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Genetic variation in susceptibility of Atlantic salmon *(Salmo salar L.)* to salmon lice, *Lepeophtherius salmonis*, from field and challenge tests

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ABSTRACT

Sea lice, *Lepeophtheirus salmonis (L. salmonis)* is the major threats for Norwegian salmon farming industry and the cost of the parasite was estimated to be around five billion NOK per year. Chemical treatments have been used to control sea lice infection in the last two decades, however, negative impacts on the economic, environment and animal welfare has also been reported. Results from several Atlantic salmon breeding programs suggest that there is substantial additive genetic variation in resistance to the sea lice. The aim of this study was to estimate the genetic variation in resistance to sea lice (*L. salmonis*) with dataset from field and tank challenges in marine harvest breeding populations. The specific objectives of the study were to estimate genetic (co) variation between repeated lice challenges of *i*) the same year class of families, *ii*) between different year classes, and *iii)* the importance on the use of lice count per fish (LC) as compared to lice density (LD; i.e. LC adjusted for body size of the fish). To achieve this, a total of 15457 individuals with complete phenotype information and 14269 individuals with genotype information from 1339 families of 644 sire and 1305 dams were measured for LC and LD across several year-classes of Atlantic salmon. Challenge tests were conducted in sea net-cages and tanks. The average LC in net cages ranged from 4.6 (SD = 2.9) to 15.5 (SD = 8.0) and in tank it was 17.2 (SD = 12.5) and 23.9 (SD = 15.5) for *Matre* and *Veso*, respectively. In net cages heritability estimates of LC was 0.05 to 0.18 and 0.06 to 0.15 using pedigree and genomic relationship matrix, respectively. In tanks, the heritability estimates for LC was 0.19 and 0.30 and 0.16 and 0.24 in *Matre* and *Veso*, using pedigree and genomic relationship matrix, respectively. LD heritability estimates ranged from 0.04 to 0.16 in net cages while in tanks it was 0.22 in *Matre* and 0.25 in *Veso*. Genetic correlation between LC and LD was 0.67 to 0.99 across all the year classes.

The genetic correlation between the two independent counts for year class 2015 and 2016 was found to be positive and significantly different from zero. In addition to this, we have found a moderate genetic correlation between the three locations of 2017- year class both for LC and LD. Phenotypic correlation of body weight with LC or LD were -0.05 to 0.2 and -0.05 to -0.41 for LC and LD respectively; suggesting that selection for increased body weight in Atlantic salmon would not cause unfavourable correlated response in lice resistance.

SAMMENDRAG

Lakselus, *Lepeophtheirus salmonis (L. salmonis)* er den største trusselen for norsk laks oppdrettsindustri, og kostnaden forbudet med parasitten er anslått til rundt fem milliarder kroner per år. Mange ulike behandlingsmetoder er i bruk for å kontrollere problemet, men alle med negativ virkninger på miljø og dyrevelferd. Studier har vist at det er betydelig additiv genetisk variasjon i resistens mot lakselus og at det derfor er mulig å gjøre utvalg for økt resistens i et avlsarbeid. Målet med denne studien var å undersøke størrelsen på den genetiske variasjonen for resistens hos laks mot *L. salmonis* og den genetiske korrelasjonen mellom resistens mellom gjentatte målinger av resistens på samme familier i en årsklasse og mellom familier i ulike årsklasser; og i hvilken egenskap, antall lus per fisk (LC, lice count) eller tetthet lus (LD, dvs. LC justert for fisken størrelse), en bør bruke år en skal gjøre utvalg for resistens mot lus i avlsarbeidet. For å undersøke dette analyserte vi LC data fra 15457 laks etter 644 fedre og 1305 mødre og som ble registrert på fisk i både i merder og kar. Gjennomsnittlig LC i merdene varierte fra 4,6 (SD = 2,9) til 15,5 (SD = 8,0) og i kar 17,2 (SD = 12,5) og 23,9 (SD = 15,5) for henholdsvis Matre og Veso. I merdene varierte arvegraden for LC fra 0,05 til 0,18 ved bruk av klassisk slektskapsmatrise, og fra 0,06 til 0,15 ved bruk av genomisk slektskapsmatrise. I kar var de tilsvarende estimatene 0,19 og 0,30 for Matre og 0,16 og 0,24 for Veso. De genetisk korrelasjonene mellom LC og LD varierte fra 0,67 til 0,99, noe som tyder på at korrigering av LC for størrelsen på fisken hadde relativ liten betydning i dette fiskematerialet. Den genetiske korrelasjonen mellom LC i uavhengige tester i samme årsklasse ble funnet å være positive. I tillegg ble det funnet en moderat genetisk korrelasjon mellom de tre smittetestene i samme årsklasse. Fenotypisk korrelasjon mellom kroppsvekt var -0,05 til 0,2 for LC og -0,05 til -0,41 for LD; noe som tyder på at utvalget for økt kroppsvekt i atlantisk laks ikke ville forårsake ugunstig korrelert respons i resistens mot lus.

Table of Contents

List of Tables

List of Figures

List of Appendices

1. BACKGROUND

The demand and consumption of fish has seen a significant growth around the world and it is the primary source of animal protein for over a billion people, which account for ~17% of the global intake of animal protein (FAO, 2016). The aquaculture industry has grown dramatically over the last few decades and this has helped to reduce the further depletion of natural fish stocks.

Atlantic salmon (*Salmo salar*), an anadromous species found primarily in the northern Atlantic Ocean, is the most widely farmed species in the aquaculture industries with an estimated production of more than 2 million metric tons and in 2016 the global economic value of this species was estimated at approximately 8-10 billion US dollar (FAO, 2016). The industry of salmon farming is led by Norway, which produced ~1.31 million tons of Atlantic salmon with an economic value of 44.3 billion NOK in 2015. Other countries like Chile, UK, Canada, the Faroe Islands and Australia are also considered as main producers' of Atlantic salmon(*Statistics Norway* 2016).

One of the biggest challenge to the Atlantic salmon industry is sea lice (Torrissen et al., 2013), although many other diseases (pancreas disease, infectious pancreas necrosis, cardiomyopathy syndrome, etc) affect the industry as a whole. The success and the sustainability of the Atlantic salmon industry is largely dependent on the control of diseases (Yanez et al., 2014).

Sea lice are small marine ectoparasites that belongs to the order copepod in the genera of *Lepeophtherius* and *Caligus*. Sea lice has a life cycle of 10 developmental stages, of which two are planktonic stages and eight are parasitic on the host fish (Kabata, 1988; *World of Copepods*, 2012). *Lepeophtheirus salmonis (L. salmonis)* and *Caligus rogercresseyi (C. rogercresseyi)* are the two most dominant and concerned species in the aquaculture industries and both affect wild and farmed salmonids (Johnson et al., 2004; Pike & Wadsworth, 2000). *L. salmonis* is the major parasite of concern for European salmon industry and although *L. salmois* is prevalent both in Atlantic and Pacific Ocean costs, the Pacific and Atlantic form of *L. salmonis* are genetically distinct (Boulding et al., 2009; Yazawa et al., 2008). *C. rogercresseyi* is the most prevalent parasite in the South Hemisphere and is the major parasite in Chile affecting about 99% of cultured cages (Boxshall & Bravo, 2000; Carvajal et al., 1998). *Lepeophtheirus salmonis* has been found on Atlantic salmon, sea trout, rainbow trout, chinook and Coho salmon (Gjerde & Saltkjelvik, 2009; Johnson & Albright, 1992).

Even though sea lice usually do not cause high mortality, they represent a significant challenge for the salmon aquaculture industry because of the negative economic, animal welfare and

environment impacts. Skin lesions, osmotic imbalance and increased susceptibility to other infections are the symptoms that are manifested on the fish infested by sea lice. Furthermore, large economic losses occur directly due to loss of products and treatment costs, while indirect production losses occur through reduced growth, low feed efficiency and increased indirect mortality. The annual losses attributed to control of sea-lice is estimated to be $480 million dollars(Costello, 2009). Reports from Norway revealed that cost of sea lice has gradually increased as the estimated cost has increased from 2.45 NOK per Kg in 2011 (Jensen, 2013) to 4.25 NOK per kg of salmon produced. The annual cost of sea-lice in Norway was estimated as 5 billion NOK (Iversen et al., 2017).

During the last 10-15 years' chemical and mechanical treatments have been used to control sea-louse infections. These control measures result in large direct costs, as well as possible damage to the environment (Tsai et al., 2016). Several attempt are being made to develop vaccines, however, it's not likely the vaccine will be available for the market in the nearest future (Gjerde et al., 2010). Farmers also using cleaner fish (lumpfish and wrasse) as an alternative method of biological control, although there is doubt about the effectiveness of cleaner fish (Kolstad et al., 2005). An alternative or addition control measure is through selective breeding. Several studies there is additive genetic variation for resistance to sea lice for *Lepeophtheirus salmonis* (Gjerde et al., 2010; Glover et al., 2005; Kolstad et al., 2005) and *C. rogercresseyi* (Correa et al., 2017b; Tsai et al., 2016). Lhorente et. al (2012) estimated heritability (h^2) of 0.32 for resistance to *C. rogercresseyi* whereas Yanez et al. (2014) obtained h^2 of 0.12 for the same trait using pedigree and molecular information. These low to moderate heritabilities indicate that it is feasible to include resistance to these sea lice as a trait in the breeding program of Atlantic salmon.

The breeding programs of Atlantic salmon are the most advanced programs of all the aquaculture species and genomic information are routinely incorporated to construct pedigrees, and to improve selection accuracy via marker-assisted (MAS) or genomic selection (GS) (Lhorente et al., 2012). Studies conducted by Correa et al. (2017) and Houston et al. (2014) on *C. rogercresseyi* and *L. salmonis* using 50 K and 200 K SNP array respectively, proved that the genetic architecture of the salmon lice resistance trait has a polygenic inheritance (Correa et al., 2017b; Houston et al., 2014). For a trait that has a polygenic nature like sea-lice, genomic selection is an appropriate method of selection.

The overall objective of this study is to obtain reliable estimates of the genetic parameters of resistance to *L. salmonis* in the Marine Harvest nucleus populations of Atlantic salmon using filed and tank challenge tests. Specifically, we computed the genetic (co)variation between

repeated challenges of *i*) the same year class of families, *ii*) between different year classes, and *iii)* the importance including lice density as compared to lice count per fish in the model for estimation of genetic (co)variation. Lastly, we also estimated the genetic correlation across all year-class using genomic information.

2. LITERATURE REIVEW

2.1 Biology of sea lice

Copepods are a group of small aquatic crustaceans and they are referred to as the most abundant metazoans on earth (Humes, 1994). There are over 250 described families, 2600 genera and 21,000 species which are categorized in ten orders, classified under this subclass (Copepoda) (Walter TC, 2008). They have a diversified ecological habitat, and some of the species are both? planktonic and benthic, and play also a great role to maintain the aquatic food chain, but one-third of marine copepod species are expected live as associates, commensals or parasites on invertebrates and fishes (Humes, 1994). Sea lice is a collective name given to parasitic copepods in the genera *Lepeophtherius* and *Caligus* that are commonly found on farmed and wild marine finfishes, (Costello, 2006; Johnson et al., 2004; Pike & Wadsworth, 2000) and 129 and 245 species are found on both genera, respectively (Kabata, 1988; *World of Copepods*, 2012).

L. salmonis and *C. elongates* account for the most of infestation occurred in farmed and wild salmonids in the North Atlantic Ocean, while in the eastern north Pacific Ocean *L. salmonis* and *Caligus clemensi* are the dominant species (Johnson et al., 2004; Pike & Wadsworth, 2000). Even though *L. salmonis* are prevalent in both the Atlantic and Pacific oceans cost, recent and earlier studies strongly suggest that the sea lice on these two ocean costs are genetically distinct species due to the separation of the Atlantic and Pacific oceans that occurred over 2.5 to 11 million years ago, and the parasites are described to herein as the Pacific and Atlantic forms of *L. salmonis*, respectively (Boulding et al., 2009; Yazawa et al., 2008). In the South Hemisphere *C. rogercresseyi* species is the most prevalent parasite and it's the major concern of parasite in Chile salmonid aquaculture and found on the 99% of the affected cultured cages (Boxshall & Bravo, 2000; Carvajal et al., 1998).

Lepeophtheirus and *Caligus* species are morphologically distinguished and can also be differentiated from each other by their life cycle pattern and host range. *L. salmonis* has a life cycle of ten developmental stages, while *C. elongatus* and *C. rogercresseyi* almost have a similar life cycle of ? development stages, but appear to have lack of pre-adult stages (Gonzalez & Carvajal, 2003; Piasecki & Mackinnon, 1995).

2.1.1 Host range

L.salmonis has mainly affected the salmonids but the infestation of the parasite also has been reported from non-salmonids hosts, including sticklebacks, that co-occur with salmon (Jones et al., 2006). On the other hand, some of the *Caligus* species have a wide range of host that affects salmonids and non-salmonids (Costello, 2006; Johnson et al., 2004). Atlantic salmon and sea trout are the hosts on which *L. salmonis* has a great impact, followed by rainbow trout, chinook and coho salmon (Gjerde & Saltkjelvik, 2009; Johnson & Albright, 1992). However, rainbow trout are more susceptible than Atlantic or coho salmon to *C. rogecresse* (Gonzalez et al., 2000). Thus *L. salmonis* and Caligus species display clear differences on morphology, life cycle and host range.

2.1.2 Life cycle of sea lice

Generally, Copepods of *Lepeophtheirus* and *Caligus* species have a similar developmental cycle and no intermediate hosts are involved. *L. salmonis* life cycle has 10 developmental stages which consist of three planktonic stages (two uninfective nauplii and one infective copepodid) and eight parasitic stages (one copepodid, four chalimus (C1, 2, 3 and 4), two pre-adult and one adult) (Johnson & Albright, 1991).

The cycle begins with extruding of egg strings into the water column from mature female (1cm), after which the first nauplii stage are released and moult to the second nauplii stage, then to copepodids, which are the infective stage that attaches to the skin of fish and remain immobile until the motile stages. In spite of possessing a rudimentary gut, the preceding stages are non-feeding and their energy source is depending on endogenous lipid. Reduction on infectivity among 3- to 7-day old ages of copepodids is associated with declining of energy reserves, and after 7 to 20 days of hatch around 95% of their mean endogenous lipid content is shown to be lost (Cook et al., 2010; Tucker et al., 2000b). Therefore, viability and infectivity of free-swimming copepodids are highly depended on the rate of consumption of endogenous lipids. Once the Copepodid (0.7mm) settled on the host, it moults to the first of four chalimus stages that are attached to the skin of the host by the frontal filaments and remains non-motile until the fourth Chalimus stage moults in to preadult stages (3-4mm), which can able to move around on the surface of the fish and also swim in the water column, and finally moult to the reproductive adult stage (Brauner et al., 2012). While in some species of *Caligus* the preadult

stages might be absent, or reduced to one stage, additional chalimus stages might occur (Gonzalez & Carvajal, 2003; Piasecki & Mackinnon, 1995). The sexes can be distinguished in the fourth stage chalimus and later stages (Johnson & Albright, 1991; Kabata, 1972; Kabata, 1988; Piasecki & Mackinnon, 1995). Hence life history of sea lice involves two distinct phases, an earlier free-living phase and a later parasitic phase, and having an understanding of factors that involved in the process of both phases will assist to formulate management strategies (Jones & Johnson, 2014).

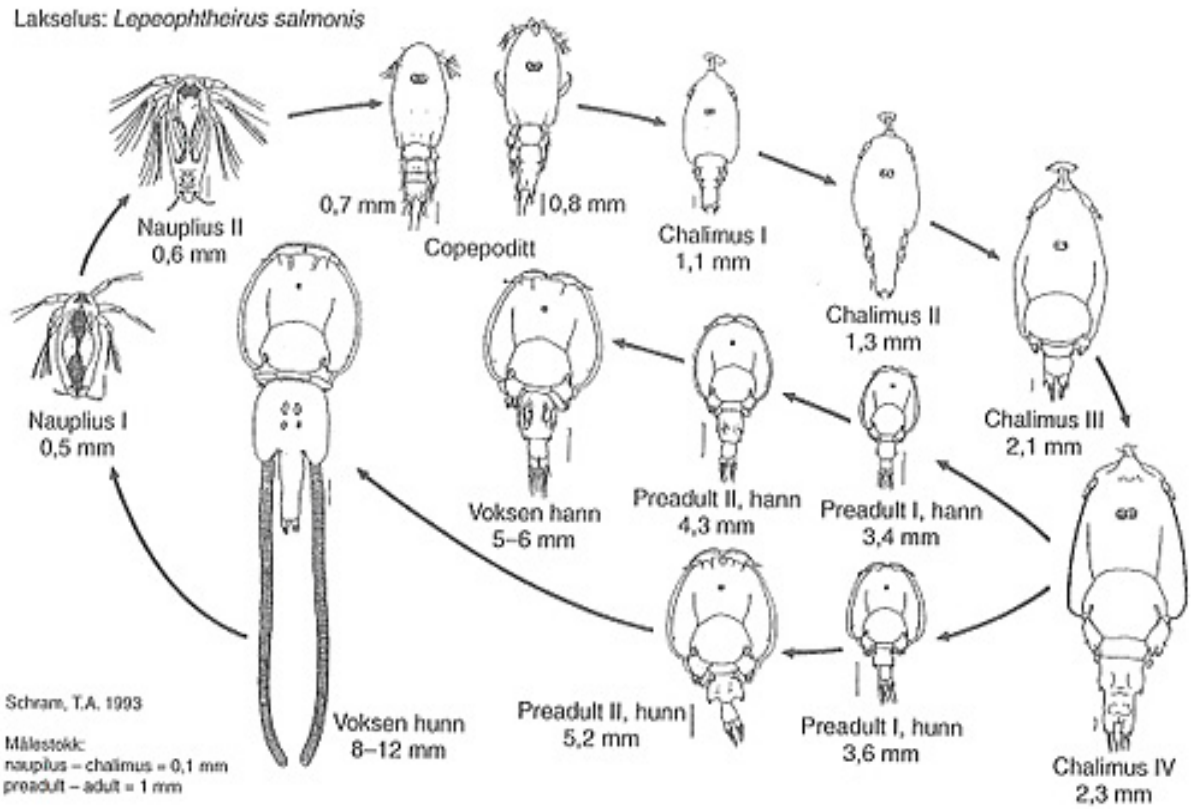

Figure 1. The life cycle of *L salmonis*

2.1.3 Reproduction of sea lice

Adult male *L. salmonis* become sexual mature approximately a day earlier than the adult female (Todd et al., 2005). The male *L. salmonis* is attracted to pheromones secreted by preadult II females (Ingvarsdottir et al., 2002) and will guard the female until the final moult, after which mating occurs. A single mating is sufficient in a lifetime to fertilize all the eggs that are produced (Ritchie, 1997). In case of *L. salmonis* reports indicate that a fertilized female can

produce 11 pairs of egg strings, and mating with multiple males have been confirmed when fertilized female lose one or both spermatophores (Heuch et al., 2000). Male *C. elongates* are expected to die after mating (Piasecki & Mackinnon, 1995) and studies revealed that female *L. salmonis* has a lifespan of 191 days in the laboratory, but the authors suggested a longer survival may be possible under natural conditions (Heuch et al., 2000).

The length of egg string has a direct relationship with the number of embryos that develops in the string and factor that govern this are described as follows. a) Batch number: the length of egg string is increased with the batch numbers following mating (Heuch et al., 2000; Pike & Wadsworth, 2000); b) Host: lice attached to wild salmon has longer egg strings than farmed salmon (Jackson & Minchin, 1992) and even though the finding was not confirmed species of salmon and season of sample collection are also expected to have effects on the length of egg strings. Lice attached to a susceptible host (Atlantic salmon) have longer egg stings than lice attached to resistance salmon species (Chinook salmon), and samples of female lice collected in winter have longer egg strings than lice collected during summer. Differences are also frequently observed on proportions of viable embryos between sequentially extruded egg strings, but the reasons behind these are not well known (Heuch et al., 2000; Pike & Wadsworth, 2000).

2.1.4 Factors affecting the development of sea lice

Temperature

The time from infection to production of the first egg strings is 20 days at 12.2 ^{o}C and 79 days at 7.1 ^{o}C (Heuch et al., 2000). The rate of embryonic development is affected by temperature, and hatching of nauplii takes 45.1, 35.2, 27.6 and 21.6 days at 2, 3, 4 and 5 ^{o}C, respectively and 8.7 days at 10 ^{o}C (Boxaspen & Naess, 2000). The generation time of *L. salmonis* is temperature dependent and ranges from 4 weeks at 18 ^{o}C to 8-9 weeks at 6 ^{o}C (Hayward et al., 2011). Tully (1992) observed that the rate of development and generation times for *C. elongatu*s is strongly temperature dependent and similar relationship is also expected for *C. clemensi* (Tully, 1992).

The development and survival of parasitic stages of *L. salmonis* are regulated by temperature. Stien et al., (2005) suggested that there are sex-specific rates of development to chalimus or preadult stages; preadult males are developed more rapidly than preadult females. In addition, *L. salmonis* and *C. elongatus* were both found to be larger and more fecund in colder water

(Hogans, 1995; Tully, 1989). However, moult success was reduced at 2 ^{o}C, infectivity of *L. salmonis* persist over-winter on farmed salmon (Chang et al., 2011; Hogans, 1995; Tully, 1989).

Salinity

The salmon louse is a stenohaline copepod whose larval stages, in particular, possess a limited capacity for osmoregulation (Jones & Johnson, 2014). Although many fields and laboratory reports showed that adult *L. salmonis* survived several days in fresh water, *L. salmonis* survival and development is optimal in high salinity seawaters (Piasecki & Mackinnon, 1995). The salmon louse needs salinity greater than 23 ‰ (ppt) to complete its life history and viable copepodids are developed when the salinity is at least 30 ‰ (Jones & Johnson, 2014). In a laboratory study, it is observed that eggs are failed to develop at 10 ‰ and even if eggs develop at 15 ‰, nauplii could not hatch. Copepodid will survive more than one day when the salinity is greater than 10 ‰ (Johnson & Albright, 1991). In addition to reduced survival, poor infectivity of *L. salmonis* is also strongly associated with lowered salinity (Tucker et al., 2000a).

2.2 Impact of sea lice

2.2.1 Clinical signs at individual levels

Skin lesions, osmotic imbalance, physiological stress, anaemia, reduced feeding and growth, exposed for secondary microbial infection, reduced disease resistance and increased mortality are symptoms manifested on the fish infested by sea lice. On severely infested fish mortality may occur within 10-20 days of exposure to lice larvae when the lice develop into preadult and adult stage. Reduced swimming performance of the fish may occur at sub-lethal infestation of lice (Finstad et al., 2011; Finstad et al., 2012; Thorstad et al., 2015).

Atlantic salmon are facing a challenge to increased salinity when they migrate from fresh water to sea water and problem with the salt balance induced by salmon lice may ultimately lead to mortality. Osmotic and ionic imbalance caused by sea lice is happened due to damage of skin, mucous surfaces and dermal tissue that impairs the physical barriers of the body and as a result, increase the leakage of water from the body (Thorstad et al., 2015). Condition factors and body mass of salmon infected with sea lice is reduced compared to the uninfected fish and this may be due to physiological stress response, dehydration and reduced feeding activity (Finstad et al., 2011; Finstad et al., 2012; Wagner et al., 2003; Wagner et al., 2004).

2.2.2 Effects of salmon lice on wild salmon population

Several studies that done in Ireland, Scotland and Norway have showed that the infestation of salmon lice from intensive fish farms were attributed for declines and collapses of wild Atlantic salmon and sea trout fish population (Thorstad & Finstad, 2018). The Norwegian Scientific Advisory Committee for Atlantic salmon has done a survey both in farm-free and farm-intensive areas that covered the entire country to assess the effect of salmon lice at a population level (Anon, 2017). For a period 2010-2014 the annual loss of wild salmon from Norwegian rivers due to salmon lice was estimated 50 000 adult salmon and this resembled on a national level that 10% of wild salmon were lost annually due to salmon lice. A large-scale experiment also conducted to quantify the returns of spawner to the river by releasing two groups of smolts; one chemically protected from salmon lice and another of unprotected fish. The result revealed that the average risk ratio of protected fish returns to the river to spawn compared with unprotected fish ranged from 1.14:1 to 1.4:1, and a meta-analysis from Norwegian studies indicated a 1.18:1 overall risk of ratio (Anon, 2017).

2.2.3 Economic loss

In general, diseases are accountable for loss of production directly or indirectly through growth reduction, low feed efficiency and increased mortality. Economic impact of diseases might also be substantial and aquaculture farmers may face these direct and immediate economic losses (Asche et al., 2009; Aunsmo et al., 2010; Costello, 2009; Liu & Bjelland, 2014; Menzies et al., 2002; Mustafa et al., 2001). Sea lice is a major global threat for the salmon farming industry and the global cost of the parasite was estimated to be around $423 million US dollar (Costello, 2009). For example, in Norway in 2011 the cost of sea lice was estimated to be 0.79 NOK per kg of salmon produced (Anon, 2012). If this was applied to all salmon producer in Norway the cost would be about 130 million US dollar (790 million NOK). Current reports indicated that due to increased occurrence of sea lice and expense of treatment; the cost of sea lice in 2013 getting three times bigger than 2011 which is estimated around 2.45 NOK per kg of salmon (Jensen, 2013) and a report from this year indicate the cost could be reached up to five billion NOK (Iversen et al., 2017).

Severe infestation of contagious diseases has the potential to decrease the quality of marketable products (low market price) (Aunsmo et al., 2010; McVicar, 1997; Mustafa et al., 2001), changing the consumption pattern and behavior of the consumer when consumers are very

concerned about environmental impacts, seafood safety and human health. As a result, these may gradually lead to a decline in demand of farmed salmon (Israngkura & Sae-Hae, 2002). However, combating diseases requires additional resources, in other words, prevention and treatment measures require additional effort and investment which likely increase production cost and undermine production. Therefore, the economic effects of diseases on the aquaculture sector, especially at a farm level, can be measured by changes in productivity and profitability (Liu & Bjelland, 2014).

2.3 Prevention and control management

Even though a total eradication of sea lice from salmon farm is not likely possible it needs to be substantially reduced through different combination of treatments and management strategies. In addition to chemo-therapeutant treatments, different management measures have been implemented to control sea lice and one of the management decision that has been proposed and developed in major producing countries is Integrated Pest Management (IPM). IPM is a regional management coordination which may take the following measures; coordinating stocks with year classes, follow-up farm sites, and synchronizing treatment strategies within the fjords and between different aquaculture companies. For instance, in Norway to reduce the overall pressure of sea lice infestation during wild salmonids spring run, a mandatory and synchronized delousing strategy was proposed and implemented in most of the Norwegian coastline during late autumn and early spring. IPM is playing a great role on prevention and control of sea lice by contributing decision tools and evaluating the economic performance of different sea lice control strategies (Liu & Bjelland, 2014).

Norwegian authorities have imposed strict regulations on the number of sea lice per treated fish because of the potential effect on wild salmon stocks and the latest regulation implemented in 2009 includes a limit of on average 0.5 adult female or 3 mobile lice per fish from January 1 to August 31, and 1 adult female or 5 mobile per fish from September 1 to December 31. A routine monitoring with sampling is compulsory, and the treatments are required when the lice number exceeds these limits (Liu & Bjelland, 2014).

2.3.1 Chemical bath and treatment

Chenical treatment of sea lice in Norway can be done in two ways either by giving drugs in-feed pellet (oral treatment) or chemical bathing. The oral in-feed treatments traditionally have been through products like SLICE and *Ektobann* in which the main active ingredients of are

Ememectinbenxoate and *Teflubenzuron,* respectivel (Liu & Bjelland, 2014).

The second chemical treatment option is delousing using a bath. Chemical bathing is performed in enclosed system which is detached from the surrounding water. The main compounds for bathing are *Deltamethrin, Azamethiphos* and H_2O_2 and in the market known as *Alphamax, Salmosan* and *Hydrogen peroxide*, respectively. An enclosed system is done by lifting the net to a shallow depth (<5m) and enclosed with a skirt/tarpaulin or simply using the wells of a well boat. Pre-mixed chemical in water is added by directly tipping or through a leaky pipe (Liu & Bjelland, 2014).

2.3.2 Biological control

Wrasses are moderate-sized finfish species that can eat massive quantities of sea lice directly from the infected salmon in cages and generally are released into cages along with smolt and stay there for the entire production cycle (Liu & Bjelland, 2014). The use of wrasse as cleaner fish for salmon lice control was developed in the late 1980s (Bjordal, 1988a; Bjordal, 1988b; Bjordal, 1990) and the most frequently species used in Norway are goldsinny wrasse, ballan wrasse and corkwing wrasse (Blom, 2010). The success of using cleaner fish is depending on the health of the fish and cleanness of the cages. In addition, this wrasse require shelter for well-being and readily seek alternativfeed sources if the nets are overgrown. Stocking density of approximately 4 wrasses per 100 salmon is commonly used but for smaller wrasses like goldsinny slightly higher densities are used while lower densities is sufficient for larger wrasses like ballan wrasses (Torrissen et al., 2013).

Ballan wrasse is the largest European wrasse which reaches maximum size up to 60 cm and suitable to be kept in the cages with large salmon (3-6 kg). Due to their wider geographical distribution they are tolerating a wide range of environmental conditions and can even survive over winter in Norway. Interest of farming wrasses in the last few years is growing specially focusing on ballan even though it is challenging to get them to eat a formulated diet (Torrissen et al., 2013).

Recent studies also showed that lumpfish *(Cyclopterus lumpus L.)* has a high potential to graze sea lices and their production in Norway over the last three years was increased dramatically. Compared to large sized lumpfish the smaller sized (20-54g) were showed to consume more lices and a 40-97% lowered burden were recorded when cages infested with sea lices were stocked with small sized lumpfish. However, there is still a challenge to optimize their use as

biological delousing agent since they have strong opportunistic feeding behaviour and as a new species also needs more scientific record (Imsland et al., 2015; Imsland et al., 2016).

2.3.2 Selective breeding

Genomic selection involves the prediction of individual breeding values for complex traits by combining statistical methods with genomic data (SNP, i.e. single nucleotide polymorphism) (Tsai et al., 2016). Nowadays GS is become a widely used approached particularly for traits that cannot be recorded on the live breeding candidates (disease resistance and carcass quality). For example, in recent years' a successful genomic selection was performed on the following pathogens to improve the fish health such as *Aeronomonas salmonicida* (Furunculosis), infectious salmon anaemia virus (ISAV) and infectious pancreatic necrosis virus (IPNV) (Lhorente et al., 2012).

The feasibility of GS schemes depends on the availability of a high quality SNP genotyping platform and on the extensive trait records collected in the reference (training) population (Tsai et al., 2016). To include and select efficiently in genomic selection breeding program, the traits has to be exhibited a significant genetic variation and studies done on Atlantic salmon infected with sea lice species showed that there is genetic variation in host resistance to sea lice (Correa et al., 2017a; Gjerde et al., 2010; Glover et al., 2005; Kolstad et al., 2005; Tsai et al., 2016). Therefore, selective breeding to improve the host resistance to sea lice is an alternative method for control of sea lice and proposed as a feasible option to improve disease resistance in several livestock and aquaculture species.

2.4 Genetic parameters for resistance to the sea lice

Today, Aquaculture breeding program becomes more advanced and complex in which several traits are included in the breeding goal such as body weight, early sexual maturity, fillet quality (colour & fat) and other quality traits and several disease resistance traits. Having reliable information on the genetic parameters is very crucial to include traits in the breeding goal and to select the individual efficiently for several traits simultaneously. Heritability and genetic correlations are the two most parameters that quantify genetic variation among the population.

The magnitude of the heritability is telling us to what degree the trait is repeatable in the next generations of families, whereas the sign and magnitude of genetic correlation tell us to what degree the traits can be selected simultaneously or not. Low to moderate heritabilities resistance

against lice infestation were reported from the previous studies. Studies done on *C. rogercresseyi* the heritability was estimated between 0.1 to 0.32 (Correa et al., 2017b; Lhorente et al., 2012; Yanez et al., 2014) and between 0.07 to 0.33 heritability were estimated from the studies done on *L. salmonis spp* (Gjerde et al., 2011; Glover et al., 2005; Kolstad et al., 2005; Ødegård et al., 2011; Tsai et al., 2016).

Fast growth has always been a primary breeding goal in salmon industries, but few studies have reported on the genetic correlation of resistance to *L. salmonis* with growth or other traits. Reported from Gjerde et al., (2011), Glover et al (2005), Gjerde and Saltkjelvik (2009) showed that, although lice count was increased with increased body weight, the genetic correlation between lice count and body weight was poor. However, Kolstad et al., 2005 was estimated slightly higher genetic correlation (0.37±0.1) between LC and body weight, Yanez et al., 2014 was reported a significant and moderate negative genetic correlation. Gjerde et al., 2011 and Tsai et al., 2016 were estimated a low correlation of 0.08 to 0.29 (±0.11) and -0.06 to 0.1, respectively. The relationship between resistance to *L. salmonis* and body weight is more appropriately explained by lice density (i.e., number of lice per fish per unit of body weight or surface area) than lice count. The genetic correlation LD with body weight was estimated almost close to zero (Gjerde et al., 2011) and LD is independent of body weight, this will imply that there is a possibility to improve both body weight and resistance to *L. salmonis* by including both simultaneously in the breeding program. So far to our knowledge we haven't seen many studies that are done to see the genetic correlation of resistance to lice with other disease trait except, Yanez et al., 2014 which they try to estimate the genetic correlation between *C. rogercresseyi* and *Piscirickettsia salmonis* and found a very low and non-significant correlation around -0.02 ±0.17.

3. MATERIALS AND METHODS

3.1 Fish materials

The fish used in this study consisted of a total of 15,457 Atlantic salmon (*Salmon salar*) of three-year classes (2015, 2016 and 2017, year-class refers to the time the fish is expected to be sent to sea as 1+) from the breeding population of Marine Harvest AS, Norway. The start feeding date of the 2015, 2016 and 2017 year-classes were in April 2014, 2015 and 2016, respectively. Because of the 4-years generation interval, the breeding population consist of four sub-populations (year-classes), the data sued in this study came from three of the sub-populations. Because Marine harvest does not have family tanks, fertilized eggs of each fullsibs family are kept in separate trays until start feeding after which a given number of fry from each family are pooled. The fish from all families are therefore start feed in a common tank until they reached an average pre-smolt body weight of 20 grams at which they were PIT tagged and their individual body weight and length recorded. Detailed description of the population is presented below in table 1 and figure 2.

Year class 2015 consisted of 4842 individuals with complete phenotype and 4633 genotype information derived from 345 full-sib families the offspring of 172 sires and 345 dams. The average number of fish per family was 14.3 and ranged from 5 to 21 individuals. This group of fish were sent to sea in one net cage in May 2015 and they had mean smolt weight of ~59.7 grams.

Year class 2016 was composed of 4236 individuals with phenotype and 3901 with genotype information from 340 full-sib families. The offspring were from 188 sires and 333 dams with an average number of 12.5 fish per family. This population were smoltified and sent to sea in May 2016.

Year class 2017 consisted of 6379 individuals that were reared in sea-cages or in tanks. The first group of fish were reared in sea-cages at *Alsåkervik, Norway* (broodstock farm of marine harvest) in May 2017 and the consisted of 275 full-sib families produced from 98 sires and 188 dams. The number of fish with recorded phenotype was 3461. The second of fish were raised in tanks at *Matre, Norway* and consisted of 1462 individuals from 190 full-sib families produced from 82 sires and 181 dams. The last group of the 2017 year-class were raised in tanks at *Veso, Vikan, Norway.* A total of 1456 from 189 full-sib families produced from 81 sires and 180 dams were used. The was complete overlap in the sires and dams of the net-cage and

tank challenges.

Table 1: Number of recorded fish, number of sires and dams, average mating ratio and smolt weight for each year-class.

Year class	Cage	Number of				Matin g ratio	mean smolt weight (g)
		observation	families	sires	dams		
2015	-[1]	4842	345	172	345	2.00	59.68
2016	-[1]	4236	340	188	333	1.77	125.87
2017	Alsåkervik[1]	3461	275	121	266	2.20	89.10
	Matre[2]	1462	190	82	181	2.20	85.43
	Veso[2]	1456	189	81	180	2.20	84.79

[1] *– fish were reared in sea-cages and challenge test was performed*
[2] *- fish were reared in tanks and challenge test was performed at these locations*

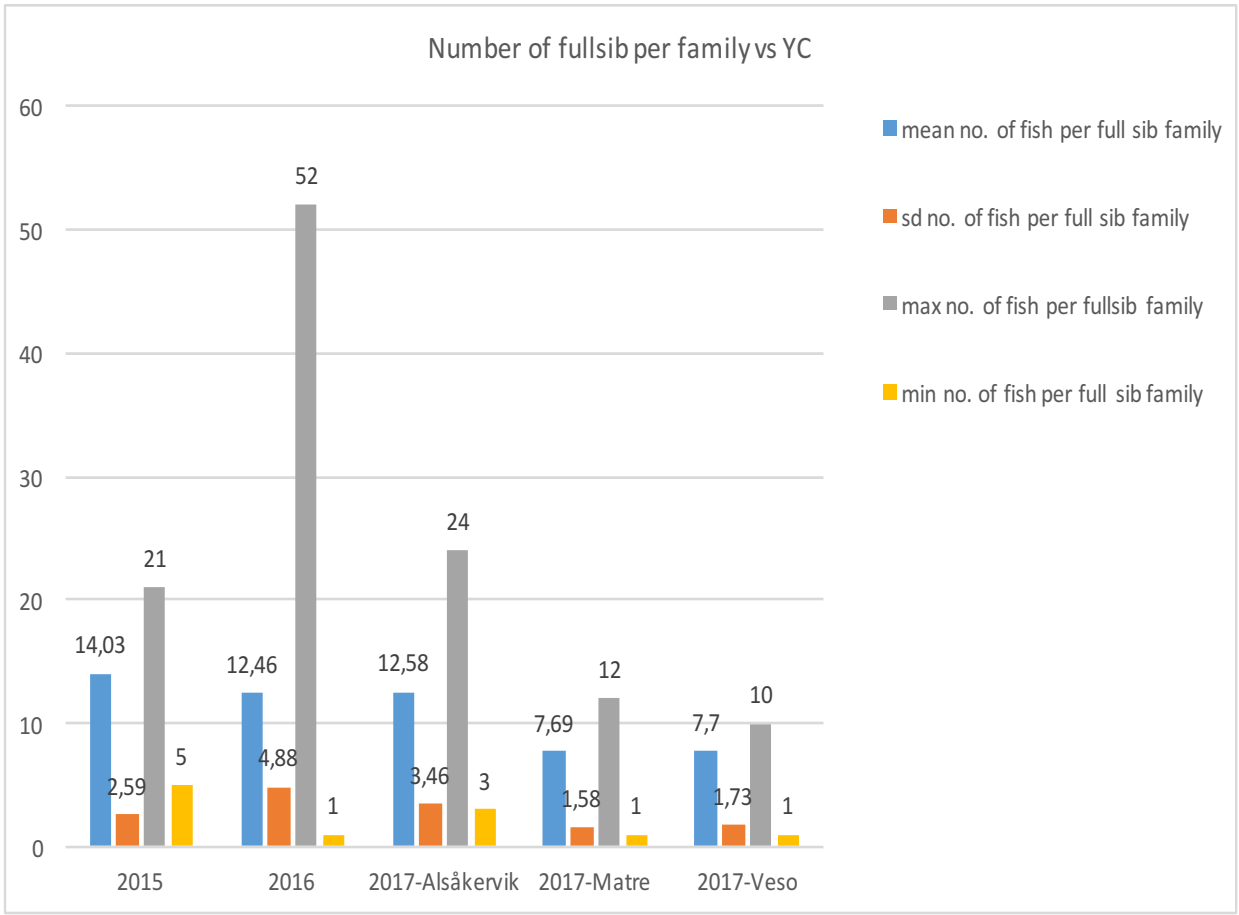

Figure 2. Descriptive statistics of number of fish per fullsibs family for each year class

3.2 Copepodids production and challenge test

Production of copepods and the challenge protocol followed the same procedures for all year classes. Egg sacs were harvested from host fish either by picking egg-bearing females directly from fish in a tank or from anaesthetized individuals. Egg strings were incubated in a large container (bucket) with a capacity to hatch 50 pairs of egg strings. The temperature of the incubator was adjusted 9.5 -12.6 ^{o}C and fed with particle filtered salt water with an average salinity of 34.5 ppt. Egg strings were kept in the incubators until majority of the hatched individuals had reached the copepodid stage, which took around 14 days. Copepodids were counted using zooplankton counting chamber and those who were non-viable were not counted.

Lice infestation

In net-cage

Before the infection process was performed a 4-meter-deep tarpaulin was put around the net of the cage and the net was raised to the bottom of the tarpaulin and oxygen was added to the water through an aerator. A camera was used to observe that the fishes were swimming properly. The infestation was done by pouring a 25-litter large bucket of water with lice copepodids into the cage from a small boat inside the net-cage that was rotated circularly. One bucket per circle was applied, the fish were challenged with an average of 45 larvae per fish. This infestation procedure lasted for around 30 min. The fish started to jump 5 minutes after the first bucket of copepodids was added and the jumping continued until about 1-2 hours. After the frequency of jumping was reduced and fish swam normally, the net was lowered to the depth of the tarpaulin. The tarpaulin was removed on the next day and the net lowered to its normal depth of 6-meter. Salinity and temperature measurements were taken periodically before, during and just after the infection. The lice were monitored daily until most of the lice had reached into the chalimus I stage.

In tanks

The challenge tests were undertaken in tanks at *Matre, Norway* and *Veso, Vikan Norway*. Forty-five copepodid larvae per fish were added to each tank and covered for 2-3 hours until the infestation settled. Parameters for temperature, oxygen and salinity were taken regularly. After the infestation, each tank was monitored routinely every day until most of the lice had reached chalimus I stage.

Lice counting

A small sample of fish were checked for lice every 4-7 days post lice challenge. The lice counting of all fish were performed when the lice had reached the chalimus III stage. The counting was done within 2- 4 days on anesthetized fish, at which also the body weight and length of each fish was measured. For year-class 2015 and 2016 two independent lice challenge tests and lice counts were performed. with the fish were deloused with freshwater after each lice count and the second lice count was carried out after 8 and 3 months for 2015 and 2016 year classes respectively, while for 2017-year class only one challenge test and thus one lice count was performed. The number of lice counters in year class 2017 was 10 at *Alsåkervik,* 9 at *Matre* and 4 at *Veso*, and in 2016-year class, 5 persons counted the lice for both lice counts, while in the 2015-year class, 4 and 5 persons countered the lice at the first infection and second infection respectively.

Sex of the fish

Sex of all the fish in this study was determined using the sdY gene after genotyping the population. The probes for the sdY gene (developed by (Houston et al., 2014)) are included in the SNP panel used in this study. The Y-specific sex-determining gene shows mean intensity values of the probes for males higher than females.

Lice density

To account for the fact that longer and bigger fish have larger surface area and therefore could have a lot more lice count than smaller fish, we computed lice density. Additionally, lice density is expected to be less skewed and more normally distributed than lice count because the correlation lice count to body weight is expected to be removed. Lice density was calculated according to Gjerde et al. (2011) as:

$$\mathrm{LD} = \frac{LC}{\sqrt[3]{BW^2}}$$

where LC is the number of lice counted per fish, BW is the weight of the fish in grams during counting. The formulae $\sqrt[3]{BW^2}$ is an approximate measure for the surface area of the fish.

Prediction of body weight using length

Only length was recorded for fish at *Alsåkervik* (2017 year-class) and the second infestation of the 2016 year-class, thus, we transformed the length records into weight to be able to compute lice density for these populations. We followed Fulton's equation as:

$$\boldsymbol{K} = \frac{W*100}{L^3}$$

where ***K*** is the Fulton's condition factor, *W* is weight and *L* is length. We used both weight and length records from the fish recorded at *Veso, Vikan* (2017 year-class) to obtain ***K***. We obtained a value of 0.95 for ***K***, thus the approximate weight of the fish from *Alsåkervik* and of the second infestation of the 2016 year-class group was computed as:

$$\boldsymbol{W} = \boldsymbol{K}\,\boldsymbol{L}^3$$

3.3 Statistical analysis

A linear mixed animal model was carried out to estimate the variance and covariance components of the study traits, lice count (LC) and lice density (LD) using the ASREML software (Gilmore et al., 1999). The following fixed effects were used in the model: date of scoring, sex, counter and haul (fetching/scooping of fish from the sea-cages using a large net mounted on a crane) and the time of hour of recording. It takes some time to count lice on all fish that are hauled up, therefore we modelled an interaction between haul and the hour of recording which was treated as a covariable. Therefore, the interaction was a nested effect of hour of recording within haul.

$$\mathbf{Y = Xb + Zu + e}$$

Where **Y:** is a vector of observation for LC1, LC2, LD1 and LD2

b: is a vector of the overall mean, sex, counter and a nested effect of hour of recording within haul.

u: is a vector of random additive genetic effects

e: is a vector of random residuals for each trait

X and **Z:** are the appropriate incidence matrices for each trait

First a univariate analysis was run for each trait (LC1, LC2, LD1, LD2) of the five experimental groups (Model 1). Thereafter, a bivariate animal model was applied separately for the two groups of fish in the 2015 and 2016 year-class with repeated lice count records and for each of the lice traits LC1, LC2 and LD1, LD2 (Model 2):

$$\begin{bmatrix}\mathbf{Y_1}\\ \mathbf{Y_2}\end{bmatrix} = \begin{bmatrix}X_1 & 0\\ 0 & X_2\end{bmatrix}\begin{bmatrix}b_1\\ b_2\end{bmatrix} + \begin{bmatrix}Z_1 & 0\\ 0 & Z_2\end{bmatrix}\begin{bmatrix}u_1\\ u_2\end{bmatrix} + \begin{bmatrix}e_1\\ e_2\end{bmatrix}$$

Finally, a multi-trait animal model (Model 3) was used for the three groups of the 2017-year class *Alsaakervik, Matre* and *Veso,* and separately for the LC and the LD trait:

$$\begin{bmatrix}\boldsymbol{Y_1}\\ \boldsymbol{Y_2}\\ \boldsymbol{Y_3}\end{bmatrix} = \begin{bmatrix}X_1 & 0 & 0\\ 0 & X_2 & 0\\ 0 & 0 & X_3\end{bmatrix}\begin{bmatrix}b_1\\ b_2\\ b_3\end{bmatrix} + \begin{bmatrix}Z_1 & 0 & 0\\ 0 & Z_2 & 0\\ 0 & 0 & Z_3\end{bmatrix}\begin{bmatrix}u_1\\ u_2\\ u_3\end{bmatrix} + \begin{bmatrix}e_1\\ e_2\\ e_3\end{bmatrix}$$

The additive genetic and residual effects were assumed to be distributed as ~N (**0, G$_0$⊗A**) and ~N (**0, R$_0$⊗I$_N$**), respectively. Where **A** is the additive genetic relationship matrix among the animals, **I** is an identity matrix with dimension equal to the number of animals, and ⊗ indicate the direct product operator. **G$_0$** and **R$_0$** are the co(variance) matrices of genomic and residual effects, respectively. The random effect associated with the full-sib family was excluded from the model as it was found to be not significant (P>0.05) based on a likelihood ratio test.

Heritability for each trait was calculated as:

$h^2 = \frac{\sigma_a^2}{(\sigma_a^2)+(\sigma_e^2)}$, where σ_a^2: additive genetic variance and σ_e^2: is residual variance.

The genetic correlation (r_{xy}) between two traits was calculated based on the following formula (Falconer & Mackay, 1996):

$$r_{xy} = \frac{\sigma_{a_x,a_y}}{\sqrt{\sigma_{a_x}^2 \sigma_{a_y}^2}},$$

3.4 Genetic correlation between year-classes with genomic information

We estimated the genetic correlation of lice count and lice density between year-classes using genomic information. All animals were fin clipped and DNA extracted in Identigen, Ireland (https://identigen.com/). After extracting the DNA, all samples were genotyped with a 55K Affymetrix array developed by Nofima, Marine Harvest and Salmobreed. After genotype calling, we performed quality check using the following parameters: Samples and SNP markers with call rate <90% were removed, SNP markers that deviated largely from Hardy Weinberg equilibrium (marker with Fisher exact p-values < 10^{-25} were discarded) and had minor allele frequency (MAF) <0.01 were discarded. After performing the quality check, the number of

samples available for each dataset was 4633 (2015 year-class), 3901 (2016 year-class), 2851 (2017 year-class, *Alsåkervik*), 1434 (2017 year-class, *Matre*) and 1450 (2017 year-class, *Veso*). And after merging all the samples, the number of markers that were used in further analysis was 50890.

All the genomic variance component analysis was performed with WOMBAT (Meyer, 2007). The model parameters were the same as the model above, except that, the numerator relationship matrix (computed with pedigree information) was replaced with a genomic relationship matrix (computed with marker information). In addition, we run a 7-trait multivariate model with all the year-class instead of the within year-class analysis that was done with pedigree information. Due to the very limited genetic ties (pedigree grand-parental ties, only few grand-parents the year classes shared) between the year-classes, it was not possible to perform the 7-trait analysis with pedigree information.

The genomic relationship matrix used in this study was constructed using the approach of (Wientjes et al., 2017). The genomic relationship matrix within year-class was similar to (VanRaden, 2008) as

$$\frac{(M_Y - 2P_Y)(M_Y - 2P_Y)'}{2\times \sum_{i=1}^{50,890} p_{Y_i}(1-p_{Y_i})}$$

where the subscript y refers to the year-class, M was the genotype matrix coded as 0 (AA), 1 (AB) and 2 (BB), P was a matrix with allele frequency of each marker, p_{y_i} is the allele frequency of marker i in population y. The genomic relationship matrix between year-classes was computed as

$$\frac{(M_Y - 2P_Y)(M_T - 2P_T)'}{\sqrt{2\times \sum_{i=1}^{50,890} p_{y_i}(1-p_{y_i})}\ \sqrt{2\times \sum_{i=1}^{50,890} p_{T_i}(1-p_{T_i})}}$$

where the subscript Y and T refers to year-class 1 and 2. The rest of the notations have been described earlier.

4. RESULT

4.1 Descriptive statistics

Lice count

Descriptive statistics for the studied traits for each year class are presented in Table 2. The mean number of counted lice varied among year classes and between first and second lice count. The average lice count ranged from 4.6 to 23.9, and the coefficient of variation (CV) ranged from 38.4% to 72.7%. The largest %CV was obtained in the tank challenges (64.9% for Matre and 72.7% for Veso). Among the field challenges test and for the first infection, the %CV ranged from 38% to 63% and the largest %CV was found in the 2015 year-class, followed by 2017 year-class (Alsåkervik) and then 2016-year-class. For the second infections in the field test, the %CV ranged from 46.3% to 51.6%. The distribution of lice count for all the year-class and populations is presented in figure 3 and 4. In all the dataset, lice count is skewed to the right, however, the largest skewness was observed in the tank challenges.

Lice density

Lice density was computed with direct weight records on the fish or length that was converted to weight using Fulton's approximation ($weight = 0.95 \times Length^3$). The average lice density based on actual weight ranged from 0.042 to 1.156 with the standard deviation ranging from 0.023 to 0.727. When length was converted to weight, the average lice density ranged from 0.005 (SD=0.003) to 0.006 (0.003). The %CV ranged from 43.6% to 79.6% and the largest %CV was obtained from the tank challenge tests.

Body weight and Length

As expected the weight of the fish in the tank challenges were the lowest compared to the field test. The average weight of the fish in the tank challenges was 123.2 g (SD=35.4 g) and 98.2 g (SD=21.8g) for Matre and Veso of the 2017 year-class, respectively. For the field test, the average weight ranged from 1177 g (SD=279 g) to 6388 g (SD=1278g). For the two populations that weight was not measured, the average length was 48.5 cm (SD=3.5 cm) and 59.1 cm (SD=4.6 cm) for Alsåkervik (2017 year-class field challenge) and the second infection of the 2016 year-class, respectively. The expected weight of these fish would be 1310 g (length = 49 cm) and 2270 g (length = 59cm) for the two population.

Table 2. Descriptive statistics for the recorded traits of the five experimental groups.

Year class	Location	No. of observation	Lice count	No. of counting days	No. of counter	Lice count				Lice density[3]		Body weight (g)		Body length (cm)	
						Mean	SD	Min	Max	Mean	SD	Mean	SD	Mean	SD
2015	-[1]	4842	1	3	4	4.6	2.9	1.0	19.0	0.042	0.027	1177.0	279.0	-	-
	-[1]		2	4	5	15.5	8.0	1.0	59.0	0.045	0.023	6387.8	1278.6	-	-
2016	-[1]	4236	1	4	5	16.4	6.3	1.0	57.0	0.110	0.048	1670.7	403.7	-	-
	-[1]		2	3	5	14.9	6.9	1.0	59.0	0.005	0.003	-	-	59.1	4.6
2017	Alsåkervik[1]	3461	1	3	10	13.5	5.9	1.0	51.0	0.006	0.003	-	-	48.5	3.5
	Matre[2]	1462	1	2	9	17.2	12.5	1.0	262.0	0.739	0.588	123.2	35,4	-	-
	Veso[2]	1456	1	4	4	23.9	15.5	2.0	161.0	1.156	0.727	98.2	21.8	21.5	1.5

[1] *– fish were reared in sea-cages and challenge test was performed*

[2] *- fish were reared in tanks and challenge test was performed at these locations*

[3] *– Lice density* $= \frac{lice\ count}{\sqrt[3]{bodyweight^2}}$

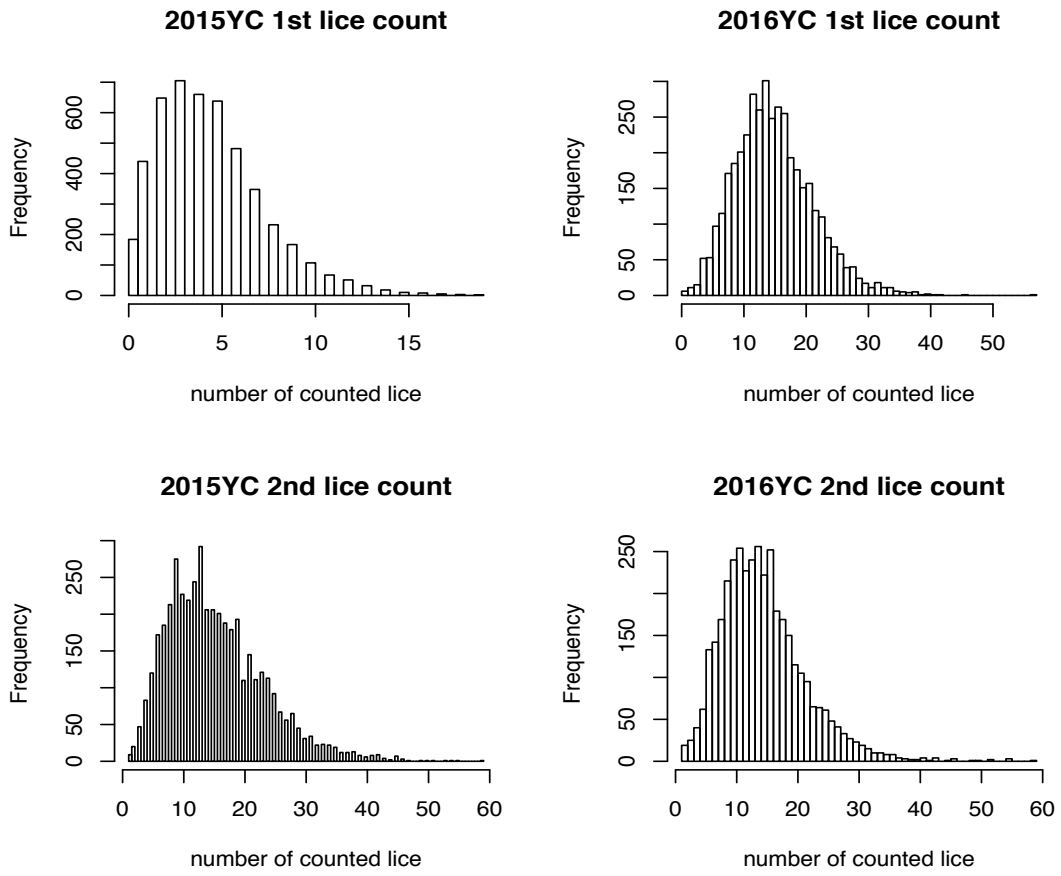

Figure 3. Distribution of 1st and 2nd lice count for year class 2015 and 2016.

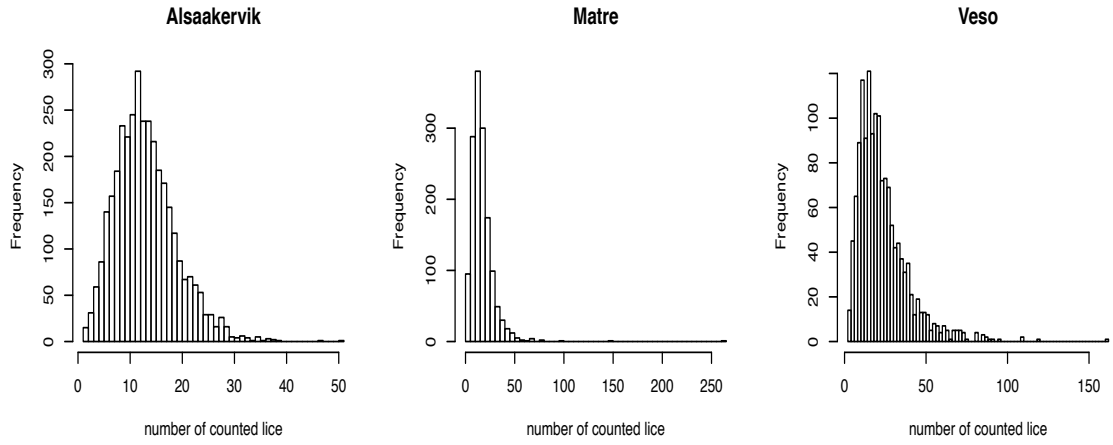

Figure 4. Distribution of lice count in three cages of 2017-year class population.

4.2 Phenotypic relationship of body weight with lice count and lice density

Relationship between lice count and body weight/length are shown in Table 3 and appendix 2 and 3. In general, the relationship between lice count and body weight or length was weakly positive (phenotypic correlation < 0.20) except for Matre (2017-year class tank challenge) and second infection of the 2016-year class. Accounting for bodyweight (by computing lice density) lead to a negative relationship between lice density and body weight or length.

Table 3. Phenotypic correlation LC and LD with body weight/length for each year class

Trait	Year Class				
	2015	2016	2017		
			Alsaakervik	Matre	Veso
LC1 ~ Body_wt	0.18	0.19	0.14*	-0.04	0.2
LC2 ~ Body_wt	0.08	-0.05*	-	-	-
LD1 ~ Body_wt	-0.06	-0.22	-0.23*	-0.30	-0.05
LD2 ~ Body_wt	-0.11	-0.41*	-	-	-

** indicates the correlations are between the trait and body length, not with body weight.*

4.3 Effects of sex on distribution of lice

The difference in LC and LD between male and female are shown in Table 3. The overall effect of sex on lice count was significant (P<0.01) for all year classes. However, the effect of sex on LD was not significant in all year classes except for 2015-year class (P<0.01). Male salmon had higher lice count than female in all year classes, but in 2016-year class for LD1 &LD2 females have higher lice density than males. For 2015 and 2017 year classes the lice density in male was higher than females.

4.4 Effect of lice counter and date of counting

Lice counter and the date of counting had a significant effect (P<0.05) on both lice count and lice density for all year classes. The mean lice count with standard deviation for each counter and date of counting for all year classes is presented in figure 5-8.

Table 4. Sex differences (male – female ± standard errors) in lice count (LC) and lice density (LD).

Year class	Location	Lice count no.	LC	LD
2015		1	0.64±0.10	0.004±0.001
		2	1.38± 0.22	0.0004±0.001
2016		1	0.46 ± 0.01	-0.002 ± 0.00
		2	0.45 ± -0.001	-0.0002 ± 0.00
2017	**Alsaakervik**	1	0.70 ± 0.18	0.00003 ± 0.0001
	Matre	1	1.03 ± 0.61	0.032 ± 0.03
	Veso	1	0.63 ± 0.78	0.002 ± 0.04

**2015 unknown sex LC1 = -0.2083, LC2 = -0.3147, LD1 = -0.0003738, LD2 = 0.0001053.*

**2016 unknown sex LC1 = 16.74, LC2 = 13.79, LD1 = 0.128, LD2 = 0.004289.*

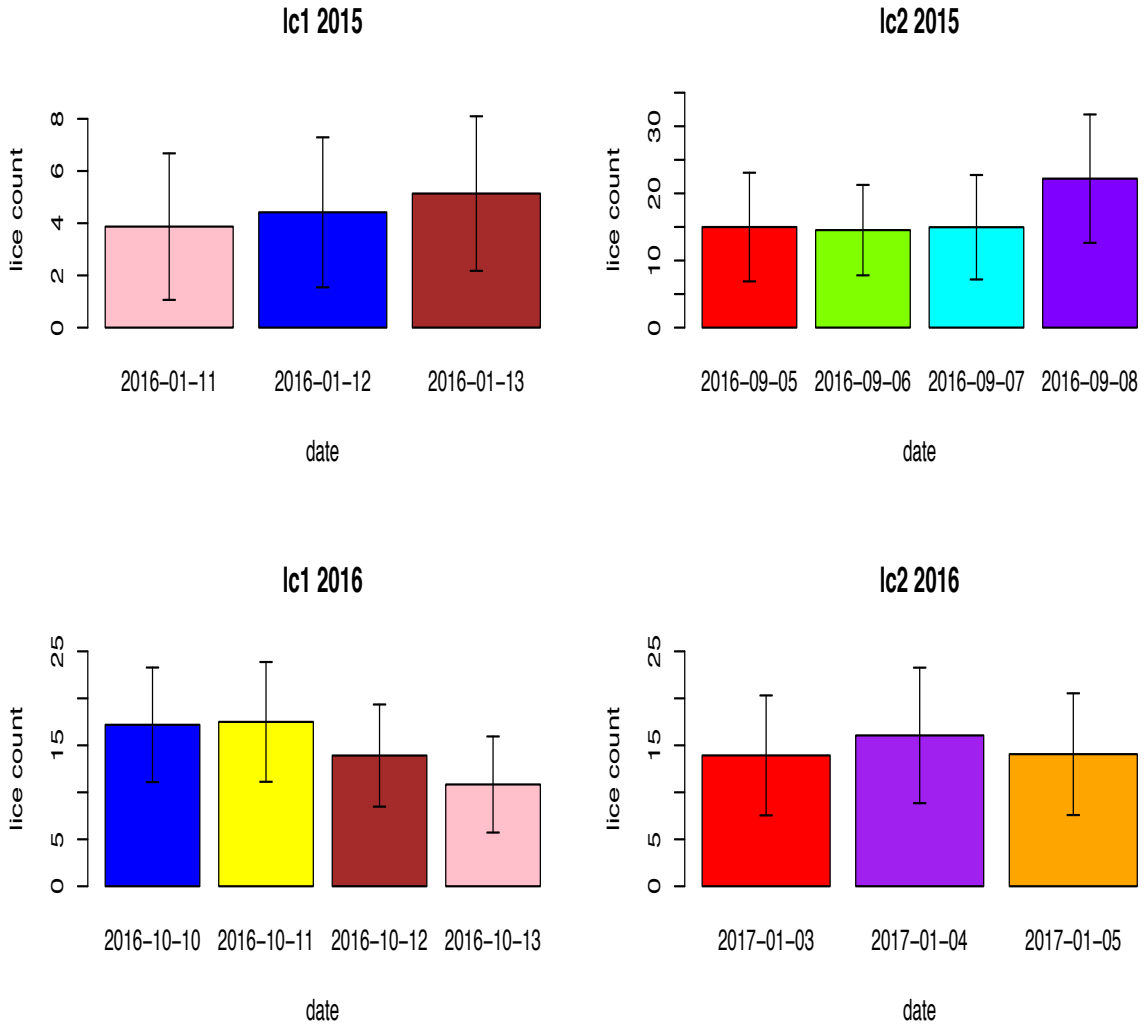

Figure 5. *Mean lice count with standard deviation for each date of counting for the 2015 and 2016 year classes.*

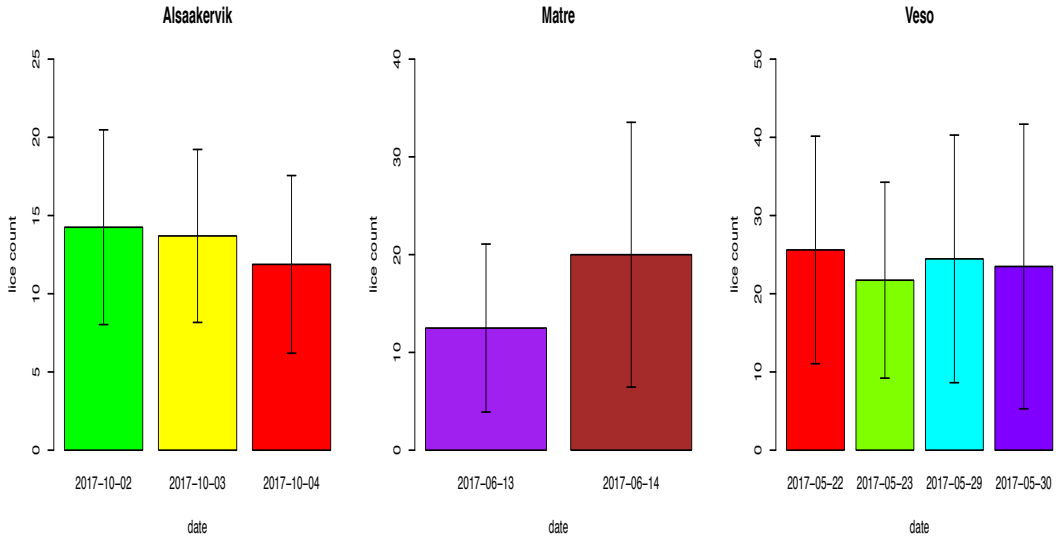

Figure 6. *Mean lice count with standard deviation for each date of counting for the 2017-year class.*

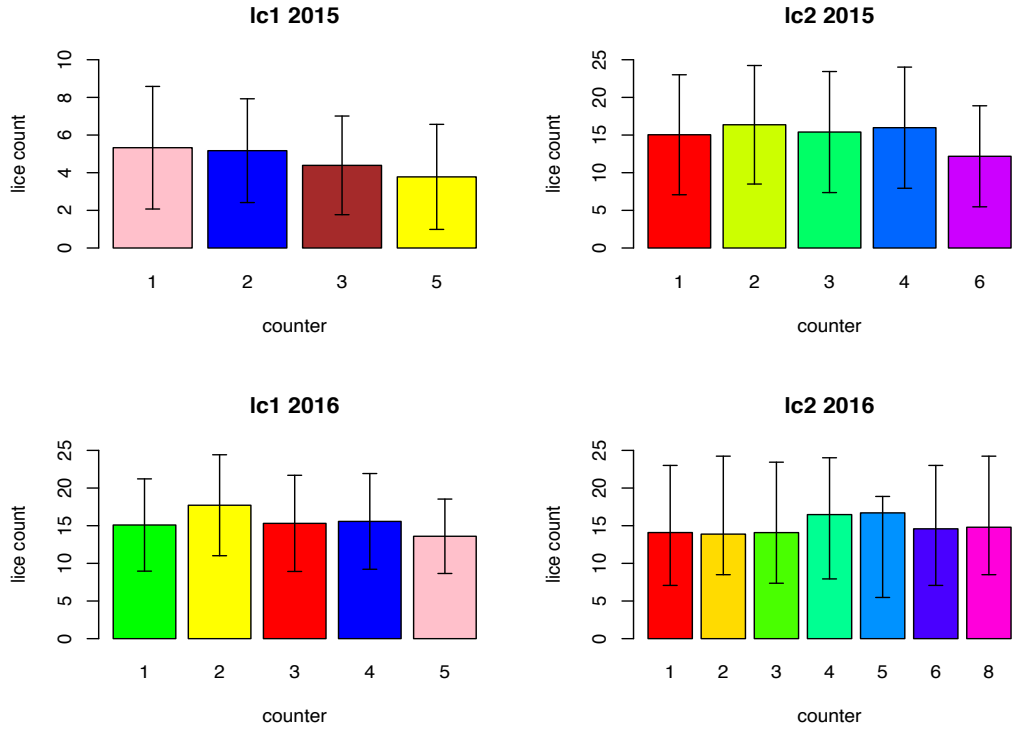

Figure 7. *Mean lice count with standard deviation for each counter for the 2015 and 2016 year classes.*

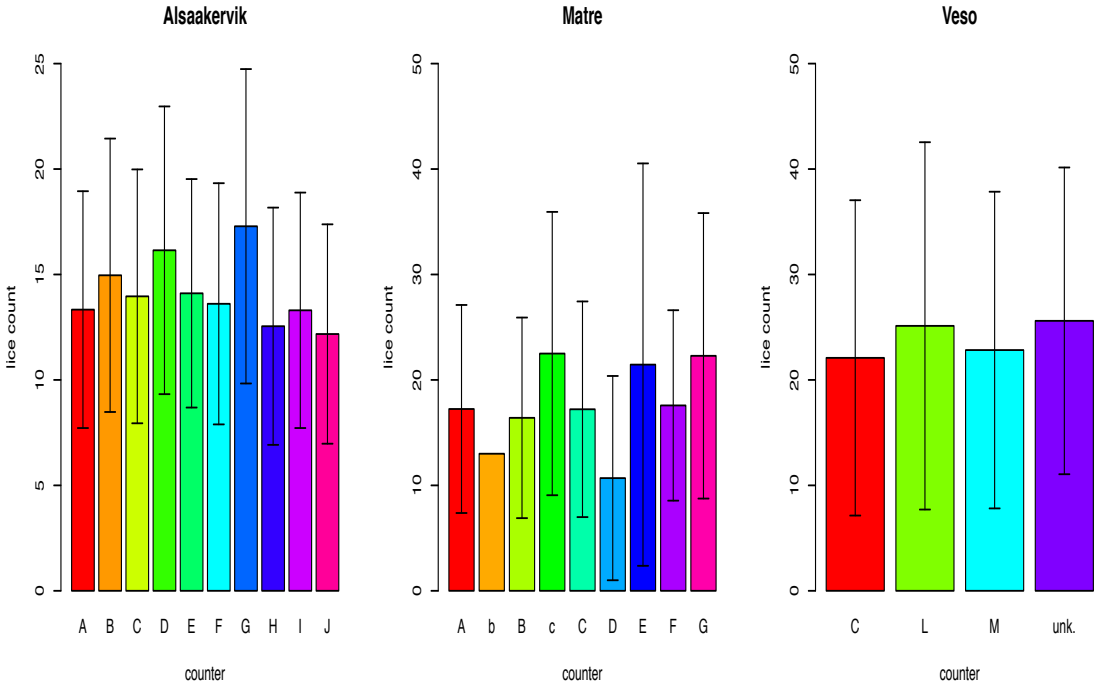

Figure 8. *Mean lice count with standard deviation for each counter for the year class 2017.*

4.5 Heritability estimates from pedigree information

Estimated heritabilities of lice count and lice density of each year class are presented in Table 5. Low to moderate heritabilities were obtained for LC and LD using pedigree information. The estimated heritability of lice count of the first infection ranged from 0.05 to 0.13 in the field test, while higher values were obtained in the tank challenges (h^2 ranged from 0.19 to 0.30). The estimated heritability (h^2 ranged from 0.13 to 018) of the second infection from the field tests were higher than from the first infections. Using lice density, heritability estimates were similar or slightly higher than using lice count except for *Alsåkervik* and *Veso* of the 2017-year class. Heritability estimates ranged from 0.04 to 0.25 and the largest estimate were also from the tank challenge test.

Table 5. . Estimated genetic, residual, phenotypic and heritability with associated standard errors for lice count and lice density of each year class using pedigree relationship matrix

Year Class	Location	Trait	σ_a^2	σ_e^2	σ_p^2	h^2
2015		LC1	0.45	7.3	7.7	0.05 ± 0.02
		LC2	7.3	47.3	54.7	0.13 ± 0.02
		LD1	0.04	0.6	0.6	0.06 ± 0.02
		LD2	0.07	0.4	0.5	0.14 ± 0.02
2016		LC1	4.3	29.5	33.8	0.13 ± 0.02
		LC2	8.05	36.5	44.6	0.18 ± 0.03
		LD1	0.3	2.0	2.0	0.16 ± 0.03
		LD2	0.001	0.005	0.006	0.16± 0.03
2017	Alsåkervik	LC1	2.9	26.4	29.2	0.1 ± 0.02
		LD1	0.005	0.1	0.1	0.04 ± 0.02
	Matre	LC1	25.9	112.4	138.2	0.19 ± 0.05
		LD1	70.0	240.0	310.0	0.22 ± 0.06
	Veso	LC1	71.9	173.8	245.27	0.3 ± 0.06
		LD1	140.0	400.0	530.0	0.25 ± 0.06

σ_a^2 - genetic variance, σ_e^2- residual variance, σ_p^2- phenotypic variance and h^2- heritability

4.6 Phenotypic, genetic and residual correlation between LC and LD

The Phenotypic and genetic correlation between LC and LD was high across all the year classes and the magnitude of r_e and r_g are reported on Table 6. The genetic correlation between lice count and lice density ranged from 0.67 to 0.93, while the residual correlation ranged from 0.91

to 0.96. These estimates suggest that, accounting for body by computing lice density changes the rank of families only slightly in the second infection (rg > 0.86) but moderately in the first infection (rg < 0.76).

Table 6. Estimated genetic (rg), residual (re) and phenotypic (rp) correlation between LC and LD for each year class

Trait	Year Class				
	2015	2016	2017		
			Alsåkervik	Matre	Veso
LC1 ~ LD1					
r_e	0.96± 0.00	0.91± 0.00	-	-	-
r_g	0.76± 0.07	0.67± 0.07	-	-	-
r_p	0.96	0.89	0.84	0.94	0.96
LC2 ~ LD2					
r_e	0.95± 0.00	0.92± 0.00			
r_g	0.93± 0.02	0.86± 0.03			
r_p	*0.95*	*0.91*			

-Convergence was not achieved

4.7 Genetic correlation between first and second infection

Estimated genetic and residual correlations between the first and second infection for lice count (LC1, LC2) and lice density (LD1, LD2) are shown in Table 7. The genetic correlation between LC1 and LC2 was slightly lower in 2015 (0.44) than in 2016 (0.59) year-class. However, for both year-classes the genetic correlation between LD1 and LD2 were higher.

Table 7. Estimated genetic (r_g) and residual (r_e) correlation (±SE) of lice count (LC) and lice density (LD) between first and second infection for 2015 and 2016-year classes.

Trait	Year class	
	2015	2016
LC1 ~ LC2		
r_e	0.04±0.02	0.02±0.02
r_g	0.44±0.14	0.59±0.11
LD1 ~LD2		
r_e	0.003±0.02	0.11±0.02
r_g	0.63±0.13	0.68±0.09

4.8 Estimated genetic correlation between field and tank challenges

We estimated genetic correlation of LC and LD between the three locations of year-class 2017 (Table 8). A moderate correlation was found between the three locations and slightly higher genetic correlation was estimated between *Matre* and *Veso*

Table 8. Estimated genetic (r_g) correlation (±SE) of lice count (LC, above diagonal) & lice density (LD, below diagonal) between the three locations of the 2017 year class.

Year-class	Location	Alsåkervik	Matre	Veso
2017	Alsåkervik		0.33 ± 0.20	0.57 ±0.15
	Matre	0.20 ± 0.25		0.64 ± 0.14
	Veso	0.35 ± 0.22	0.57 ± 0.15	

4.9 Parameter estimates using genomic information

Estimated parameters for all year classes using genomic information are presented in Table 9. The heritability estimates of LC reported from the SNP data was very similar with the estimates of pedigree relationship matrix and ranged from 0.06 to 0.24. A moderate h^2 value of LC were obtained in *Matre* (0.16) and *Veso* (0.24), and a strong genetic correlation was also observed between these two groups of population. However, the model parameters did not converge for LD, therefore the results from that estimations are presented in appendix 1.

Generally, the genetic correlation between lice count 1 from field test of the 2015 year-class with lice count 1 of all other year-class was low (≤0.40). Similar but slightly higher genetic (≤0.53) correlations were observed for lice count 1 of the 2016 year-class field test with lice count 1 of other populations. Low to moderate genetic correlation (r_g = 0.16 to 0.53) was observed with the field test population (*Alsåkervik*) of the 2017 year-class. This suggest that the genetic correlation of lice count 1 from the field test poorly reflected lice count 1 in the other populations. Lastly, we obtained moderate genetic correlation of lice count 1 between the two 2017 year-class population that was challenge tested. We also observed that, lice count 2 was also poorly correlated to lice 2 in other populations (rg = 0.17 to 0.40). Additionally, the genetic correlation between lice count 1 and lice count 2 from the field test were generally low to moderate (r_g = 0.09 to 0.58), except for the genetic correlation between lice count 2 of the 2015 year-class and lice count 2 of the 2016 year-class (r_g=0.74).

Table 9. Estimated genetic variance, heritabilities (diagonal) and genetic correlation (below the diagonal) for lice count of each year class using genomic information.

Trait	LC1-2015	LC2-2015	LC1-2016	LC2-2016	LC1_2017_A	LC1_2017_M	LC12017_V
LC1-2015	0.06 ± 0.01						
LC2-2015	0.40 ± 0.12	0.13 ± 0.02					
LC1-2016	0.32 ± 0.18	0.74 ± 0.13	0.13 ± 0.02				
LC2-2016	0.20 ± 0.19	0.17 ± 0.15	0.52 ± 0.09	0.15 ± 0.02			
LC1_2017_A	0.16 ± 0.30	0.09 ± 0.24	0.53 ± 0.23	0.58 ± 0.22	0.07 ± 0.02		
LC1_2017_M	0.32 ± 0.27	0.45 ± 0.22	0.50 ± 0.21	0.51 ± 0.20	0.24 ± 0.18	0.16 ± 0.11	
LC1_2017_V	0.13 ± 0.24	0.32 ± 0.19	0.20 ± 0.18	0.21 ± 0.18	0.44 ± 0.15	0.73 ± 0.11	0.24 ± 0.04

LC1-2015, LC2-2015 are lice count 1 and 2 of the 2015 year-class
LC1-2016, LC2-2016 are lice count 1 and 2 of the 2016 year-class
LC1-2017_A, LC1-2017_M and LC1_2017_V are lice count 1 of the 2017 year-class for Alsåkervik (field test) and Matre (Challenge test) and Veso (Challenge test).

5. DISCUSSION

In the current study lice count, pedigree and SNP genomic data from Atlantic salmon of three different year-class (2015, 2016 and 2017) of Marine Harvest breeding nucleus, were used to estimate genetic parameters for host resistance to sea lice.

In this study, we observed that males were significantly *i*) heavier and *ii*) had more lice count than females. However, the effect was negligible when lice density was used. Therefore, since males are heavier this translated to the observed difference in lice count.

The year-class 2015 and 2016 lice count data were obtained from two repeated and independent (freshwater treatment after the first lice counting) challenge tests performed in a net cage, while the year-class 2017 data were obtained from three challenge tests with sibs from the same families; one in a net cage at *Alsåkervik* and the two others in tanks at *Matre* and *Veso*, respectively. The average lice count in net cages was ranged from 4.6 (±2.9) to 16.4 (±6.3) and in the tank challenges was average lice count ranged from 17.2 (±12.5) to 23.9 (±15.5). In net cages, the estimated heritability for resistance to lice ranged from 0.05 to 0.18 for lice count and from 0.04 to 0.16 for lice density (LD). In the tanks challenges, a moderate heritability was estimated 0.19 and 0.3 for lice count and 0.22 and 0.25 for lice density in *Matre* and *Veso*, respectively. The lower heritabilities in cages than in tanks because a higher fraction of the lice falls off when netting and handling the larger fish from cages than the smaller fish from tanks. Environmental variation may also play a great role in sea-cage experiments, e.g. due to less consistency in performing challenge tests in a sea cage than in a tank.

Our estimated heritabilities are in good agreement with what has been reported in literature about the genetic variation for lice resistance. Ødegård et al., 2011 reported heritabilities ranging from 0.13 to 0.14 in a challenge test carried out on sea net-cages. Results from the tank challenges were also consistent with estimates by Gharbi et al., (2015) (~0.3), Gjerde et al., (2011) and Tsai et al., (2016) (~0.2 to 0.3).

The genetic correlation between first and second infection was positive and significantly different from zero for both populations. We estimated genetic correlation between first and second infection of 0.44 ± 0.14 and 0.59 ± 0.11 for the 2015 and 2016 year classes, respectively. Kolstad et al (2005) also reported a positive (0.35) genetic correlation between firt and second infection, although their estimate was not statistically significant. First infection is expected to trigger innate immunity while subsequent infections is expected to trigger adaptive immunity.

Such development of acquired/adaptive immunity in fish after infection with copepod parasites was reported by (Woo & Shariff, 1990). Both the heritability estimates and the moderate estimate of genetic correlation between first and second infection suggests that these two infections are slightly different traits. However, it is important to verify these correlations in tank challenges.

A relatively low positive (~0.14 to 0.2) phenotypic correlation was recorded in this study between lice count per fish and body weight and this was in good agreement with the reports of previous studies. However, we found a very low to close to zero negative phenotypic correlation of body weight with lice density from the second infection of 2016 year class and Matre population. In contrast to our findings, Kolstad et al., (2005) estimated slightly higher genetic correlation (0.37±0.1) between LC and body weight. Yáñez et.al, (2014) have also reported significant and moderate negative genetic correlation between body weight and lice count when fish was challenge tested with *C. rogercresseyi*.

Since the phenotypic correlation between lice density and body weight get close to zero for some of the populations, it suggests that, the relationship between bodyweight and lice count is slightly removed when lice density is computed. However, in some of the population the relationship between lice density and bodyweight was largely negative (-0.30), although the phenotypic correlation between lice count and bodyweight was slightly negative (-0.04). It is difficult to explain why this happens, however, this suggest that care must be taken when lice density is to be used as a trait in the breeding goal. When the genetic correlation of LD with LC was computed, this estimate was moderate to high and similar results have been reported by Gjerde et.al., (2011) (r_g 0.89 ± 0.03). Higher genetic correlations between lice count and lice density suggest that there is limited re-ranking of animals and families, therefore lice count could be used as the trait of interest instead of lice density.

The magnitude of the genetic correlation between field and tank challenge tests was low to moderate (0.33 to 0.57) for lice count and was low (0.20 and 0.35) for lice density. These results mean that, tank challenge test does not reflect field tests, however, it important to note that, phenotypes obtained from field test can be less accurate (due to netting and handling fish) which might have influenced the estimated genetic correlation. Interestingly, the genetic correlation between the tank challenges was surprisingly lower than expected (0.64 for lice count and 0.57 for lice density). These estimates were also lower than the repeated infections of lice (first and second infection). The reason for this difference can be because of genotype by environment

(different counters, etc.) interaction as these two tank challenges were conducted in different locations.

In this study we obtained very similar estimate of heritability using pedigree and genomic information. Genomic variance component has been observed to be lower, similar and higher than estimate from pedigree information in several studies (Erbe et al., 2013; Robledo et al., 2018) The reasons for these differences range from: a) genetic markers only capture part of the variants (other variant include structural variation, etc) in the genome b) markers are not causal variant and therefore might be in weaker linkage disequilibrium with the causal variant which means that they will capture a percentage of genetic variance. The concordance between the genomic and pedigree heritability estimate also means that, you can estimate heritability in populations when parental genotypes are not available to reconstruct pedigrees. We obtained low to moderate genomic correlations between populations. These estimated genomic correlations between populations suggest that, across population genomic predictions will be difficult and most importantly, it needs to be done such that accuracy of selection of sea-lice resistance within population will not be reduced.

6. CONCULSION

The current study shows that there is significant additive genetic variation in Atlantic salmon in resistance to *L. salmonis* in the marine harvest population. Lower heritability estimates were found when the resistance to the lice was measured in challenge tests in sea net-cages than in tanks. High genetic correlation was found between lice count (LC) and lice density (LD) as expected from the relative low and close to zero phenotypic correlation of body weight and LC meaning that LC and LD both can be used as trait for resistance to sea lice.

Heritability estimate based on the classical additive genetic relationship matrix was very close with the findings of those obtained from genomic relationship matrix. However, the genomic correlation between population was low to moderate suggesting that, genomic prediction should be done within population since across population accuracies is expected to be low.

Further studies on using genomic information to improve accuracy of selection of breeding candidate compared to pedigree information is this populations needs to be studies. Lastly, accuracy of selection using multi-population or across population reference population should also be studied.

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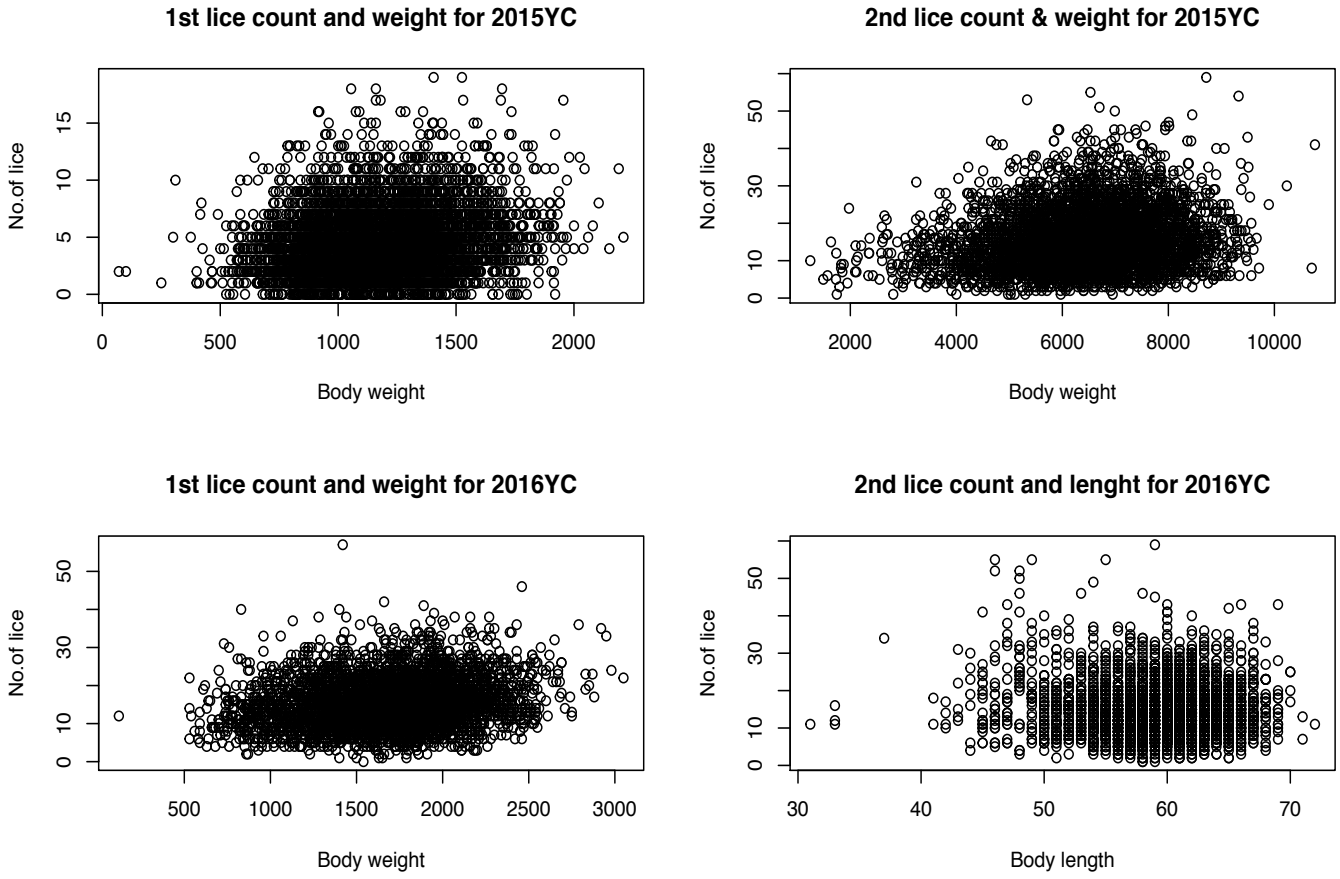
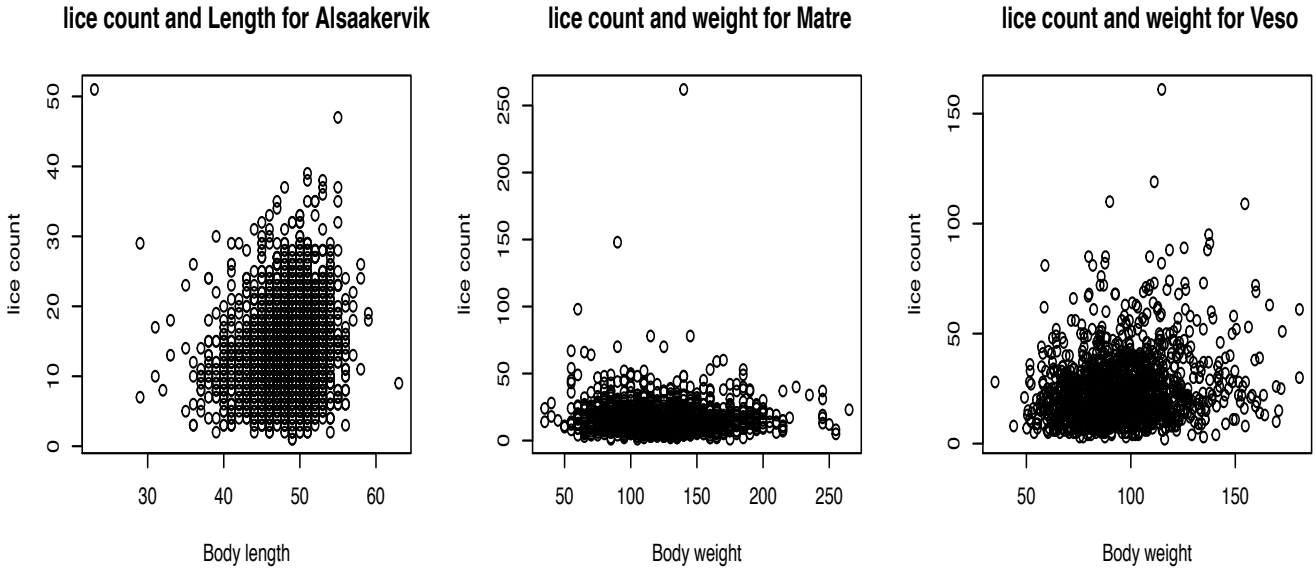
8. APPENDIX

Appendix 1. Estimated heritabilities (diagonal) and genetic correlation (below the diagonal) for lice density of each year class using G-matrix relationship

Trait	LC1-2015	LC2-2015	LC1-2016	LC2-2016	LC1_2017_A	LC1_2017_M	LC1_2017_V
LC1-2015	0.06						
LC2-2015	0.407	0.13					
LC1-2016	0.26	0.52	0.13				
LC2-2016	0.05	0.15	0.76	0.16			
LC1_2017_A	0.02	0.05	0.30	0.49	0.04		
LC1_2017_M	0.12	0.20	0.10	0.17	0.03	0.20	
LC1_2017_V	0.03	0.10	0.04	0.10	0.17	0.54	0.23



Appendix 2. Relationship between weight/length and lice count per fish for 2015 and 2016-year class



Appendix 3. Relationship between weight/length and lice count per fish for 2017-year class.

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