ISBN 978-82-575-1002-2
ISSN 1503-1667

Nofima - The Norwegian Institute of Food, Fisheries and Aquaculture Research,
Osloveien 1,
NO-1430 Ås, Norway
www.nofima.no, e-mail: nofima@nofima.no

Norwegian University of Life Sciences
NO–1432 Ås, Norway
Phone +47 64 96 50 00
www.umb.no, e-mail: postmottak@umb.no

Tale Marie Karlsson Drangsholt

Norwegian University of Life Sciences • Universitetet for miljø- og biovitenskap
Department of Animal and Aquacultural Sciences
Philosophiae Doctor (PhD) Thesis 2011:39

Philosophiae Doctor (PhD) Thesis 2011:39

Quantitative genetics of traits related to disease resistance and effects of vaccination in Atlantic salmon (*Salmo salar*)

Kvantitativ genetikk for egenskaper relatert til sykdomsresistens og effekter av vaksinering i Atlantisk laks (*Salmo salar*)

Tale Marie Karlsson Drangsholt

Quantitative genetics of traits related to disease resistance and effects of vaccination in Atlantic salmon (*Salmo salar*)

Kvantitativ genetikk for egenskaper relatert til sykdomsresistens og effekter av vaksinering i Atlantisk laks (*Salmo salar*)

Philosophiae Doctor (PhD) Thesis

Tale Marie Karlsson Drangsholt

Dept. of Animal and Aquacultural Sciences
Norwegian University of Life Sciences

Ås 2011

Thesis number 2011: 39
ISSN 1503-1667
ISBN 978-82-575-1002-2

PhD supervisors

Bjarne Gjerde
Nofima Marin
P.O. Box 5010, 1432 Ås, Norway

Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences (UMB)
P.O. Box 5003, 1432 Ås, Norway

Jørgen Ødegård
Nofima Marin
P.O. Box 5010, 1432 Ås, Norway

Department of Animal and Aquacultural Sciences
Norwegian University of Life Sciences (UMB)
P.O. Box 5003, 1432 Ås, Norway

PhD Evaluation Committee

Associate professor Henk Bovenhuis
Wageningen University, Animal Breeding and Genomics Center
P.O. Box 338, NL 6700 AH Wageningen, The Netherlands

Dr. Nick Elliott
CSIRO Marine and Atmospheric Research
Castray Esplanade, GPO Box 1538, Hobart TAS 7000, Australia

Professor Odd Vangen
Department of Animal and Aquacultural Sciences,
Norwegian University of Life Sciences
P.O. Box 5003, 1432 Ås, Norway

Acknowledgements

This study was founded through The Research Council of Norway (grant 179009/S40), The Fishery and Aquaculture Industry Research Fund and PHARAMQ AS. Thanks to SalmoBreed AS for supplying all fish used in the experiments and additional data. Thanks to PHARMAQ for providing funding, vaccines and skilled personnel for evaluation of vaccine induced side effects.

I would like to thank my main supervisor Bjarne Gjerde for his skilful and thorough supervision. You enthusiasm has inspired me, and I am grateful for our interesting discussions and for your encouragement. I would also like to thank my co-supervisor Jørgen Ødegård for valuable inputs and fruitful discussions, and for helping me through the mysteries of the DMU-program. Thanks to Hans B. Bentsen for bringing in valuable perspectives and inputs and for supporting me. Thanks to co-authors and members of the project group Frode Finne-Fridell and Øystein Evensn for interesting discussions and suggestions. Thanks to staff at Nofima Sunndalsøra and Averøy where most of my experiments were carried out. A special thanks to Kjellrunn Gannestad for your skilful help with the experiments and for always answering my questions.

Thanks colleagues at Nofima and at the Department of Animal and Aquacultural sciences (IHA) at UMB. Thanks to my (former) officemate Carloz Lozano for all good talks and computer help. Thanks to PhD-students Bente, Siri, Katrine, Hilde H. and everyone else at the 3rd floor at IHA for all your support, interesting (and funny) discussions, and the much welcome coffee breaks. Thanks to group leader Kari Kolstad and everyone in the breeding and genetics group in Nofima for creating a great work environment filled with interesting discussions and humour. Thanks to Celeste Jacq for helping me improve my language. It has meant a lot to me to have great colleagues around me!

Thanks to my friends and family for your support and the good times we have had together away from work. Last but not least I would like to thank my dear husband Anders for all your love, patience, and support. You are not only my partner and best friend but also a great scientist – thank you for all your advice and interesting discussions. I am very grateful for having you by my side!

Ås, May 2011
Tale Marie Karlsson Drangsholt

Contents

SUMMARY 3

SAMMENDRAG 4

LIST OF PAPERS 5

SYNOPSIS 7

1 Introduction 9
1.1 Background 9
1.2 The main disease and testing methods 10
1.3 Vaccination and side effects 12
1.4 Genetic parameters 14
1.5 Selection for improved disease resistance 15
1.6 Breeding goal and structure of breeding programs 17

2 Objectives 20

3 Material and methods 21
3.1 Family material 21
3.2 Subsamples and traits 21
3.3 Vaccines 22
3.4 Challenge tests 23
3.5 Vaccine-induced side effects 23
3.6 Genetic parameter estimation 23

4 Main results 24
4.1 Paper I: Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.) 24
4.2 Paper II: Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*). 24
4.3 Paper III: Genetic correlation among disease resistance, vaccine-induced side effects and harvest body weight 25

5 Discussion 26
5.1 Challenge test data 26
5.2 Side effects of vaccination 27
5.3 Disease resistance in the breeding goal 28

6 Concluding remarks 32

7 References 34

PAPER I
PAPER II
PAPER III

Papers I-III have individual page numbers

SUMMARY

Disease resistance is of major importance to the fish farming industry as disease outbreaks have negative effects on the industry's economy, its reputation and on fish welfare. Today, almost all fish in the Norwegian salmon industry are vaccinated against a number of diseases, while selection for increased resistance to specific diseases is based on survival of unvaccinated fish in challenge tests. The main aim of this doctorial thesis was to obtain a better understanding of how to select for increased resistance to furunculosis in Atlantic salmon and how this relates to side effects of vaccination, taking into account that most fish in the industry are currently vaccinated. Resistance to furunculosis (survival) was recorded by challenge testing fish from 150 families (unvaccinated and vaccinated fish). Vaccine-induced side effects (adhesions of internal organs and melanin deposits) were recorded on samples of the 150 families at three points in time: after three months in freshwater (high temperature), and six and 12 months after sea transfer.

The first objective was to estimate the magnitude of the genetic (co)variation in survival of unvaccinated and vaccinated Atlantic salmon challenged with *A. salmonicida*, the bacteria causing furunculosis. The results showed a low genetic correlation between resistance to furunculosis in unvaccinated and vaccinated fish. The second objective was to estimate the magnitude of genetic variation of the negative side effects of vaccination. Intermediate heritabilities were obtained for adhesions and melanin deposits. However, the results also showed that an alternative vaccine reduced the side effects compared to the standard vaccine. The third objective was to estimate the magnitude of the genetic correlation between disease resistance, side effects of vaccination, and harvest body weight. These traits were not genetically correlated; though a possible exception is harvest body weight and survival of vaccinated fish, where a weak and unfavorable correlation was reported.

Today's breeding strategy of testing unvaccinated fish is optimal if the long term goal is a reduced need for vaccination. Selection based on vaccinated fish is likely to be the most effective short term strategy, as all fish in the industry today are vaccinated. However, this strategy is not very relevant for furunculosis as the vaccine is highly effective. Vaccine-induced side effects (adhesions and melanin deposits) could be reduced through selective breeding, but it is likely to be more appropriate to focus on other measures such as vaccine development. Selection for increased disease resistance, vaccine-induced side effects, or harvest body weight are not expected to lead to unfavorable correlated responses in any of these traits, with the possible exception of survival of vaccinated fish and harvest body weight.

SAMMENDRAG

Forbedret sykdomsresistens hos Atlantisk laks er viktig for oppdrettsnæringen ettersom sykdomsutbrudd har negativ påvirking på næringens økonomi og omdømme, og på fiskens velferd. I dag vaksineres det aller meste av fisken mot en rekke sykdommer. Samtidig pågår det et avlsarbeid for økt sykdomsresistens basert på resultater fra smittetester med uvaksinert fisk. Hovedmålet med dette doktorgradsarbeidet var å få bedre forståelse for hvordan man bør selektere for økt resistens mot bakteriesykdommen furunkulose (forårsaket av bakterien *A. salmonicida)*, og hvordan dette er relatert til bivirkninger av vaksinering. Resistens ble målt som overleving i smittetester hos fisk fra 150 familier. Vaksinebivirkninger, sammenvoksinger av organ i bukhulen og melaninflekker på organ og bukvegg, ble målt på et tilfeldig utvalg av fisk fra de 150 familiene på tre ulike tidspunkt: Etter tre måneder i ferskvann (høy temperatur) og seks og tolv måneder etter sjøutsett.

I smittetest med furunkulose ble det funne høyere genetisk variasjon for uvaksinert enn vaksinert fisk og en relativ lav genetisk korrelasjon mellom furunkuloseresistens i uvaksinert og vaksinert fisk. For sammenvoksinger og melaninflekker ble det funnet middels store arvegrader. En alternativ vaksine gav reduserte vaksinebivirkninger sammenlignet med standardvaksinen. Egenskapene sykdomsresistens, vaksinebivirkninger og slaktevekt ble funnet å ikke være genetisk korrelert, med et mulig unntak mellom slaktevekt og overlevelse av vaksinert fisk i smittetest hvor det ble funnet en svak, ugunstig korrelasjon.

Dagens avlsstrategi basert på smittetester med uvaksinert fisk er optimal hvis det langsiktige avlsmålet er å redusere bruken av vaksinering. Seleksjon basert på vaksinert fisk er likevel den optimale strategien på kort sikt ettersom all fisk i næringen vaksineres, men en liten aktuell strategi for furunkulose ettersom dagens vaksine mot furunkulose er svært effektiv. Vaksinebivirkninger (sammenvoksninger og melaninflekker) kan reduseres gjennom alvsarbeid, men det er mest sannsynlig mer hensiktsmessig å fokusere på andre tiltak som for eksempel vaksineutvikling. Seleksjon for økt sykdomsresistens og slaktevekt og reduserte vaksinebivirkninger forventes ikke å gi ugunstige korrelerte responser i noen av de andre egenskapene, men overlevelse av vaksinert fisk og slaktevekt kan være et unntak.

LIST OF PAPERS

Paper I:

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Fridell, F., Evensen, O., Bentsen, H.B. Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). *Heredity, available online.*

Paper II:

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Fridell, F., Bentsen, H.B. Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*). *Aquaculture, 318(3-4)**:** 316-324*

Paper III:

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Fridell, F., Evensen, O., Bentsen, H.B. Genetic correlation among disease resistance, vaccine-induced side effects and harvest body weight. *Manuscript*

SYNOPSIS

1 Introduction

1.1 Background

Aquaculture is the fastest growing food-producing sector with a total production of 5.2 billion tonnes in 2008 (fish, shellfish and crustaceans) of which around 60% is produced in China. The annual aquaculture production of Atlantic salmon is about 1.5 million tonnes at a value of US$ 7.2 billion (FAO). Interestingly, nearly 100% of this production is based on genetically improved stock (Rye *et al.*, 2010), to a large degree a consequence of the positive results of the first family breeding program for Atlantic salmon that started in Norway in the 1970's (Gjedrem, 2010). Today Norway is the world's largest producer of Atlantic salmon accounting for around 50% of the global production. Disease resistance is of major importance to the fish farming industry, as disease outbreaks have negative effects on both the industry's economy and its reputation. In the first years of production in the 1970s, the breeding goal focused mainly on growth. In the 1990s disease resistance based on survival in challenge tests was included after substantial genetic variation was detected for survival to furunculosis (Gjedrem *et al.*, 1991). Later, the viral diseases infectious salmon anaemia (ISA) and infectious pancreatic necrosis (IPN) were also included in the breeding goal (Gjedrem, 2010; Storset *et al.*, 2007). Today, the breeding objective of most breeding programs of Atlantic salmon includes from one to three specific disease resistance traits in addition to growth and one or several carcass quality traits (Rye *et al.*, 2010).

The use of antibiotics was very high in the late 1980s in the Norwegian salmon farming industry due to frequent outbreaks of diseases like furunculosis, vibriosis and cold water vibriosis, and there was a high demand for preventive measures. Selective breeding for increased disease resistance was therefore regarded as an important contribution for reducing mortalities due to specific diseases. At the same time, effective vaccines were developed and vaccination against furunculosis began in 1990 (Håstein *et al.*, 2005). Thus, both selection and vaccination can act as measures for preventing disease outbreaks; yet to what degree these strategies are complimentary or conflicting remains to be studied.

The following parts of the introduction will present relevant background information for this thesis. Firstly, the principal disease to be studied (furunculosis), the most common

testing methods for disease resistance (challenge tests), and general information about survival in the seawater phase will be presented. Vaccination is involved in most of the results of this thesis and an introduction to vaccination and associated side effects will also be given. Literature on genetic parameters related to disease resistance and other traits relevant for this study will then be presented, followed by an introduction to analysis methods and selection theory relevant for disease resistance, as special consideration is required when using challenge tests Lastly, the breeding goal and the structure of a typical breeding program and is presented, as these have a vital role for implementation of the results of this thesis.

1.2 The main disease and testing methods

1.2.1 Furunculosis

Furunculosis (classical or typical) is a bacterial disease caused by *Aeromonas salmonicida*. *salmonicida* and is present in most aquaculture areas in the Northern Hemisphere. In salmon farming, infection with *A. salmonicida* occurs most often in seawater, and mortalities of unvaccinated fish are generally large. Furunculosis is believed to have been introduced to Norway in 1985 from Scotland and has subsequently caused severe losses to the industry and led to heavy use of antibiotics (Gjedrem *et al.*, 1991; Håstein *et al.*, 2005). In the 1990s, an effective vaccine became available, and this proved to be successful in combating the disease. The vast majority of farmed salmon produced in Norway are vaccinated against this and other diseases, and furunculosis is therefore not considered a threat to the salmon industry today (Håstein *et al.*, 2005). Substantial genetic variation in resistance to furunculosis in unvaccinated fish has been reported (e.g. Gjedrem *et al.*, 1991) and an effective vaccine is available. Thus, furunculosis can serve as a good model for estimating the magnitude of the genetic variation in survival of unvaccinated and vaccinated fish, and of the genetic correlation between these two traits.

1.2.2 Challenge tests

Challenge tests of fish in a controlled environment are widely used to test for disease resistance. This allows for full exposure to the infection in question and generally results in high mortalities. Further, most challenge tests can be performed on small fish and thus the economical value of each individual is relatively low. Different methods of

infection can be used for challenge tests. A cohabitation infection model closely mimics natural outbreaks, as the cohabitant shedders are injected with the pathogen in question and subsequently spread the disease through the natural route of infection to the non-infected fish in the same tank (Nordmo, 1997). This method has been used for challenge tests with *A. salmonicida* for nearly 20 years (Gjedrem *et al.*, 1991; Gjøen *et al.*, 1997; Ødegård *et al.*, 2007b). Another challenge model is intra peritoneal (i.p) injection where the pathogen is directly administered to non-infected fish and will thus circumvent the anatomical barriers of infection (e.g., gills, mucus and skin). This model has been used for challenge tests with *V. salmonicida* (cold water vibriosis) which can not otherwise be tested for in fresh water (Gjedrem and Gjøen, 1995). Challenge tests with ISA were earlier performed using i.p injections (Gjøen *et al.*, 1997), but lately a cohabitation model has been developed and taken into use for this disease (Wetten *et al.*, 2007; Ødegård *et al.*, 2007b). Challenge tests can also be performed through immersion (bath challenges), where the pathogen is added to the water. This method also mimics to some extent natural outbreaks, and has been used for challenge tests with the IPN-virus (causing IPN) (Kjøglum *et al.*, 2008; Storset *et al.*, 2007). To assess genetic variation in disease resistance, the experimental infection should mimic the natural infection route as closely as possible. It is therefore believed that infection through cohabitants or immersion/bath challenges are most appropriate. Challenge tests are also widely used to test the effectiveness of vaccines.

1.2.3 Survival in the seawater phase

The annual production loss of farmed Atlantic salmon in the seawater phase is high in Norway (23% in 2008), while even higher losses are reported in Scotland (28%) and in particular Chile (43%) (Gullestad *et al.*, 2011). Conversely, the Faroe Islands have managed to reduce their production losses to about 5-10% (Gullestad *et al.*, 2011). General survival of fish in aquaculture is important from economic, animal welfare and ethical strand points, and increased survival could both decrease the production cost and increase the income. It has been estimated that a one percent unit reduction of production losses could decrease the cost by 25 million NOK (3.2 million €) and increase gross income by 200 million NOK (25.5 million €) for the salmon farming industry in Norway (Gullestad *et al.*, 2011). There are thus huge economic incentives for increasing the overall survival. However, selection for increased survival based on crude mortality does not seem to be effective, due in part to variable causes of death over time (Vehvilainen *et al.*, 2008). Furthermore, field records of infectious diseases in

aquaculture are generally unavailable as the cause of death is rarely recorded. One of the reasons for this lack in records is due to poor methodology for determining specific causes of death. However, Aunsmo et al. (2008a) developed such a method for Atlantic salmon and showed that it is possible to obtain cause-specific mortalities from commercial grow-out farms; yet this requires frequent investigation of dead fish and thus a high demand for resources. The development of a national system for better health and disease recording has been slow (Gullestad *et al.*, 2011). Further, fish in commercial production are not individually tagged, but individual information on cause of death could potentially be obtained from the breeding companies' test fish as well as from the breeding candidates. Little information exists regarding the correlation between survival to specific diseases in challenge tests and survival in the field, especially for vaccinated fish. Such research is, to the best of my knowledge, currently limited to one study on furunculosis (Gjøen *et al.*, 1997) and one study on IPN (Wetten *et al.*, 2007) (see 1.4.2 Genetic correlations between traits).

1.3 Vaccination and side effects

In comparison to most vertebrates, the immune system of fish is poorly studied; but it is clear that fish also have an adaptive immune system. This is a prerequisite for effective vaccine development as the vaccine exposes the fish to a specific pathogen and consequently induces specific immunity that protects the fish against the pathogen in the future (Press and Jørgensen, 2002). The first commercially available vaccine for fish was developed in the 1970s for enteric redmouth disease and vibriosis, and today vaccines are available for a number of diseases and species (Sommerset *et al.*, 2005). In farmed Atlantic salmon, vaccination is one of the most important tools to prevent outbreaks of a number of bacterial and viral diseases, and is largely responsible for a reduction in the use of antibiotics in the 1990s (Ellis *et al.*, 1997; Gudding *et al.*, 1999; Håstein *et al.*, 2005). The use of vaccination against vibriosis started in 1977 followed by cold water vibriosis in 1987 and furunculosis in 1990 (Håstein *et al.*, 2005). Today, farmed Atlantic salmon are routinely vaccinated before sea transfer against a number of bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral (IPN, ISA, pancreas disease (PD) diseases (McLoughlin and Graham, 2007).

Fish can be immunized by oral-, immersion- or injection vaccines, and injections are most common in commercial production (Gudding *et al.*, 1999). For some diseases such as furunculosis, an oil-adjuvant is needed in the vaccine in order to obtain long-lasting protection, as other adjuvants (aluminium salt and glucans) have shown inferior success (Gudding *et al.*, 1999; Midtlyng *et al.*, 1996). Most i.p. vaccines protect against a number of diseases (polyvalent), and as oil-adjuvant is needed for furunculosis, other antigens are normally added to this vaccine e.g., a vaccine containing six different antigens (ALPHA JECT® 6-2 (PHARMAQ, Oslo, Norway)).

Although oil-adjuvant vaccines are effective; they can lead to vaccine-induced side effects. The side effects can be seen as adhesions between intra-peritoneal organs and melanin deposits on internal organs and on the abdominal wall (Midtlyng *et al.*, 1996). In the most severe cases these vaccine-induced side effects can lead to reduced fish welfare and downgrading of the fish slaughter quality (Midtlyng, 1996; Poppe and Breck, 1997). The vaccines can also reduce fish appetite and growth (Aunsmo *et al.*, 2008b; Berg *et al.*, 2006; Midtlyng and Lillehaug, 1998; Sørum and Damsgård, 2004). Adhesions and melanin deposits are seen in almost all vaccinated fish, and in a study by Lund et al. (1997), adhesions proved to be a good marker for distinguishing between vaccinated and unvaccinated fish, whereas melanin deposits were a poor marker.

Adhesions and melanin deposits are associated with prolonged inflammation caused by persistent antigens from the vaccine (Mutoloki *et al.*, 2004). The severity of vaccine-induced intra-abdominal adhesions may be affected by the time (of year) of vaccination, vaccine formulation, water temperature and the size of fish at vaccination (Aucouturier *et al.*, 2001; Berg *et al.*, 2007; Berg *et al.*, 2006). It has also been shown that oil-adjuvant vaccines can provoke systemic autoimmune reactions; and that adhesions and granulomas seen in the abdominal cavity of vaccinated fish are related to both chronic inflammation, and autoimmune reactions (Haugarvoll *et al.*, 2010; Koppang *et al.*, 2008). Genetics appears to play a role in the development of vaccine-induced side effects. In a pilot study, intermediate heritabilities (h^2 = 0.18 - 0.19) were found for susceptibility to intra-abdominal adhesions (Gjerde *et al.*, 2009).

1.4 Genetic parameters

1.4.1 Genetic variation in disease resistance

Genetic variation in disease resistance is a prerequisite for response to selection, and can be estimated from challenge test data. Substantial genetic variation has been found for several diseases in Atlantic salmon; with heritability estimates for survival in challenge tests ranging from 0.43 to 0.62 for *A. salmonicida* (Gjedrem *et al.*, 1991; Gjøen *et al.*, 1997; Kjøglum *et al.*, 2008; Ødegård *et al.*, 2007b); from 0.19 to 0.32 for ISA-virus (Gjøen *et al.*, 1997; Ødegård *et al.*, 2007b); and from 0.31 to 0.55 for IPN-virus (Kjøglum *et al.*, 2008; Wetten *et al.*, 2007). Recently, a QTL explaining a very large proportion of the genetic variation in IPN resistance has been detected in both Scottish and Norwegian populations of Atlantic salmon (Houston *et al.*, 2009; Moen *et al.*, 2009). Genetic variation has also been found in resistance to parasites such as sea lice (*Lepeophtheirus salmonis*) (Gjerde *et al.*, 2011; Kolstad *et al.*, 2005), flatworms (*Gyrodactylus salaris*) (Salte *et al.*, 2010) and amoebae (*Neoparamoeba perurans*) (Taylor *et al.*, 2009). The genetic relationship between resistance to different diseases, both bacterial and viral, seems to be neutral or slightly favorable (Kjøglum *et al.*, 2008; Ødegård *et al.*, 2007b). Because of biosecurity, the survivors in the challenge tests cannot be used as breeding candidates, and therefore selection must be based on records of their full- and half-sibs (family selection). The documentation of response to selection for increased disease resistance in selective breeding programs is limited to the results by Storset et al. (2007) who reported a substantial response in survival to IPN based on challenge test data.

1.4.2 Genetic correlations between traits

Many examples of unfavorable genetic correlations among traits and unfavorable correlated selection responses can be found in selective breeding experiments and programs of livestock, particularly in traits not included in the breeding objective (Rauw *et al.*, 1998). Hence, reliable estimates of genetic correlations between traits are important so that measures can be taken to prevent such unfavorable correlated responses when selecting for other traits. Growth until harvest size (measured as body weight at harvest size), and survival to specific diseases in challenge tests, are two important traits in the breeding goal of Atlantic salmon; and the magnitude of the genetic correlation between these two traits is of great importance. However, few reliable estimates of genetic correlations between growth and other traits are available,

with none showing strongly unfavorable correlations (Gjedrem *et al.*, 1991; Standal and Gjerde, 1987). Vaccine-induced side effects are currently not commonly included in the breeding goal of Atlantic salmon, and Gjerde et al. (2009) found no evidence of unfavorable genetic correlations of vaccine-induced side effects with survival to furunculosis and ISA in challenge tests. However, unfavourable genetic correlations of harvest body weight with both adhesion scores (-0.45 ± 0.10) and melanin scores (-0.27 ± 0.11) were reported. When considering the correlation between survival in challenge tests and survival after outbreaks in the field, a very large genetic correlation has been reported for furunculosis survival (0.95) (Gjøen *et al.*, 1997). When it comes to the correlation between survival in challenge tests and survival after outbreaks in the field, a very high genetic correlation has been reported for survival of to furunculosis (0.95) (Gjøen *et al.*, 1997). These results were in all likelihood based on fish vaccinated with a vaccine without an oil-based adjuvant, which gave a weak and transient protection against furunculosis (Arne Storset, pers. comm.), although this was not explicitly stated in the paper. A large genetic correlation between field and challenge test data was also found for survival to IPN in two year-classes, where the field data of one year class was from vaccinated fish (r_g=0.78 ± 0.16) and the other from unvaccinated fish (0.83 ± 0.07) (Wetten *et al.*, 2007). Both year-classes had a large degree of mortalities suggesting that the IPN-vaccine was not very effective.

1.5 Selection for improved disease resistance

1.5.1 Statistical models for analysing survival data

There are several ways of analyzing survival data from challenge tests, and the issue has recently been addressed in a review by Ødegård et al. (2011a). A linear mixed model is a simple model based on survival at termination of the test or at an overall 50% survival of the population; such models were used in some of the earliest studies (Gjedrem *et al.*, 1991), seeming to predict breeding values accurately (Gitterle *et al.*, 2006; Ødegård *et al.*, 2006; Ødegård *et al.*, 2007a). These models assume a normal distribution of data, even though the observations are binary (dead or alive). A mixed threshold model takes into account the binary distribution of data, and assumes that the binary trait is fully explained by an underlying continuous trait (liability). Heritabilities estimated with linear models and threshold models can therefore not be directly compared as they are calculated on two different scales, and heritabilities on the underlying scale (threshold

models) are generally greater than on the linear scale (linear models). However, ranking of families is often similar using linear and threshold models (Gitterle *et al.*, 2006; Ødegård *et al.*, 2006; Ødegård *et al.*, 2007a). An alternative to these cross-sectional models is longitudinal models, where the time of death is considered rather than the binary notation of "dead or alive" at a given point in time. Proportional hazard models, where risk of mortality is a function of time, have been suggested as an appropriate model for lifetime data (Ducrocq and Casella, 1996). The survival score model is an approximation of this model, where individuals are scored as dead or alive in different sub-periods (e.g. days) (Ødegård *et al.*, 2011a); results have shown that these can give a slightly more accurate estimation of breeding values compared to simpler cross-sectional models (Gitterle *et al.*, 2006; Ødegård *et al.*, 2006; Ødegård *et al.*, 2007a).

An important issue in challenge testing is when to terminate the test. Common practice is to terminate the test when the cumulative mortality reaches about 50%, as this maximizes phenotypic variance for a binary trait. It is assumed that all individuals are susceptible and will die given sufficient time. However, if a non-susceptible (or resistant) fraction exists amongst the survivors, this assumption is violated and may bias both the cross-sectional and longitudinal models if the test is terminated before mortalities cease. In a study by Salte et al. (2010), where Atlantic salmon were challenged with the parasite *Gyrodactylus salaris*, a small genetic correlation was estimated between time until death and survival at the termination of the test (when mortalities had ceased). Thus, severe re-ranking of families would have occurred if the test had been terminated at 50% mortality. Selection for disease resistance should ideally be for reduced susceptibility rather than for a greater time until death (endurance). If a non-susceptible fraction exists, it may be possible to select for this by continuing the challenge tests until mortalities naturally cease and when most susceptible fish are assumed to be dead. A cure model has been developed in order to account for both susceptible and non-susceptible fish in a challenge test, and such a model has been applied to survival data of *Penaeus vannamei* challenge tested with taura syndrome virus (TSV) (Ødegård *et al.*, 2011b). In the aforementioned study, endurance and susceptibility were seemingly two different traits (genetic correlation did not significantly deviate from zero) (Ødegård *et al.*, 2011b), thus selection for susceptibility rather than endurance will then be possible. Similar studies on other species and diseases are necessary, yet currently lacking.

1.5.2 Selection theory

The goal of selection is to obtain (favorable) genetic changes in the traits, which can be defined as:

$$\Delta G = \frac{\text{intensity} \times \text{accuracy} \times \text{genetic variation}}{\text{generation interval}}$$

Fish are typically highly prolific species; therefore, large full- and half-sib family groups can be used for challenge tests. Survivors of the tests are considered potential disease carriers, and thus they are not themselves used as selection candidates. However, records of their full- and half-sib can be used to select breeding candidates (i.e. family selection). As family selection relies solely on information from between-family variation and not within-family variation (half of the total genetic variance), this method reduces accuracy. The maximum accuracy that can be obtained (with large sib-groups) is thus $\sqrt{0.5}$, and the intensity of selection is reduced in family selection as selection is restricted to random individuals from the highest ranking families rather than the best individuals across families (Ødegård *et al.*, 2011a). However, if the within-family variation could be exploited (i.e., by selecting surviving fish) both the accuracy and the intensity of selection would increase.

In order to facilitate individual selection, indirect selection based on immunological markers correlated to disease resistance has been proposed as an alternative to challenge test data. Fjalestad et al. (1993) showed that the genetic correlation between the maker traits and survival needs to be large in order to be more efficient than direct selection for survival. Attempts to find such markers have not been very successful, as low genetic correlations have generally been reported (Gjedrem and Thodesen, 2005; Lund *et al.*, 1995).

1.6 Breeding goal and structure of breeding programs

1.6.1 Breeding goal

Selective breeding is often a relatively slow process due to long generation intervals (three-four years in Atlantic salmon), and the current breeding goal must therefore remain relevant for at least 10-20 years in the future. However, in order to keep up with changes in the industry and markets, the breeding goal has to be adjusted over time. It is therefore relevant to talk about long and short term breeding goals. Short term breeding

goals generally try to solve a present problem or demand, while the long term breeding goals try to foresee what can be important in the future. In general, the breeding goal of the nucleus has to be long term, while a narrower and short term breeding goal can be applied on the multiplier level.

The breeding goal consists of all traits directly or indirectly selected for in a selection program, and is primarily of an economic nature (e.g. maximum profit) for most farm animals. The breeding goal for Atlantic salmon in Norway typically includes traits related to growth, quality and health. Health generally includes less deformities and increased resistance toward specific diseases like furunculosis, ISA and IPN (Rye *et al.*, 2010; www.aquagen.no; www.salmobreed.no). The breeding goal of Atlantic salmon was initially only focused on increased growth, and this led to a significant increase in this trait. Age at sexual maturation was the second trait to be included and disease resistance and quality traits followed (Gjedrem, 2010). Growth will probably be important both in the long and short term, while quality traits (e.g. filet fat) may change over time as consumer preference changes. Further, to some degree there is an optimal level for quality traits and other traits such as age at sexual maturation, thus suggesting that such traits may be less important in the breeding goal in the long term. Survival is generally considered a long term breeding goal, as this is crucial for production from economic, animal welfare and ethical points of view (Gjedrem, 2005).

The relative economic weight for each trait in the breeding goal must be considered, and a common approach in farm animals is to derive economic values using profit equations (Nielsen *et al.*, 2011). However, in fish breeding a desired or restricted gain approach is most commonly used (Brascamp, 1984; Nielsen *et al.*, 2011; Olesen *et al.*, 2000). In this method, the breeding goal is to obtain a desired or restricted genetic gain for each included trait, and the weights are then derived in order to reach that goal.

1.6.2 Structure of breeding programs

Selective breeding programs for fish typically consist of several levels. Firstly, a breeding nucleus contains test fish and breeding candidates. Secondly, at the multiplier level, offspring of selected fish from the nucleus are reared to sexual maturation from which eyed eggs, fry, or fingerlings are supplied to producers for the grow-out stage (Gjerde, 2005). .For species with very high fecundity (e.g. Atlantic cod, *Gadus*

morhua), the multiplier level may be skipped; but for Atlantic salmon, a multiplier is needed in order to produce enough eggs and fry to supply the market (Skagemo et al., 2010). In order to meet the different demands of the producers, brood stock groups on the multiplier level can be especially selected for a single or a limited number of traits like disease resistance quality traits and growth; thus developing different products to suit the specific requirements of different producers. The broodstock groups are developed from a few families selected based on breeding values for the desired traits (e.g. disease resistance). The resulting females are then mated to either males from the breeding nucleus or males from the same or a different broodstock group, and the resulting eyed eggs or fry are sold to the grow-out producers. Many of the traits selected for cannot be recorded on the selection candidates (e.g. disease resistance), and family selection is then used both at the nucleus and the multiplier level. The multiplier level allows for higher selection intensity than can be practiced in the nucleus, as (long term) inbreeding is not a concern.

Recently, marker assisted selection has been implemented in some breeding programs of Atlantic salmon (Moen, 2010), following the discovery of a QTL explaining a very large fraction of the genetic (and phenotypic) variation in IPN-resistance in both Scottish (Houston *et al.*, 2009) and Norwegian (Moen *et al.*, 2009) populations. In particular, this offers opportunities to select for specific traits in the broodstock groups at the multiplier level. Currently, at least one breeding company offers fry from broodstock tested for the IPN-QTL (Moen *et al.*, 2009; www.aquagen.no) and others plan to offer similar products (www.salmobreed.no).

2 Objectives

Today almost all fish in the Norwegian salmon aquaculture industry are vaccinated against a number of diseases, but the use of oil-adjuvant vaccines leads to vaccine-induced side effects. In parallel with vaccination, selection for increased disease resistance is performed for several diseases. However, selection is based on survival of unvaccinated fish in challenge tests. There is therefore a great need to establish the genetic variation of traits related to disease resistance and vaccination, and the correlation between them.

The objectives of the thesis were:

- To estimate the genetic correlation between survival of unvaccinated and vaccinated Atlantic salmon challenged with *A. salmonicida*, the bacteria causing furunculosis.
- To estimate the genetic variation in vaccine-induced side effects.
- To estimate the genetic correlations between survival (of both unvaccinated and vaccinated fish), vaccine-induced side effects and harvest body weight.

3 Material and methods

3.1 Family material

The fish used throughout this study were from 150 full sib families (the offspring of 85 sires and 150 dams) from the breeding nucleus of the Norwegian breeding company SalmoBreed AS. The families were produced in November 2006, and the first feeding of the families occurred during February 5 to April 17, 2007. Selection for increased resistance to furunculosis and ISA had been performed for one generation (on parents of fish used in this study), and the breeding goal also included growth, fillet fat and fillet colour.

3.2 Subsamples and traits

The main traits analyzed were survival, vaccine-induced side effects and body weight. These traits were recorded on defined subsamples (Table 1) of 10-15 fish from each of the 150 families. Table 1 gives an overview of the groups, treatments, and in which papers they are included.

Table 1: Overview of traits, sample groups, treatments and in which papers they are included.

Trait/Group	Challenge agent	Environment and duration	Type of vaccine	Time of vaccination	Paper I	Paper II	Paper III
Survival							
Fur	*A. salmonicida*	Challenge test, 21 days	N/A		x		x
ISA	ISV-virus	Challenge test, 22 days	N/A		x		x
IPN	IPN-virus	Challenge test, 39 days	N/A		x		
FUR-SV[†]	*A. salmonicida*	Challenge test, 60 days	Commercial	October 2007	x		x
FUR-RD	*A. salmonicida*	Challenge test, 60 days	Reduced dose	October 2007	x		
Vaccine-induced side effects*							
FW		Freshwater, 3 months (17°C)	Commercial	October 2007		x	
SW6		Seawater, 6 months	Commercial	March 2008		x	
SW6-RV		Seawater, 6 months	Experimental	March 2008		x	
SW12		Seawater, 12 months	Commercial	March 2008		x	x
Body weight							
SW12		Seawater, 12 months	Commercial	March 2008			x

[†] FUR-SV group is named FUR-V in paper III
*Adhesions in the abdominal cavity and melanin deposits
Vaccines are described in section 3.3 Vaccines.

3.3 Vaccines

All three vaccines used in this study (Table 1) contained mineral oil as adjuvant and were administered through i.p. injections. The vaccines included antigens of *A. salmonicida*, *Vibrio anguillarum* serotype O1 and O2, *Vibrio salmonicida*, *M. viscosa* and IPN virus (protection against furunculosis, classical vibriosis, cold water vibriosis, winter ulcer and IPN, respectively). The commercial vaccine was ALPHA JECT® 6-2 (0.1 ml pr fish). The reduced dose vaccine contained 60% less *A. salmonicida* antigen than the commercial ALPHA JECT 6-2 and was administered with a reduced injection volume (0.05 ml pr fish). The experimental vaccine was administered with a reduced injection volume (0.05 ml pr fish) and is now a commercial vaccine (ALPHA JECT® micro 6). All vaccines were produced by PHARMAQ. The vaccination time is given in Table 1.

3.4 Challenge tests

Challenge tests were performed at VESO Vikan (Namsos, Norway) between October 2007 and January 2008, and a cohabitation challenge model was used for all challenge tests. Challenge tests of unvaccinated fish (Fur, ISA, IPN) were part of SalmoBreeds routine genetic evaluation, and each of these diseases was tested in a separate tank. The vaccinated fish (Fur-SV and Fur-RD) were tested in another tank.

3.5 Vaccine-induced side effects

Vaccine-induced side effects were subjectively evaluated by trained personnel from PHARMAQ. Observations of the FW group were made at Nofima Marin Sunndalsøra in January 2008 and observations of the SW6, SW6-RD and SW12 groups occurred at Nofima marine, Averøy in November 2008 and June 2009. Adhesions were scored in three separate regions of the abdominal cavity (region 1, 2 and 3) using a scale from 0 – 6 and an interval of 0.5; where 0 = no adhesions and 6 = extremely severe adhesions (Midtlyng *et al.*, 1996). Region 1 was anterior, region 2 was dorsal and posterior, and region 3 was the ventral part of the abdominal cavity. Melanin deposits on internal organs and the abdominal wall were scored on a scale from 0 – 3 with an interval of 1.0; where 0 = no visible melanin and 3 = severe melanin spots.

3.6 Genetic parameter estimation

Estimates of heritabilities were obtained for survival (five traits), adhesions and melanin (each on four subsamples) and body weight (one trait). The genetic correlations between these traits were also estimated. Survival traits were analyzed using threshold models, and linear models were used for all other traits. The DMU software was used for all genetic analysis (Madsen and Jensen, 2007).

4 Main results

4.1 Paper I: Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.)

Substantial genetic variation was found in resistance to furunculosis in both the unvaccinated (heritabilities of 0.51 ± 0.05) and vaccinated (0.39 ± 0.06) fish. However, the genetic correlation between resistance to furunculosis in the two groups was low (0.32 ± 0.13), indicating a weak genetic association between resistance in the two groups. Significantly favourable genetic correlations were evident between resistance to furunculosis and resistance to both IPN and ISA in unvaccinated fish. This pattern was less pronounced for vaccinated fish.

4.2 Paper II: Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*).

The SW6-RV group had lower adhesion scores (0.93 vs. 1.68) and melanin scores (1.04 vs. 1.49) than the parallel SW6-group. For the fish groups administered with the commercial vaccine, the heritability estimates for adhesion scores were 0.31 ± 0.05 (FW), 0.19 ± 0.04 (SW6) and 0.16 ± 0.05 (SW12), and for melanin scores 0.27 ± 0.05 (FW), 0.28 ± 0.05 (SW6) and 0.30 ± 0.05 (SW12). For the SW6-RV group, the heritabilities were lower; 0.08 ± 0.03 (SW6-RV) for the adhesion score and 0.11 ± 0.03 (SW6-RV) for the melanin score. The genetic correlation between adhesion scores and melanin scores within groups was intermediate for the FW group (0.52 ± 0.11) but higher for the SW6 (0.89 ± 0.06) and SW12 groups (0.87 ± 0.06). The genetic correlation between the SW6 and SW12 groups was large for both adhesion (0.89 ± 0.07) and melanin (0.92 ± 0.11) scores. However, the genetic correlations between FW group and the SW6 and SW12 groups were smaller for adhesions (0.62 ± 0.12 and 0.48 ± 0.14, respectively) and for melanin (0.84 ± 0.08 and 0.61 ± 0.11, respectively). These results show that vaccine-induced side effects can be reduced through selective breeding, but that a reduction can also be achieved by other factors such as improvement of the vaccine.

4.3 Paper III: Genetic correlation among disease resistance, vaccine-induced side effects and harvest body weight

The genetic correlations between disease resistance traits and vaccine-induced side effects did not significantly deviate from zero. Likewise, the genetic correlation between body weight at harvest and vaccine-induced side effects did not significantly deviate from zero. Genetic correlations between harvest body weight and disease resistance (survival) were also weak; -0.18 ± 0.17 (furunculosis) and 0.05 ± 0.17 (ISA) for unvaccinated fish and -0.36 ± 0.16 (furunculosis) for vaccinated fish. These results suggest that selection for one trait is not likely to produce an unfavourable correlated response in the other traits, with a possible exception being for harvest body weight vs. survival of vaccinated fish.

5 Discussion

The main aim of this thesis was to get a better understanding of how to select for increased resistance to furunculosis in Atlantic salmon and how this relates to side effects of vaccination, taking into account that most fish in the industry are currently vaccinated. Paper I focused on the magnitude of the genetic (co)variation in survival of unvaccinated and vaccinated fish in challenge tests, and found that survival from furunculosis in unvaccinated and vaccinated fish was weakly correlated genetically. Paper II addressed the genetic variation of the negative side effects of vaccination and found that both adhesions and melanin deposits were heritable. Paper III examined the genetic correlation between disease resistance, side effects of vaccination and growth measured as body weight after 12 months at sea and found that these traits were generally not genetically correlated. The discussion will address issues relevant for selective breeding based on the above mentioned traits and results.

5.1 Challenge test data

Challenge tests are the most important tool when it comes to selection for improved disease resistance in aquaculture. However, as the surviving fish from these tests cannot be considered as breeding candidates due to biosecurity (fear of spreading the disease), the genetic variation within the family is not exploited. However, use of survivors as breeding candidates would also require measures to obtain records on these fish for other traits, especially other diseases. Therefore, use of genomic information is most likely a better approach to exploit the within family variation, either through marker assisted or genomic selection (Sonesson, 2007; Sonesson and Meuwissen, 2009). However, implementation of marker assisted selection would require knowledge about more QTLs associated with resistance to disease (excepting IPN); and genotyping a large number of fish is still quite costly.

All challenge tests in this study (Paper I and Paper III) were performed by cohabitation. This method mimics a natural outbreak, albeit at a greater infection pressure, and can therefore potentially capture genetic variation on both innate immunity (including anatomical barriers) and more specific immune reactions (adaptive immunity). One possible explanation for the positive genetic correlation found between the bacterial disease furunculosis and the viral disease ISA (Paper I) can thus be that some features

of innate immunity may be relevant for both diseases. Previous studies reporting neutral or negative genetic correlations have partially been based on methods (i.p.) circumventing the anatomical barriers (Gjøen *et al.*, 1997; Kjøglum *et al.*, 2008).

The vaccinated fish challenged in this study had mounted a specific immune response prior to the challenge, and a large pathogen load was needed in order to obtain sufficient mortalities. Innate immunity may thus play a less important role and the specific immunity a larger role in these fish, compared with the unvaccinated fish challenged in a different tank.

In our study, challenge tests with vaccinated fish (Fur-SV and Fur-RD) were run until a plateau was reached; whereas the tests with unvaccinated fish (Fur, ISA and IPN group) were terminated at an earlier point (close to 50% mortality), as this was common practice at the time of testing. The early termination of these tests may have affected the results to some extent, as it may obscure differences between potentially non-susceptible fish and susceptible fish still alive at the end of the testing period. However, it is more likely that a non-susceptible fraction would be most prominent in the vaccinated groups, and for these groups the testing period was continued until mortality naturally plateaued. Neither a cross-sectional nor a longitudinal survival model is expected to remove possible bias due to ignorance of potentially non-susceptible individuals. Furthermore, the two types of models did not give significantly different genetic correlations between survival of vaccinated and unvaccinated fish (Paper I). Unvaccinated and vaccinated fish were tested in different tanks, and could alternatively have been tested in one common tank. However, in this scenario, the pathogen load would have to be very high to achieve sufficient mortality among the vaccinated fish, which may not be optimal for simultaneous testing of unvaccinated fish in the same tank.

5.2 Side effects of vaccination

Vaccination has been performed in parallel with selection for disease resistance, yet the vaccines used against furunculosis (oil-adjuvant vaccine) are known to cause side effects in terms of adhesions of internal organs and melanin deposits. Results from this study show that it is possible to select for less severe side effects, as significant genetic

variation was found for these traits (Paper II). The results also show that a vaccine with improved composition can effectively reduce the severity of the side effects (Paper II). The latter result can be implemented immediately, whereas selective breeding is a long-term strategy. Hence, in my view it is unlikely that side effects of vaccination will be included in the breeding goal of commercial populations, as inclusion of yet another trait would necessarily lead to less gain in other more important traits. The genetic correlations between side effects and survival in challenge tests were found to be non-significant, and this was also found for side effects and body weight (Paper III). This shows that severity of these side effects is not expected to be effected by selection for increased disease resistance (in both vaccinated and unvaccinated fish) or selection for increased growth. Little genetic change in vaccine-induced side-effects should thus be expected as a result of selection on other traits.

5.3 Disease resistance in the breeding goal

Increased resistance (survival) to specific diseases is considered a long term breeding goal. However, vaccination in parallel with selection for increased disease resistance complicates this situation. The relatively weak (albeit favorable) genetic correlation between resistance to furunculosis in unvaccinated and vaccinated fish strongly indicates that there is a need to distinguish between a long and short term breeding goal for resistance to furunculosis and to determine what should be the main focus. A short term breeding goal would be to improve survival of vaccinated fish (as mass vaccination is likely to be required in the near future). However, through the current practice of vaccination, field mortality due to furunculosis is already very low (Håstein *et al.*, 2005). The last three years showed no reported outbreaks of this disease in Norway (Veterinærinstituttet, 2008, 2009, 2010), but some unreported mortalities may exist. Improving genetic resistance to furunculosis in vaccinated fish would therefore have little additional practical value. However, if the aim is to make vaccination programs redundant in the future, a long term breeding goal should aim at improving furunculosis resistance of unvaccinated fish (i.e., as is the current practice for all diseases selected for). This selection strategy will lead to a gradual increase in disease resistance in the population, and vaccination of a decreasing fraction of the fish could be a potential strategy. However, partial vaccination has epidemiological implications and would need further investigation. Selection based on unvaccinated fish is expected to

give little, if any, observable improvement of field survival for the industry in the near future, due to the weak genetic correlation between resistance in vaccinated and unvaccinated fish and continued vaccination resulting in low prevalence of furunculosis in commercial salmon farming (due to vaccination).

Disease resistance is important in the breeding goal both from an economical and animal welfare point of view. However, how to assign economic weight to specific disease resistance traits as compared to other traits included in the breeding objective is a complicated issue. Desired or restricted gain is the most utilized method for determining economic weights for different traits in fish breeding schemes. If the long term breeding goal is a fish not in need of vaccination, disease resistance must be heavily emphasised in the breeding goal. The substantial heritability estimates for disease resistance reported in this study (Paper I) and several others (reviewed in Ødegård *et al.*, 2011a) imply that selection for disease resistance may be effective. However, as surviving fish cannot be considered as selection candidates, this will lower the genetic progress, and make the weight assigned to disease traits even more important. Even though health is becoming more important in the breeding goal of Atlantic salmon, growth is still heavily emphasised (30-50%) (Håvard Bakke, SalmoBreed, pers. comm.). Assigning relatively more weight to disease resistance will necessarily give less genetic gain for body weight (and other traits), even though there is no unfavourable genetic correlation between the traits (Paper III).

Disease outbreaks do not only cause big losses to the industry and effect profitability directly, but they are also of concern from an animal welfare perspective. Disease resistance thus has a non-market value in addition to a market value (Olesen *et al.*, 2000), suggesting that the desired genetic gain for disease resistance should exceed the purely economic value of the trait. Selection on the multiplier level for a single or a limited number of traits (e.g. better disease resistance) is being practised by some breeding companies (e.g., AquaGen, SalmoBreed). This may be beneficial, as this can provide the market with fish with a higher genetic level of disease resistance without compromising too much on other traits (e.g., growth). A simulation study for fish species with a high fecundity showed that this could give substantial increased genetic gain for the grow-out producers as compared to random selection (Skagemo *et al.*, 2010). This strategy enables breeding companies to supply customers with special

products, maintaining a stable and long term breeding goal in the nucleus. However, the additional genetic gain on the multiplier level is temporary (all offspring of selected broodfish will be slaughtered) while the gain in the nucleus is cumulative. Genetic gain obtained at the multiplier level would hence be limited to the gain in the breeding nucleus. The focus of the breeding nucleus therefore needs to be both on long term genetic progress and on maintaining genetic diversity.

Increased growth has been the main focus of selective breeding of Atlantic salmon, but disease resistance has been in included in the breeding goal for over 20 years (Gjedrem, 2010) and has become more important during the last few years. Unfavorable genetic correlations between growth and disease resistance would complicate selective breeding, but only low genetic correlations were found between growth and resistance to both furunculosis (unvaccinated and vaccinated) and ISA (Paper III). Only the genetic correlation between furunculosis resistance in vaccinated fish and growth was significantly different from zero, and selection for increased growth may thus lead to a slight decrease in disease resistance of vaccinated fish. However, this effect is unlikely to have any practical consequences, as the vaccine against furunculosis is very effective (Håstein *et al.*, 2005; Veterinærinstituttet, 2008, 2009, 2010).

It has earlier been shown that vaccination can have a negative effect on harvest weight (Aunsmo *et al.*, 2008b; Midtlyng and Lillehaug, 1998), but the reduced growth of vaccinated fish is not necessarily connected to the level of adhesions or melanin deposits; thus suggesting that reduced growth is a another side effect of oil-adjuvant vaccines (Aunsmo *et al.*, 2008b). This is in agreement with results from Paper III, where small phenotypic and genetic correlations of growth with adhesions and melanin were reported; suggesting that genetic predisposition to growth does not affect the level of adhesions or melanin deposits. However, the new vaccine that reduced the level of adhesions and melanin also resulted in increased body weight (six months after sea transfer) (Paper II). A similar effect comparing two different vaccines with different injection volumes and from different manufactures has been reported earlier (Sørum and Damsgård, 2004). The negative effect of vaccination on body weight (unvaccinated and vaccinated with full and reduced dose) of 10-20% was found both at six and 12 months after sea transfer (Bjarne Gjerde, pers. comm.). This can be used as an argument to

assign more weight to disease resistance in the breeding goal, in order to reduce or avoid vaccination.

6 Concluding remarks

The main findings from this study are:

- Resistance to furunculosis in unvaccinated and vaccinated Atlantic salmon are only moderately positive genetically correlated and should therefore be looked upon as different genetic traits.
- Selection for increased specific disease resistance based on vaccinated fish has no practical effect when an effective vaccine is available for the disease.
- Therefore, if the long term breeding goal is to reduce or avoid vaccination, selection for increased resistance to furunculosis should be based on unvaccinated fish
- Side effects of vaccination (melanin and adhesions) are heritable traits, which are not significantly genetically correlated to disease resistance or growth. Side effects of vaccination can be reduced through new vaccine formulation.
- For unvaccinated fish resistance against furunculosis and ISA are both weakly genetically correlated with growth. Thus, selection for increased growth is not expected to result in unfavourable correlated responses in resistance to these diseases.
- Resistance to furunculosis, ISA and IPN in unvaccinated fish were favourably genetically correlated, suggesting that some genetic variation exists for non-specific disease resistance in Atlantic salmon when all diseases are assessed using a cohabitation challenge model.

Based on these results my recommendation is continued selection for increased resistance to furunculosis based on unvaccinated fish, which in the long run may result in a population of fish where a decreasing fraction has to be vaccinated. However, in order to speed up this progress larger emphasis should placed on disease resistance in the breeding goal and thus less emphasis on other traits. Furunculosis is no longer considered a problem under farming conditions using vaccinated fish (Håstein *et al.*, 2005), but efficient vaccines are still lacking for other diseases like IPN and PD (Biering *et al.*, 2005). Similar studies with unvaccinated and vaccinated Atlantic salmon should be carried out for these diseases, and potentially also for other diseases and species where vaccination and selection are parallel prophylactic measures.

When it comes to vaccine-induced side effects (adhesions and melanin deposits) my recommendation is that these are not included in the breeding goal, but should be reduced through other measures such as vaccine development and optimization of vaccination procedures.

7 References

Aucouturier J, Dupuis L, Ganne V (2001). Adjuvants designed for veterinary and human vaccines. *Vaccine* **19**(17-19)**:** 2666-2672.

Aunsmo A, Bruheim T, Sandberg M, Skjerve E, Romstad S, Larssen RB (2008a). Methods for investigating patterns of mortality and quantifying cause-specific mortality in sea-farmed Atlantic salmon Salmo salar. *Dis Aquat Organ* **81**(2)**:** 99-107.

Aunsmo A, Larssen RB, Valle PS, Sandberg M, Evensen Ø, Midtlyng PJ *et al* (2008b). Improved field trial methodology for quantifying vaccination side-effects in farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture* **284**(1-4)**:** 19-24.

Berg A, Rødseth OM, Hansen T (2007). Fish size at vaccination influence the development of side-effects in Atlantic salmon (*Salmo Salar* L.). *Aquaculture* **265**(1-4)**:** 9-15.

Berg A, Rødseth OM, Tangerås A, Hansen T (2006). Time of vaccination influences development of adhesions, growth and spinal deformities in Atlantic salmon *Salmo salar*. *Dis Aquat Organ* **69**(2-3)**:** 239-248.

Biering E, Villoing S, Sommerset I, Christie KE (2005). Update on viral vaccines for fish. In: Midtlyng PJ (ed) *Developments in biologicals* Karger. Vol. 121, pp 97-113.

Brascamp EW (1984). Selection indexes with constraints. . *Animal Breed Abstr* **52:** 645–654.

Ducrocq V, Casella G (1996). A Bayesian analysis of mixed survival models. *Genet Sel Evol* **28**(6)**:** 1-25.

Ellis AE, Gudding R, Lillehaug A, Midtlyng P, Brown F (1997). Immunization with bacterial antigens: Furunculosis. *Dev Biol Stand* **90:** 107-116.

Fjalestad KT, Gjedrem T, Gjerde B (1993). Genetic improvement of disease resistance in fish: an overview. *Aquaculture* **111**(1-4)**:** 65-74.

Gitterle T, Ødegård J, Gjerde B, Rye M, Salte R (2006). Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in *Penaeus (Litopenaeus)* vannamei using different statistical models. *Aquaculture* **251**(2-4)**:** 210-218.

Gjedrem T (2005). Breeding plans. In: Gjedrem T (ed) *Selection and Breeding Programs in Aquaculture*. Springer: Dordrecht pp 251-277.

Gjedrem T (2010). The first family-based breeding program in aquaculture. *Reviews in Aquaculture* **2**(1)**:** 2-15.

Gjedrem T, Gjøen HM (1995). Genetic variation in susceptibility of Atlantic salmon, *Salmo salar L.*, to furunculosis, BKD and cold water vibriosis. *Aquacult Res* **26**(2)**:** 129-134.

Gjedrem T, Salte R, Gjøen HM (1991). Genetic variation in susceptibility of Atlantic salmon to furunculosis. *Aquaculture* **97**(1)**:** 1-6.

Gjedrem T, Thodesen J (2005). Selection. In: Gjedrem T (ed) *Selection and Breeding Programs in Aquaculture*. Springer: Dordrecht pp 89-111.

Gjerde B (2005). Design of breeding programs In: Gjedrem T (ed) *Selection and Breeding Programs in Aquaculture*. Springer: Dordrecht pp 173-195.

Gjerde B, Evensen Ø, Bentsen HB, Storset A (2009). Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). *Aquaculture* **287**(1-2)**:** 52-58.

Gjerde B, Ødegård J, Thorland I (2011). Estimates of genetic variation in the susceptibility of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis*. *Aquaculture* **In Press, Corrected Proof**.

Gjøen HM, Refstie T, Ulla O, Gjerde B (1997). Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture* **158**(3-4)**:** 277-288.

Gudding R, Lillehaug A, Evensen O (1999). Recent developments in fish vaccinology. *Vet Immunol Immunopathol* **72:** 203 - 212.

Gullestad P, Bjørgo S, Gudding R, Osland AB, Eithun I, Hansen H *et al* (2011). Effektiv og bærekraftig arealbruk I havbruksnæringen – arealbruk til begjær. *Ministry of Fisheries and Costal affairs,Oslo*

Haugarvoll E, Bjerkås I, Szabo NJ, Satoh M, Koppang EO (2010). Manifestations of systemic autoimmunity in vaccinated salmon. *Vaccine* **28**(31)**:** 4961-4969.

Houston RD, Haley CS, Hamilton A, Guy DR, Mota-Velasco JC, Gheyas AA *et al* (2009). The susceptibility of Atlantic salmon fry to freshwater infectious pancreatic necrosis is largely explained by a major QTL. *Heredity* **105**(3)**:** 318-327.

Håstein T, Gudding R, Evensen Ø (2005). Bacterial vaccines for fish - an update of the current situation worldwide. In: Midtlyng PJ (ed) *Developments in Biologicals* Karger. Vol. 121, pp 55-74.

Kjøglum S, Henryon M, Aasmundstad T, Korsgaard I (2008). Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. *Aquacult Res* **39**(5)**:** 498-505.

Kolstad K, Heuch PA, Gjerde B, Gjedrem T, Salte R (2005). Genetic variation in resistance of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis*. *Aquaculture* **247**(1-4)**:** 145-151.

Koppang EO, Bjerkås I, Haugarvoll E, Chan EKL, Szabo NJ, Ono N *et al* (2008). Vaccination-induced systemic autoimmunity in farmed Atlantic salmon. *The Journal of Immunology* **181**(7)**:** 4807-4814.

Lund RA, Midtlyng PJ, Hansen LP (1997). Post-vaccination intra-abdominal adhesions as a marker to identify Atlantic salmon, *Salmo salar* L., escaped from commercial fish farms. *Aquaculture* **154**(1)**:** 27-37.

Lund T, Gjedrem T, Bentsen HB, Eide DM, Larsen HJS, Røed KH (1995). Genetic variation in immune parameters and associations to survival in Atlantic salmon. *J Fish Biol* **46**(5)**:** 748-758.

Madsen P, Jensen J. (2007). University of Aarhus, Faculty Agricultural Sciences (DJF), Dept. of Genetics and Biotechnology, Research Centre Foulum: Tjele.

McLoughlin MF, Graham DA (2007). Alphavirus infections in salmonids - a review. *J Fish Dis* **30**(9)**:** 511-531.

Midtlyng PJ (1996). A field study on intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. *Fish Shellfish Immunol* **6**(8)**:** 553-565.

Midtlyng PJ, Lillehaug A (1998). Growth of Atlantic salmon *Salmo salar* after intraperitoneal administratin of vaccines containing adjuvants. *Dis Aquat Organ* **32:** 91-97.

Midtlyng PJ, Reitan LJ, Speilberg L (1996). Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. *Fish Shellfish Immunol* **6**(5)**:** 335-350.

Moen T (2010). Breeding for Resitance to Viral Diseases in Salmonids. In: Bishop SC, Axford RFE, Nicholas FW and Owen JB (eds) *Breeding for Disease Resistance in Farm animlas* CABI London p166.

Moen T, Baranski M, Sonesson A, Kjoglum S (2009). Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* **10**(1)**:** 368.

Mutoloki S, Alexandersen S, Evensen Ø (2004). Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish Shellfish Immunol* **16**(5)**:** 633-644.

Nielsen H, Olesen I, Navrud S, Kolstad K, Amer P (2011). How to Consider the Value of Farm Animals in Breeding Goals. A Review of Current Status and Future Challenges. *Journal of Agricultural and Environmental Ethics***:** In press, available online.

Nordmo R (1997). Strengths and weakness of different challenge methods. In: Gudding R LA, Midtlyng PJ, Brown F (ed) *Fish vaccinology. Dev Biol Stand*. Karger: Basel, Switzerland. Vol. 90.

Olesen I, Groen AF, Gjerde B (2000). Definition of animal breeding goals for sustainable production systems. *J Anim Sci* **78**(3)**:** 570-582.

Poppe TT, Breck O (1997). Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized wityh oil-adjuvanted vacccine. A case report. . *Dis Aquat Organ* **29:** 219-226.

Press CM, Jørgensen TØ (2002). Fiskens immunsytem. In: Poppe TT (ed) *Fiskehelse og fiskesykdommer*, 2. edn. Universitetsforlaget: Oslo, pp 38-47.

Rauw WM, Kanis E, Noordhuizen-Stassen EN, Grommers FJ (1998). Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* **56**(1)**:** 15-33.

Rye M, Gjerde B, Gjedrem T. (2010).Genetic Improvement Programs For Aquaculture Species In Developed Countries. *In:* Proceedings to *9th World Congress on Genetics Applied to Livestoc Production,* Leipzig, Germany, 1-6 August, 2010.

Salte R, Bentsen HB, Moen T, Tripathy S, Bakke TA, Odegard J *et al* (2010). Prospects for a genetic management strategy to control *Gyrodactylus salaris* infection in wild Atlantic salmon (*Salmo salar*) stocks. *Can J Fish Aquat Sci* **67**(1)**:** 121-129.

Skagemo V, Sonesson AK, Meuwissen THE, Rye M (2010). Increased profits in aquaculture through optimised dissemination schemes. *Aquaculture* **300**(1-4)**:** 65-72.

Sommerset I, Krossøy B, Biering E, Frost P (2005). Vaccines for fish in aquaculture. *Expert Review of Vaccines* **4**(1)**:** 89-101.

Sonesson AK (2007). Within-family marker-assisted selection for aquaculture species. *Genet Sel Evol* **39**(3)**:** 301-317.

Sonesson AK, Meuwissen THE (2009). Testing strategies for genomic selection in aquaculture breeding programs. *Genet Sel Evol* **41**.

Standal M, Gjerde B (1987). Genetic variation in survival of Atlantic salmon during the sea-rearing period. *Aquaculture* **66**(3-4)**:** 197-207.

Storset A, Strand C, Wetten M, Kjøglum S, Ramstad A (2007). Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **272**(Supplement 1)**:** 62-S68.

Sørum U, Damsgård B (2004). Effects of anaesthetisation and vaccination on feed intake and growth in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **232**(1-4)**:** 333-341.

Taylor RS, Kube PD, Muller WJ, Elliott NG (2009). Genetic variation of gross gill pathology and survival of Atlantic salmon (*Salmo salar* L.) during natural amoebic gill disease challenge. *Aquaculture* **294**(3-4)**:** 172-179.

Vehvilainen H, Kause A, Quinton C, Koskinen H, Paananen T (2008). Survival of the Currently Fittest: Genetics of Rainbow Trout Survival Across Time and Space. *Genetics* **180**(1)**:** 507-516.

Veterinærinstituttet (2008). Helsesituasjonen hos oppdrettsfisk 2008. *http://www.vetinst.no/nor/Forskning/Publikasjoner/Fiskehelserapporten*

Veterinærinstituttet (2009). Fiskehelserapporten 2009. *http://www.vetinst.no/nor/Forskning/Publikasjoner/Fiskehelserapporten*

Veterinærinstituttet (2010). Helsesituasjonen hos laksefisk 2010. *http://www.vetinst.no/nor/Forskning/Publikasjoner/Fiskehelserapporten*

Wetten M, Aasmundstad T, Kjøglum S, Storset A (2007). Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **272**(1-4)**:** 111-117.

www.aquagen.no (18th of March 2011)

www.salmobreed.no (18th of March 2011)

Ødegård J, Baranski M, Gjerde B, Gjedrem T (2011a). Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. *Aquacult Res* **42:** 103-114.

Ødegård J, Gitterle T, Madsen P, Meuwissen T, Yazdi M, Gjerde B *et al* (2011b). Quantitative genetics of Taura syndrome resistance in Pacific white shrimp (*Penaeus vannamei*): A cure model approach. *Genet Sel Evol* **43**(1)**:** 14.

Ødegård J, Olesen I, Gjerde B, Klemetsdal G (2006). Evaluation of statistical models for genetic analysis of challenge test data on furunculosis resistance in Atlantic salmon (*Salmo salar*): Prediction of field survival. *Aquaculture* **259**(1-4)**:** 116-123.

Ødegård J, Olesen I, Gjerde B, Klemetsdal G (2007a). Evaluation of statistical models for genetic analysis of challenge-test data on ISA resistance in Atlantic salmon (*Salmo salar*): Prediction of progeny survival. *Aquaculture* **266**(1-4)**:** 70-76.

Ødegård J, Olesen I, Gjerde B, Klemetsdal G (2007b). Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). *Aquaculture* **271**(1-4)**:** 173-177.

PAPER I

Heredity (2011), 1–7

www.nature.com/hdy

ORIGINAL ARTICLE

Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.)

TMK Drangsholt[1,2], B Gjerde[1,2], J Ødegård[1,2], F Finne-Fridell[3], Ø Evensen[4] and HB Bentsen[2]
[1]*Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (UMB), Ås, Norway;* [2]*Nofima Marin, Ås, Norway;* [3]*PHARMAQ AS, Oslo, Norway and* [4]*Norwegian School of Veterinary Science, Oslo, Norway*

Furunculosis (*Aeromonoas salmonicida*) is an important disease in Atlantic salmon (*Salmo salar*) farming. Vaccination and selective breeding for increased resistance to the disease on the basis of challenge tests of unvaccinated fish are used as complementary prophylactic methods. An important issue is whether genetic predisposition to infection is consistent across vaccinated and unvaccinated fish. Hence, the main objective of this study was to determine the magnitude of the genetic associations (correlations) between resistance to furunculosis in vaccinated and unvaccinated fish, and to estimate the magnitude of the correlation of resistance to furunculosis with resistance to the viral diseases infectious pancreatic necrosis (IPN) and infectious salmon anaemia (ISA). Sub-samples of unvaccinated and vaccinated salmon from 150 full-sib families were subjected to separate cohabitation challenge tests. Substantial genetic variation was found in resistance to furunculosis in both the unvaccinated (heritabilities of 0.51 ± 0.05) and vaccinated (0.39 ± 0.06) fish. However, the genetic correlation between resistance to furunculosis in the two groups was low (0.32 ± 0.13), indicating a weak genetic association between resistance in the two groups. Hence, the current selection strategy on the basis of challenge tests of unvaccinated fish is likely to produce low genetic improvement in resistance to furunculosis under field conditions, where fish are vaccinated with an effective vaccine. Evidence was found of significantly favourable genetic associations of resistance to furunculosis in unvaccinated (but less so for vaccinated) fish with resistance to both IPN and ISA (unvaccinated fish), indicating that vaccination 'mask' genetic associations between resistance to different diseases.
Heredity advance online publication, 11 May 2011; doi:10.1038/hdy.2011.34

Keywords: vaccine; heritability; resistance; Atlantic salmon; furunculosis; challenge test

Introduction

Vaccination is important in preventing outbreaks of infectious diseases (NOHA, 2010), both for humans and livestock. In farmed fish, especially salmonids, vaccination has been the single most efficient prophylactic measure and has contributed to a significant reduction in the use of antibiotics in the Norwegian salmon industry in the 1990's (Ellis, 1997; Gudding *et al*., 1999; Håstein *et al*., 2005) Currently, farmed Atlantic salmon are routinely vaccinated as pre-smolts against a number of diseases, both bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral (infectious pancreatic necrosis (IPN), pancreas disease, infectious salmon anaemia (ISA)).

At the same time, several studies have provided ample evidence that there is genetic variation in resistance against bacterial and viral diseases in Atlantic salmon. For example, heritability estimates for survival in challenge tests range from 0.43 to 0.62 for *Aeromonas salmonicida* (subsp. *salmonicida*), the causative agent of furunculosis (Gjedrem *et al*., 1991; Kjøglum *et al*., 2008; Ødegård *et al*., 2007b). These findings have motivated breeding companies to include disease resistance in their breeding goals (Kjøglum *et al*., 2008). However, genetic variation in disease resistance has been based on unvaccinated fish and we were thus interested in examining to what extent the same genetic traits are involved in resistance to challenge in vaccinated and unvaccinated fish. We hypothesized that different parts of the immune system have a role in protection against disease in vaccinated and unvaccinated fish. Through vaccination, the fish have mounted an immune response before infection, which may reduce the relative importance of the innate defence mechanisms, although anatomical barriers and innate immune responses are likely to have a role also in the vaccinated fish. The details of immune responses to vaccination have not been studied in fish, nor are they well understood in higher vertebrates (Nakayama *et al*., 2010). Hence, the genetics underlying disease resistance may be quite different in the two situations, and to date, there are no data available to determine if genetic improvement of disease resistance on the basis of unvaccinated fish would also lead to enhanced disease resistance in vaccinated fish (beyond the effect of vaccination).

Correspondence: TMK Drangsholt, Nofima Marin, PO Box 5010, 1432 Aas, Norway.
E-mail: tale.drangsholt@nofima.no
Received 7 October 2010; revised 21 January 2011; accepted 31 March 2011

In terrestrial farmed species, estimates of genetic variation in the response to an infectious disease are generally based on individual field records, where the exposure to the pathogen can be incomplete and often result in low frequency of the disease (Bishop and Woolliams, 2010). In aquaculture species, however, challenge tests exposing all individuals to the infection can be performed by either intraperitoneal injection (i.p.), bath or cohabitation challenge tests. The latter method infect the test fish through the natural route of infection (Nordmo, 1997), and the genetic correlation for survival tested by experimental challenge and in the field was found to be close to unity (0.95) for furunculosis (Gjøen *et al*., 1997) and also high (0.78–0.83) for IPN (a double-stranded RNA virus) (Wetten *et al*., 2007).

Furunculosis was the first disease to be included in the breeding goal for Atlantic salmon. Currently, an effective vaccine is available for this disease, thus representing a valuable model to study disease resistance in vaccinated and unvaccinated animals. The main objective of this study was to estimate the genetic association of protection against a bacterial disease (furunculosis) in unvaccinated and vaccinated Atlantic salmon (including two different vaccines). The secondary aim was to estimate the genetic associations between resistance against furunculosis and two viral diseases (ISA and IPN) currently included in breeding programs for Atlantic salmon.

Materials and methods

Family material

The fish in this study were all from the breeding nucleus population of SalmoBreed AS. In total, 279 full-sib families (offspring of 140 sires and 279 dams) were included in the analysis. The families were produced by Bolaks, Eikelandsosen, Norway in November 2006. In January 2007, they were transported as eyed eggs to Nofima Marin, Sunndalsøra, Norway, where they were reared in separate trays. From first feeding (5 February to 17 April 2007), they were kept in separate 0.75 m^3 tanks, until they were tagged with PIT (Passive Integrated Transponder) tags in July and September 2007 (Table 1). The environmental conditions were standardized during the hatchery and rearing period until tagging to minimize environmental differences between families. Additive genetic relationships were available between the individuals, as well as among their parents.

In the present population, selection for increased resistance against furunculosis on the basis of challenge test survival data (with unvaccinated fish) had been performed for one generation. The breeding goal also included resistance against ISA, maximum growth, minimum fillet fat and improved fillet colour.

Five fish groups were included in this study; two groups from a random sample of 150 of the 279 families (groups 1 and 2; the offspring of 87 sires and 150 dams; as considered adequate for estimation of genetic parameters) and three groups from all the 279 families (groups 3, 4 and 5; as they were part of the standard genetic evaluation programme of SalmoBreed AS; Bolaks). The different groups of fish all consisted of a random sample of 15 fish per family and were tested as follows:

Table 1 Number of individuals (N) and mean and standard deviation (s.d.) for body weight (in grams) and age (in days from first feeding) of the fish in the five groups

Group and trait	N	*Mean*	*s.d.*
Fur-SV			
Body weight at tagging	2151	11.5	3.4
Body weight at vaccination	2151	36.2	10.8
Age at vaccination	2151	206	15
Age at start of challenge	2151	256	15
Fur-RD			
Body weight at tagging	2139	11.6	3.5
Body weight at vaccination	2139	36.7	11.2
Age at vaccination	2139	205	15
Age at start of challenge	2139	256	15
Fur			
Body weight at tagging	4128	34.9	12.3
Age at start of challenge	4128	197	18
ISA			
Body weight at tagging	4179	34.4	12.2
Age at start of challenge	4179	199	18
IPN			
Body weight at tagging	3741	45.1	15.2
Age at start of challenge	3741	311	18

Abbreviations: IPN, infectious pancreatic necrosis; ISA, infectious salmon anaemia.
Fur-SV, fish vaccinated with standard vaccine and challenged with *A. salmonicida*.
Fur-RD, fish vaccinated with reduced dose vaccine and challenged with *A. salmonicida*.
Fur, unvaccinated fish challenged with *A. salmonicida*.
ISA, unvaccinated fish challenged with ISA virus.
IPN, unvaccinated fish challenged with IPN virus.

Group 1 (Fur-SV): Fish vaccinated with a commercial multi-component vaccine and challenged with *A. salmonicida*.

Group 2 (Fur-RD): Fish vaccinated with an experimental multi-component vaccine with a reduced antigen dose and challenged with *A. salmonicida*.

Group 3 (Fur): Unvaccinated fish challenged with *A. salmonicida*.

Group 4 (ISA): Unvaccinated fish challenged with ISA virus.

Group 5 (IPN): Unvaccinated fish challenged with IPN virus.

The Fur-SV and Fur-RD groups were tagged in July 2007 and the Fur, ISA and IPN groups were tagged in September 2007.

Vaccines and vaccination

The Fur-SV and Fur-RD fish were vaccinated at the pre-smolt stage from 2 October to 4 October 2007. The Fur-SV fish were i.p. injected with 0.1 ml of the commercial vaccine ALPHA JECT 6–2 (AJ6–2) (PHARMAQ, Oslo, Norway). AJ6–2 includes antigens of *A. salmonicida*, *Vibrio anguillarum* serotype O1 and O2, *Vibrio salmonicida*, *M. viscosa* and IPN virus, and protects against furunculosis, classical vibriosis, cold-water vibriosis, winter ulcer and IPN, respectively. The Fur-RD fish were vaccinated with an experimental AJ6–2 of 0.05 ml per fish, containing 60% less *A. salmonicida* antigen than the commercial AJ6–2 used in the Fur-SV group.

The vaccines were prepared by PHARMAQ AS and are water-in-oil emulsions, where the dispersal water phase contains the inactivated viral and bacterial antigens. A reduced antigen dose of *A. salmonicida* in the vaccine given to the Fur-RD fish was used for two reasons: first, to see if fish given a reduced antigen dose showed different genetic variation in survival compared with fish given the commercial AJ6–2, and second, to ensure mortality in at least some vaccinated fish post-challenge. Individual body weights of the vaccinated fish were recorded at the time of vaccination.

Challenge tests

Fur group, ISA group and IPN group: Pre-smolts of the Fur-SV and Fur-RD groups were transported from Sunndalsøra to VESO Vikan (Namsos, Norway) on 15 November 2007, and the challenge test started on 22 November 2007. The Fur-SV and Fur-RD groups were challenged in the same tank, containing 3 m^3 of 12 °C freshwater at an exchange rate of approximately 0.8 l kg^{-1} fish. The challenge was carried out by cohabitation, where naive Atlantic salmon were injected i.p., with the respective pathogens to act as shedders. The number of shedders was 20% of the total number of fish in the tank and they were added at three different time points to prevent the mortality from plateauing, before 50% control mortality was reached; 29% of the total number of shedders were added at day one of test (i.p. injected with 5.0×10^3 cfu of *A. salmonicida*), 29% at day five (i.p. injected with 3.0×10^1 cfu of *A. salmonicida*) and the remaining (42%) at day 32 (i.p. injected with 3.0×10^1 cfu of *A. salmonicida*). A total of 100 unvaccinated fish were randomly sampled from the 150 Fur-SV/Fur-RD families, ink tagged and used as an unvaccinated control to monitor the challenge pressure.

Fur group: Pre-smolts were transported from Sunndalsøra to VESO Vikan on 29 September 2007, and the challenge test started at 2 October 2007. All fish were tested in a single test tank, containing 3 m^3 of 12 °C freshwater at an exchange rate of about 0.8 l kg^{-1} fish. In this test, shedders (i.p. injected with 5.0×10^3 cfu of *A. salmonicida*) comprising 7% of the total number of fish were used, all added on the first day of the test.

ISA group: Pre-smolts were transported from Sunndalsøra to VESO Vikan on 29 September 2007, and the challenge test started on 5 October 2007. Fish were tested in a single test tank, containing 3 m^3 of 12 °C freshwater at an exchange rate of about 0.8 l kg^{-1} fish. In this test, shedders (i.p. injected with 6.4×10^3 $TCID_{50}$ of ISA virus comprising 24% of the total number of fish were all added on the first day of the test.

IPN group: Post-smolts were transported from Sunndalsøra to VESO Vikan on 21 January 2008. The fish were transferred to seawater the day after arrival, and the challenge test started on 25 January 2008. Because of a larger biomass, fish were randomly allocated to two different tanks, each of 5 m^3 of 12 °C seawater, with an exchange rate set to give a minimum oxygen level of 5–6 mg O_2/l in the outlet water. In this test, shedders (i.p. injected with 0.4 ml solution with approximately 1×10^7 $TCID_{50}$/ml of IPN virus) comprising 28% of the total number of fish were used. The shedders were added at two time points: 50% on day one and 50% on day five.

Dead fish were recorded daily in all tests. Mortality diagnostics were performed in order to verify the causative agent of mortality. Bacteriological tests were used to detect *A. salmonicida*, clinical signs were used to diagnose ISA and an enzyme linked immunosorbent assay was used to detect IPN virus.

Data recording

Survival in challenge tests 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 challenge tests for groups Fur, ISA and IPN were terminated at intermediate mortalities (day 21, 33 and 30 of the challenge test, respectively). This has been the standard procedure for such tests as the phenotypic variance for the observed binary trait is at its maximum at this point. The challenge tests for the groups Fur-SV and Fur-RD were terminated when mortalities had essentially ceased (day 60).

Statistical analysis

Models using survival at end of test were chosen. More complex longitudinal survival models (using test-day survival) were tested, but did not give significantly different genetic associations between the disease traits (results not shown). Earlier studies have shown that simple cross-sectional models and more advanced longitudinal models give very similar ranking of families for this type of data (Gitterle *et al*., 2006; Ødegård *et al*., 2007a), which was consistent with our results.

To find the most appropriate model, linear single trait sire-dam models were first run for the traits Fur-SV, Fur-RD, Fur, ISA, IPN and body weight at vaccination. The full univariate model for each trait was:

$$y_{ijkl} = \mu_i + b_i \cdot x_j + \text{sire}_k + \text{dam}_l + \text{family}_l + e_j$$

where: y_{ijkl} = survival or body weight (trait i) for fish j, progeny of sire k and dam l, μ_i = the overall mean, x_j = tank in challenge for fish j (only for IPN-group) b_i = fixed regression coefficient of age, sire_k = random additive genetic effect of sire k, dam_l = random additive genetic effect of dam l, family_l = random effect common to full sibs of dam l (only for body weight at vaccination), e_j = random residual for fish j.

Likelihood ratio tests (Lynch and Walsh, 1998) were used to determine whether the family effect was significant in addition to the additive genetic sire and dam effects, that is, whether one should use the full or reduced model. Body weight at vaccination was the only trait where family effect were significant ($P > 0.05$), and the family effect was thus only included for this trait in the further multi-trait models. For the software used, likelihood ratio tests can not be used for threshold models, but testing the effect of including a family effect on a linear, single trait model serves as a good approximation, even for binary traits.

Then a multi-trait linear model, including the body weight trait and the five binary disease traits (Fur-SV, Fur-RD, Fur, ISA, IPN), was run to obtain estimates of variance components on the observed linear scale for all

traits. In addition, a similar multi-trait model, including a linear model for the body weight trait and a threshold (probit) model for each of the five binary traits, was used to obtain estimates of variance components on the underlying liability scale for the binary traits and covariance components among the traits. The model for each binary trait can be written as:

$$\Pr(S_{ijkl} = 1) = \Phi(\mu_i + b_i \cdot x_j + \text{sire}_k + \text{dam}_l)$$

where S_{ijkl} = survival trait i for fish j, of sire k, dam l, $\Phi(\,)$ = cumulative standard normal distribution, and the other parameters are as described above.

$$\text{Further } \mathbf{u} = \begin{bmatrix} \mathbf{u}_{\text{weight}} \\ \mathbf{u}_{\text{Fur-SV}} \\ \mathbf{u}_{\text{Fur-RD}} \\ \mathbf{u}_{\text{Fur}} \\ \mathbf{u}_{\text{ISA}} \\ \mathbf{u}_{\text{IPN}} \end{bmatrix} \mathbf{e} = \begin{bmatrix} \mathbf{e}_{\text{weight}} \\ \mathbf{e}_{\text{Fur-SV}} \\ \mathbf{e}_{\text{Fur-RD}} \\ \mathbf{e}_{\text{Fur.}} \\ \mathbf{e}_{\text{ISA}} \\ \mathbf{e}_{\text{IPN}} \end{bmatrix}, \mathbf{u}$$

$$\sim N(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})\mathbf{e} \sim N(\mathbf{0}, \mathbf{R} \otimes \mathbf{I})$$

where **u** is a vector of sire and dam effects and **e** is the residual vector for all six traits, **A** is the additive relationship matrix among the sire and dams, **I** is an identity matrix of appropriate size, **G** is the additive genetic (co)variance matrix and **R** is the residual variance-covariance matrix among the traits, defined as:

$$\mathbf{R} = \begin{bmatrix} \sigma^2_{\text{weight}} & \sigma_{\text{weight,Fur}-SV} & \sigma_{\text{weight,Fur}-RD} & 0 & 0 & 0 \\ \sigma_{\text{weight,Fur}-SV} & 1 & 0 & 0 & 0 & 0 \\ \sigma_{\text{weight,Fur}-RD} & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

As the last five traits were binary, recorded on different fish and fitted by a probit threshold model, the lower 5×5 corner of **R** was restricted to be an identity matrix. Furthermore, vaccine weights were only measured in vaccinated fish. Hence, only three parameters of the **R** matrix needed to be estimated: $\sigma^2_{\text{weight},}$(residual variance for weight), $\sigma_{\text{weight, Fur}-\text{SV}}$ (residual covariance between weight and Fur-SV) and $\sigma_{\text{weight, Fur}-\text{RD}}$ (residual covariance between weight and Fur-RD).

The DMU software (Madsen and Just 2007) was used in all statistical analyses.

Heritability

For all traits, heritability was calculated as:

$$h^2 = \frac{4\sigma^2_u}{2\sigma^2_u + \sigma^2_c + \sigma^2_e}$$

where; σ^2_μ is the additive genetic sire-dam variance (1/4 of the total additive genetic variance), σ^2_c is the common family variance (non-significant and therefore set to zero for all survival traits) and σ^2_e is the (underlying) residual variance (per definition set to 1.0 for all binary traits).

Results

Descriptive statistics

Table 1 contains descriptive statistics for body weight (at tagging and vaccination) and age (days from first feeding) of fish in the five groups. At the start of the challenge tests, the average weight of fish (based on two subsamples of 50 fish) was 46 g for the Fur-SV/Fur-RD group, 30 g for each of the Fur and ISA groups and 85 g for the IPN group.

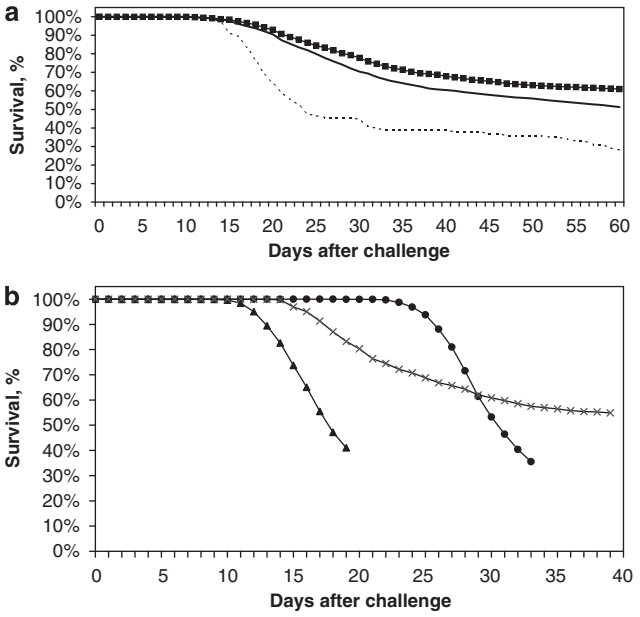

Figure 1 (**a**) Survival curves for the two vaccinated fish groups (Fur-SV (■) and Fur-RD (—)) and for the unvaccinated control group (Control (- - -)) during challenge with *A. salmonicida* in the same tank. (**b**)Survival curves for the three unvaccinated fish groups (Fur (▲), ISA (•) and IPN (x) during challenge with *A. salmonicida*, ISA virus and IPN virus. Challenges were carried out in three different tanks.

Challenge tests survival curves

Survival curves for the two vaccinated fish groups and the unvaccinated control fish group (furunculosis) are shown in Figure 1a, and the survival curves for the three unvaccinated fish groups (furunculosis, IPN and ISA) are shown in Figure 1b. For the Fur-SV and Fur-RD groups, onset of mortality started at day 12 post challenge and remained relatively low, although higher for Fur-RD than for Fur-SV. By day 35–40, daily mortality for both these groups plateaued and the test was terminated on day 60. At the end of the test, Fur-SV showed an approximately 10% higher survival than Fur-RD (61 versus 51 %). For the unvaccinated Fur group, onset of mortality was at day eight post challenge and continued at a high rate until the test was terminated on day 21, giving a survival of 28% (Figure 1b). For the ISA group, the first mortalities were observed on day 22 post challenge and continued at a high rate until the test was terminated on day 33 at 36% survival (Figure 1b). For the IPN group, mortality started on day 12 and daily mortalities continued at an intermediate rate until the test was terminated on day 39 post challenge, resulting in 55% survival (Figure 1b).

Heritabilities and genetic correlations

The estimated heritabilities (observed and underlying scale) are shown in Table 2. Estimated underlying heritabilities of Fur-SV and Fur-RD were high (0.39 ± 0.06 and 0.34 ± 0.06, respectively), but clearly lower than for the unvaccinated Fur group (0.51 ± 0.05). Estimated underlying heritabilities for ISA and IPN (0.33 ± 0.05 and

0.39 ± 0.05, respectively) were similar to the heritabilities for Fur-SV and Fur-RD. As expected, the estimated heritabilities on the observed scale (obtained with a linear model) were generally lower than those on the underlying liability scale. The estimated heritability for body weight at vaccination was relatively high ($h^2 = 0.29 \pm 0.09$) with a significant, albeit small, environmental effect common to full-sibs (0.09 ± 0.09, $P < 0.05$).

Estimated genetic correlations between the five survival traits and body weight at vaccination are shown in Table 3. All genetic correlations among survival traits (except between furunculosis in vaccinated fish and IPN) were significantly larger than zero and thus favourable. The highest genetic correlation (0.90 ± 0.06) was between survival and furunculosis in the two vaccinated groups (Fur-SV and Fur-RD), whereas genetic correlations between survival and furunculosis in vaccinated (Fur-SV and Fur-RD) and unvaccinated (Fur) fish were medium to low (0.32–0.54), and of the same magnitude as the genetic correlations among different diseases in unvaccinated fish (Fur, ISA and IPN). The estimated genetic correlations between body weight at vaccination and the survival traits were all positive, but low and not significantly different from zero ($P > 0.05$).

Table 2 Estimated heritabilities ($h^2 \pm$ standard errors) for survival after challenge with different disease agents on the underlying scale (threshold model) and observed scale (linear model)

Trait	*Heritability*	
	Underlying scale	*Observed scale*
Fur-SV	0.39 ± 0.06	0.25 ± 0.04
Fur-RD	0.34 ± 0.06	0.22 ± 0.04
Fur	0.51 ± 0.05	0.33 ± 0.04
ISA	0.33 ± 0.05	0.22 ± 0.03
IPN	0.39 ± 0.05	0.20 ± 0.05
Body weight at vaccination	Not applicable	0.29 ± 0.09

Abbreviations: IPN, infectious pancreatic necrosis; ISA, infectious salmon anaemia.
Fur-SV, fish vaccinated with standard vaccine and challenged with *A. salmonicida*.
Fur-RD, fish vaccinated with reduced dose vaccine and challenged with *A. salmonicida*.
Fur, unvaccinated fish challenged with *A. salmonicida*.
ISA, unvaccinated fish challenged with ISA virus.
IPN, unvaccinated fish challenged with IPN virus.

Discussion

The results in this study strongly suggest that heritability of resistance against furunculosis decreases as a result of vaccination. Hence, resistance against furunculosis that is induced by vaccination appears to 'mask' some of the innate genetic resistance against the disease. However, the heritability estimates for survival to furunculosis were relatively high and accurate, suggesting that there is substantial additive genetic variation in resistance against furunculosis in both unvaccinated and vaccinated fish, which can be used to improve resistance to furunculosis in Atlantic salmon through selective breeding. For the unvaccinated fish, the heritability estimates for the three studied diseases are of the same magnitude as those reported in previous studies (Ødegård *et al*., 2007b; Kjøglum *et al*., 2008; Gjerde *et al*., 2009; Guy *et al*., 2009). To our knowledge, estimates of heritability for disease resistance in vaccinated farmed fish or livestock have not been published earlier.

For the two vaccinated groups (Fur-SV and Fur-RD) the heritabilities for survival to furunculosis were very similar suggesting that the experimental vaccine, which provided lower protecting compared the commercial vaccine, was sufficient to 'mask' innate genetic resistance to furunculosis to the same degree as the commercial vaccine. This is also confirmed by the high genetic correlation ($r_g = 0.90$) between resistant to furunculosis in the two groups, implying that the genetic resistance is largely the same trait irrespective of the injection volume and the antigen dose of *A. salmonicida* in the vaccines. In contrast, the genetic correlation between survival of vaccinated and unvaccinated fish was positive, but moderate to low ($r_g = 0.32$–0.54), and suggests that resistance in vaccinated and unvaccinated fish should be regarded as partly different genetic traits. These results disagree with the results from a study of Marek's disease in chickens, where a linear relationship was observed between disease resistance in unvaccinated and vaccinated chickens from seven genetically different strains (not fullsib families as in the present study) (Gavora *et al*., 1990).

Our results suggest that selection for improved genetic resistance to furunculosis on the basis of testing of unvaccinated animals will also lead to some degree of improved disease resistance in vaccinated animals (the present situation in the industry). However, the moderate genetic correlation between survival in vaccinated

Table 3 Genetic correlations ($r_g \pm$ standard errors) between the four survival traits and body weight at vaccination

	Fur-SV	*Fur-RD*	*Fur*	*ISA*	*IPN*
Fur-SV	1				
Fur-RD	0.90 ± 0.06	1			
Fur	0.32 ± 0.13	0.54 ± 0.11	1		
ISA	0.33 ± 0.13	0.43 ± 0.13	0.52 ± 0.09	1	
IPN	0.08 ± 0.13	0.23 ± 0.13	0.35 ± 0.10	0.23 ± 0.11	1
Weight at vaccination	0.21 ± 0.13	0.10 ± 0.14	0.03 ± 0.13	0.24 ± 0.14	0.16 ± 0.13

Abbreviations: IPN, infectious pancreatic necrosis; ISA, infectious salmon anaemia.
Fur-SV, fish vaccinated with standard vaccine and challenged with *A. salmonicida*.
Fur-RD, fish vaccinated with reduced dose vaccine and challenged with *A. salmonicida*.
Fur, unvaccinated fish challenged with *A. salmonicida*.
ISA, unvaccinated fish challenged with ISA virus.
IPN, unvaccinated fish challenged with IPN virus.

and unvaccinated fish raises the question if the testing procedure agrees with the breeding goal for the trait resistance to furunculosis. And for such a trait the issue of short- or long-term breeding goal is of particular interest and needs to be clarified. The current challenge testing procedure using unvaccinated fish is only optimal if the long-term breeding goal is to produce a resistant or tolerant fish without vaccination. This selection strategy will also provide some increased resistance in vaccinated fish through indirect selection, but to a large extent, dependent on the economic weight given to the trait relative to other traits selected for. The most likely scenario is, however, that vaccination has to be practised by the industry in the foreseeable future (short to medium term). Therefore, selection for improved survival based on testing of vaccinated fish would be more efficient and relevant for the salmon farming industry today.

Differences in challenge methods (duration) may also have affected the genetic correlation between resistance to furunculosis in vaccinated and unvaccinated fish. The unvaccinated fish in this study were tested using a standard challenge test procedure (SalmoBreed breeding program), where the test is terminated at an intermediate and still high mortality, whereas the vaccinated fish groups were tested over a much longer time period (with repeated challenges using additional shedders) and the test terminated when mortality had essentially ceased. End-survival variation may be explained by differences in susceptibility to the disease (given that the population contains a fraction of non-susceptible fish) and variation in individual hazard rates (time until death), given that the fish are susceptible. These two traits may be governed by differing genetic factors, as has previously been estimated in challenge tests with the ectoparasite *Gyrodactylus salaris* in Atlantic salmon (Salte *et al*., 2010) and taura syndrome virus in Pacific white shrimp (*Penaeus vannamei*) (Ødegård *et al*., 2011). In this case, the relative importance of susceptibility status and hazard rate (given susceptibility), with respect to final survival, will depend on the chosen endpoint of the test. Generally, earlier termination of the test (at a still high mortality) will increase the relative importance of individual hazard rates and decrease the relative importance of susceptibility. In addition, early termination of the test can potentially lower the specificity, in the sense that some non-healthy individuals will be classified as healthy (survivors) even though they would have died given sufficient challenge time. Low specificity can often be seen in field data due to incomplete exposure to the pathogen (Bishop and Woolliams, 2010), whereas challenge tests aim to expose all individuals to the specific infection. Exposure to the pathogen is needed for an individual to express its genotype for resistance, and in the challenge test with vaccinated fish in this study, shedders were added several times to ensure this. In challenge tests with unvaccinated fish, the disease will spread faster and shedders were thus only added at the first day of challenge. In the present study, all families were kept in the same tank in each challenge test, and a genetic correlation close to unity (0.95) has been estimated between survival to furunculosis in challenge and field test with unvaccinated fish (Gjøen *et al*., 1997). It cannot be ruled out that differences in pathogenic pressure may have some effect on ranking of families, but performing the test in replicated tank was not possible due to financial constraint as such tests are expensive to perform.

The genetic correlation between resistance to furunculosis and ISA in unvaccinated fish was moderately positive and is concordant with results from Ødegård *et al*. (2007b), who reported a positive, but lower genetic correlation between the two traits. Conversely, other studies have found this genetic correlation to be not significantly different from zero (Kjøglum *et al*., 2008), or low and even negative (Gjøen *et al*., 1997). In all previous studies, ISA virus challenges were partly or exclusively performed using i.p. injections, whereas cohabitation challenges were used for *A. salmonicida*. In the current study, cohabitation challenge was used for both furunculosis and ISA. Cohabitation challenge, which mimics the natural route of infection, will provide a better estimate of the genetic variation underlying protective responses, whereas i.p. injections, where natural barriers are surpassed, will likely reveal a restricted profile of the underlying genetic variation. Estimates of positive genetic correlations across diseases may be due to the innate defence mechanisms and are therefore likely more precise when the pathogen exposure mimics natural infection as close as possible. In contrast, such a correlation may be less obvious using artificial pathogen exposure, for example, through the i.p. route. The challenge model is thus likely to influence the magnitude of the genetic correlations between resistance to different diseases.

The heritability estimate for IPN was intermediate (0.39 ± 0.05) and is concordant with previous studies (Wetten *et al*., 2007; Kjøglum *et al*., 2008). A quantitative trait loci explaining a very large proportion of the genetic variation in IPN resistance has been detected in Atlantic salmon (Houston *et al*., 2009; Moen *et al*., 2009). In our study, evidence was found of significantly favourable genetic correlations of resistance to furunculosis in unvaccinated fish with resistance to both IPN and ISA (unvaccinated fish). Kjøglum *et al*. (2008) reported a non-significant genetic correlation between these two traits, but i.p. injections and immersions were used as challenge models and IPN resistance was tested using fry and not pre-smolt. In our study, the correlations between IPN and ISA were lower for the vaccinated fish groups, indicating that vaccination (in this case against furunculosis) 'mask' genetic associations between resistant to different diseases.

The present findings indicate that genetic resistance against furunculosis in vaccinated and unvaccinated fish is controlled by partially different genetic mechanisms, thus questioning selective breeding for furunculosis resistance in vaccinated fish based on testing of unvaccinated fish. Furthermore, potential compromises between vaccination programs and selective breeding programs for enhanced resistance against infectious diseases should be investigated for other diseases, where vaccines are widely used.

Conflict of interest

Frode Finne-Fridell is a full-time employee at PHARMAQ AS. The remaining authors declare no conflict of interest.

Acknowledgements

This study was funded by grant 179009/S40 from the Research Council of Norway, the Fishery and Aquaculture Industry Research Fund and PHARMAQ AS through the project 'Does selection for increased disease resistance increase the immune response, the incidence of vaccine injuries and the effect of vaccination?' SalmoBreed AS provided the fish material and the challenge test data of the three unvaccinated fish groups. The authors are grateful to Celeste Jacq (Nofima) for proof reading the manuscript.

References

Bishop SC, Woolliams JA (2010). On the genetic interpretation of disease data. *PLoS ONE* **5**: e8940.

Ellis AE (1997). Immunization with bacterial antigens: Furunculosis. *Dev Biol Stand* **90**: 107–116.

Gavora JS, Spencer JL, Okada I, Grunder AA (1990). Correlation of genetic-resistance of chickens to Mareks-disease viruses with vaccination protection and invivo response to phytohemaglutinin. *Genet Sel Evol* **22**: 457–469.

Gitterle T, degård J, Gjerde B, Rye M, Salte R (2006). Genetic parameters and accuracy of selection for resistance to White Spot Syndrome Virus (WSSV) in *Penaeus (Litopenaeus)* vannamei using different statistical models. *Aquaculture* **251**: 210–218.

Gjedrem T, Salte R, Gjøen HM (1991). Genetic variation in susceptibility of Atlantic salmon to furunculosis. *Aquaculture* **97**: 1–6.

Gjerde B, Evensen Ø, Bentsen HB, Storset A (2009). Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). *Aquaculture* **287**: 52–58.

Gjøen HM, Refstie T, Ulla O, Gjerde B (1997). Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture* **158**: 277–288.

Gudding R, Lillehaug A, Evensen O (1999). Recent developments in fish vaccinology. *Vet Immunol Immunopathol* **72**: 203–212.

Guy DR, Bishop SC, Woolliams JA, Brotherstone S (2009). Genetic parameters for resistance to Infectious Pancreatic Necrosis in pedigreed Atlantic salmon (*Salmo salar*) post-smolts using a Reduced Animal Model. *Aquaculture* **290**: 229–235.

Håstein T, Gudding R, Evensen (2005). Bacterial vaccines for fish - an update of the current situation worldwide. *Dev Biol* **121**: 55–74.

Houston RD, Haley CS, Hamilton A, Guy DR, Mota-Velasco JC, Gheyas AA *et al*. (2009). The susceptibility of Atlantic salmon fry to freshwater infectious pancreatic necrosis is largely explained by a major QTL. *Heredity* **105**: 318–327.

Kjøglum S, Henryon M, Aasmundstad T, Korsgaard I (2008). Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. *Aquacult Res* **39**: 498–505.

Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates: Sunderland.

Madsen PJ, Just J (2007). DMU a package for analysing multivariate mixed models. Department of Genetics and Biotechnology, Research Centre Foulum, University of Aarhus, Faculty Agricultural Sciences (DJF): Tjele.

Moen T, Baranski M, Sonesson A, Kjoglum S (2009). Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* **10**: 368.

Nakayama Y, Plisch EH, Sullivan J, Thomas C, Czuprynski CJ, Williams BRG *et al*. (2010). Role of PKR and Type I IFNs in viral control during primary and secondary infection. *PLoS Pathog* **6**: e1000966.

NOAH 2010. Vaccination of farm animals. Briefing Document No. 22. National Office of Animal Health http://www.noah.co.uk/issues/briefingdoc/bd22pdf.

Nordmo R 1997. Strengths and weakness of different challenge methods. In: Gudding R LA, Midtlyng PJ, Brown F (eds) *Fish vaccinology. Dev Biol Stand*, Vol. 90 Karger: Basel, Switzerland.

Salte R, Bentsen HB, Moen T, Tripathy S, Bakke TA, Odegard J *et al*. (2010). Prospects for a genetic management strategy to control *Gyrodactylus salaris* infection in wild Atlantic salmon (*Salmo salar*) stocks. *Can J Fish Aquat Sci* **67**: 121–129.

Wetten M, Aasmundstad T, Kjøglum S, Storset A (2007). Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L. *Aquaculture* **272**: 111–117.

Ødegård J, Gitterle T, Madsen P, Meuwissen T, Yazdi M, Gjerde B *et al*. (2011). Quantitative genetics of Taura syndrome resistance in Pacific white shrimp (*Penaeus vannamei*): A cure model approach. *Genet Sel Evol* **43**: 14.

Ødegård J, Olesen I, Gjerde B, Klemetsdal G (2007a). Evaluation of statistical models for genetic analysis of challenge-test data on ISA resistance in Atlantic salmon (*Salmo salar*): prediction of progeny survival. *Aquaculture* **266**: 70–76.

Ødegård J, Olesen I, Gjerde B, Klemetsdal G (2007b). Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). *Aquaculture* **271**: 173–177.

PAPER II

Aquaculture 318 (2011) 316–324

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*)

Tale Marie K. Drangsholt [a,b,*], Bjarne Gjerde [a,b], J. Ødegård [a,b], F. Fridell [c], Hans B. Bentsen [b]

[a] Norwegian University of Life Sciences (UMB), PO Box 5003, 1432 Ås, Norway
[b] Nofima, PO Box 5010, 1432 Ås, Norway
[c] PHARMAQ AS, PO Box 267 Skøyen, 0213 Oslo, Norway

ARTICLE INFO

Article history:
Received 22 December 2010
Received in revised form 20 May 2011
Accepted 27 May 2011
Available online 6 June 2011

Keywords:
Vaccine
Side effects
Adhesions
Melanin
Atlantic salmon
Salmo salar heritability

ABSTRACT

Vaccine-induced side effects (adhesions and melanin deposits) were subjectively evaluated in this study for 150 full-sib families of Atlantic salmon from Salmo Breed AS (year-class 2007). There were four experimental fish groups of which three received a commercial six-component vaccine with an injection volume of 0.1 ml per fish. The groups are described as follows: 1) Pre-smolts kept in freshwater at high temperature (17 °C) and evaluated three months after vaccination (FW); 2) Post-smolts evaluated six months after sea transfer (SW6); 3) Post-smolts evaluated 12 months after sea transfer (SW12); 4) Post-smolts that received an experimental vaccine with a reduced injection volume of 0.05 ml per fish and evaluated six months after sea transfer (SW6-RV). Adhesions between intraperitoneal organs and melanin deposits on internal organs were scored. The FW group had the largest adhesion scores (2.06, SD = 0.49) ($P<0.05$) but the smallest melanin scores (0.95, SD = 0.44) ($P<0.05$). The SW6 group had greater adhesion scores (1.68, SD = 0.65) than the SW12 group (1.46, SD = 0.56) ($P<0.05$), but similar melanin scores (1.49 vs. 1.45; $P=0.12$). The SW6-RV group had smaller adhesion scores (0.93 vs. 1.68) and melanin scores (1.04 vs. 1.49) ($P<0.05$) than the parallel SW6-group. For the fish groups given the commercial vaccine, the heritability estimates for adhesion scores were 0.31 ± 0.05 (FW), 0.19 ± 0.04 (SW6) and 0.16 ± 0.05 (SW12), and for melanin scores 0.27 ± 0.05 (FW), 0.28 ± 0.05 (SW6) and 0.30 ± 0.05 (SW12). For the SW6-RV group the heritabilities were smaller; 0.08 ± 0.03 (SW6-RV) for adhesion score and 0.11 ± 0.03 (SW6-RV) for melanin score. The genetic correlation between adhesion scores and melanin scores within groups was intermediate for the FW group (0.52 ± 0.11) but greater for the SW6 (0.89 ± 0.06) and SW12 groups (0.87 ± 0.06). The genetic correlation between the SW6 and SW12 groups was large for both adhesion (0.89 ± 0.07) and melanin (0.92 ± 0.11) scores. However, the genetic correlations between FW group and the SW6 and SW12 groups were smaller for adhesions (0.62 ± 0.12 and 0.48 ± 0.14, respectively) and for melanin (0.84 ± 0.08 and 0.61 ± 0.11, respectively). These results show that vaccine-induced side effects can be reduced through selective breeding, but that a reduction can also be achieved by other factors such as improvement of the vaccine.

1. Introduction

Vaccination is one of the most important tools to prevent outbreaks of a number of bacterial and viral diseases in farmed Atlantic salmon (*Salmo salar*), and was largely responsible for reducing the use of antibiotics in the 1990s (Ellis et al., 1997; Gudding et al., 1999; Håstein et al., 2005). Farmed Atlantic salmon is today routinely vaccinated before sea transfer against a number of bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral (infectious pancreatic necrosis, (IPN); infectious salmon anemia, (ISA); pancreas disease, (PD)) diseases (McLoughlin and Graham, 2007).

Oil-adjuvant vaccines are needed to obtain long lasting protection, for example against furunculosis (Midtlyng et al., 1996). However, a disadvantage of this type of vaccine can be intra-peritoneal side effects and further reduced appetite and growth (Berg et al., 2006; Midtlyng and Lillehaug, 1998; Sørum and Damsgård, 2004). These side effects can be seen as adhesions between intraperitoneal organs and melanin deposits on internal organs and on the abdominal wall (Midtlyng et al., 1996). In the most severe cases these vaccine-induced side effects can lead to reduced fish welfare and downgrading of the fish slaughter quality (Midtlyng, 1996; Poppe and Breck, 1997). The side effects are associated with prolonged inflammation caused by persistent antigens from the vaccine (Mutoloki et al., 2004). Further, vaccine-induced side effects appear to increase from the time of vaccination until approximately six months after vaccination, before decreasing (Mutoloki et al., 2004). The severity of vaccine-induced intra-abdominal adhesions may be affected by time of

* Corresponding author at: Nofima, PO Box 5010, 1432 Ås, Norway. Tel.: +47 454 72414; fax: +47 64 94 95 02.
E-mail address: tale.drangsholt@nofima.no (T.M.K. Drangsholt).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.05.044

vaccination, vaccine formulation, water temperature and fish condition (Aucouturier et al., 2001; Berg et al., 2006; 2007). Vaccination of small fish and at high temperatures seems to increase the severity of intra-abdominal adhesions (Berg et al., 2006; 2007). It has also been shown that oil-adjuvant vaccines can provoke systemic autoimmune reactions, and that adhesions and granulomas seen in the abdominal cavity of vaccinated fish are related to both chronic inflammation and autoimmune reactions (Haugarvoll et al., 2010; Koppang et al., 2008).

Genetics seem to play a role in the development of vaccine-induced side effects. In a pilot study, intermediate heritabilities ($h^2 = 0.18–0.19$ on the observed scale) were found for susceptibility to develop intra-abdominal adhesions (Gjerde et al., 2009). Since then, vaccines have been substantially improved, which have reduced the severity of the side effects. If susceptibility to vaccine-induced side effects are to be included in the breeding goal, the optimal time of sampling needs to be established, due to the fact that the severity is known to develop over time (Mutoloki et al., 2004). Further, whether vaccine-induced side effects should be included in the breeding goal or not depends on the severity of side effects of future vaccines and on the magnitude of genetic variation of the side effects of these vaccines and of the genetic correlations between the side effects and other important traits. The objective of this study was to estimate the magnitude of the heritability of vaccine-induced side effects in Atlantic salmon in a freshwater test and under conditions similar to commercial conditions in experimental cages at six and 12 months after sea transfer. This was evaluated on fish vaccinated intra peritoneal (i.p.) with a commercial and an experimental vaccine. Further, the magnitude of genetic correlation coefficients between two types of vaccine-induced side effects (adhesions and melanin deposits) and between each of these effects at different ages of the fish were estimated.

2. Material and methods

2.1. Fish material

The Atlantic salmon were from 150 full-sib families; i.e., the offspring of 85 sires and 150 dams from the breeding nucleus of SalmoBreed AS. They were hatched in November 2006 at Eikelandsosen and in January 2007 they were transported as eyed eggs to Nofima Marin, Sunndalsøra where they were reared in separate trays. The first feeding of the families occurred from 5 February to 17 April, 2007. From first feeding they were kept in separate tanks (diameter: 0.60 m, water volume: 0.1 m^3) until a body size suitable for tagging (July 2007, Table 1) with PIT-tags (2.12×12.0 mm) from Trace ID Systems AS (Stavanger, Norway). The environmental conditions (e.g. water temperature, water flow, oxygen conditions, number of individuals per tank) were standardized during the hatchery and rearing period until tagging to minimize environmental differences between the families.

Selection for increased resistance to furunculosis and ISA had been performed for one generation (on parents of fish used in this study), and the breeding goal also included growth, filet fat and filet color.

2.1.1. Vaccinated groups and vaccines

The vaccine-induced side effects were recorded on four experimental groups of fish of which the first three (FW, SW6, SW12) received a commercial six-component vaccine (ALPHA JECT® 6–2 from PHARMAQ AS (Oslo, Norway)) with an injection volume of 0.1 ml per fish of an oil-in-water adjuvant emulsion containing antigens of *Aeromonas salmonicida*, *Vibrio anguillarum*, *V. salmonicida*, *Moritella viscosa* and IPN-virus which protects against furunculosis, classical vibriosis, cold water vibriosis, winter ulcer and IPN, respectively. The fourth group (SW6-RV) consisted of post-smolts that received an experimental vaccine with a reduced injection volume of 0.05 ml per fish of an oil-in-water adjuvant emulsion containing the same antigens as the commercial vaccine. The intention with the experimental vaccine was to obtain a reduction of the negative side effects of vaccination and hence improve animal welfare as well as the growth of the vaccinated fish. Both vaccines were produced by PHARMAQ AS, and the experimental vaccine is now a commercial vaccine (ALPHA JECT® micro 6). According to the producer the two vaccines has the same efficacy. Information on the exact composition of the two vaccines, e.g. in terms of relative proportion of oil and water and the relative proportion of the six different antigens, is proprietary of PHARMAQ AS and not available for disclosure.

The FW group was vaccinated 18–19 October 2007 (at a freshwater temperature of 13 °C), while the SW6, SW6-RV and SW12 groups were vaccinated in 4–5 March 2008 (at a freshwater temperature of 7 °C); two and a half months prior to sea transfer. Average age and weight at vaccination is shown in Table 1.

Table 1
Number of individuals (N) and mean and standard deviation (SD), minimum and maximum for body weight (in grams) and age at vaccination (in days from first feeding) of the Atlantic salmon in the four groups.[a]

Trait and fish group	N	Mean (SD)	Min	Max
Body weight at tagging				
–All	7104	11 (4)	5	28
Body weight at vaccination				
–FW	1690	44 (14)	7	97
–SW6, SW6-RV, SW12	5343	153 (44)	6	371
Age at vaccination				
–FW	1690	221 (15)	203	256
–SW6, SW6-RV, SW12	5343	360 (15)	342	394
Body weight at sampling				
–FW	1630	235 (58)	36	528
–SW6	1627	1007 [b] (293)	160	2380
–SW6-RV	1699	1085[b](308)	140	2220
–SW12	1567	2366 (733)	370	5485

[a] FW = fish in freshwater; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.
[b] Body weight of fish in SW6 and SW6-RV groups were significantly different $P<0.01$.

2.1.2. Unvaccinated control groups

Small unvaccinated control groups were included at each sampling event in order to get an indication of the level of adhesions and melanin deposits in unvaccinated fish. The control groups were tagged on 11 October 2007 and i.p. injected with saline on 19 October 2007.

The control group in fresh water (FW-control) consisted of 65 fish randomly sampled from a random sample of 50 of the families. The

Table 2
Mean adhesion score within regions and mean of average adhesion score across regions for each fish group[a] with standard deviations in parenthesis.

	FW	SW6-RV	SW6	SW12
N	1630	1699	1627	1567
Region 1	2.00 (0.71)^{Aa}	0.49 (0.66)^{Ba}	1.34 (1.04)^{Ca}	0.99 (0.95)^{Da}
Region 2	1.70 (0.49)^{Ab}	0.89 (0.71)^{Bb}	1.67 (0.82)^{Ab}	1.68 (0.75)^{Ab}
Region 3	2.49 (0.80)^{Ac}	1.42 (0.75)^{Bc}	2.01 (0.82)^{Cc}	1.70 (0.73)^{Db}
Average	2.06 (0.49)^{A}	0.93 (0.54)^{B}	1.68 (0.65)^{C}	1.46 (0.56)^{D}

[1]FW = fish in fresh water; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.
Different upper case letters denote significant ($P<0.05$) differences between groups and different lower case letters denote significant differences between regions within groups.

fish were then kept together with the FW group and evaluated for side effects (assessed blind) at the same time (January/February 2008).

The unvaccinated controls for the seagoing groups consisted of a random sample of 62 fish from 17 of the families. After tagging these fish were kept together with the vaccinated fish both in freshwater and seawater. The small number of individuals and families of this control was due to a human error at tagging. A total of 34 control fish were evaluated six months after sea transfer (SW6-control) and 28 control fish 12 months after sea transfer (SW12-control).

2.2. Rearing and sampling

The FW group was transferred in November 2007 to two replicate freshwater tanks (diameter: 3 m, water volume: 7.3 m^3) where the water temperature was gradually increased to 17 °C over the course of seven days, and was kept at approximately this temperature for three months (84 days). This elevated temperature has previously been shown to be a stable laboratory model in order to mimic the worst-case scenario with respect to vaccine-induced side effects (PHARMAQ AS). Unfortunately, there were several short lasting temperature drops due to technical problems with the water pump, but whether this had any effect on the results is not known. The FW group was examined for vaccine-induced side effects over the course of three days in the last week of January 2008; four months after vaccination.

The SW6, SW6-RV and SW12 groups were transferred to an experimental net-cage (400 m^3) in the sea in May 2008; two and a half months (460 day degrees) after vaccination. The SW6 and SW6-RV groups were examined for vaccine induced side effects in November 2008 (eight months after vaccination, six months after sea transfer). The SW12 group was examined in June 2009 (15 months after vaccination, 12 months after sea transfer). Fish were fed commercial feed. In total, 12–15 fish from each of the 150 families were sampled at each sample point except for SW12 where 144 families were sampled. This reduction of number of families was due to removal of six families for an unrelated experiment. Random sampling of fish into the SW6, SW6-RV and SW12 groups was ensured by assigning a random number to all fish IDs prior to each sampling. Thus all fish had to be screened at each sampling event.

2.3. Data recording

At each sampling, a landing net was used to catch fish from the tanks (FW) or the cage (SW6, SW6-RV, SW12). All fish were anesthetized (FW fish with metacain, SW6 and SW6-RV fish with FINQUEL®, SW12 fish with CO_2-gas) before slaughter. Fish ID, body weight and sex (male, female or unknown based on gonad inspection after gutting) were recorded for each fish. Further, the fish were examined for vaccine induced side effects as described below. Fish recorded as sexually mature (FW and SW6) or maturing (SW12) were excluded from the data (21 fish in FW, 15 in SW6 and SW6-RV, none in SW12) due to the low number of such fish.

2.4. Evaluation of vaccine induced side effects

At each stage of recording, fish were evaluated by two (FW and SW12) or four (SW6, SW6-RV) trained persons from PHARMAQ. Information on family and vaccine type was not known to these persons. Adhesions in three separate regions of the abdominal cavity (regions 1, 2 and 3) were scored using a 13 point scale (0 = no adhesions, 6 = extremely severe adhesions: interval 0.5) (Midtlyng et al., 1996). Region 1 was anterior, region 2 was dorsal and posterior, and region 3 was the ventral part of the abdominal cavity. Melanin deposits on internal organs and the abdominal wall were scored on a four point scale (0 = no visible melanin, 3 = severe melanin spots: interval 1.0).

2.5. Statistical model and methods

The traits analyzed were adhesion score (average across the three regions) and melanin score (average across organs and abdominal wall). Differences between and within the groups (FW, SW6, SW6-RV, SW12, control groups) were tested by use of a *t*-test (SAS, 2004).

Estimates of (co)variance components for the studied traits were obtained from multivariate animal models using the DMU software (Madsen and Jensen, 2007). For each trait *i*, the model had the following general characteristics:

$$y_i = X_i\beta_i + Z_i a_i + e_i$$

where

- y_i is the vector of the observations for the trait *i*,
- β_i is a vector of fixed effects of trait *i*, including effects of person responsible for the recording of the adhesions and melanin deposits, tank/net-cage by sex and regression coefficient of age of the fish (to correct for the fact that the families were produced over a period of 72 days) nested within tank/net-cage and sex,
- a_i is a vector of additive genetic effects of each individual fish for trait *i*,
- e_i is a vector of random residuals for trait *i* and
- X_i and Z_i are appropriate incidence matrices.

Initially, a random effect common to full-sibs [i.e., an environmental (tank) effect due to the separate rearing of the families until tagging and non-additive genetic effects common to fullsibs], was also included in the animal model above. The significance of this was tested using a likelihood ratio test (Lynch and Walsh, 1998), using single-trait models for each trait. This effect was not significant ($P>0.05$) for any of the traits and was thus not included in the final analysis.

In preliminary analyzes a multivariate model with adhesion scores recorded at each of the three regions as separate traits was used for each of the four fish groups and is given in the Appendix. Similarly, a bivariate model with melanin scores was also used, treating melanin scores recorded at internal organs and the abdominal wall as separate traits. The estimated genetic correlations were large between melanin score in organs and the abdominal wall and also relatively large between adhesion scores in the three regions, except for the genetic correlation between regions 1 and 3 for the SW12-group which was lower (Appendix). Due to these results it was decided to base the final analysis on average scores (across regions) for both adhesions and melanin.

Estimates of (co)variance components for the studied traits in the four groups (FW, SW6, SW6-RV, SW12) were obtained through two separate multivariate models: one for average adhesion score (four traits: a trait for each of the four groups) and one for average melanin score (four traits: a trait for each of the four groups). The residual correlations were set to zero for these models, as the traits were measured on different individuals from the four groups. Estimates of co-variance between adhesion score and melanin score within each group (FW, SW6, SW6-RV, SW12) were obtained through four bivariate models (one model for each group).

For all traits, heritability was calculated as the additive genetic variance divided by the sum of additive genetic and residual variance.

3. Results

3.1. Descriptive statistics

3.1.1. Fixed effects

The fixed effects were included in the model if they were significant for at least one of the groups. Sex had the largest effect

and was significant for all groups and traits. The effect of age of fish was generally small but significant for all groups and traits except for adhesions and melanin deposits in the SW6-RV group and melanin deposits in the SW12 group. The effect of person on adhesions and melanin score was small, but significant for all groups except for adhesions in the SW12 group.

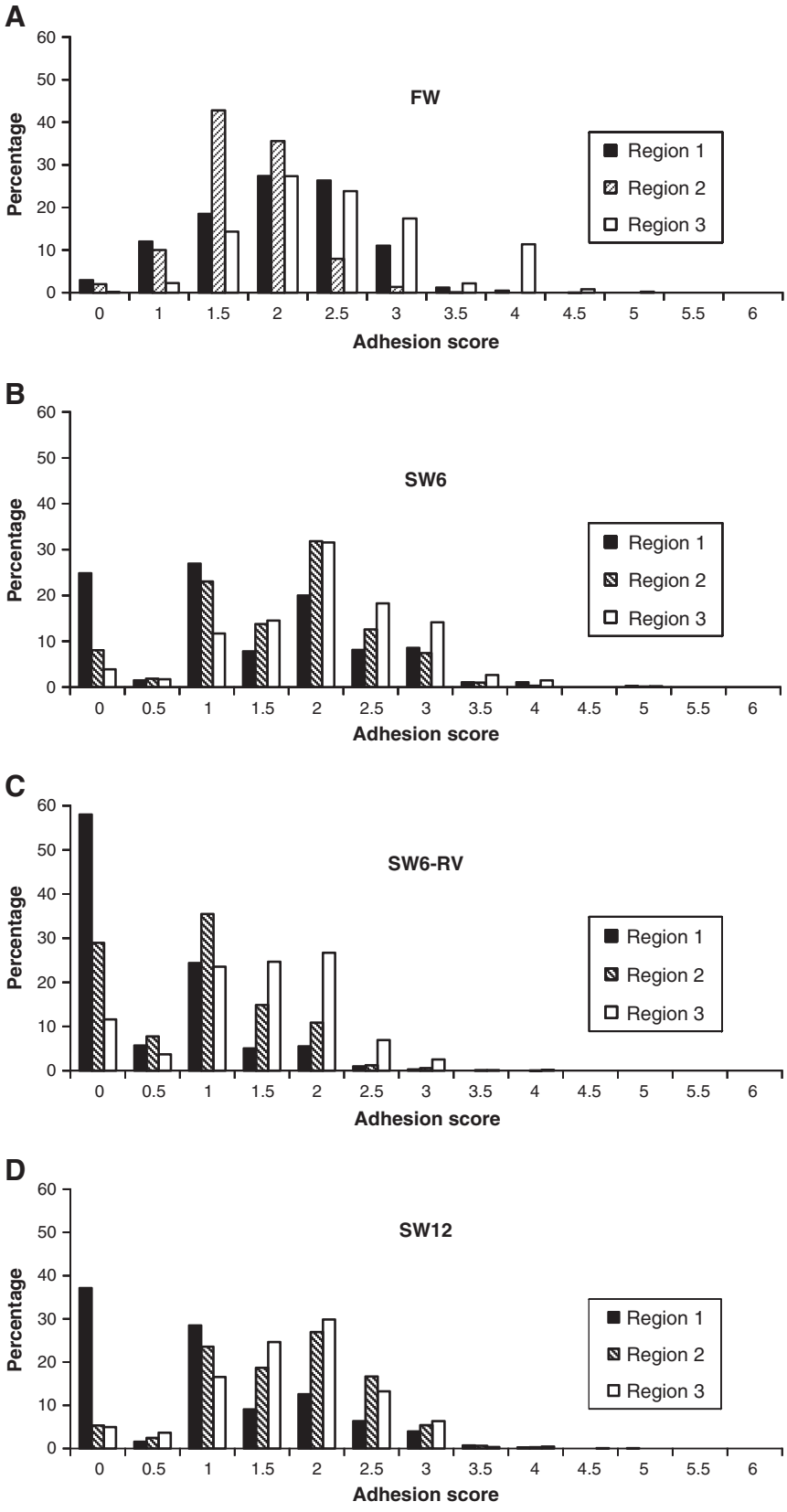

Fig. 1. Percent fish with different adhesion scores in all three regions for fish in A. freshwater (FW), B sampled six months after sea transfer vaccinated with commercial vaccine (SW6), C. sampled after six months vaccinated with the experimental vaccine with reduced injection volume (SW6-RV) D. sampled 12 months after sea transfer (SW12).

Table 3
Mean melanin score for organs and abdominal wall and mean of average melanin score across organs and abdominal wall for each fish group[1] with standard deviations in parenthesis.

	FW	SW6-RV	SW6	SW12
n	1630	1699	1627	1567
Organs	1.37 (0.53)^{Aa}	1.23 (0.53)^{Ba}	1.80 (0.68)^{Ca}	1.68 (0.70)^{Da}
Abdominal wall	0.53 (0.53)^{Ab}	0.85 (0.66)^{Bb}	1.19 (0.78)^{Cb}	1.22 (0.83)^{Cb}
Average	0.95 (0.44)^{A}	1.04 (0.49)^{B}	1.49 (0.62)^{C}	1.45 (0.69)^{C}

[1]FW = fish in freshwater; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.
Different upper case letters denote significant ($P<0.05$) differences between groups and different lower case letters denote significant differences between organs and abdominal wall within groups.

3.1.2. Body weight and age

Table 1 shows descriptive statistics for body weight at tagging, at vaccination and at each sampling for each group, as well as the age at vaccination (in days from first feeding) of the fish in the four groups. Fish from groups SW6, SW6-RV and SW12 were vaccinated five months later than the FW group and were thus older and heavier at vaccination.

3.1.3. Average scores—commercial vaccine

Within group, the mean adhesion scores were significantly different for all regions and largest in region 3, except for the SW12 group where no significant difference between regions 2 and 3 was observed (Table 2). Distribution of the frequency of adhesion scores in each of the three regions is shown in Fig. 1 for the FW group (1A), SW6 group (1B), SW6-RV group (1C), and SW12 group (1D). There were no scores of zero in any regions for FW fish, but this was more frequent for SW6 and SW12 fish, especially in region 1 (25–37% with score = 0).

Within each group, the melanin score was generally greater on internal organs than on the abdominal wall (Table 3). As shown in Fig. 2, melanin deposits were present on internal organs of almost all fish in all groups, but not on the abdominal wall (score = 0 in 15–48% of the fish). A score of zero was less frequent in the SW6 and SW12 groups compared to the FW group.

Average adhesion scores (average of regions 1, 2 and 3) for fish groups given a commercial vaccine (all groups except SW6-RV) were all significantly different from each other, and were largest for the FW group and lowest for the SW12 group (Table 2). Average melanin scores (average of internal organs and abdominal wall) were lower for the FW group than for the SW6 and SW12 groups, but no significant difference was observed for average melanin scores between the SW6 and SW12 groups (Table 3).

3.1.4. Experimental vs. commercial vaccine

The fish given the experimental vaccine with reduced injection volume (SW6-RV) were significantly heavier ($P<0.01$) and had lower average adhesion scores ($P<0.05$) compared with the fish given the commercial vaccine (SW6) (Tables 1 and 2). As seen in Fig. 1C, 58% of the SW6-RV fish had no visible adhesions (score = 0) in regions 1 and 2, which is substantially larger than for SW6 fish (8–25%) in the same regions (Fig. 1B). Melanin score was also significantly lower for SW6-RV fish compared to SW6 fish (Table 3). Almost all SW6 and SW6-RV fish had visible melanin deposits on internal organs (score ≥ 1), while melanin deposits on the abdominal wall were not visible (score = 0) on 15% of the SW6 fish and 28% of the SW6-RV fish (Fig. 2B and C).

3.1.5. Unvaccinated control groups

Adhesion scores were very low in the FW-control group (mean score 0.04, SD = 0.14), and 89% of the fish received a score of zero for all regions. The level of melanin in the FW-control group was also low (mean score 0.24, SD = 0.25) and 52% of the fish had no visible melanin deposits. The SW6-control group had similar low scores (mean adhesion score 0.08, SD = 0.27 and mean melanin score 0.06, SD = 0.16), with 91% and 88% of the fish having no visible adhesions or melanin deposits, respectively. The scores were also low in the SW12-control group (mean adhesion score 0.13, SD = 0.21, mean melanin score 0.25, SD = 0.44), with 57% of the fish in this group having no visible adhesions and 68% with no visible melanin deposits. Adhesions were mainly observed in regions 2 and 3, and melanin deposits mainly on internal organs. Fore adhesion and melanin score all control groups were significantly different from the corresponding vaccinated groups ($P<0.001$). This was also the case when comparing only the 17 families of vaccinated fish that were also present in the unvaccinated control groups.

3.1.6. Fish with no side effects

The number of fish with no adhesions in any region (average score = 0) was three in the FW group, 36 in the SW6 group, 136 in the SW6-RV group, and seven fish in the SW12 group. The fish in the SW6-RV group were from 85 different families and no more than four fish per family received an average score of zero. These can be true observations of vaccinated fish. However, there is also a chance that these fish did not receive the vaccine due to human or technical error. Melanin deposits were absent on the abdominal wall and internal organs (average score = 0) in five fish in group FW, seven in group SW6, 53 in group SW6-RV and 15 fish in group SW12. Melanin deposition is considered a complex trait that can also be seen in unvaccinated fish due to the perforation of the abdominal wall with a needle. Hence lack of adhesions was more common than lack of melanin deposits and therefore additional analyses excluding fish with zero score for adhesions were performed. The results from these analyses did not affect the results significantly. However, unlike for the full dataset, the genetic correlation between adhesions in SW6 and SW6-RV was significantly different from zero (0.50 ± 0.22, $P<0.05$) when fish with no adhesions were removed. Final analysis and results presented in the rest of this paper include fish with no adhesions as it cannot be determined whether they were actually vaccinated or not, and their influence on the results was marginal.

3.2. Genetic (co)variances

3.2.1. Commercial vaccine

Table 4 shows the estimated variance components and heritabilities for average adhesion and melanin scores for each group. The heritability for adhesion scores was intermediate for the FW group (0.31 ± 0.05), and lower for the SW6 (0.19 ± 0.04) and SW12 (0.16 ± 0.04) groups. The genetic variance was similar among these groups, but the FW group had a lower residual variance resulting in a larger heritability. The heritability for average melanin score was intermediate, and similar in all three groups. Variance components were similar for the SW6 and SW12 groups, but both genetic and residual variances were somewhat lower for the FW group.

The genetic correlations between average adhesion and melanin scores measured at the same time were large for the SW6 and SW12 groups, and moderate for the FW group (Table 4).

Table 5 shows the genetic correlations between average adhesion score, and between average melanin scores, measured in the three groups given the commercial vaccine (FW, SW6, SW12) and the group

Fig. 2. Percent fish with different melanin scores on internal organs and abdominal wall for A. Fish in freshwater (FW), B. Fish sampled six months after sea transfer vaccinated with commercial vaccine (SW6) C. Fish sampled after six months vaccinated with the experimental vaccine with reduced injection volume (SW6-RV) and D. Fish sampled 12 months after sea transfer (SW12).

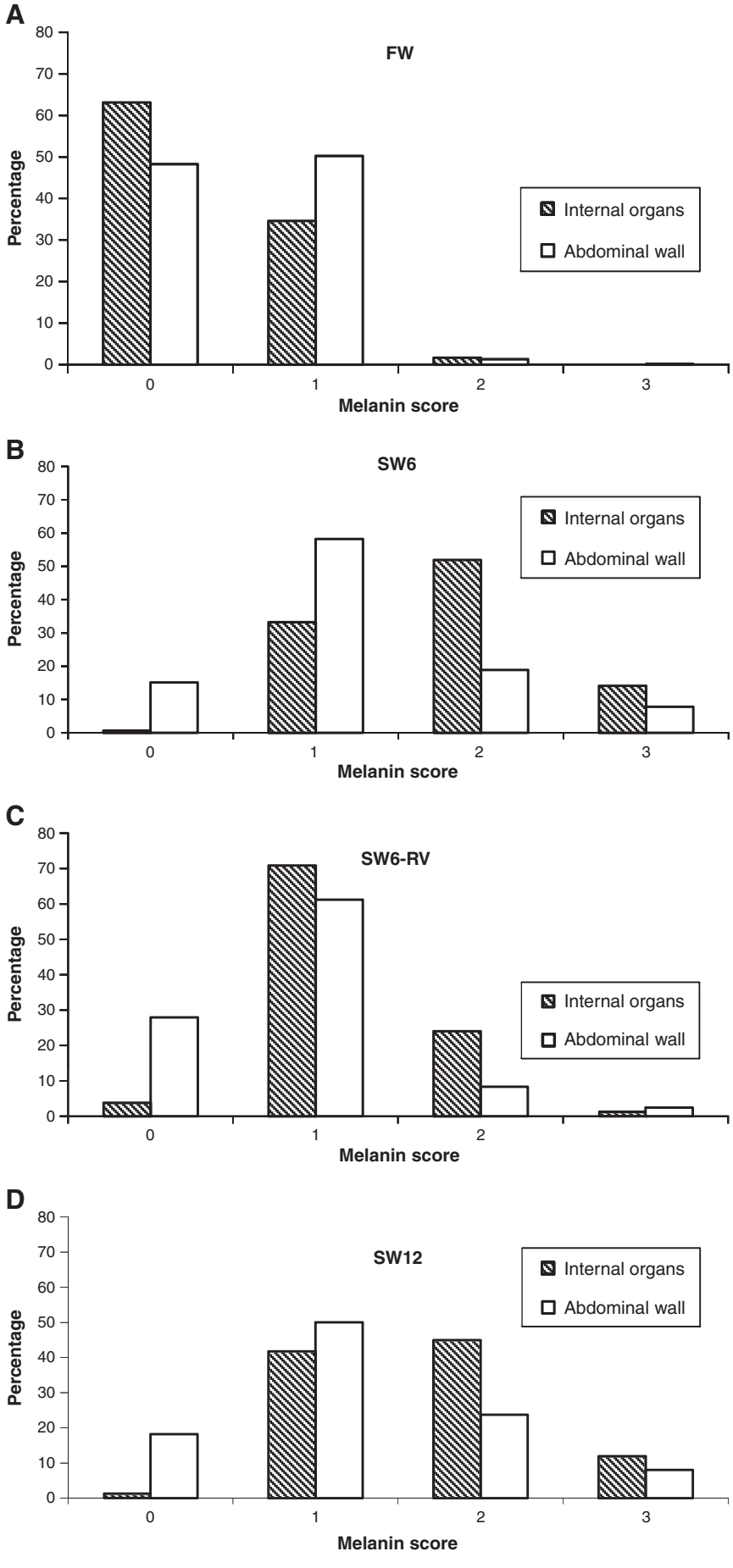
A
FW
Percentage
Melanin score
Internal organs
Abdominal wall
B
SW6
C
SW6-RV
D
SW12

Table 4
Genetic variance ($\sigma^2_g \pm$ standard error), residual variance ($\sigma^2_e \pm$ standard error) and heritabilities ($h^2 \pm$ standard error) for adhesion and melanin scores at sampling for each group[a]. Genetic correlations ($r_g \pm$ standard error) and residual correlations ($r_e \pm$ standard error) between adhesion and melanin scores at sampling within group.[a]

	FW	SW6-RV	SW6	SW12
Adhesion scores				
σ^2_g	0.07 ± 0.01	0.02 ± 0.01	0.07 ± 0.02	0.05 ± 0.01
σ^2_e	0.15 ± 0.01	0.23 ± 0.01	0.32 ± 0.02	0.26 ± 0.01
h^2	0.31 ± 0.05	0.08 ± 0.03	0.19 ± 0.04	0.16 ± 0.04
Melanin scores				
σ^2_g	0.05 ± 0.01	0.02 ± 0.01	0.11 ± 0.02	0.14 ± 0.01
σ^2_e	0.14 ± 0.01	0.21 ± 0.01	0.28 ± 0.02	0.33 ± 0.01
h^2	0.27 ± 0.05	0.11 ± 0.03	0.28 ± 0.05	0.30 ± 0.05
Adhesion vs. melanin scores				
r_g	0.52 ± 0.11	0.76 ± 0.14	0.89 ± 0.06	0.87 ± 0.06
r_e	0.24 ± 0.04	0.32 ± 0.03	0.37 ± 0.03	0.52 ± 0.03

[a] FW = fish in freshwater; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.

given the experimental vaccine (SW6-RV). In general, the magnitude of the genetic correlation between the same traits between groups decreased with increasing time interval between the recordings. For adhesion scores the genetic correlation between the SW6 and SW12 groups was positive and large, while only moderately positive between the FW group and the SW6 and SW12 groups. For melanin score the genetic correlation was large between the FW and SW6 groups and between the SW6 and SW12 groups, but moderate between the FW and SW12 groups (Table 5).

3.2.2. Experimental vaccine

For the SW6-RV group the heritabilities for average adhesion and melanin scores were lower than for the parallel SW6 group (Table 4). The genetic correlation between adhesion and melanin scores was relatively large (0.76 ± 0.14) and similar to the genetic correlation between the same two traits for the SW6 group. The genetic correlation between adhesion scores for the SW6-RV and SW6 groups was low and not significantly different from zero (0.25 ± 0.21), while the genetic correlation between average melanin scores for the two groups was large (0.83 ± 0.13) (Table 5).

4. Discussion

4.1.1. Commercial vaccine

As expected, the greatest adhesion scores were observed in the FW group as this group was tested using a laboratory model (high temperature) designed to induce a worst case scenario with respect to vaccine related side effects. Fish in this group were also vaccinated earlier and were thus smaller than fish in the other groups and the temperature at vaccination was higher. These factors may have contributed to the larger average adhesion and melanin score in the FW group. Adhesion scores were larger six months (SW6) compared to 12 months after sea transfer (SW12), which supports findings from Mutoloki et al. (2004) suggesting that adhesions peak approximately six months after vaccination (five months after sea transfer). Melanin deposits seem to develop slower, which may explain why the FW group had the lowest score for melanin (measured just over three months after vaccination). Melanin scores did not seem to peak as there was no difference in average melanin score between the SW6 and SW12 groups.

Unvaccinated control fish had very low average scores for adhesions and melanin suggesting that the vaccine was the main cause of these observations. Control fish were PIT-tagged like all vaccinated groups and injected with saline which may explain why some unvaccinated fish had scores above zero for both adhesions and melanin. Although rare, adhesions have previously been observed in unvaccinated fish injected with saline (Lund et al., 1997). Melanin deposits appear to be a complex trait that can also occur in unvaccinated fish (Mørkøre, 2008), and seem to be a poor marker for discriminating between unvaccinated and vaccinated fish (Lund et al., 1997). The influence of factors other than vaccine for the cause of adhesions and melanin deposits observed on fish in this study can therefore not be ruled out.

To determine if the PIT-tags contributed to melanin or adhesion score in vaccinated fish, Atlantic salmon were vaccinated (same vaccine as the FW, SW6 and SW12 groups) and either PIT-tagged or not tagged in March 2008. These fish were reared in seawater together with the experiment fish and evaluated six and 12 months after sea transfer. PIT-tagging increased the level of adhesions (1.61 vs. 1.31) and melanin deposits (1.46 vs 1.14) slightly (unpublished results). Consequently, a fair comparison of experimental groups is only possible if all are PIT-tagged or (if possible) untagged.

Our results show that susceptibility to vaccine-induced side effects (adhesions and melanin deposits) are heritable traits in Atlantic salmon, thereby confirming the findings of Gjerde et al. (2009) suggesting that adhesions and melanin deposits can be reduced through selective breeding. In our study adhesions show a trend of larger heritability in the FW group compared to the SW6 and SW12 groups, primarily because of a much lower residual variance in the FW group. For melanin scores both residual and genetic variances were lower in the FW group than in the SW6 and SW12 groups, resulting in similar heritabilities in these three groups. Factors that can help explain the lower residual variance in FW includes the shorter experimental period and more controlled environment, and thus more stable environment than for the fish kept in an experimental cage in sea water. Compared with Gjerde et al. (2009), the current estimates of heritability for the SW6 and SW12 groups were similar for adhesion scores (0.19 ± 0.04 and 0.16 ± 0.04 vs. 0.19 ± 0.03), but larger for melanin scores (0.28 ± 0.05 and 0.30 ± 0.05 vs. 0.18 ± 0.03).

The genetic correlations between adhesion and melanin scores were large within the SW6, SW12 and SW6-RV groups, suggesting that development of adhesions and melanin deposits are to a large extent influenced by the same genetic factors and can thus be considered as the same trait. A large genetic correlation was also found between adhesions measured in the SW6 and SW12 groups and this was also the case for melanin scores. However, the genetic correlations were only moderate between adhesions measured in the FW group and the SW6 and SW12 groups and the same trend was seen for melanin scores. These results suggest that vaccine-induced side effects are somewhat different traits when measured in a high temperature freshwater test designed to induce such effects under conditions similar to commercial conditions in seawater. Side effects evaluated after six and 12 months in seawater on the other hand appear to be closely related genetically.

Table 5
Genetic correlations (± standard error) between the three groups[a] given commercial vaccine and between the two groups[a] sampled after six months for average adhesion scores and melanin scores.

	Adhesion score	Melanin score
FW vs. SW6	0.62 ± 0.12	0.84 ± 0.08
FW vs. SW12	0.48 ± 0.14	0.61 ± 0.11
SW6 vs. SW6-RV	0.25 ± 0.21	0.83 ± 0.13
SW6 vs. SW12	0.92 ± 0.11	0.89 ± 0.07

[a] FW = fish in freshwater; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.

4.1.2. Experimental vs. commercial vaccine

The experimental vaccine gave significantly lower scores for both adhesion and melanin compared to the commercial vaccine measured six months after sea transfer. Midtlyng et al. (1996) showed that the combination of mineral oil and antigens caused the most severe side effects, but also that combining the two gave the best protection against furunculosis. In this study, the injected volume of adjuvant in the experimental vaccine was half of that in the commercial vaccine. The results indicate that reducing the injection volume can be a successful way of reducing the degree of negative side effects of a vaccine seen as adhesions and melanin spots. This is also supported by the fact that the SW6-RV group had significantly larger body weights compared to the SW6 group. However, it cannot be ruled out that changes in e.g. the relative proportions of oil and water and of the six different antigens in the experimental vaccine may have had a positive effect in terms of the reduced occurrence of adhesion and melanin scores and also the observation of increased growth.

The genetic correlation between adhesion scores in the SW6-RV and SW6 groups was low and not significantly different from zero (0.25 ± 0.21) showing that adhesions resulting from these two vaccines should be regarded as different genetic traits. However, if the few fish with no adhesions were omitted from the data (8% in SW6-RV and 2% in SW6) a moderately positive genetic correlation (0.50 ± 0.22) was estimated. This may indicate that presence/absence of adhesions (most important in the SW6-RV group) may be a different aspect of vaccine-induced side effects than degree of adhesions (given that adhesions are present). The genetic correlation between melanin scores in the SW6-RV and SW6 groups was large (0.83 ± 0.13), suggesting a common genetic background for this trait independent of vaccine type. The apparent lack of coherence between estimates of genetic correlation between and within the SW6-RV and SW6 groups for adhesion and melanin scores remains to be explained.

The relatively low genetic correlations between adhesion scores in the SW6 and SW6-RV groups and between fish reared in fresh (FW) and seawater (SW6 and SW12) may indicate that different immunological factors and/or different degrees of autoimmune reactions (Haugarvoll et al., 2010; Koppang et al., 2008) are involved for different vaccines and under different environmental conditions. However, in this study only one vaccine was tested in different environments and a reliable estimate for the vaccine by environment interaction can therefore not be obtained. The magnitude of the genetic variation in autoimmune reactions relative to chronic inflammation also requires further study.

4.1.3. Practical implications

Vaccine-induced side effects are mainly a problem in the grow-out phase in seawater. Therefore, the rather low genetic correlations of vaccine-induced side effects in the freshwater test with those after six and 12 months in the sea indicate that side effects observed on pre-smolts in freshwater at high temperatures are not an appropriate indicator for accurate ranking of families with respect to susceptibility to side effects under close to commercial conditions in the sea. Whether the freshwater test is appropriate to discriminate between different types of vaccines needs further research, with different vaccines tested both in the freshwater test and in the grow out period in seawater so that the magnitude of the interaction between vaccine type and test environment can be estimated. As side effects measured after six and 12 months in the seawater are largely correlated, side effects relevant for the whole sea period can be assessed at slaughter as this would be the simplest and most cost-efficient way of including these traits in a breeding program.

Inclusion of additional traits in the breeding goal will necessarily reduce the genetic gain for other traits. Due to the relatively low heritabilities for adhesion and melanin scores obtained with the experimental vaccine, genetic gain for reduced vaccine-induced side effects is expected to be low, unless given an unrealistically large economic weight in the total merit index. Alternative approaches for reducing vaccine side effects should therefore be given a high priority; e.g. improvement of management factors such as age and body size of fish and water temperature at vaccination (Berg et al., 2006; 2007), and through development of improved vaccines. The low and uncertain estimates of genetic correlations between adhesion scores in fish given the two types of vaccine suggests that selection based on a particular vaccine may be inefficient in the long run, as new vaccines are continuously being developed.

The effects of including vaccine-induced side effects in the breeding goal also depend on the magnitude of the genetic correlation with other traits such as disease resistance traits and body weight and carcass quality traits. This needs to be further investigated.

Acknowledgements

This study was funded by grant 179009/S40 from the Research Council of Norway and The Fishery and Aquaculture Industry Research Fund and PHARMAQ AS. Fish used in the project were provided free of charge from SalmoBreed AS. The authors thank Kjellrun Gannestad and staff at Nofima, Sunndalsøra and Nofima, Averøy for their skilful assistance during data collection. We are grateful to Celeste Jacq (Nofima) for proof reading the manuscript.

Appendix

Genetic correlation (±standard error) between adhesion scores measured in three regions and between melanin scores measured on internal organs and on abdominal wall for FW, SW6, SW6-RV and SW12[1].

	Adhesion scores			Melanin scores
	Region 1 vs. 2	Region 1 vs. 3	Region 2 vs. 3	Organs vs. abdominal wall
FW	0.71 ± 0.14	0.74 ± 0.12	0.91 ± 0.08	0.68 ± 0.11
SW6	0.89 ± 0.12	0.69 ± 0.17	0.83 ± 0.13	0.95 ± 0.05
SW6-RV	0.99*	0.73 ± 0.27	0.99 ± 0.27	0.75 ± 0.17
SW12	0.56 ± 0.22	0.35 ± 0.24	0.92 ± 0.10	0.99*

*Genetic correlation fixed at 0.99.

[1]FW = fish in freshwater; SW6-RV = six months after sea transfer vaccinated with the experimental vaccine with reduced injection volume; SW6 = six months after sea transfer; SW12 = 12 months after sea transfer. FW, SW6 and SW12 were vaccinated with the commercial vaccine.

For adhesion score in the SW6-RV group, genetic co-variances between regions 1, 2 and 3 from a three variate animal models could not be obtained due to lack of convergence, and therefore bivariate models were used. However, the bivariate model with regions 1 and 2 still did not converge, but converged when the genetic correlation was fixed to 0.99. Similarly, the bivariate model for melanin scores on organs and abdominal wall in the SW12 group did initially not converge, but converged when the genetic correlation was fixed at 0.99.

References

Aucouturier, J., Dupuis, L., Ganne, V., 2001. Adjuvants designed for veterinary and human vaccines. Vaccine 19, 2666–2672.

Berg, A., Rødseth, O.M., Tangerås, A., Hansen, T., 2006. Time of vaccination influences development of adhesions, growth and spinal deformities in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 69, 239–248.

Berg, A., Rødseth, O.M., Hansen, T., 2007. Fish size at vaccination influence the development of side-effects in Atlantic salmon (*Salmo salar* L.). Aquaculture 265, 9–15.

Ellis, A.E., Gudding, R., Lillehaug, A., Midtlyng, P., Brown, F., 1997. Immunization with bacterial antigens: furunculosis. Developments in Biological Standardization 90, 107–116.

Gjerde, B., Evensen, Ø., Bentsen, H.B., Storset, A., 2009. Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). Aquaculture 287, 52–58.

Gudding, R., Lillehaug, A., Evensen, O., 1999. Recent developments in fish vaccinology. Veterinary Immunology and Immunopathology 72, 203–212.

Håstein, T., Gudding, R., Evensen, Ø., 2005. Bacterial vaccines for fish—an update of the current situation worldwide. In: Midtlyng, P.J. (Ed.), Developments in Biologicals Karger, pp. 55–74.
Haugarvoll, E., Bjerkås, I., Szabo, N.J., Satoh, M., Koppang, E.O., 2010. Manifestations of systemic autoimmunity in vaccinated salmon. Vaccine 28, 4961–4969.
Koppang, E.O., Bjerkås, I., Haugarvoll, E., Chan, E.K.L., Szabo, N.J., Ono, N., Akikusa, B., Jirillo, E., Poppe, T.T., Sveier, H., Tørud, B., Satoh, M., 2008. Vaccination-induced systemic autoimmunity in farmed Atlantic salmon. The Journal of Immunology 181, 4807–4814.
Lund, R.A., Midtlyng, P.J., Hansen, L.P., 1997. Post-vaccination intra-abdominal adhesions as a marker to identify Atlantic salmon, *Salmo salar* L., escaped from commercial fish farms. Aquaculture 154, 27–37.
Lynch, M., Walsh, B., 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland. XVI, 980s. pp.
Madsen, P., Jensen, J., 2007. DMU a package for analysing multivariate mixed models 6.4.7, University of Aarhus, Faculty Agricultural Sciences (DJF). Dept. of Genetics and Biotechnology, Research Centre Foulum Tjele.
McLoughlin, M.F., Graham, D.A., 2007. Alphavirus infections in salmonids—a review. Journal of Fish Diseases 30, 511–531.
Midtlyng, P.J., 1996. A field study on intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish & Shellfish Immunology 6, 553–565.
Midtlyng, P.J., Lillehaug, A., 1998. Growth of Atlantic salmon *Salmo salar* after intraperitoneal administratin of vaccines containing adjuvants. Diseases of Aquatic Organisms 32, 91–97.
Midtlyng, P.J., Reitan, L.J., Speilberg, L., 1996. Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish & Shellfish Immunology 6, 335–350.
Mørkøre, T., 2008. Melanin i lakesfilet. Nosk Fiskeoppdrett 33, 62–64.
Mutoloki, S., Alexandersen, S., Evensen, Ø., 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. Fish & Shellfish Immunology 16, 633–644.
Poppe, T.T., Breck, O., 1997. Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil-adjuvanted vaccine. A case report. Diseases of Aquatic Organisms 29, 219–226.
SAS, 2004. SAS Online Doc® 9.1.3. SAS Institute Inc, Cary, NC, USA.
Sørum, U., Damsgård, B., 2004. Effects of anaesthetisation and vaccination on feed intake and growth in Atlantic salmon (*Salmo salar* L.). Aquaculture 232, 333–341.

PAPER III

Genetic correlation between disease resistance, vaccine-induced side effects and harvest body weight in Atlantic salmon (*Salmo Salar*).

T. M. K. Drangsholt[1,2], B. Gjerde[1,2], J. Ødegård[1,2], F. Finne-Fridell[4], Ø. Evensen[3], H. B. Bentsen[2]

[1]Norwegian University of Life Sciences (UMB), Dept. of Animal and Aquacultural sciences, PO Box 5003, 1432 Ås, Norway
[2]Nofima Marin, PO Box 5010, 1432 Ås, Norway
[3]Norwegian School of Veterinary Science, PO Box 8146 Dep, 0033 Oslo, Norway
[4]PHARMAQ AS, PO Box 267 Skøyen, 0213 Oslo, Norway

MANUSCRIPT

Abstract

Farmed Atlantic salmon are vaccinated as presmolts to prevent disease outbreaks at later stages of the production. This causes vaccines induce side effects characterized as abdominal adhesions and melanin deposits. Alongside with vaccination, selective breeding is practised both for increased disease resistance and growth until harvest size. However, reliable genetic correlations between these traits are not known. The objective of this study was to estimate the genetic correlations between resistance to furunculosis (of vaccinated and unvaccinated fish) and infectious salmon anaemia (ISA) (unvaccinated fish), vaccine-induced side effect and growth in Atlantic salmon. Four subsamples of individually tagged fish from 150 full-sib families from SalmoBreed AS were used: 1) Vaccinated and 2) unvaccinated fish challenged *Aeromonas salmonicida* (causing furunculosis); 3) Unvaccinated fish challenged with ISA-virus; and 4) Vaccinated fish sampled 12 months after sea transfer and assessed for adhesion- and melanin scores and body weight. The genetic correlations between disease resistance traits and vaccine-induced side effects were not significantly different from zero. Genetic correlation between body weight at harvest and vaccine-induced side effects was also not different from zero. These results indicate that selective breeding for disease resistance (whether in vaccinated or unvaccinated fish) and/or growth will not lead to more severe vaccine-induced side effects. Genetic correlations between harvest body weight and disease resistance (survival) were also weak; -0.18 ± 0.17 (furunculosis) and 0.05 ± 0.17(ISA) for unvaccinated fish and -0.36 ± 0.16 (furunculosis) for vaccinated fish. These results suggest that selection for one of the trait is not likely to result in unfavourable correlated responses in the other, with a possible exception for harvest body weight vs. survival of vaccinated fish. However, to improve each of these traits through selective breeding they have to be directly selected for.

Key words: disease resistance, vaccination, side effects, growth, Atlantic salmon, genetic correlation

Corresponding author: Tale Marie Karlsson Drangsholt,
Phone: +47 454 72414, Fax: +47 64 94 95 02, E-mail: tale.drangsholt@nofima.no

1 Introduction

Vaccination is the single most important tool to prevent outbreaks of a number of diseases in farmed Atlantic salmon, and all fish in the Norwegian salmon industry are routinely vaccinated against a number of diseases (furunculosis, vibriosis, cold water vibriosis, winter ulcer, pancreas disease (PD) and infectious pancreatic necrosis (IPN)) (McLoughlin and Graham, 2007; Ramstad et al., 2007; Sommerset et al., 2005). Oil-adjuvanted vaccines delivered intraperitoneally give lasting protection, but can also lead to side effects at the injection site seen as adhesions between organs in the abdominal cavity and melanin deposits on internal organs and on the abdominal wall (Midtlyng et al., 1996). These side effects can in the most severe cases lead to downgrading of slaughtered fish and is a concern for fish welfare. The side effects are seemingly associated with persistent antigens of oil-based vaccines which stimulates and maintain inflammatory reactions at the injection site (Mutoloki et al., 2004). Further, oil-adjuvanted vaccines have been associated with systemic autoimmune reactions (Haugarvoll et al., 2010; Koppang et al., 2008). However, the benefit of vaccination is obviously that fish acquire a long-term immunity.

In Atlantic salmon, selective breeding for increased disease resistance has been performed in parallel with vaccine development. Selection is based on survival data from challenge tests, and substantial additive genetic variation in survival has been reported for a number of diseases like the bacterial disease furunculosis, the viral diseases infectious salmon anemia (ISA) and IPN and parasites like sea lice (*Lepeopheirus salmonis*) and *Neoparamoeba perurans* causing amoebic gill disease (Ødegård et al., 2011a). Genetic variation has also been found for resistance against furunculosis in vaccinated fish although this heritability was lower than for unvaccinated fish in the same study (Drangsholt et al., 2011b).

In selective breeding experiments- and programs there are many examples of unfavorable genetic correlation between traits and of unfavorable correlated selection responses particularly in traits not included in the breeding objective (Rauw et al., 1998). Hence, reliable estimates of genetic correlations between traits are important so that appropriate actions can be taken to prevent such unfavorable effects. The genetic relationship between different diseases, both bacterial and viral, seems to be neutral or

slightly positive (Drangsholt et al., 2011b; Gjøen et al., 1997; Kjøglum et al., 2008; Ødegård et al., 2007; Ødegård et al., 2011a). Similarly, the genetic relationship between survival of unvaccinated and vaccinated fish in challenge tests are found to be low to moderate (Drangsholt et al., 2011b). These results suggest that partly different immunogenetic factors are involved in development of disease resistance depending on vaccine status and type of disease. However, simultaneous selection for resistance against various diseases seems to be feasible (no unfavorable genetic correlations). In general, reliable estimates of genetic correlations between growth and disease resistance are few, and in most cases low or not significantly different from zero (Gjedrem et al., 1991; Henryon et al., 2002; Silverstein et al., 2009; Standal and Gjerde, 1987).

Vaccine-induced side effects seems to be affected by many factors including time of vaccination, vaccine formulation and injection volume (Drangsholt et al., 2011a), as well as water temperature and fish size (Aucouturier et al., 2001; Berg et al., 2007; Berg et al., 2006). For adhesions and melanin deposits significant heritable components have also been detected (Drangsholt et al., 2011a; Gjerde et al., 2009). Further, in a pilot study by Gjerde et al. (2009) no evidence was found of unfavorable genetic correlations between vaccine-induced side effects and survival of unvaccinated fish in challenge tests (furunculosis and ISA), indicating that the genetic variation in these traits can be attributed to different components of the immune systems. However, unfavourable genetic correlations were found between harvest body weight and adhesions scores (-0.45 ± 0.10) and melanin scores (-0.27 ± 0.11).

The main objective of this study was to obtain reliable estimates of the genetic correlation between vaccine-induced side-effects and resistance to furunculosis in vaccinated and unvaccinated fish and resistance to ISA in Atlantic salmon, and further to obtain genetic correlations between the mentioned traits and body weight at harvest.

2 Material and methods

2.1 Fish material

The Atlantic salmon used in this study were from 279 families; i.e., the offspring of 140 sires and 279 dams from SalmoBreed's breeding nucleus (2007 year-class). The fish had been selected for increased resistance to furunculosis and ISA based on challenge

tests with unvaccinated fish for two generations (grandparents and parents of the studied fish) but also for increased growth, improved fillet color and reduced fillet fat. The families were produced in November 2006 by Bolaks, Eikelandsosen, Norway and transferred to Nofima Marin, Sunndalsøra, Norway in January 2007. The families were kept in separate tanks until four subsamples were PIT-tagged (July - September 2007), and families were mixed after this.

This study included four groups (subsamples):

- Vaccinated fish challenged with *Aeromonas salmonicida* (causing furunculosis) (FUR-V)
- Unvaccinated fish challenged with *A. salmonicida* (FUR)
- Unvaccinated fish challenged with ISA-virus (causing ISA) (ISA)
- Vaccinated fish sampled 12 months after sea transfer and assessed for adhesions- and melanin scores and harvest body weight (SW12).

Each group consisted of a random sample of 10-15 fish per family. The unvaccinated FUR and ISA groups included fish from all the 279 families, and were tested as a part of SalmoBreed's breeding program, while the vaccinated FUR-V group was from a random subsample of 150 of the 279 families; i.e., the offspring of 150 dams and 85 sires. The SW12 group included fish from 144 families as fish from six families were used for another, unrelated experiment. Descriptive statistics of each group are shown in Table 1.

2.2 Vaccination

The FUR-V group was vaccinated in October 2007, and the SW12 group in March 2008. The same vaccine was used at both vaccinations; a commercial water-in-oil emulsion (ALPHA JECT 6-2) containing antigens of *A. salmonicida*, *Vibrio anguillarum* serotype O1 and O2, *V. salmonicida*, *Moritella viscosa* and infectious pancreatic necrosis (IPN) virus, which protects against furunculosis, classical vibriosis, cold water vibriosis, winter ulcer and IPN, respectively. Vaccines were produced by PHARMAQ AS (Oslo, Norway), and all fish were vaccinated manually. Body weight and age at vaccination is shown in Table 1.

2.3 Challenge tests

Challenge tests were performed by co-habitation at VESO Vikan (Namsos, Norway) in October 2007 (FUR, ISA) and in November and December 2007 (FUR-V) (Drangsholt et al., 2011b). The FUR-V group was tested for disease resistance in one tank where shedders; i.e., naive fish injected intra-peritoneally (i.p.) with a lethal dose of *A. salmonicida,* were added into the same tank as the vaccinated fish at three different time points (day one, day five and day 32) during the challenge period. The unvaccinated FUR and ISA groups were challenged using the same method in separate tanks and shedders i.p. injected with *A. salmonicida* and ISA-virus, respectively, were added at day one of the test. Individual records of whether the fish was dead or alive at the end of the challenge tests were used in the analysis as a measure of disease resistance. The challenge tests for the FUR and ISA groups were terminated at intermediate mortalities as this has been the standard procedure used in the breeding program (at maximum phenotypic variance for a binary trait like survival). The challenge test with the FUR-V group was terminated when mortalities had essentially ceased. Survival curves for the three groups are shown in Figure 1 and endpoint test survivals are shown in Table 1.

2.4 Vaccine-induced side effects and harvest body weight records

The SW12 group was transferred to an experimental net-cage (400 m^3) in the sea at Nofima Marin, Averøy in May 2008 (two and a half months after vaccination). In June 2009 (12 months after sea transfer, 14 months after vaccination) vaccine-induced side-effects and body weights were recorded. Adhesions were scored in three separate regions of the abdominal cavity (anterior, dorsal and posterior, and ventral part of abdominal cavity) using a scale from 0-6 (0 = no adhesions, 6 = extremely severe adhesions, interval 0.5) (Midtlyng et al., 1996). Melanin deposits of internal organs and abdominal wall were scored on a scale from 0-3 (0 = no visible melanin, 3 = severe melanin spots, interval 1.0). The evaluation was performed by four trained persons from PHARMAQ AS. In the analysis, adhesions score was defined as the average score across the three regions and melanin score as the average score across the organs and abdominal wall (Drangsholt et al., 2011a).

2.5 Statistical models

Estimates of (co)variance components for the six studied traits were obtained from linear sire-dam models using the DMU software (Madsen and Jensen, 2007). For each trait *i* the model had the following general characteristics:

$$\mathbf{y}_i = \mathbf{X}_i\boldsymbol{\beta}_i + (\mathbf{Z}_{Si} + \mathbf{Z}_{Di})\mathbf{a}_{SDi} + \mathbf{e}_i$$

where

$\mathbf{y}_i$ is the vector of the observations for the trait *i*,

$\boldsymbol{\beta}_i$ is a vector of fixed effects of trait *i*, including effects of person responsible for recording (for adhesions and melanin), tank/net-cage by sex (for adhesions, melanin and body weight), regression coefficient on age of the fish, nested within tank/net-cage and sex (nested only for adhesions, melanin and body weight)

$\mathbf{a}_{SDi}$ is a vector of additive genetic sire-dam effects for trait *i*

$\mathbf{e}_i$ is a vector of random residuals for trait *i* and

$\mathbf{X}_i$ is the incidence matrix for the fixed effects for trait *i*,

$\mathbf{Z}_{Si}$ is the incidence matrix for the additive sire effect for trait *i*,

$\mathbf{Z}_{Di}$ is the incidence matrix for the additive dam effect for trait *i*,

Four sub-models were used due to convergence problems when all six traits were included simultaneously. The first model included adhesions score, melanin score and harvest body weight (SW12-group). The next three models included the challenge test survival traits (the FUR-V, FUR, ISA groups), one at the time, in addition to adhesions score, melanin score and harvest body weight (SW12-group).

Initially an effect common to full sibs, i.e. an environmental (tank) effect due to the separate rearing of the families until tagging and non-additive genetic effects common to full sibs, was included in the analyses. A likelihood ratio test (Lynch and Walsh, 1998) of models with and without this effect showed that it was not significantly different from zero ($P>0.05$) for any of the traits. However, for harvest body weight (SW12-group) the effect common to full sibs was 0.06 ± 0.10 ($P=0.10$). The effect common to full sibs was therefore only included for harvest body weight in the final analysis.

A test on whether a genetic correlation was significantly different from zero was obtained using a likelihood ratio test of two models where the genetic correlation was unconstrained and restricted to zero, respectively (Lynch and Walsh, 1998).

Heritability was calculated as:

$$h^2 = \frac{4\sigma^2_{sd}}{2\sigma^2_{sd} + \sigma^2_c + \sigma^2_r}$$

Where; σ^2_{sd} is the additive genetic sire-dam variance, σ^2_c is the common family variance (only included for body weight) and σ^2_e is the residual variance.

3 Results

3.1 Descriptive statics

Table 1 shows body weight at tagging, body weight and age at vaccination, age at start of challenge test, end of test survival, body weight at harvest, adhesions- and melanin scores.

The FUR and ISA groups were not vaccinated and could therefore be challenge tested at a slightly younger age compared to the FUR-V group. The SW12 group was older and heavier at vaccination than the FUR-V group.

Table 1. Mean and standard deviation (SD) for body weight at tagging, vaccination and harvest, age (days from first feeding) at vaccination and start of challenge, and adhesion and melanin score at harvest for each of the four fish groups.

	FUR-V		**FUR**		**ISA**		**SW12**	
Traits	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight at tagging (g)	11.5	3.4	34.9	12.3	34.4	12.2	11.5	3.4
Body weight at vaccination (g)	36.2	10.8	-	-	-	-	154	44
Age at vaccination (d)	206	15	-	-	-	-	361	15
Age at start of challenge (d)	256	15	198	18	199	18	-	-
End-of-test survival	61 %	-	28 %	-	36 %	-	-	-
Body weight at harvest (g)	-	-	-	-	-	-	2366	733
Adhesion scores (0-6)	-	-	-	-	-	-	1.68	0.65
Melanin scores (0-3)	-	-	-	-	-	-	1.49	0.62

FUR-V = Fish vaccinated with standard vaccine and challenged with *A. salmonicida*, N=2151
FUR = Unvaccinated fish challenged with *A. salmonicida*, N=4128
ISA = Unvaccinated fish challenged with ISA-virus N=4179
SW12 = Vaccinated fish evaluated for side effects 12 months after sea transfer, N=1567
- = not applicable

Figure 1 shows the survival curves for the fish groups in challenge tests. At the termination of the challenge tests there were 28% survivors in the FUR group, 36% in the ISA group and 61% in the FUR-V group.

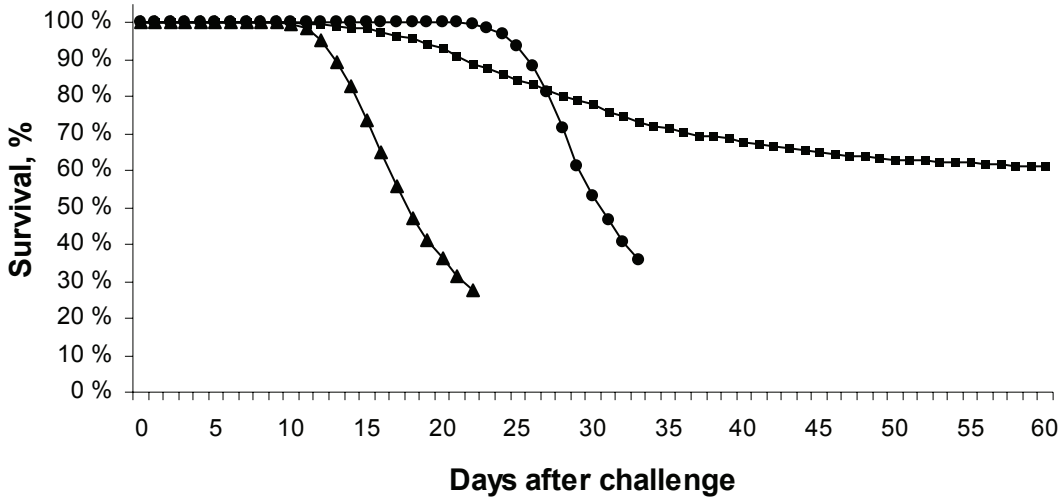

Figure 1. Survival curves for the two unvaccinated fish groups FUR (▲), ISA (●) and for the vaccinated group FUR-V (■) during challenge with *A. salmonicida* (FUR and FUR-V) and ISA virus (ISA). Challenges were carried out in three different tanks.

3.2 (Co) variance components

Table 2 shows estimates of the additive genetic sire and dam and residual variances and the heritabilities for the six studied traits. The magnitude of the heritability estimates were similar to previous estimates based on the same material (Drangsholt et al., 2011a; Drangsholt et al., 2011b).

Table 2. Additive genetic sire-dam variance (σ^2_{sd}), variance common to full-sibs (σ^2_c), residual variance (σ^2_e) and heritabilaties (h^2) with standard errors for each of the studied traits.

Trait	σ^2_{sd}	σ^2_c	σ^2_e	h^2
Suvival FUR-V	0.01 ± 0.003	-	0.21 ± 0.01	0.24 ± 0.04
Survival FUR	0.02 ± 0.002	-	0.17 ± 0.004	0.33 ± 0.04
Survival ISA	0.01 ± 0.002	-	0.21 ± 0.005	0.23 ± 0.03
Adhesion score	0.01 ± 0.003	-	0.29 ± 0.01	0.16 ± 0.04
Melanin score	0.03 ± 0.003	-	0.40 ± 0.01	0.29 ± 0.06
Body weight	38977 ± 15350	33298 ± 21352	414870 ± 15349	0.30 ± 0.10

FUR-V = Fish vaccinated with standard vaccine and challenged with *A. salmonicida*
FUR = Unvaccinated fish challenged with *A. salmonicida*
ISA = Unvaccinated fish challenged with ISA-virus
- = not applicable

Table 3 shows the genetic correlations between the traits. The genetic correlation between harvest body weight and resistance to furunculosis in vaccinated fish was low but negative (-0.36 ± 0.16) and thus unfavourable. The high genetic correlation (0.87) and the medium phenotypic correlation (0.53) between adhesion score and melanin score is in accordance with previous estimates based on the same material (Drangsholt et al., 2011a). None of the other genetic correlations were significantly different from zero ($P>0.05$). The phenotypic correlation of adhesions score with body weight was nearly zero and low and was negative (-0.17) for melanin score with body weight.

Table 3. Genetic and residual correlations (± se) between the studied traits.

	Adhesion	**Melanin**	**Body weight**
Genetic correlations			
Survival, Fur-V	0.063 ± 0.16	0.03 ± 0.15	-0.36 ± 0.16*
Survival, FUR	0.13 ± 0.16	-0.16 ± 0.14	-0.18 ± 0.17
Survival, ISA	0.24 ± 0.16	-0.0001 ± 0.15	0.05 ±0.17
Adhesions	-	-	0.05 ± 0.19
Melanin	0.87 ± 0.06	-	-0.08 ± 0.17
Residual correlations			
Adhesion	-	-	-0.02 ± 0.03
Melanin	0.56 ± 0.02*	-	-0.20 ± 0.03*
Phenotypic correlations			
Adhesion	-	-	-0.01
Melanin	0.49	-	-0.11

*Significantly different from zero ($P<0.05$)
FUR-V = Fish vaccinated with standard vaccine and challenged with *A. salmonicida*
FUR = Unvaccinated fish challenged with *A. salmonicida*
ISA = Unvaccinated fish challenged with ISA-virus
- = not applicable
se = standard error

4 Discussion

4.1 Survival and side effects

Our results show that the genetic correlations between side effects of vaccination (abdominal adhesions and melanin deposits) and survival from furunculosis and ISA of unvaccinated fish were not significantly different from zero and in agreement with Gjerde et al. (2009). Furthermore, it was also found that the genetic correlation between vaccine-induced side-effects and survival from furunculosis of vaccinated fish were not significantly different from zero. The results suggest that the immunological reactions causing side-effects of vaccination are genetically different from those of both the innate and the adaptive immune response against the disease challenge.

4.2 Body weight and survival

The genetic correlations between harvest body weight (12 months after sea transfer) and survival of unvaccinated fish (the FUR and ISA groups) were not significantly different from zero, and thus in agreement with Gjerde et al. (2009). However, our results indicate a low but negative and thus unfavourable genetic correlation between survival of vaccinated fish (FUR-V) and body weight at harvest (-0.36 ± 0.16). The difference in correlations between survival of unvaccinated and vaccinated fish and growth is supported by earlier findings (based on the same family material) that showed that survival of unvaccinated (FUR) and vaccinated (FUR-V) fish were partially different traits (r_g = 0.32 ± 0.13) (Drangsholt et al., 2011b). It can further be hypothesised that the most resistant fish after vaccination are directing more resources into immunological reactions and less into growth and this may help explain the negative genetic correlation between survival of vaccinated fish and body weight at harvest. This issue should be further investigated thru other studies.

It has been shown that whether an animals is susceptible or not (susceptibility status) and endurance (time until death of susceptible animals) can be genetically different traits (moderately genetically correlated) (Salte et al., 2010; Ødegård et al., 2011b). The challenge tests performed with unvaccinated fish in this study (the FUR and ISA groups) were terminated before mortality had ceased, while in the vaccinated groups mortality had essentially stopped. Thus, the observed survival of vaccinated fish might to a large extent be determined by susceptibility status while survival of unvaccinated fish possibly is mainly determined by the fish ability to survive until the termination of the test (endurance). This may have influenced the genetic correlations obtained. Further studies may be needed to optimize challenge testing as a tool in breeding programs.

4.3 Body weight and side effects

The genetic correlations between harvest body weight and adhesions or melanin deposits were close to and not significantly different from zero, indicating that growth rate and susceptibility to vaccine-induced side-effects are genetically independent traits. In addition, the phenotypic correlation of body weight with adhesions and with melanin was low. These results are in agreement with the results of Aunsmo et al. (2008) who found that neither adhesions score nor melanin score were phenotypically associated

with body weight recorded eight and 16-20 months after sea transfer (harvest). However, our results are in contradiction to the negative genetic and phenotypic correlations between harvest body weight and adhesions (r_g = -0.45 ± 0.10; r_p = −0.26 ± 0.02) or melanin (r_g = -0.27 ± 0.11; r_p= -0.18 ± 0.02) scores reported by Gjerde et al. (2009). For adhesion score, the disagreement may be associated to the much higher average adhesion score in the latter study (2.5 vs. 1.46 in this study and 1.7 in (Aunsmo et al., 2008). Lower adhesions scores indicates less severe inflammatory responses and it is thus likely that the katabolic cost of this response, which can affect growth negatively, was lower. However, the average melanin score in the present study (1.45) was higher than the average score (1.0) in Gjerde at al. (2009) and Aunsmo et al. (2008). The type of vaccine used must also be taken into consideration and will likely affect the results. Consequently, our results are therefore comparable to what was reported by Aunsmo et al. (2008) where the same vaccine was used (ALPHA JECT 6-2). The results from Gjerde et al. (2009) are less comparable as a different vaccine was used in that study.

4.4 Practical implications

Our results indicate that the genetic correlations between the traits studied (resistance to furunculosis and ISA, vaccine-induced side effects and growth until harvest) are not significantly different from zero (resistance of unvaccinated fish) or low (resistance of vaccinated fish). Therefore it is to be expected that the currently selection approach for increased resistance to furunculosis and ISA based on challenge test survival data of unvaccinated fish will not cause unfavourable correlated response in neither in harvest body weight nor vaccine-induced side effects. . An alternative and logical approach would be to select for increased disease resistance using vaccinated fish as most fish in the industry today are vaccinated and survival to furunculosis of unvaccinated and vaccinated fish seems to be different traits (Drangsholt et al., 2011b). The pro and contra of these two selection strategies are discussed in (Drangsholt et al., 2011b).

Acknowledgements

This study was funded by grant 179009/S40 from the Research Council of Norway and The Fishery and Aquaculture Industry Research Fund and PHARMAQ AS. Fish used in the project and challenge test data of the three unvaccinated fish groups were provided free of charge from SalmoBreed AS. The authors thank Kjellrun Gannestad at Nofima, Sunndalsøra and staff at Nofima, Averøy for their skilful assistance during data collection.

References

Aucouturier, J., Dupuis, L., Ganne, V., 2001. Adjuvants designed for veterinary and human vaccines. Vaccine 19, 2666-2672.

Aunsmo, A., Larssen, R.B., Valle, P.S., Sandberg, M., Evensen, Ø., Midtlyng, P.J., Østvik, A., Skjerve, E., 2008. Improved field trial methodology for quantifying vaccination side-effects in farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 284, 19-24.

Berg, A., Rødseth, O.M., Hansen, T., 2007. Fish size at vaccination influence the development of side-effects in Atlantic salmon (*Salmo Salar* L.). Aquaculture 265, 9-15.

Berg, A., Rødseth, O.M., Tangerås, A., Hansen, T., 2006. Time of vaccination influences development of adhesions, growth and spinal deformities in Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 69, 239-248.

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Fridell, F., Bentsen, H.B., 2011a. Quantitative genetics of vaccine-induced side effects in farmed Atlantic salmon (*Salmo salar*). Aquaculture, In press.

Drangsholt, T.M.K., Gjerde, B., Ødegård, J., Fridell, F., Evensen, O., Bentsen, H.B., 2011b. Quantitative genetics of disease resistance in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). Heredity, In press.

Gjedrem, T., Salte, R., Gjøen, H.M., 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture 97, 1-6.

Gjerde, B., Evensen, Ø., Bentsen, H.B., Storset, A., 2009. Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). Aquaculture 287, 52-58.

Gjøen, H.M., Refstie, T., Ulla, O., Gjerde, B., 1997. Genetic correlations between survival of Atlantic salmon in challenge and field tests. Aquaculture 158, 277-288.

Haugarvoll, E., Bjerkås, I., Szabo, N.J., Satoh, M., Koppang, E.O., 2010. Manifestations of systemic autoimmunity in vaccinated salmon. Vaccine 28, 4961-4969.

Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209, 59-76.

Kjøglum, S., Henryon, M., Aasmundstad, T., Korsgaard, I., 2008. Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. Aquacult. Res. 39, 498-505.

Koppang, E.O., Bjerkås, I., Haugarvoll, E., Chan, E.K.L., Szabo, N.J., Ono, N., Akikusa, B., Jirillo, E., Poppe, T.T., Sveier, H., Tørud, B., Satoh, M., 2008. Vaccination-induced systemic autoimmunity in farmed Atlantic salmon. The Journal of Immunology 181, 4807-4814.

Lynch, M., Walsh, B., 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, XVI, 980 s. pp.

Madsen, P., Jensen, J., 2007. DMU a package for analysing multivariate mixed models 6.4.7, University of Aarhus, Faculty Agricultural Sciences (DJF), Dept. of Genetics and Biotechnology, Research Centre Foulum Tjele

McLoughlin, M.F., Graham, D.A., 2007. Alphavirus infections in salmonids - a review. J. Fish Dis. 30, 511-531.

Midtlyng, P.J., Reitan, L.J., Speilberg, L., 1996. Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish Shellfish Immunol. 6, 335-350.

Mutoloki, S., Alexandersen, S., Evensen, Ø., 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. Fish Shellfish Immunol. 16, 633-644.

Ramstad, A., Romstad, A.B., Knappskog, D.H., Midtlyng, P.J., 2007. Field validation of experimental challenge models for IPN vaccines. J. Fish Dis. 30, 723-731.

Rauw, W.M., Kanis, E., Noordhuizen-Stassen, E.N., Grommers, F.J., 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Production Science 56, 15-33.

Salte, R., Bentsen, H.B., Moen, T., Tripathy, S., Bakke, T.A., Odegard, J., Omholt, S., Hansen, L.P., 2010. Prospects for a genetic management strategy to control *Gyrodactylus salaris* infection in wild Atlantic salmon (*Salmo salar*) stocks. Can. J. Fish. Aquat. Sci. 67, 121-129.

Silverstein, J.T., Vallejo, R.L., Palti, Y., Leeds, T.D., Rexroad, C.E., III, Welch, T.J., Wiens, G.D., Ducrocq, V., 2009. Rainbow trout resistance to bacterial cold-water disease is moderately heritable and is not adversely correlated with growth. J. Anim Sci. 87, 860-867.

Sommerset, I., Krossøy, B., Biering, E., Frost, P., 2005. Vaccines for fish in aquaculture. Expert Review of Vaccines 4, 89-101.

Standal, M., Gjerde, B., 1987. Genetic variation in survival of Atlantic salmon during the sea-rearing period. Aquaculture 66, 197-207.

Ødegård, J., Olesen, I., Gjerde, B., Klemetsdal, G., 2007. Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). Aquaculture 271, 173-177.

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011a. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquacult. Res. 42, 103-114.

Ødegård, J., Gitterle, T., Madsen, P., Meuwissen, T., Yazdi, M., Gjerde, B., Pulgarin, C., Rye, M., 2011b. Quantitative genetics of Taura syndrome resistance in Pacific white shrimp (*Penaeus vannamei*): A cure model approach. Genet. Sel. Evol. 43, 14.