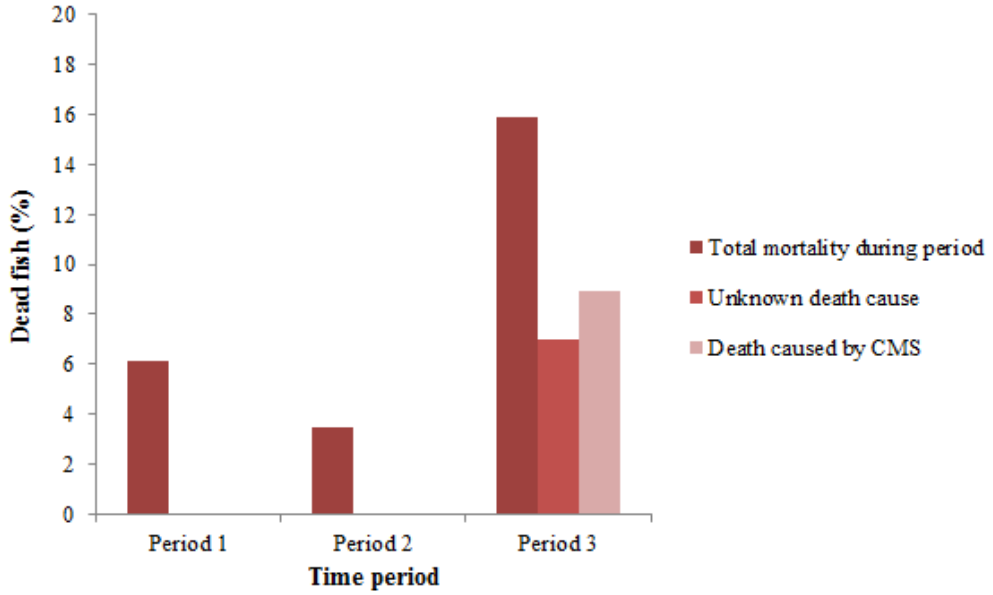

Figure 7. Fish mortality during the different periods, in percent out of total PIT-tagged fish (n=4667).

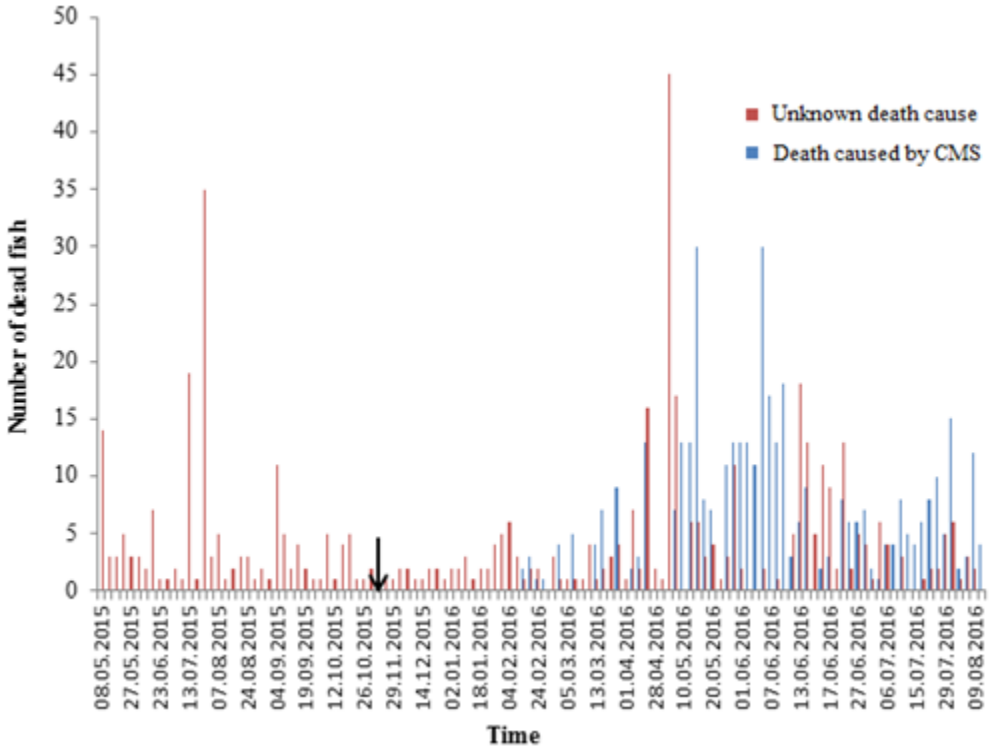

Figure 8. Number of dead fish per day during period 2 (from 05.05.2015 to 12.11.2015, N=164) and period 3 (from 13.11.2015 to 25.08.2016, N=741) in the sea phase. In period 3 death caused by CMS (n=414) and unknown death cause (n=327) are represented in blue and red columns, respectively.

In the sea phase, in total 905 fish died, 491 by unknown death cause and 414 by CMS. Highest mortality was observed between splitting and slaughter (period 3), which was also the longest period. Signs of CMS was observed from February 2016, and caused higher mortality than unknown death causes in period 3, with 414 diagnosed in retrospect with CMS compared to 327 with unknown death cause.

4.2 Regression analysis

Figure 9 shows the regression of ventricle weight on body weight. Both the first and the second degree polynomial of body weight were significant ($p<0.001$) and explained altogether 80.4% of the variation in ventricle weight.

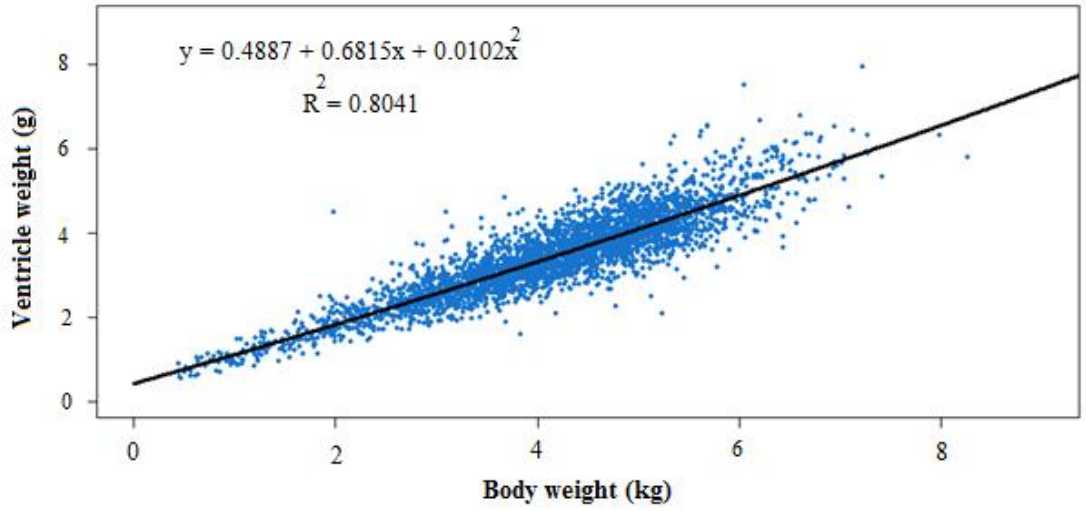

Figure 9. Scatterplot showing the relationship between body weight (kg) and ventricle weight (g) of all fish that survived until slaughter (n=3423).

Figure 10 shows the regression of heart index on body weight. Both the first and second degree polynomial of body weight were significant ($P<0.001$) and explained altogether 16.3% of the variation on heart index.

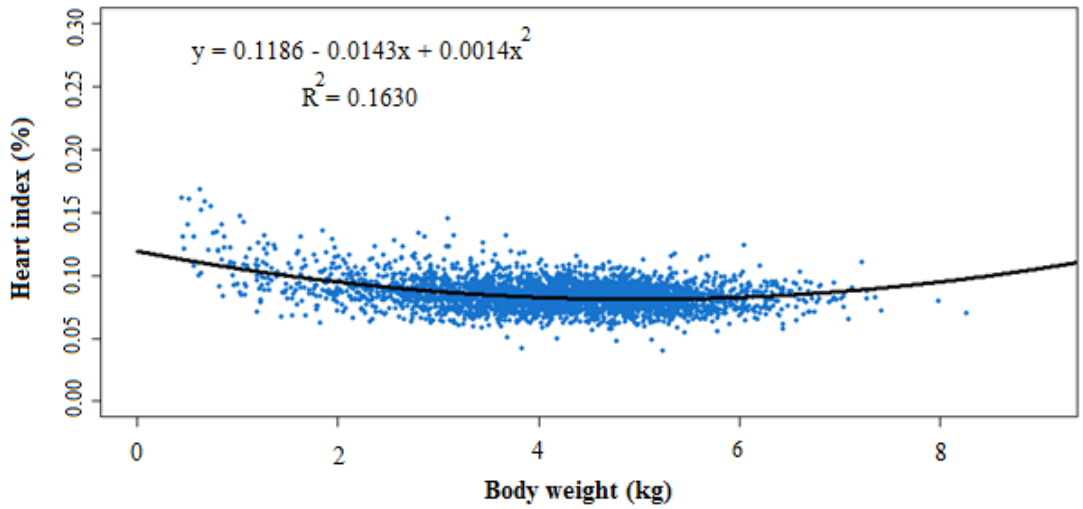

Figure 10. Scatter plot showing the relationship between body weight (kg) and heart index (%) of all fish that survived until slaughter (n=3423).

Differences in ventricle weight and heart index between male and female fish adjusted for the cage effect is shown in figure 11.

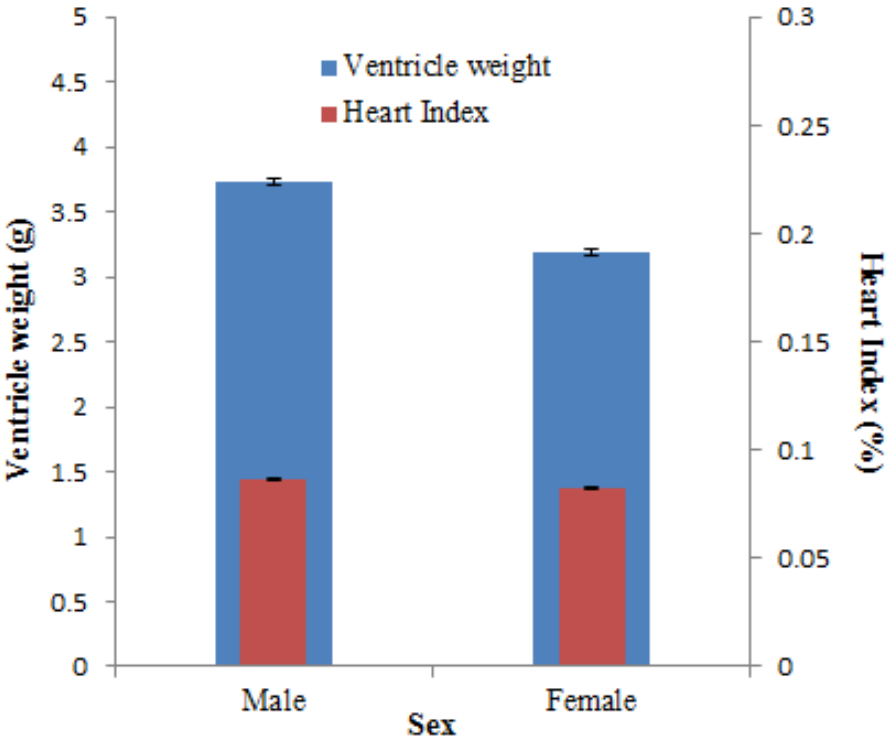

Figure 11. Least squares means of ventricle weight and heart index ± SE for male (n=1712) and female (n=1711) fish.

Male fish had on average heavier hearts than female fish, 3.728 g and 3.193 g respectively (Figure 11). When adjusted for body weight, as in heart index, this difference was considerably reduced, with an average heart index of 0.086% for males and 0.082% for females. The difference between the sexes was significant ($p<0.05$) for both ventricle weight and heart index.

4.3 Heritabilities and Correlations

In table 4 estimates of heritabilities (h^2) for the different traits are listed, and for smolt weight the effect common to fullsibs other than additive genetics (c^2).

Table 4. Estimates of heritabilities (h^2) and of the effect common to fullsibs other than additive genetics (c^2) for the different traits.

Trait	Heritability (h^2) ± SE	Effects common to fullsibs (c^2) ±SE
Smolt weight	0.54 ± 0.07	0.02 ± 0.01
Body weight	0.30 ± 0.04	
Ventricle weight	0.34 ± 0.04	
Heart Index	0.20 ± 0.03	
CMS, harvest[2]	0.09 ± 0.04	
Survival		
- Period 1	0.07 ± 0.04[1]	
- Period 2	0.03 ± 0.04[1]	
- Period 3	0.17 ± 0.04	
- Unknown	0.05 ± 0.03[1]	
- CMS	0.25 ± 0.05	

[1]Not significant ($p>0.05$) [2]CMS (score 1-3) or not CMS (score 0) at harvest

Estimated heritability for smolt weight was relatively high (0.54). For body weight (0.30), ventricle weight (0.34), and heart index (0.20) the estimated heritability was moderate, while for survival (0.17) and CMS at harvest (0.09) it was low. The effect common to fullsibs was only significantly different from zero for smolt weight and of very low magnitude (0.02).

For survival in the different periods the estimated heritability was low, and not significant in period 1 and 2. The heritability for survival of unknown death causes was low and not significant, while survival of CMS was moderate (0.25).

Estimated heritability, genetic correlation, residual correlation and phenotypic correlation (Pearson's correlation coefficient) between traits are presented in table 5.

Table 5. Estimated heritability (diagonal), genetic correlation (above diagonal), residual correlation and phenotypic correlation (Pearson's correlation coefficient) (below diagonal) between traits, and their standard errors. Residual correlation and phenotypic correlation are separated by /.

	Survival[2]	**Smolt weight**	**Body weight**	**Ventricle weight**	**Heart index**	**CMS,harvest[3]**
Survival[2]	0.17± 0.04	0.08 ± 0.14[1]	0.32 ± 0.15	0.34 ± 0.14	0.09 ±0.16[1]	
Smolt weight	-0.35/0.12	0.54 ± 0.07	0.44 ± 0.08	0.42 ± 0.08	-0.01 ±0.10[1]	0.18 ± 0.20[1]
Body weight	-0.04	0.33/0.37	0.30 ±0.04	0.92 ± 0.02	-0.23 ± 0.11	0.33 ± 0.22[1]
Ventricle weight	-0.02	0.29/0.34	0.88/0.89	0.34 ± 0.04		0.51 ± 0.22
Heart index	0.05	-0.09/-0.07	-0.37/-0.32	/0.09	0.20 ± 0.03	0.48 ± 0.23
CMS, harvest[3]		0.06	0.04	0.03	-0.02	0.09 ± 0.04

[1]Not significant (p>0.05) [2]Survival period 3 [3]CMS (score 1-3) or not CMS (score 0) at harvest

Estimated genetic correlation between ventricle weight and body weight was very high (0.92), moderate between ventricle weight and smolt weight (0.42), between ventricle weight and survival (0.34) and between CMS scored at harvest and heart index (0.48). There was a moderate negative genetic correlation between heart index and body weight (-0.37), while for heart index and survival (0.09) and smolt weight (-0.01), the genetic correlation was very low and non-significant (p>0.05). Estimated residual correlation between body weight and ventricle weigh was high (0.88), moderate or low for all the other traits. The genetic correlation analysis did not converge for survival of CMS.

Heart index is a function of ventricle weight and body weight; hence there will be an autocorrelation between heart index and the respective traits, which greatly affects the correlation between them. For ventricle weight and heart index, the genetic correlation analysis did not converge.

Phenotypic correlation (Pearson's) between body weight and ventricle weight was high (0.89), moderate between smolt weight and body weight and between smolt weight and ventricle weight, while low for the other traits. For body weight and heart index there was a negative correlation (-0.32). For survival, only phenotypic correlation was calculated for smolt weight, due to no registered observations on the dead fish for the remaining traits.

4.4 Breeding Values

Table 6 shows descriptive statistics for estimated breeding values (EBV) for body weight, ventricle weight, heart index and survival of CMS by sire. Figure 12 shows EBVs for the respective traits for each of the 173 sires.

Table 6. Descriptive statistics for estimated breeding values by trait by sire.

Trait	N	Mean	S.D	Min	Max	CV
Body weight (kg)	173	4.16	0.42	3.15	5.25	10.1
Ventricle weight (g)	173	3.46	0.37	2.58	4.46	10.8
Heart index (%)	173	0.084	0.003	0.076	0.098	3.9
Survival of CMS (%)	173	72.3	10.5	35.1	90.6	14.6

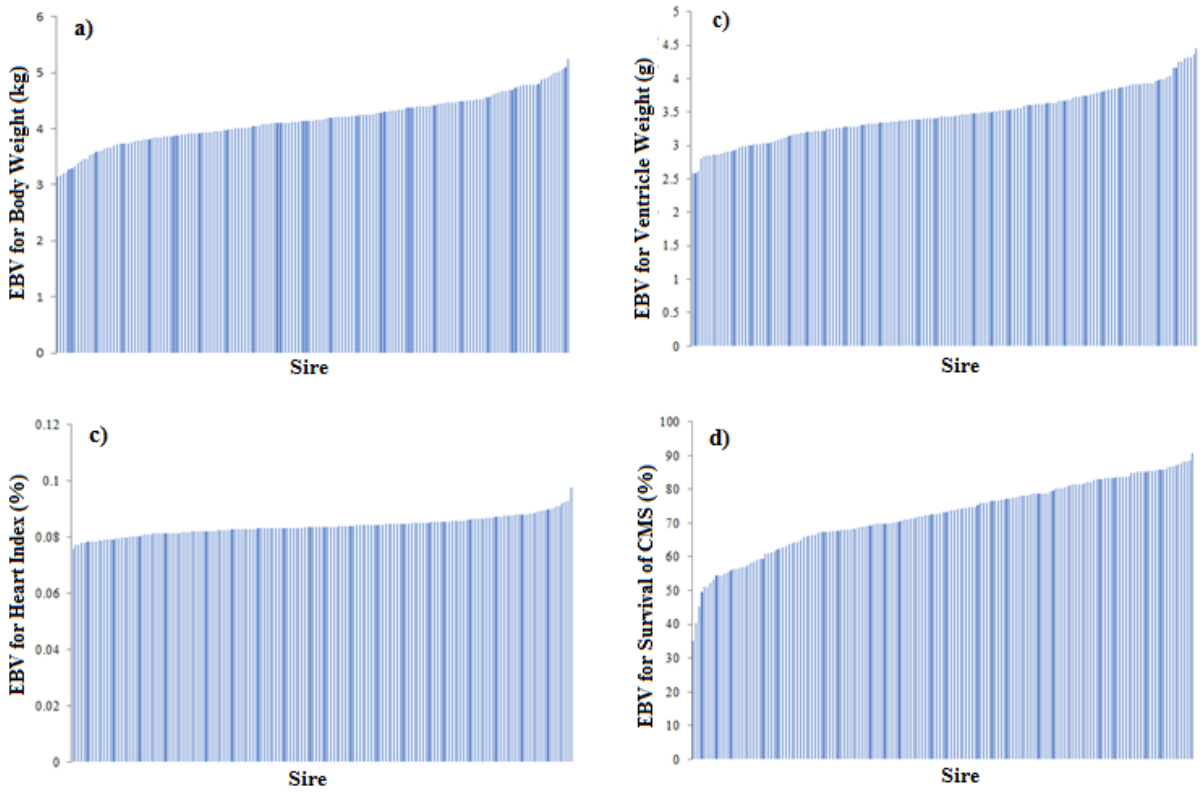

Figure 12. Estimated breeding values (EBV) for a) body weight (kg), b) ventricle weight, c) heart index and d) survival of CMS by sire.

For body weight the EBV ranged from 3.15 to 5.25 kg, for ventricle weight from 2.6 to 4.5 g, for heart index from 0.076 % to 0.098 %, and for survival of CMS from 35.1% to 90.6 %. The coefficient of correlation (CV) for body weight and ventricle weight were nearly similar, 10.1 and 10.8, respectively. Heart index had low CV (3.9), while for survival of CMS it was considerably high (14.6).

Pearson's correlation coefficients for the EBV's for the different traits were calculated (table 7), as the genetic correlation analysis for survival of CMS did not converge.

Table 7. Pearson's correlations coefficient between estimated sire breeding values for different traits.

	Ventricle weight	Heart index	Survival of CMS
Body weight	0.92	-0.22	0.23
Ventricle weight		0.16	0.35
Heart index			0.31

The correlations showed positive relationships between the breeding values among body weight, ventricle weight, heart index and survival of CMS, except between body weight and heart index.

4.5 Viral load, visual- and histological scoring of CMS

Of the 47 dead fish from which a tissue sample was obtained during summer 2016, 32 were diagnosed with CMS, while 15 had unknown death cause. Figure 13 shows the average PMCV-Ct value for each group.

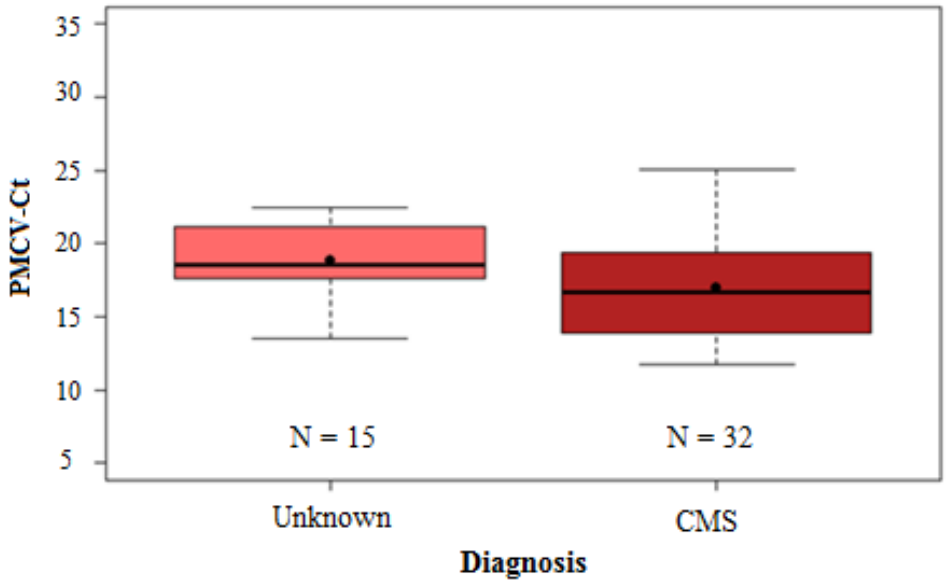

Figure 13. Box-plots showing PMCV-Ct values summary statistics for fish dead due to CMS and unknown causes. The black line of the box is the median (the 50th percentile), the point is the mean, the box extends from the lower to upper quartile (the 25th to 75th percentile, respectively) and the whiskers represent minimum and maximum values, as long as they lie within 1.5 interquartiles (box-lengths).

CMS-diagnosed fish had an on average lower PMCV-Ct value, that is to say higher viral load, than fish with unknown death cause ($P<0.05$). Fish diagnosed with CMS had also more extreme minimum and maximum values than the fish with unknown death cause.

Of the slaughtered fish, 3204 fish showed no visual signs of CMS (score 0), 244 fish fell within score 1, 19 fish within score 2, and 10 fish within score 3. 41, 19 and 10 samples from score 1, 2 and 3, respectively, plus samples from 360 fish with no CMS observations were analyzed with qPCR for CMS. Figure 14 shows the average PMCV-Ct value for each group.

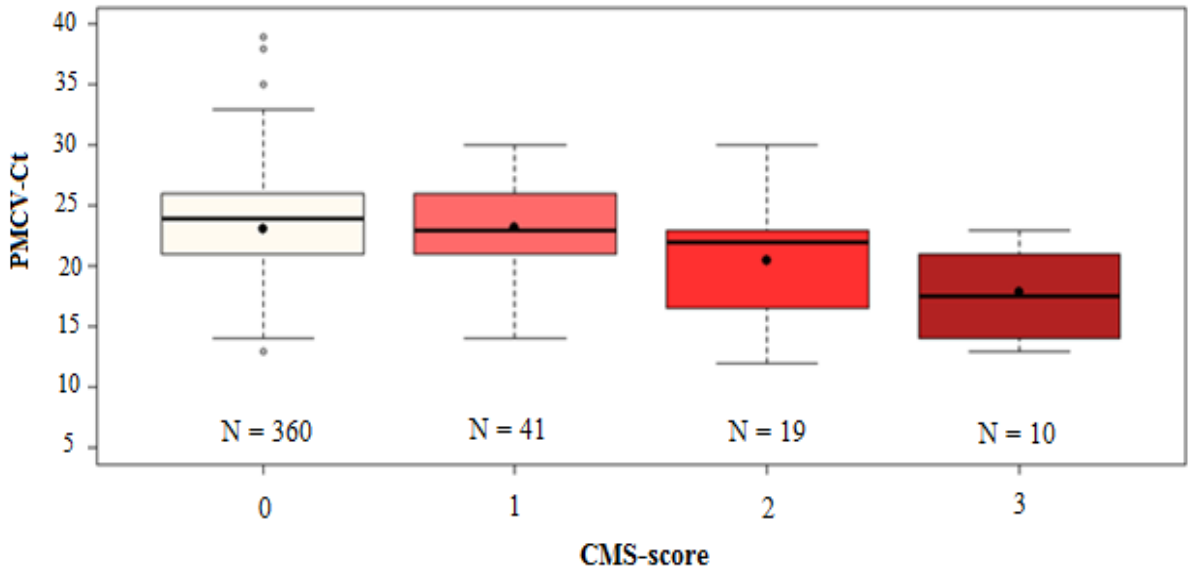

Figure 14. Box-plots showing PMCV-Ct values summary statistics for fish visually scored for CMS during slaughter (see table 2). The black line of the box is the median (the 50th percentile), the point is the mean, the box extends from the lower to upper quartile (the 25th to 75th percentile, respectively) and the whiskers represent minimum and maximum values, as long as they lie within 1.5 interquartiles (box-lengths). The points outside the whiskers are outliers, and lie outside the range of 1.5 interquartiles (box-lengths).

Fish with CMS-score 3 had the highest viral load. Fish with score 2 had second highest, while fish with score 0 and score 1 had nearly equal and the highest PMCV-Ct values, and thereby the lowest viral load. There was a significant difference ($p<0.05$) in viral load between score 3 and 0, score 3 and 1, and between score 2 and 0.

Pearson's correlation coefficient of CMS-score and PMCV-Ct value was -0.20 and significant ($P<0.05$), indicating that higher CMS-score gives higher viral load. Correlation coefficient of CMS-score with ventricle weight (-0.02) and heart index (0.02) were very low and not significantly different from zero ($P>0.05$).

Figure 15 shows the visual CMS-score as a function of bodyweight, ventricle weight and heart index, respectively.

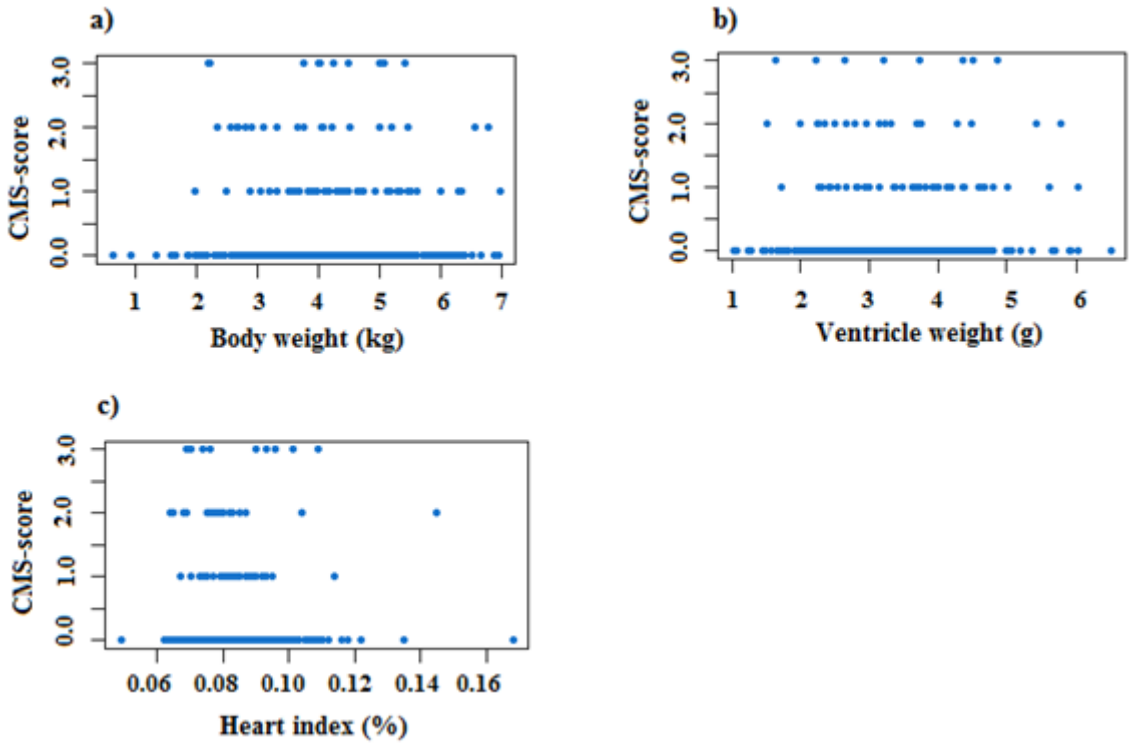

Figure 15. Scatterplots showing the relationship between CMS-score and a) body weight, b) ventricle weight and c) heart index.

Neither body weight, ventricle weight or heart index explained any significant variation in CMS-score ($P>0.05$).

Histology scores of CMS in the spongiosum were on average higher than in the compactum for all sample groups (figure 16). The sample groups with high CMS-scores and lowest PMCV-Ct values had higher histology scores than the sample group with the highest PMCV-Ct value, showing more severe inflammations and lesions in the ventricle of the two former groups.

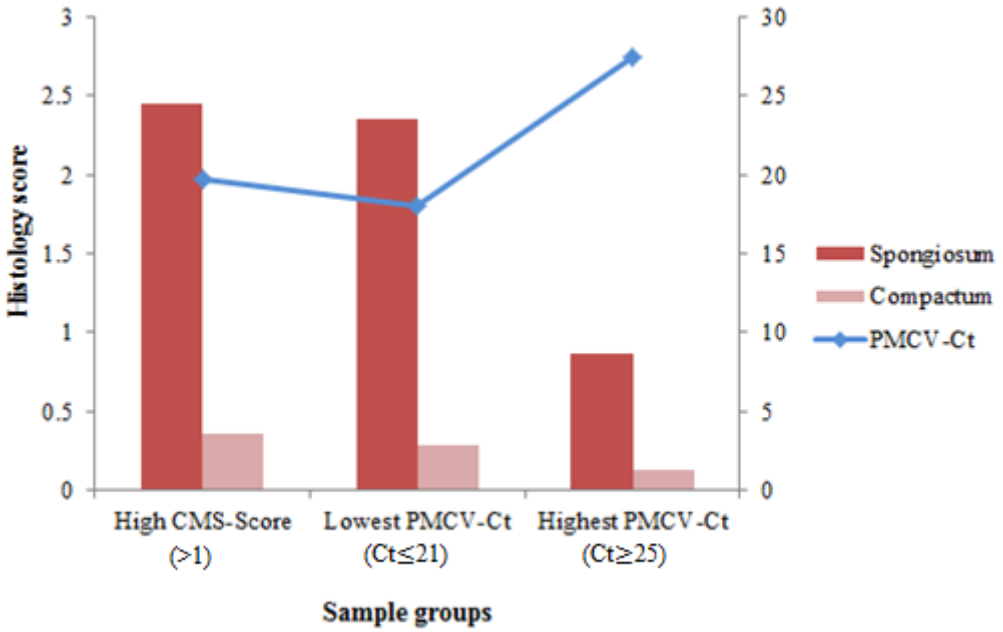

Figure 16. Average histological scores of spongiosum and compactum from ventricles with high CMS-score, and high and low Ct-values from PCR from slaughter fish. Average Ct-values for each sample group are also presented.

The correlation coefficient between compacta and spongiosa scores was low (0.3). Correlation coefficient of PMCV-Ct value with histological scores in spongiosum was high (-0.79, $P<0.05$), while that with histological scores of compactum was much lower (-0.28, $P<0.05$). This indicates that histology scoring in spongiosum is a better predictor of CMS than scoring in compactum.

5 Discussion

5.1 Mortality/Survivability

In the dataset, overall mortality was 25.5%. In the sea phase the mortality was 19.4%, which is consistent with The Norwegian Veterinary report 2016 (Hjeltnes et al. 2017). Hjeltnes et al. (2017) reported approximately 20 % annual losses during the sea phase. Mortality tends to be highest the first months after smolt is transferred to sea or during disease outbreaks (MarineHarvest 2016). In this study the mortality was highest in the last period, especially from the end of May (figure 8), after a sea-lice treatment, and was probably a result of vulnerable fish due to CMS.

Information about the magnitude of genetic variation in survival is scarce. In general, the heritability of survival in Atlantic salmon is low (0.0-0.16), and most studies are performed at young fish (Gjedrem & Baranski 2010; Rye et al. 1990; Standal & Gjerde 1987). Similarly, low heritabilities (0.08-0.17) were found for rainbow trout from 10 different year classes reared at three test stations, when survival was treated as a single trait. However, a wide range of heritabilities (0.04-0.71) were found when each generation and specific test station were tested alone (Vehviläinen et al. 2008). In the present study the estimated heritability of overall survival (period 3) was 0.17, which is in the upper range of what earlier literature has reported for Atlantic salmon, whereas lower compared to some of the findings for rainbow trout by Vehviläinen et al. (2008). The heritability at different life stages showed large variation (0.03-0.17), with the highest heritability in period 3. Low heritability estimates for survival indicate that low selection response will be obtained when selecting for survival (fish survived until harvest). Standardized challenge tests are often performed to study genetic variation in survival (resistance) against viruses, bacteria and parasites, instead of studying the overall survival (Gjedrem 2005). This can reveal potential hidden genetic variation within the overall survival trait as overall survival may be due to different causes.

Estimated heritability for survival of CMS was moderate (0.25±0.05), which indicates that this is a heritable trait and could possibly be improved by selective breeding, as also seen by the relatively large variation in EBV for survival of CMS among sires (figure 12d). This might be comparable to a study by Norris et al. (2008), who found an estimated heritability for survival to a natural PD-challenge of 0.21±0.005. Norris et al. (2008) argued that by including mortality by other causes, the residual variance would increase and heritability thereby decrease. This is evident for the current study, as the estimated heritability for overall

survival was 0.17, when unknown death causes was included in the multivariate analysis in addition to death caused by CMS. Kjøglum et al. (2008) estimated heritabilities for resistance against other diseases, such as ISA (0.37), IPN (0.55) and furunculosis (0.43-0.62). These studies were, however, based on standardized challenge tests.

Genetic correlations between survival and body weight and survival and ventricle weight were 0.32 and 0.34, respectively. This suggests that selection for body weight and/or ventricle weight will have a positive, but small, effect on survival. The standard errors for the respective correlations were considerably high, as explained previously in the chapter, and constitute an uncertainty to the analysis of the survival correlations.

Pearson's correlation coefficient for EBV for survival of CMS with heart index was 0.31 (table 7), and slightly higher than Pearson's correlation coefficient for total survival in the sea phase with heart index (0.23, not shown in the result). This may indicate that a big heart is more important for survival of CMS than for other death causes, although no significant genetic correlation was found between survival and heart index.

5.2 Body weight

Moderate heritabilities for body weight (0.2-0.4) have been found for Atlantic salmon in various age classes (Gjerde & Gjedrem 1984; Gjerde 1986; Gjerde et al. 1994; Rye & Refstie 1995). In the current study, the estimated heritability of body weight at slaughter was 0.30±0.04 and in accordance with previous studies, while for smolt weight (0.54±0.07) it was a little higher than previous studies. The estimated genetic correlation between smolt weight and final body weight was moderate (0.44±0.08), and in agreement with Gjerde et al. (1994). Body weight measured at different life stages seems to be different traits with different genetic variation in Atlantic salmon.

5.3 Ventricle weight

Estimated heritability for ventricle weight was moderate (0.34±0.04) and in accordance with the only previously published estimate of this trait in Atlantic salmon (Shehzad 2009). The very high genetic correlation between ventricle weight and body weight (0.92±0.02) indicates that the two traits are to a large degree controlled by the same genes. As the correlation is positive, the ventricle weight increases with increased body weight, which also the regression in figure 9 illustrates. This is in agreement with (Poupa & Lindstrom 1983) who suggested that heart weight in fish was almost directly proportional to body weight. However, as the

genetic correlation is less than unity, direct selection for increased body weight, as performed in today's breeding programs (Gjedrem & Baranski 2010), may over time due to a correlated response in ventricle weight result in a relatively smaller ventricle as further discussed in next chapter.

5.4 Heart index

The ventricle weight itself does not tell anything about whether the ventricle is small or large relative to the body weight. For this purpose, the trait heart index was used. According to Farrell and Jones (1992) the heart index of fish varies conciderably among species, from 0.035% in flounder (*Pleuronectes platessa*) to 0.38% in skipjack tuna (*Katsuwonus pelamis*), but also intraspecifically. The heart index in the current study ranged from 0.040% to 0.168 %, with an average of 0.084 % (CV=14.3), pursuant with the findings by Farrell and Jones (1992). This is also in accordance to a Farrell et al. (1988), who observed that heart index in rainbow trout (*Salmo gairdneri*) ranged between 0.08 % and 0.13 %. These results indicate that the heart of Atlantic salmon is relatively small compared to the body weight, and that there can be large variations within one species, even within the same population.

Significantly higher ventricle weight and heart index were observed for male compared to female fish in this study. This is also reported for sexually mature males compared to mature females (Franklin & Davie 1992; Graham & Farrell 1992; Shehzad 2009), but few studies include information about differences between sexes in immature fish, as this study shows. Consequently, when studying ventricle weight and heart index the observation should be accounted for sex and sexual maturity.

The estimated heritability for heart index in the current study was moderate (0.20±0.03) and thus substantially lower than for ventricle weight, and with a much lower CV (table 1). Thus, by expressing ventricle weight relative to body weight, the genetic variation in heart index is much reduced as compared to the genetic variation in ventricle weight. This is due to the high genetic correlation between the two traits, as also seen by the low variation in heart index between sires (figure 12c). As expected and due to the autocorrelation, the genetic correlation between heart index and body weight was negative and low (-0.23±0.11). This indicates that selection for increased body weight, and not simultaneously for heart index, will give a reduced heart index at slaughter, due to a lower correlated increase in heart weight relative to the increase in body weight, as discussed above.

The regression line of heart index on body weight (figure 10) showed, as expected, an inverse relationship between heart index and body weight. This was in agreement with the estimated negative genetic correlation coefficient (table 5). However, fish heavier than 6 kg had increasing heart index. The coefficient of determination (R^2=0.163) was low and indicated that body weight explained only 16.3 % of the variation in heart index.

The observed inverse relationship between heart index and body weight indicated that body weight increased faster than the ventricle weight, and thereby, faster than the circulatory system. A disproportionate relationship between these two traits may result in an imbalance between heart capacity and oxygen demand of the fish (Castro et al. 2011). This could lead to fish that are vulnerable to stressing situations, as the metabolic demand is high under such circumstances. This potential scenario might be a result of the relatively intensive selection for high body weight over many generations.

Direct selection for higher heart index could make it difficult to decide whether an improvement in heart index is a result of increased heart weight, decreased body weight or a modification of both traits. Furthermore, selection for a ratio trait is not effective as the realized heritability for the trait is low (Webb & King 1983), and the heritability of a ratio trait does not estimate a statistic that can be used to predict genetic change for that ratio (Gunsett 1986).

Theoretically, the selection differential will be largest for the more variable trait of the two traits in the ratio heart index (Gjedrem 2005). In this study the coefficient of variation was almost similar for the estimated breeding values for body weight (CV=10.1) and ventricle weight (CV=10.8). This indicates that when selection for heart index there will be a nearly equal selection pressure on body weight and ventricle weight. Both traits are also sensitive to environmental variations (Gamperl & Farrell 2004; Gjedrem & Baranski 2010), another confounding feature that will make it difficult to interpret the selection response of heart index.

The estimated genetic correlations among heart index, smolt weight and survival were low and not significant, suggesting that there is no favorable or unfavorable genetic relationship between the traits. However, as body weight, heart weight and heart index were missing for the dead fish in the current study, the estimated genetic correlations between survival and the other mentioned traits are likely biased and also have considerably higher standard errors than for the other genetic correlations (table 5). The degree of bias can be studied through

stochastic simulation and should be done before a firm conclusion about any favorable or unfavorable genetic relationship of heart weight, heart index and other traits is drawn.

5.5 Viral load, visual- and histopathological scoring of CMS

Estimated heritability for visual CMS at harvest was low (0.09). An unfavorable relationship between CMS at harvest and body weight was found, indicating that selection for increased body weight will give higher frequency of fish with CMS. As body weight is an economic important trait in the breeding goal for Atlantic salmon, direct selection also for increased resistance to CMS should therefore be implemented in the selection program.

A moderate genetic correlation (0.48) between CMS at harvest and heart index was found; implying that a relatively large heart may not always be favorable. The residual correlation was very low between the traits, suggesting that there were no compensation mechanisms (Gamperl & Farrell 2004; Mikalsen 2016) involved aiming to replace destroyed cardiac tissues or other responses to environmental- or physical demands which led to larger hearts for fish scored with CMS.

Dead fish diagnosed with CMS had in average significantly higher PMCV load than fish with unknown death cause. However, the viral load in fish with unknown death cause still had abundant viral load (Ct<29), suggesting that fish with few or no obvious pathological changes also might have been infected with PMCV. These fish might therefore have been classified wrongly; hence the CMS-category was underestimated. Post-mortem examination was performed by non-veterinarian personnel, and consequently pathological changes might have been overseen. This could also have underestimated the scope of the disease. However, some fish might have died by unknown death causes, but still being infected by PMCV. This could be due to resistance or tolerance against CMS, or perhaps they might have recovered after the disease.

Viral load can be a useful indicator of the severity of CMS, as viral load has been shown to be highly correlated with histopathological scores of the heart, kidney and spleen (Haugland et al. 2011; Timmerhaus et al. 2011). This was also true for the present study, with a high correlation between PMCV-Ct and histology scores in the spongiosum (-0.79). The much lower correlation between PMCV-Ct and histology scores in the compactum (-0.28) indicates that histology scoring in spongiosum was a better predictor of CMS than scoring in compactum. This may be related to that the compact layer of the ventricle usually is less

affected by CMS, and lesions occur at later stages of the disease than in the spongiosum (Ferguson et al. 1990; Fritsvold et al. 2009).

The significant differences between viral load and the visual score 3 and 0, score 3 and 1, and between 2 and 0 (figure 16), suggested that it was easier to differentiate between low and high pathological changes rather than no or low changes. Still, some fish diagnosed with lower scores had high amounts of virus. This could be due to resistance or recovery of CMS, as discussed above. The two traits were negatively correlated (-0.20), showing that fish with high CMS-scores had lower PMCV-Ct-values. Due to the correlation being low, an increase in CMS-score only gave a small increase in viral load.

Based on the current study's results for diagnosing of dead and slaughtered fish, it seems like CMS-scoring by visual examination gives an underestimation of the scope of the disease. All scores had average PMCV-Ct values below 29, indicating that many of the fish scored as healthy or with few signs of CMS actually were infected with abundant amount of viral nucleic acids. These results show that visually CMS-scoring by examination of pathological changes not always correlates to the actual viral load and lesions, and is not a good predictor of severity of the disease.

Registrations at Atlantic salmon fish farms have suggested that big fish more frequently die from CMS and cardiac problems than smaller fish (Tørud & Hillestad 2004). However, neither body weight, ventricle weight or heart index showed any significant impact on CMS-score in this study (figure 15). Since the CMS-scoring system has been shown to be unprecise, which in part could be a result of not including observations on the dead fish, these results might be incorrect. However, Haugland et al. (2011) did not find any phenotypic correlation between body size and PMCV-Ct value in their study, neither did Refstie et al. (1993) between growth and CMS.

On the other hand, Refstie et al. (1993) found a negative genetic correlation between growth and CMS, showing that families with the smallest fish had most cardiac lesions associated with CMS. This could indicate that slow-growing fish are more exposed to CMS than fast-growing fish, or that fish are growing slower due to the disease. In the current study, Pearson's correlation coefficient for EBVs for survival of CMS and body weight showed a low, positive relationship (0.24), and indicated that larger fish, to a small extent, were more tolerant against CMS. However, as discussed above, an unfavorable genetic relationship between CMS at harvest and body weight was found in this study.

As CMS might have been a stressor to the fish, potential high cortisol levels could have induced hypertrophy of the compact layer of the ventricle (Johansen et al. 2014), or other compensation mechanisms might have been induces as described in chapter 2. Due to this, the circulation system of the diseased fish might have had reduced capacity, although ventricle weight or heart index did not have any significant effect on the severity of CMS.

The last part of the study concerning visually CMS-scoring, histology-scoring and PMCV-Ct values is based on a limited data material, something which gives the results low statistical power. However, one can see tendencies which should be followed up in further work.

6 Conclusion and Implications

The current study showed that ventricle weight was moderately heritable and highly positively correlated with harvest body weight. Bigger hearts are therefore indirectly selected for via selection for high body weight at slaughter. However, as the genetic correlation between ventricle weight and body weight was less than 1, direct selection for increased body weight at harvest may over time result in smaller ventricles or heart index, as also indicated by the negative correlation between body weight and heart index.

Survival of CMS showed higher heritability than both overall survival and CMS scored at harvest. No unfavorable genetic relationships were found for survival with smolt weight, body weight, ventricle weight or heart index. The magnitude of genetic correlations of CMS at harvest with body weight, ventricle weight and heart index, however, indicated an unfavorable genetic association between these traits and CMS. Direct selection for increased resistance to CMS should therefore be implemented in the breeding goal and program to reduce economic losses and also to improve the fish welfare in the sea phase for Atlantic salmon.

Visual CMS-scoring of dead and slaughtered fish might underestimate the scope of the disease, as high viral loads and severe histopathological changes were observed in fish with no obvious, visual pathological changes.

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8 APPENDIX

Table A1 shows descriptive statistics by trait for the four specific cages (cage 7-10) the fish were split into (period 3)

Table A1. Descriptive statistics by trait for fish in cages 7-10

Trait	Number of obs.	Mean	S.D	Min. value	Max. value
Cage 7					
Smolt weight (g)	1166	72.7	18.3	23.3	165.4
Body Weight (kg)	976	4.15	1.16	0.46	8.26
Body Length (cm)	976	69.17	6.19	39.00	88.00
Ventricle Weight (g)	966	3.340	0.951	0.600	7.500
Heart Index (%)	966	0.082	0.013	0.042	0.168
Mortality (%)	190	16.3			
Cage 8					
Smolt weight (g)	1103	69.4	17.4	26.3	151.4
Body Weight (kg)	936	3.95	1.12	0.44	7.98
Body Length (cm)	936	68.16	6.56	35.00	86.00
Ventricle Weight (g)	913	3.302	0.949	0.570	6.540
Heart Index (%)	913	0.085	0.012	0.048	0.160
Mortality (%)	167	15.1			
Cage 9					
Smolt weight (g)	937	72.1	18.3	22.8	156.3
Body Weight (kg)	753	4.26	1.12	0.57	7.27
Body Length (cm)	753	69.36	5.99	40.00	82.00
Ventricle Weight (g)	746	3.598	0.968	0.740	6.650
Heart Index (%)	746	0.085	0.011	0.060	0.158
Mortality (%)	184	19.6			
Cage 10					
Smolt weight (g)	1012	68.4	18.6	20.9	140.8
Body Weight (kg)	812	4.24	1.10	0.50	7.41
Body Length (cm)	812	69.06	5.88	39.00	85.00
Ventricle Weight (g)	798	3.581	0.964	0.650	7.930
Heart Index (%)	798	0.085	0.012	0.040	0.130
Mortality (%)	200	19.8			

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