A study on the genetic relationship between salmon lice resistance and disease resistance in Atlantic salmon

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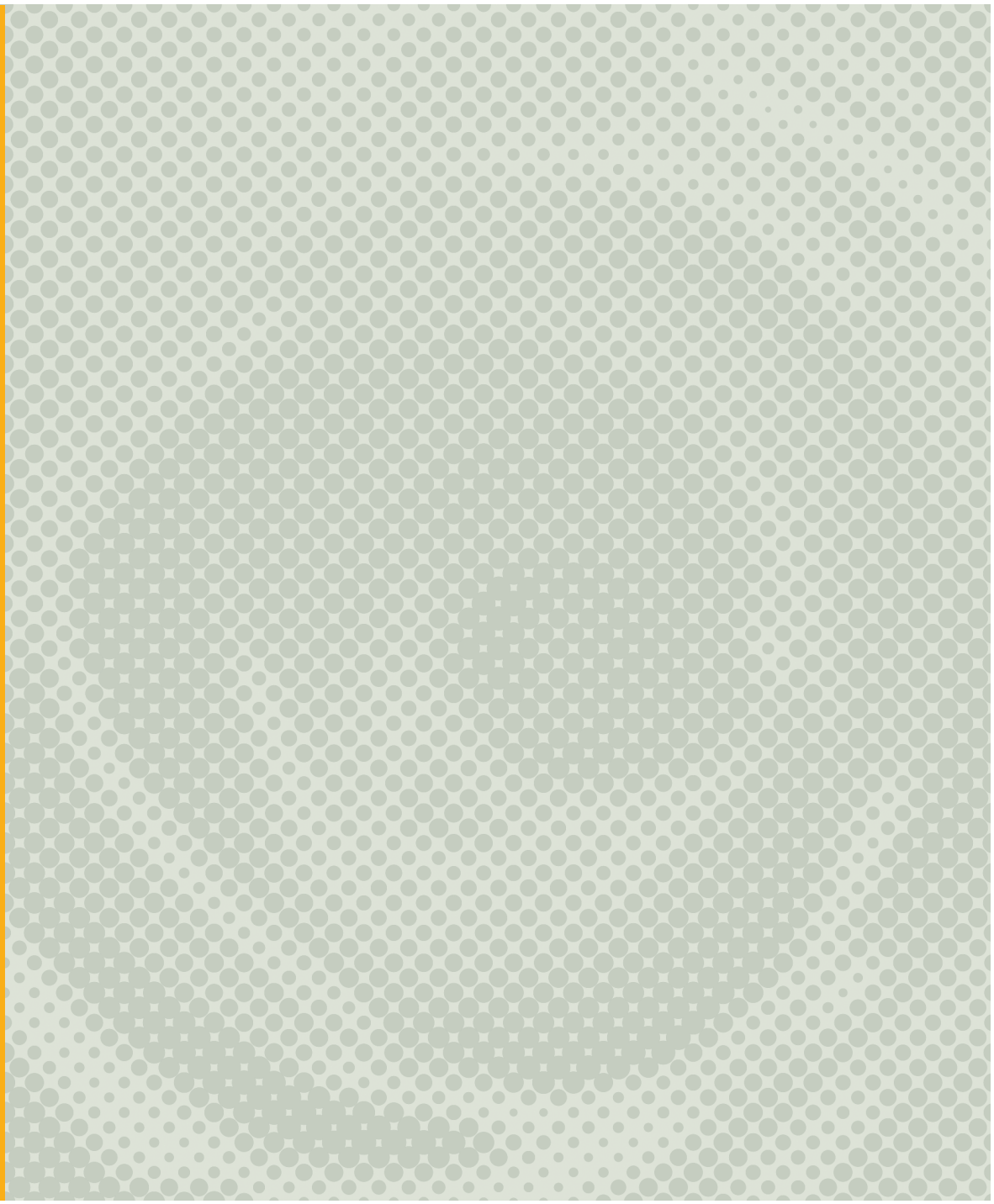

A study on the genetic relationship between salmon lice resistance and disease resistance in Atlantic salmon

Master Thesis in Aquaculture

(30 credits)

by

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Abstract

The aim of this study is to find the genetic relations between resistance to salmon lice and resistance to other diseases. Three groups of Atlantic salmon from 279 full-sib families (offspring of 140 sires and 279 dams, year-class 2007) were challenged with causative agents of furunculosis and ISA as pre-smolts and IPN as post-smolts by means of cohabitation and recorded as survival. A forth group of Atlantic salmon from 154 full-sib families (offspring of 78 sires and 154 dams) of these families were reared in two replicated tanks and infected with two levels of lice per fish (74 and 36 copepodids per fish, respectively). Sessile lice were recorded as lice number per fish (LC) and lice density per fish (LD) were calculated as LD=LC/Body weight$^{2/3}$. Harvest body weight was recorded on two subsamples of the same families as the lice infected group and additional 133 additional full-sib families (offspring of 62 sires and 133 dams). Estimated heritabilities of resistance to furunculosis (0.51), ISA (0.33), IPN (0.39), salmon lice (0.26) and harvest body weight (0.38) were all of moderate levels which indicates a great potential for improving resistance to diseases and growth rate by performing selective breeding. The genetic correlations between each two of the three survival traits were all positive and significantly different from zero (0.21 to 0.50) while genetic correlations between resistance to salmon lice and resistance to each of the three survival traits or harvest body weight were all weak and close to zero (−0.17 to 0.05). It is concluded that these studied traits can be simultaneously improved through selective breeding.

Keywords: Atlantic salmon; Salmo salar L.; Salmon lice; Lepeophtheirus salmonis; Disease resistance; Genetic correlation.

Table of Contents

1. Introduction

In 2011, the world's production of farmed Atlantic salmon amounted to 1.6 million tonnes of which the Norwegian production (1 million tonnes) was accounted for about 62.5% (Murias 2012). The farmers in Norway experience a relatively high loss of fish in the seawater phase (Gullestad 2011) due to different reasons among which specific and multifactorial disease are the most important (Anonymous 2011). Many studies have shown that the bacterial disease, furunculosis (Gjedrem et al. 1991) and viral diseases like infectious salmon anemia (ISA) (Falk et al. 1998), infectious pancreatic necrosis (IPN) (Storset et al. 2007) and pancreas disease (PD) (Taksdal et al. 2007) are serious diseases for farmed Atlantic salmon (*Salmo salar* L.). The ectoparasite sea louse also represents a problem of salmon farms (Pike 1989). The detriments of these diseases are not only the economic losses for the farmers (Gullestad 2011), people also start to concern about the fish welfare issue and the possible threat to the wild salmon (Anonymous 2011; Ford & Myers 2008; Krkošek et al. 2007).

Research has been applied to find effective methods in order to eliminate or decrease the impacts of these diseases. Vaccination is the single most efficient tool to prevent outbreaks of a number of bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral (ISA, IPN, PD) diseases (Gudding et al. 1999; Sommerset et al. 2005). At present, farmed Atlantic salmon are routinely vaccinated before sea transfer (Håstein 2005). However, for viral diseases, only a few vaccines are commercially used and no vaccines exist against parasites like salmon lice (Sommerset et al. 2005).

Sea lice infections are controlled by chemical or biological methods. The chemical methods for delousing is efficient, but the costs is considerable and the impacts on fish welfare and environment is non-negligible. The biological method is characterized of its cost-effective and environment benefits (Treasurer 2002). Cleaner fish (wrasse) are used in biological control of sea lice. However, the efficient of cleaner fish is mostly retricted by its biology and the environment conditions, i.e. water temperature,

abundance of cleaner fish and transport of cleaner fish (Anonymous 2012; Kvenseth & Kvenseth 2000).

Selective breeding programs have been used as a supplementary strategy to improve the innate resistant ability of Atlantic salmon to a number of pathogens. Resistance to furunculosis, infectious salmon anemia (ISA) and infectious pancreatic necrosis (IPN) has been included in programs for Atlantic salmon in Noway since the 1990s (Gjøen et al. 1997; Kjøglum et al. 2008). Moderate to high levels of additive genetic variation has been obtained under challenge test conditions. The heritabilities estimated for the number of lice per fish and lice density per fish under challenge test conditions were all of medium magnitude (Gjerde et al. 2011) while lower heritability was reported for number of lice per fish during natural outbreak in the field (Kolstad et al. 2005). For traits like total number of lice (Kolstad et al. 2005), surrival of furunculosis (Gjøen et al. 1997) and IPN (Storset et al. 2007; Wetten et al. 2007) high genetic correlations between challenge tests and field tests had been documented. All these above-mentioned genetic parameters show that challenge tests could be used in selective breeding to improve the resistance of Atlantic salmon to diseases.

Simultaneously improvement of the resistance to different diseases depends on the sign and magnitude of the genetic correlation among them (Rauw et al. 1998). The more favourable the correlations, the easier it is to improve the resistances simultaneously (Kjøglum et al. 2008). The magnitude and sign of the genetic correlations of resistance to the sea louse (*L. salmonis*) with bacterial diseases and viral diseases have not yet been documented. Boxaspen (2006) reviewed that according to the feeding habit of salmon louse, it may cause osmotic problems and increase the possibility of Atlantic salmon to get secondary infections with other diseases. It is also possible that salmon lice can carry pathogens of diseases and transfer among farmed fish or between farmed and wild fish, as lice in pre-adult and adult stages are mobile and can freely change host (Pike 1989; Ritchie 1997). These results suggest that for the efficient selective breeding to increase disease resistance, the magnitude and sign of the genetic correlations between resistance to salmon lice and specific diseases is of great

importance.

The main objective of this study was to estimate the magnitude of the genetic correlations of resistance to the salmon lice with the resistance to the bacterial disease furunculosis and the two viral diseases IPN and ISA. The genetic correlations between harvest body weight and resistance to the above-mentioned diseases were also estimated.

2. Literature review

2.1. The parasite - salmon louse

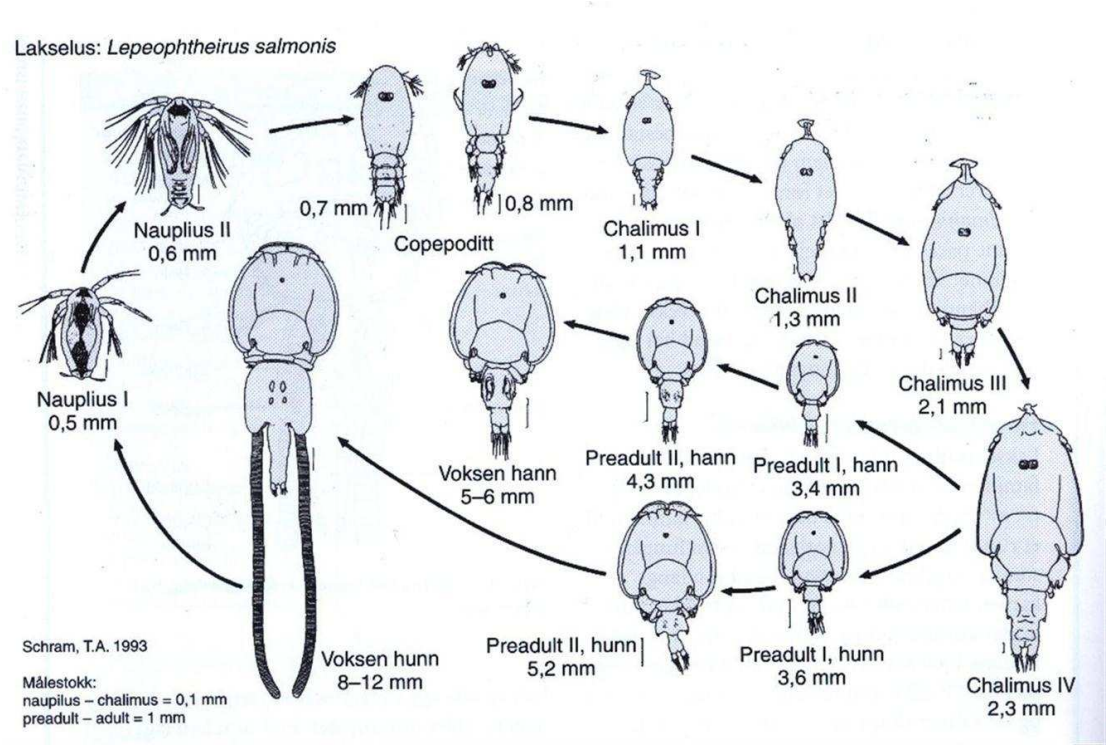
2.1.1. The biology of salmon louse

Sea louse is an ectoparasite which has a huge impact on the salmonid fish industry in brackish or marine phase. *Caligus elongatus* Nordmann and *Lepeophtheirus salmonis* Krøyer are the two main species that have been reported as crisis of the salmonid aquaculture in Northern Hemisphere (Mordue (Luntz) & Birkett 2009; Pike 1989). *C. elongatus* use over 80 different fish species as their hosts, while *L. salmonis* which also is called salmon lice, use salmonid species as their hosts, especially Atlantic salmon (*Salmo salar*) (Kabata 1979). Salmon louse (*L. salmonis*) is the most important parasite for farmed and wild salmonids while *C. elongatus* is a much less problem for these species. In Norway, salmon louse has soon become a problem for farmed Atlantic salmon since the mid-1970s (Heuch et al. 2005). *Caligus rogercresseyi* is the most important parasite that responsible for the economic losses of farmed salmon in Chile (Mordue (Luntz) & Birkett 2009).

Johnson and Albright (1991) stated that at 10ºC the life cycle of salmon lice is about 40 days for male and 52 days for female. The entire 10 life stages includes two nauplius stages, one copepodid stage, four chalimus stages, two preadult stages, one adult stage (see Fig.1) with each separated by a moult (Bellona 2009). Pike (1989) described the characters of every stage. Salmon lice are wholly free-living only in the two nauplius stages while during the eight later stages they live on their hosts. The copepodid stage is the infective larval stage. Lice in this stage start to transform and contact with the fish skin and when they come to the sessile chalimus stages they attach to the host by the frontal filament. Salmon lice can be visible by eyes at chalimus III and IV stages. Preadult and adult stages are the motile stages during which the lice can move freely on the skin of hosts. Lice in chalimus stages are less harmful to the host compared to those

in preadult and adult stages. Sexual mature adult female louse always has two egg strings which containing about 600 fertilized eggs and it can produce as many as 11 pairs of egg strings in its entire life (Heuch et al. 2000).

Fig. 1 The life cycle of salmon lice (*L. salmonis*) (Bellona 2009).



Salmon lice usually attach on the host skin, fins, gill and live by eating skin, mucus and blood. This may result in serious fin damage, skin erosion, deep open wounds and constantly bleeding on the host body. The host responses to sea lice infestation also contains changes in appetite and in the levels of haematological parameters, while the skin damage can cause osmotic problems, stress the host and make it more vulnerable to infection with other diseases (Boxaspen 2006). Johnson et al. (2004) reported that the isolation of infectious salmon anaemia virus (ISAV) (Nylund et al. 1994), furunculosis bacterium (Nese & Enger 1993) and infectious pancreatic necrosis virus (IPNV) (Jim Treasurer unpubl. data) have been successfully isolated from sea lice (*L. salmonis*) and this indicated that salmon lice may function as "vector" for the transmission and outbreaks of diseases.

A high level of infestation can cause salmon mortality. While a few salmon lice on a

large Atlantic salmon cannot result in serious damage, the same number of lice attached to a juvenile salmon may be fatal to the salmon. The juvenile salmon are especially vulnerable as a salmon of 15 grams or less will be weaken by 5 lice and 11 or more lice is found to be lethal (Anonymous 2004). However, a few lice attached to a large salmon may be fatal to the fish by gradually increasing the stress levels and weakening immune system in the long run (Anonymous 2004).

2.1.2. The impacts on wild salmonids

Over the last three decades, the dramatically decreased catch and abundance of wild salmon (Ford & Myers 2008) accompany with the rapidly increased salmon aquaculture production has enhanced the concerns about the association between diseases on farmed and wild fish (Marty et al. 2010). The abundant farmed salmon are stocked in a limited sea cage area at each farm which provides lice an ideal source of host, and finally leads to the amount of lice increase in this ocean area (Anonymous 2004). The high concentration of lice in farm region also represents a threat to the wild salmonid populations living in surrounding water. Krkošek et al. (2007) showed that salmon farm-induced *L. salmonis* infections of juvenile pink salmon have caused the reduction and tendency of local extinction of wild pink salmon populations.

Since farmed salmon are stocked in open floating net cages this implies that the lice can be easily spread with the coastal currents to farmed salmon at other farms and/or from farmed to wild salmonids and vice versa (Costello 2009). Thus farmed salmonids are a possible reservoir for lice that can infestate wild salmonid populations (Heuch et al. 2005).

In addition, as the escaped farmed salmonids may carry a plenty of adult female lice, the escaped fish remaining in the coastal waters will also represent as a reservoir of lice (Costello 2009). In the spring and summer of 2011, a large amount of escaped farmed salmon were found in the area where wild Atlantic salmon live in Norway with serious

salmon lice levels registered on migrating wild salmon smolts and on sea trout in the same area (Lyse 2011).

Marine salmon farms are typically located along the coastal regions where wild salmonids will pass by during their migration from rivers to the ocean as smolts and also homing as adults to rivers for spawning (Anonymous 2004; Ford & Myers 2008). More than 1000 fish farms are established along the Norwegian coast where over 300 million salmon are constantly reared compared to 0.5-1.0 million wild salmon return to Norwegian rivers (The deadly parasite...). Naturally, the coastal area contains few sea lice in the spring, but the fish farms form an unnatural reservoir of lice which is especially harmful to the juvenile wild salmon (Anonymous 2004). During their migration to the ocean the wild salmon smolts may pass numerous fish farms and be exposed to a large number of salmon lice. It is likely that the stresses caused by the migration of the salmon from freshwater to seawater and their small size make the smolt more susceptible to sea lice. On the west coast of Norway, some areas have encountered up to 95% mortality of migrating smolts due to sea lice (The deadly parasite...).

2.1.3. Treatments

The Norwegian regulations for lice treatment (Luseforskriften 2009) seted the limitation for the maximum average number of lice per fish is 0.5 adult female lice or 3 motile lice during January to August, and 1 adult female louse or 5 motile lice during September to December. When the set lice numbers are exceeded, delousing has to be done within two weeks (Heuch et al. 2005). There are two methods to control the salmon lice infestation: chemical method and biological method.

Vaccination can become a cost-effective method to control salmon lice infestation and avoid the disadvantages of chemical treatment, like impacts on environment and other creatures (Raynard et al. 2002). But such vaccines have not yet been successfully

developed (Raynard et al. 2002; Frost et al. 2006 cited by Gjerde et al. 2011).

Chemical treatments are divided into oral treatment and bath treatments. Bath treatments are normally specially available to lice in some certain stages and to a large extent dependent on suitable weather to be performed, while oral treatments like using SLICE® which is effective for lice in all stages, is easy to management, efficient, require little extra labor, give no additional stress on the salmon and is weather independent (Grant 2002). One main risk for chemical treatments is the fast developed drug resistances of sea lice, and it is not likely that the development of chemicals used for sea lice control can keep pace with the increasing drug resistances (Anonymous 2004; Grant 2002). In addition, chemical treatments may influence the environment and other marine animals. SLICE® has been found to accumulate in marine sediments and be harmful to nearby marine animals (Anonymous 2004).

Since 1992, cleaner fish has been used as a biological treatment of sea lice (Andersen & Kvenseth 2000). The goldsinny, corkwing and ballan wrasse are the best delousing species in northern European waters (Kvenseth & Kvenseth 2000). Goldsinny is the first choice for lice control and function best from sea release until the end of the first year in the sea (Andersen & Kvenseth 2000; Kvenseth & Kvenseth 2000). While ballan wrasse is the biggest wrasse it works best with large salmon during the second year in the sea. When the water temperature is below 8ºC, the cleaner fish will stop eating and reduce their activities gradually and will therefore result in a very poor delousing performance (Kvenseth & Kvenseth 2000). In southern Norway, the sea louse situation can be managed by cleaner fish, but in northern Norway, according to the unsuitable environment, i.e. the lower water temperature, makes it difficult to use cleaner fish for sea lice control during most of the years (EWOS 2009). The main supply of cleaner fish is still base on capture as cleaner fish farming is in its infancy while the ethical and disease related challenges caused by capture, mantaince of capacity and transport of cleaner fish could be another problem (Anonymous 2012).

2.1.4. Costs

Sea lice represents the most serious problem for the salmonid farming industry in terms of costs for treatment but also for lowered public reputation. Gjerde (2007) estimated that the direct cost for a total of 1021 sea lice treatments in 2005 to 121 mills NOK (~ € 15 mill.) in Norway (as referred by Gjerde et al. 2011).

2.1.5. Selective breeding for salmon lice resistance

Selective breeding is an eco-friendly sea lice control strategy with the aim to improve the innate lice resistance of salmon. Kolstad et al. (2005) estimated heritabilities for the number of motile lice to 0.02 ± 0.02, for the number of sessile lice to 0.12 ± 0.02 and for the total number of lice to 0.14 ± 0.02 in natural infections with very high genetic correlation ($r_g \geq 0.98$) between them. While the heritability for the number of lice was estimated to 0.26 ± 0.07 during challenge test and a high genetic correlation ($r_g = 0.88$) was found between challenge test and natural infection for the total number of lice with a relatively low number (50) of full-sib families. Gjerde et al. (2011)estimated the heritabilities for number of sessile lice per fish (LC) (0.33 ± 0.05) and the lice density per fish (LD) (0.26 ± 0.05) calculated as LD=LC/Body weight$^{2/3}$ under challenge conditions and the genetic correlation between LC and LD was different form unity (r_g = 0.89 ± 0.03). These results strongly indicated that selective breeding should be a possible supplementary strategy for sea lice control in farmed Atlantic salmon. The genetic correlation between harvest body weight and LD was not significantly different from zero, indicating that selection for improving growth rate will not increase the sea lice problem (Gjerde et al. 2011).

2.2. The bacterial and viral diseases

The bacterial diseases furunculosis, vibrosis and cold water vibrosis are efficiently controlled by effective vaccines (Gudding et al. 1999). All farmed salmon in Norway are routinely vaccinated against bacterial diseases and there was no outbreaks of furunculosis detected in 2010 (Anonymous 2011) and 2011 (Anonymous 2012).

Commercial vaccines against viral infections including infectious pancreatic necrosis (IPN) (Gudding et al. 1999), infectious salmon anemia (ISA) (Lauscher et al. 2011) and pancreas disease (PD) (Sommerset et al. 2005) are available, but efficancy are varied. The number of outbreaks of PD, ISA and IPN in Norway from 1998 to 2011 are showed in Table 2.

The outbreaks of diseases is associated with the economic losses caused by the reduction of production and the costs of medication, antibiotics and labor used for treatments (Press & Lillehaug 1995). The extensive use of antibiotics for diseases control can induce antibiotic resistance in fish pathogens and the residual antibiotics accumulated in fish and environment may result in a risk for both the environment and health of human and animals (Gudding et al. 1999; Press & Lillehaug 1995). The development of vaccines against diseases has reduced the use of antibiotic (Gudding et al. 1999). But vaccination can cause side-effects like reduced growth (Drangsholt et al. 2012), adhesions between organs in the abdominal cavity and discoloration on the internal organs and on the wall of abdominal (Midtlyng et al. 1996).

Table 2. Total number of sites detected with ISA, IPN and PD from 1998 to 2010. Include both the "suspected " and confirmed diagnoses (Anonymous 2012).

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
ISA	13	14	23	21	12	8	16	11	4	7	17	10	7	1
PD	7	10	11	15	14	22	43	45	58	98	108	75	88	89
IPN					174	178	172	208	207	165	158	223	198	154

2.2.1. Furunculosis

Furunculosis is caused by the bacteria *Aeromonas salmonicida* and has been known since the salmonid fish has been farmed (Munro 1988). It causes serious economic losses in wild and farmed salmonids both in freshwater and seawater stages (Toranzo et al. 2005). Since salmon started to be vaccinated in the early 1990s, outbreaks has seldom been recorded in Norway (Sommerset et al. 2005).

The bacterium, *Aeromonas salmonicida* can live in water for a long time thus it can be disseminate by the infected fish or the contaminated water (Austin 1997). Effective vaccines are successfully used to control funrunculosis, and in Norway it has been included as a breeding objective trait in one of the breeding programs since the 1989 (Gjøen et al. 1997). Under challenge test conditions, the estimated heritability for survival to furunculosis of unvaccinated fish ranges from 0.43 to 0.62 (Gjedrem et al. 1991; Kjøglum et al. 2008; Ødegård et al. 2007). For survival to vaccinated and unvaccinated fish, the heritability was found to be 0.39 ± 0.06 and 0.51 ± 0.05 respectively with a low genetic correlation (r_g = 0.32) between them (Drangsholt et al. 2011) which indicate that resistance to furunculosis in vaccinated and unvaccinated fish should be treated like two different traits. The genetic correlation for survival to furunculosis between challenge test and field test conditions was found to be high (r_g = 0.95) (Gjøen et al. 1997).

2.2.2. ISA

Infectious salmon anemia (ISA), a highly infectious viral disease that can be fatal mainly for farmed Atlantic salmon, was first recorded in Norway in 1984 (Thorud & Djupvik 1988). It occurs normally among farmed Atlantic salmon in seawater which rarely outbreaks on Atlantic salmon in freshwater or on other salmonid species. Wild Atlantic salmon might be less susceptible that farmed salmon (Anonymous 2011). However, during the last 2-3 decades the outbreaks of ISA in Norway have remained at

a relatively low level (see Table 2).

ISA can be transmitted via direct contact with infected fish, or by contaminated water and also by salmon lice (Nylund et al. 1994). Infectious salmon anemia virus (ISAV) also exists in blood and tissue, and fish will be infected by exposed to these organic material (Nylund et al. 1994). The horizontal transmission of ISA easily achieved within a tank or net-cage, while more slowly for salmon in different nets at a site and between farms (Anonymous 2011). ISAV can be still infectious after 20 hours in seawater and 4 days in blood and kidney tissue kept at 6ºC (Nylund et al. 1994).

The development of vaccines against ISA is still at an infancy stage (Lauscher et al. 2011; Robertsen 2011). A commercial vaccine against ISA is available in Canada and USA, but not in European countries (Sommerset et al. 2005). Encouraging results have been achieved by breaking horizontal transmission in Norway (Robertsen 2011).

Resistance of Infectious salmon anemia (ISA) has been involved in selective breeding programmes in Norway since the early 1990s (Gjøen et al. 1997; Kjøglum et al. 2008) and the estimated heritability ranges from 0.24 to 0.40 (Kjøglum et al. 2008; Ødegård et al. 2007; Ødegård et al. 2011) under challenge test conditions.

2.2.3. IPN

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease (Anonymous 2000). The IPN infection can be seen as a sudden increase in daily mortality of fry in freshwater hatcheries and among smolts shortly after sea transfer (Murray et al. 2003). Fish can be infected by IPN through the vertical transmission: from the infected parent to progeny, while it may also be infected via horizontal transmission: connected with IPN infected eggs (Munro et al. 2010).

Vaccines against IPN are commercially used for post-smolt fish but the efficacy is

variable and not well documented (Robertsen 2011; Storset et al. 2007). Resistance against IPN has been involved in selective breeding program for Atlantic salmon since 1997 (Kjøglum et al. 2008). The heritability estimated for survival to IPN ranges from 0.31 to 0.39 under challenge test conditions (Drangsholt et al. 2011; Kjøglum et al. 2008; Wetten et al. 2007). A high genetic correlation (0.78-0.83) has been found between challenge test and field test conditions for survival to IPN (Wetten et al. 2007). Moen et al. (2009) detected one major QTL for resistance against IPN, which explained 29% of phenotypic variance and 83% of the genetic variance which indicates that Atlantic salmon with much reduced risk for IPN can be produced over one generation only through marker assisted selection.

2.3. Genetic association among salmon louse and diseases

To select efficiently for several traits simultaneously, we need reliable genetic parameters for all traits, i.e. heritability, genetic correlation. The magnitude and sign of the genetic correlation will decide whether these traits can be selected simultaneously or not (Rauw et al. 1998). Many studies have focused on the genetic correlations, Gjøen et al. (1997) reported a positive genetic correlation among resistances against bacterial diseases (furunculosis, vibriosis and cold water vibriosis) and weakly negative correlations between each of these bacterial diseases and viral disease (ISA). A small but significant favorable genetic correlation (0.09-0.15) between furunculosis and ISA in Atlantic salmon were found in later studies (Dinh 2005; Ødegård et al. 2007). Kjøglum et al. (2008) estimated the genetic correlations between resistances to furunculosis and ISA or IPN in Atlantic salmon were around zero, while relatively large and positive genetic correlations were found by Drangsholt et al. (2011). Gjerde et al. (2011) found that number of lice increases with the increasing body weight but the genetic association lice density and harvest body weight is not significantly different from zero. Genetic correlation between resistance to salmon louse and bacterial diseases or viral diseases are not documented until now.

3. Materials and methods

The fish material has previously been used to study the genetic variation in resistance to the salmon louse of Atlantic salmon (Gjerde et al. 2011) and the genetic (co)variation of resistance to furunculosis in unvaccinated and vaccinated fish and resistance to the two viral diseases: infectious salmon anemia (ISA) and infectious pancreatic necrosis (IPN) (Drangsholt et al. 2011).

3.1. Fish

The Atlantic salmon from 279 full-sib families (offspring of 140 sires and 279 dams) were all from the breeding nucleus of SalmoBreed AS. The fish were produced at Eikelandsosen in November 2006 and transported to Nofima, Sunndalsøra as eyed eggs in January 2007 where they were kept in separate trays. Then fish were reared in separate 0.75 m^3 tanks from the first feeding (5 February to 17 April 2007) until they were at a body size suitable to be tagged with PIT (Passive Integrated Transponder) tags. The living conditions were standardized during the hatchery and rearing period until tagging to minimize environmental differences between families. For the present population, selection for increased resistance to furunculosis and ISA under challenge test (with unvaccinated fish) had been performed for one generation and the breeding goal also included increased growth, lower fillet fat and improved fillet colour.

Three random samples each of 15 fish from all the 279 full-sib families were used for challenge tests with furunculosis (group Fur), ISA (group ISA) and IPN (group IPN). A forth sample of 15 fish from 154 (offspring of 78 sires and 154 dams) of the 279 families were vaccinated and used for challenge tests with salmon lice (group Lice) with an age difference of maximum 53 days. Fish in these four groups were tagged in September and October 2007 (Table 3). The tagged individuals from each of the Fur, ISA and IPN groups were kept in separate 3-meter diameter tanks until disease

challenge tests could be performed (see Section 3.2).

The Lice group was kept in one 3-meter diameter tank until February 13-19, 2008 when the fish were randomly divided on two 3-meter diameter tanks with an equal number of fish per family in each tank. On 15 May 2008 the smolts were transported to Nofima, Averøy, where they were still kept in two separate 3-meter tanks with seawater.

All the fish were fed to satiation with a commercial feed prior to and throughout the experimental period.

Table 3. Number of individuals (N) and mean body weight (g) at tagging in the four groups. The Fur, ISA and IPN groups were tagged in September, Lice group was tagged in Octorber.

Group and body weight at tagging	N	Mean
Fur	4128	34.9
ISA	4178	34.4
IPN	3741	45.1
Lice	2206	54.0

A fifth group consisting of two subsamples of fish from the same154 full-sib families as the Lice group and from 133 additional full-sib families (offspring of 62 sires and 133 dams) was used for measuring the growth until harvest size (group HBw for Harvest Body weight). Between families, the age difference was maximum 80 days based on the date of start-feeding. The fish of the two subsamples were tagged with an average body weight of 18.2 g and 32.0 g and transported from Nofima, Sunndalsøra to two commercial freshwater farms (Sævareid in Hordaland and Breivik in Nordland) for rearing until smolt size, after which they were reared to harvest size in net-cages in sea at two commercial farms. In January 2008 the fish were vaccinated. The fish at Sævareid were divided on two replicated tanks, and the smolt from each these two tanks were transferred to two separated net-cages at Bolaks, Hordaland (Farm A), while the smolt at Breivik were stocked in a net cage at Salten Stamfisk AS (Farm B) for growing to harvest size. At a later stage a third group of fish from the same 287 full-sib families were tagged with a mean body weight of 45 g and kept in a separate tank at Nofima, Sunndalsøra after tagging, and were stocked in one of the two net-cages at Farm A as smolts.

3.2. Challenge tests

3.2.1. Challenge tests for furunculosis, ISA and IPN

The challenge tests of the Fur, ISA and IPN groups were carried out by cohabitation, where naive Atlantic salmon were intraperitoneal injected with the respective pathogens and acted as cohabitants. Daily recorded of dead fish were performed in all the challenge tests.

On 29 September 2007, the Fur and ISA groups were transported to VESO Vikan (Namsos, Norway) as pre-smolts and reared in separate tanks, each containing 3 m^3 of 12ºC freshwater. The challenge test of the Fur group started on 2 October 2007 and lasted for 21 days until an overall mortality of 72%. The challenge test of the ISA group started on 5 October 2007 and lasted for 33 with an overall mortality of 64%.

The IPN group were transported to VESO Vikan as post-smolts on January 2008 and randomly divided on two tanks each of 5 m^3 of 12ºC seawater. The challenge test started on 25 January 2008 and lasted for 39 days until an overall mortality of 45%.

Challenge tests were started when the average body weight of the fish was 30 g for groups Fur and ISA and 85 g for group IPN. For more details on the challenge tests of these groups see Drangsholt et al. (2011).

3.2.2. Challenge tests for salmon lice

The lice copepodids were produced at Nofima Marin, Averøy. The two tanks Atlantic salmon were infected with two different levels of lice per fish: 84,000 copepodids were added to tank 1 (74/fish) while 42,200 copepodids (36/fish) for tank 2 on 20 June 2008. For more details on the lice challenge tests see Gjerde et al. (2011).

The number of sessile lice per fish were recorded on anaesthetised fish by a visual count

when the lice were at the sessile chalimus II-III larvae stage (~2 mm long) on 30 June and 1 July for tank 1 and on 3 July and 4 July for tank 2, 2008. The lice density per fish was calculated as $LD=LC/Body\ weight^{2/3}$ where LC is the number of sessile lice per fish and Body weight is the fish body weight at counting. Given that most fish have similar body proportions, $Body\ weight^{2/3}$ is expected to be proportional to body surface.

3.2.3. Harvest Body weight group

For the HBw group, fish at Farm A were recorded alive from August 13 to 26, 2009. Sex and sexual maturity of each fish were judged into three classes based on external sex characters: sexual maturing males or females or non-maturing fish of unknown sex. Fish at Farm B were slaughtered from August 3 to 7, 2009 and their sex and sexual maturity status judged into five classes by inspection of the gonads: sexual maturing and non-maturing males, sexual maturing and non-maturing females and non-maturing fish of unknown sex.

3.3. Statistical analysis

For groups Fur, ISA and IPN, survival in challenge test was defined as a binary trait, where fish that died during the test were assigned a score of zero and fish that were still alive at the end of the test were assigned a score of one. Survival records of the different groups were treated as different traits.

The genetic correlations between LC in tank 1 and tank 2, between LD in tank 1 and tank 2 were found to be close to unity (Gjerde et al. 2011), and was therefore considered as the same trait in this study.

The variance and covariance components for the random effects of the five studied

traits: lice density (LD), harvest body weight, and survival to furunculosis, ISA, and IPN were estimated by fitting a multivariate sire-dam threshold model using the ASREML software. The estimated variance components for the three survival traits were obtained on the underlying liability scale.

A linear single trait sire-dam model can be written as:

$$y_{ijkl} = F_j + sire_k + dam_l + c_l + e_j$$

where : y_{ijkl} is the observation for trait i for fish j, progeny of sire k and dam l, F_j means fixed effects for fish j, i.e. overall mean, tank/cage, sex and age, $sire_k$ means additive genetic effects of sire k, dam_l means additive genetic effects of dam l, c_l means random effect common to full sibs of dam l, e_j means random residual effect for each individual.

As described by Gjerde et al. (2011) and Drangsholt et al. (2011) the full-sib family effect was not significant and was therefore omitted from the model. Then a multi-trait threshold model was used for trait of LD, harvest body weight and three binary traits (Fur, ISA and IPN).

$$u = \begin{bmatrix} u_{LD} \\ u_{HBw} \\ u_{Fur} \\ u_{ISA} \\ u_{IPN} \end{bmatrix}, \quad e = \begin{bmatrix} e_{LD} \\ e_{HBw} \\ e_{Fur} \\ e_{ISA} \\ e_{IPN} \end{bmatrix}$$

The additive genetic sire and dam effects (u) was assumed $\sim N(0, G \otimes A)$ and residual effect (e) was assumed $\sim N(0, R \otimes I)$. A is the additive genetic relationship matrix, G is the additive genetic (co)variance matrix, and R is the residual variance-covariance matrix among the traits.

For all the five studied traits, the heritability was calculated as:

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

Where σ_u^2 is the additive genetic sire-dam variance, which equals 1/4 of the total additive genetic variance, σ_e^2 is the (underlying) residual variance, for the three binary traits (Fur, ISA and IPN), σ_e^2 was set to 1.0.

4. Results

4.1. Descriptive statistics

The descriptive statistics for lice recording and harvest body weight are shown in Table 4. LC1 and LC2, or LD1 and LD2 are results of two different levels infestation (see section 3.2.2) in two tanks. The infestation success rate (36.7% and 38.6% when adding 75 and 36 copepodid per fish, respectively) in these two tanks is similar and only three of the recorded fish had no lice which indicated both the two infestation levels are under experimental control and 36 copepodid added per fish is close to the optimum. LC increase with increasing body weight at lice recording, while LD seems independent of body weight at lice couting. To get a more reliable estimate of the result, LD was used as a measure of lice resistance instead of LC in this study (For more information, see Gjerde et al. (2011)).

Table 4. Number of fish recorded (N), mean and standard deviation (SD) for observed lice count per fish (LC) and lice density per fish (LD) and body weight (g) at lice counting and harvest body weight (g) at Farm A and Farm B.

	Trait	N	Mean	SD	CV
Tank	Infestation test				
1	Lice count (LC1)	1094	27.1	16.4	60.5
2	Lice count (LC2)	1112	13.9	13.2	95.0
1	Lice density (LD1)	1094	0.66	0.38	57.6
2	Lice density (LD2)	1112	0.34	0.29	87.5
1	Body weight, g	1094	260.6	82.3	31.6
2	Body weight, g	1112	260.2	80.2	30.8
Cage	Growth test				
1	Body weight Farm A, g	22302	4550	1066	23.4
1	Body weight Farm B, g	4324	5009	980	19.6

4.2. Heritabilities and genetic correlations

The estimated heritabilities for the five studied traits and the estimated genetic correlations between the traits are shown in Table 5. The heritability for lice density and harvest body weight were all of medium magnitude as previously reported by

Gjerde et al. (2011) as where the heritabilities (on the liability scale) for the three disease resistant (survival) traits as previously reported by Drangsholt et al. (2011).

The estimated genetic correlation of harvest body weight with resistance to salmon lice, furunculosis or ISA were all low and negative, but not significantly different from zero (−0.08 ± 0.11 for LD, −0.06 ± 0.08 for Fur, −0.09 ± 0.09 for ISA). However, the genetic correlation between harvest body weight and resistance to IPN was positive and value was 0.17 ± 0.09. All genetic correlations of resistance to the salmon lice with resistance to the three disease resistance traits were low and close to zero (−0.08 ± 0.14 for furunculosis, −0.17 ± 0.14 for ISA, 0.05 ± 0.14 for IPN). While the genetic correlations between the three survival traits were all highly positive and value were ranged from 0.21 to 0.50 as previously reported by Drangsholt et al. (2011).

Table 5. Estimated heritabilities (h^2 ± standard errors) of lice density (LD), harvest body weight (HBw), and survival to furunculosis (Fur), ISA and IPN, and genetic correlations (r_g±standard errors) between the five traits.

		r_g ± se			
Trait	h^2 ± se	HBw	Fur	ISA	IPN
LD	0.26 ± 0.05	−0.08 ± 0.14	−0.08 ± 0.14	−0.17 ± 0.14	0.05 ± 0.14
HBw	0.38 ± 0.03		−0.06 ± 0.08	−0.09 ± 0.09	0.17 ± 0.09
Fur	0.51 ± 0.05			0.50 ± 0.09	0.35 ± 0.10
ISA	0.33 ± 0.04				0.21 ± 0.11
IPN	0.39 ± 0.05				

5. Discussion

In this study, the estimated genetic correlations of resistance to salmon lice (LD) with harvest body weight (HBw), survival to furunculosis, ISA and IPN were all slightly negative and not significantly different from zero. The genetic correlation between LD and harvest body weight estimated based on multivariable analysis is of the same magnitude as that in Gjerde et al. (2011) using bivariable analysis. There are no comparative estimates available for the genetic correlations between LD and each of the three studied survival traits in published literature. The results suggest that no strong unfavorable genetic correlations exists among the studied traits which means that it is possible to improve all these traits if they are tested and selected for. The results indicate that selection for increased growth rate or improved resistance to furunculosis, ISA or IPN will not result in unfavorable correlated effects in resistance to the salmon lice. Thus, it is not likely that the increasing salmon lice problems in the salmon industry during the last years is caused by the selections practiced for increased growth rate or improved disease resistances (furunculosis, ISA and IPN) over several generations.

Resistance to salmon lice is estimated as lice density per fish (LD) calculated from the sessile lice count per fish. Sessile lice count is a more reliable measure of lice count than motile lice count as motile lice may drop off the fish during the process of lice recording. Kolstad et al. (2005) reported a very high genetic correlation between the numbers of sessile and motile lice (0.98 ± 0.12) obtained from a relatively low number of families (50 full-sib families), while Gjerde et al. (2010) also found a high genetic correlation (0.87 ± 0.12) between sessile LD and adult LD obtained from 152 full-sib families. These results strongly indicate that resistance measured at different life stages of the lice may be regarded as the same genetic trait and that resistance to lice can be based on sessile lice counting.

The magnitude of the estimated heritability for harvest body weight and resistance to

furunculosis, ISA and IPN in this study are all in accordance with earlier studies (Kjøglum et al. 2008; Ødegård et al. 2011). The genetic correlations between the three survival traits are significantly favorable compared to the earlier results (Dinh 2005; Gjøen et al. 1997; Kjøglum et al. 2008; Ødegård et al. 2007). This difference may be explained by the different infection procedures used in challenge tests. In the present study, all traits are challenged by cohabitation method, while in the previous studies cohabitation challenges were only used for furunculosis while intraperitoneal injections or immersions were performed when testing for resistance to ISA and IPN.

The fish used in challenge tests for salmon lice and harvest body weight were vaccinated while fish challenged with furunculosis, ISA and IPN were unvaccinated. Drangsholt et al. (2011) practiced challenge tests for survival to furunculosis on both vaccinated and unvaccinated Atlantic salmon and found a low genetic correlation (0.32 ± 0.13) between these two groups which suggested that resistance to furunculosis in vaccinated and unvaccinated fish can be treated as two different traits. The low genetic correlation between salmon lice resistance and resistance to furunculosis, ISA and IPN could be influenced by the factor of vaccination.

At present, in traditional selective breeding programs sib selection is used to rank families for disease resistance traits; i.e. traits are measured on sibs of the breeding candidates. Thus, only between family selection is practiced resulting in low selection intensities and thus low genetic gain for these traits as compared to if the breeding candidates are also measured for these traits. For salmon lice, the tested individuals can be effectively deloused by chemical methods and thus be considered as breeding candidates to obtain an increased genetic gain. But according to the realistic conditions, i.e. to avoid the contamination of fish pathogens to the brood stock as transporting breeding candidates from different areas will increase this risk, the lice infected individuals are not suggested to be used as breeding candidates. For traits recorded on the sibs of the breeding candidates, marker assisted or genome wide selection is an option if marker associated with the actual traits can be found

(Sonesson 2011). Moen et al. (2009) has mapped the QTL for resistance to IPN, which explained 83% of the genetic variation and this gave a bright prospect for the possibility of applying for genomic selection. Therefore, the potential for marker assisted seletion for lice resistance should be investigated.

6. Conclusion

The magnitude of the genetic correlations between salmon lice resistance and resistance to bacterial disease (furunculosis) or viral diseases (ISA, IPN) were all quite weak and not significantly different from zero. This suggests that resistance to salmon lice, furunculosis, ISA and IPN can be improved in selective breeding programs provided that they are recorded and selected for.

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