Family-Based Genome-Wide Association Analysis of Genetic Resistance to *Lepeophtheirus salmonis* in Atlantic Salmon (*Salmo salar*) Using a 50K SNP Array: Implications for Aquatic Animal Health and Translational Genomics

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Abstract

The salmon louse, *Lepeophtheirus salmonis*, is an economically important parasite on aquaculture stocks of Atlantic salmon grown in sea cages. The aim of this study was to perform a family-based genome wide association study to identify the genomic architecture of salmon louse resistance in a commercial population of Atlantic salmon using five year classes of data. A total of 1756 fish were genotyped from 248 families, which were experimentally infected with *L. salmonis* copepodids, using a 50K SNP array designed specifically for North American Atlantic salmon. A within family-based association test for quantitative traits was run using QFAM in PLINK with salmon lice counts pre-adjusted for contemporary group as the phenotype. Salmon lice counts varied significantly among families and salmon lice resistance had an estimated heritability of 0.21 ± 0.03. After correcting for multiple testing, seven SNPs from three chromosomes surpassed a suggestive chromosome wide level. *Ssa*04 had the most suggestive SNP, and four other suggestive SNPs within a close range on the chromosome. A single SNP was suggestive on each of *Ssa*14 and *Ssa*20. The most suggestive SNP, and two suggestive tag SNPs, were located near a predicted gene on *Ssa*04 that codes for ceramide-1-phophate transfer protein which is involved with pathophysiological functions such as cell survival and inflammation. No loci with major effects were detected suggesting a polygenic trait architecture. However, several loci were detected which could be included directly in models used to estimate

genomic breeding values. Therefore, genomic selection should continue to be used to breed Atlantic salmon naturally more resistant to *L. salmonis* infection.

1. Introduction

The salmon louse, *Lepeophtheirus salmonis*, is the predominant marine ectoparasite found on wild and farmed populations of salmonids in the North Atlantic (Hogans, 1995). In farmed populations, the high density of salmon in commercial sea cages provides suitable hosts for salmon lice, resulting in high levels of infection (Torrissen et al., 2013). Salmon lice feed on the skin, mucus, and blood of Atlantic salmon causing lesions to the skin that can lead to osmoregulatory stress and secondary infection (Mustafa et al., 2001). A recent analysis suggests that salmon lice infection of salmon creates economic losses through treatment and product downgrading or loss of up to 9% of the value of the product (Abolofia et al., 2017). Further costs occur as salmon lice may be vectors for viral and bacterial diseases (Nylund et al., 1994; Barker et al., 2009). Therefore, the control of salmon louse infection is important to the aquaculture industry.

Treatments of salmon lice have not been fully effective in reducing the salmon louse problem in aquaculture. Chemotherapeutant treatments available for use are limited in number, and prolonged overuse can lead to a reduced efficacy due to the evolution of pesticide resistant salmon lice populations (Denholm et al., 2002; Westcott et al., 2004). Mechanical methods of lice control are being utilized such as high pressure water systems, and biological control appears promising such as use of cleaner fish (Powell et al., 2018). However, sea lice infections persist, so other methods of decreasing parasite load on aquaculture strains of Atlantic salmon are being investigated such as utilizing the genetic variation in salmon lice resistance within a population. Selective breeding has been utilized to select for other economically important traits in salmonid aquaculture such as growth and age of sexual maturation, through the use of traditional phenotype based selection (Gjerde 1986; Quinton et al., 2005). However, phenotype based selection can be complicated in breeding programs where phenotypes cannot be collected on individuals in the breeding nucleus, such as when the nucleus is kept in freshwater where salmon lice will not survive the low salinity (Goddard et al., 2010; Bricknell et al., 2006). Sib-testing, where traits are measured on siblings of the candidate broodstock is performed in this case, but it will only provide pedigree-based estimated breeding values (EBVs) at the full-sib family level

that are based on the mid-parent mean for candidate broodstock (Leeds et al., 2010; Ødegård et al., 2014). Breeding programs for pathogen resistance based solely on the family EBV, often will favour close relatives for breeding. As reduced inbreeding is also a breeding objective, this will reduce selection for these sib-tested traits in comparison to individual level traits (Ødegård et al., 2014; Vallejo et al., 2017). Including genomic information would allow for individual level breeding values to be calculated by connecting the genomic information of the challenged siblings and breeding candidate siblings (e.g., Schaeffer et al., 2018).

Several studies have looked at genetic variation of resistance to the salmon louse in the European subspecies of Atlantic salmon (Glover et al., 2005; Kolstad et al., 2005; Gjerde et al., 2011; Tsai et al., 2016) as well as other species of sea lice (*Caligus rogercresseyi*, Lhorente et al., 2012; Correa et al., 2017; Robledo et al., 2019). These studies have estimated a moderate heritability for sea lice resistance in their populations of Atlantic salmon ($h^2 = 0.22 – 0.33$) in highly controlled laboratory settings (reduced environmental variation). This indicates that there is enough genetic variation within these populations for selective breeding to increase the level of resistance to sea lice. Kolstad et al. (2005) determined that the genetic correlation between salmon lice resistance measured in a laboratory and resistance measured in a cage site was high (0.88 ± 0.26). Therefore, measuring salmon louse resistance in a controlled laboratory environment is a suitable substitute to measuring salmon louse resistance in natural infections at cage sites. To date only one study has been conducted on salmon lice resistance in the North American subspecies of Atlantic salmon (Rochus et al., 2018). This study determined the heritability of salmon louse counts to be 0.17 ± 0.04 using a three generation pedigree, and using approximately 3K single nucleotide polymorphisms (SNPs) from the parents of offspring challenged with salmon lice detected two significant SNPs associated with lice count (Rochus et al., 2018).

High density SNP arrays have been developed specifically for Atlantic salmon, allowing for large amounts of genotypic data to be collected on individuals (Houston et al., 2014a). This genetic information, that spans the entire genome, is useful for dissecting the genetic basis of a variety of economically important traits through the use of genome wide association (GWA) studies (Johnston et al., 2014; Correa et al., 2015; Correa et al., 2017). GWA analysis can provide insight into the genetic architecture of various disease traits, helping identify regions of the genome associated with these traits. GWA analysis utilizes SNP markers spaced across the

entire genome and phenotypes of the trait of interest to make associations between the genetic variants and the trait (Goddard and Hayes, 2007).

The aim of this study is to perform a GWA analysis for resistance to the salmon louse in the Saint John River (SJR) aquaculture population of North American Atlantic salmon to determine the genetic architecture of salmon lice resistance and identify any regions of the genome associated with resistance using genotypes of lice challenged fish for application of genomic selection.

2. Methods

2.1 Study Population

Sea cage culture in Atlantic Canada uses primarily the SJR strain of North American Atlantic salmon founded from salmon returning to below the Mactaquac Dam on the SJR (Farmer, 1991; Friars et al., 1995; Glebe, 1998; O'Flynn et al., 1999). This is due to environmental suitability as well as concerns regarding the impacts of introgression on wild Atlantic salmon stocks (Glebe, 1998). However, this aquaculture population of Atlantic salmon has been historically hybridized with a few Atlantic salmon from European origins (Glebe, 1998), and evidence of this historical admixture can still be detected within the genome (Liu et al., 2017). Atlantic salmon from European origins and from North American origins are considered to be separate subspecies (Verspoor, 1997; King et al., 2007; Bourret et al., 2013) and the two subspecies have different chromosome numbers and chromosomal arrangements (Brenna-Hansen et al., 2012). Salmon from five different year classes, indicating different hatch years (2010, 2011, 2013, 2014, 2015) were exposed to salmon louse, approximately 18 months from hatch date (see Table 1).

2.2 Disease Challenges

As the salmon louse is a saltwater parasite, challenges directly on the broodstock were not performed. Broodstock in this hatchery are kept in freshwater their entire lives for biosecurity reasons, therefore, challenges were performed on the siblings of the candidate broodstock.

All challenges were conducted at the St. Andrews Biological Station (Fisheries and Ocean Canada, St. Andrews, New Brunswick, Canada). This laboratory had access to filtered and UV sterilized ocean water with a flow through system design. Fish were smolted on site and allowed to acclimate for one week prior to infection with freshly molted *L. salmonis* copepodids. Fish were placed in tanks (2010-2013 1000L size and 2014-2015 3000L) with stocking density not exceeding 30 kg/m^3. Salmon lice egg strings were collected from New Brunswick cage sites for each year of challenge and reared to the copepodid stage in flow through ocean water hatcheries.

The methods used to infect post-smolts with the salmon louse for the 2010 and 2011 challenges can be found in Rochus et al. (2018). The 2013, 2014, and 2015 challenges followed a similar methodology. One difference was the increase in the number of lice added to the tanks to 90-100 copepodids per fish, as this was successful in the 2011 challenge. The 2010 challenge had 30 lice per fish added which was insufficient, so another 60 lice per fish were added four days post initial infection. This was done to increase the infection pressure, and ideally allow for greater visualization of the variation in lice resistance due to a wider normal distribution of phenotypes, and thus more precise ranking of resistance within families. Additionally, in years where the larger 3000L tanks were used systematic stirring of the water was done to promote lice dispersion once added to the water.

The lice were permitted to grow until reaching the chalimus II stage, the largest attached life history stage of the louse (Hamre et al., 2013), which made accurate salmon lice counts easier. Lice that were attached to the fin and the body of the fish were used to represent lice resistance/susceptibility as a quantitative trait ‘lice count’. Although lice were noted to attach within the mouth and gills, these were not included in the phenotype as these lice appear to be a product of the infection process and show little variation among families (S. Leadbeater and M. Holborn, personal observations). Any fish that showed signs of deformities such as scoliosis or lordosis, or signs of sexual maturity were removed from the dataset as these conditions may alter the lice load.

The heritability of salmon lice resistance, measured as lice count, was estimated using a Bayesian approach with data from 4699 fish with recorded lice count phenotypes from the five years of challenges. The heritability estimate was performed using a custom program (designed by L.R. Schaeffer, unpublished). The model included pedigree information from 639 ancestors

up to and including the grandparents of the 2010 year class. The heritability estimate was based on 40,000 Gibbs samples after an 8000 burn-in using the linear mixed model:

$$y_{ijk} = \mu + cg_i + s_j + a_k + e_{ijk} \quad (1)$$

where, y_{ij} is the lice count phenotype, μ is the overall mean of the population, cg_i is the fixed contemporary group effect which includes year and tank, s_j is the fixed sex effect, a_j is the random animal effect from the pedigree, and e_{ij} is the residual.

2.3 Genotyping

A sample of fin clip was taken from each fish challenged during phenotyping and stored in 95% ethanol. For each challenge, the fish were ranked according to lice count within their families using the residual of a linear regression which incorporated tank effects, and where applicable the top four and bottom four fish from each family were selected for genotyping. Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen) to produce high quality and high molecular weight DNA suitable for genotyping on a high density SNP array. The extracted DNA was sent to CIGENE (Ås, Norway) for genotyping on the NA *Ssa*50K Affymetrix Axiom SNP array, designed specifically for the North American Atlantic salmon subspecies. Some fish from the 2010 and 2011 challenges from the study by Rochus et al. (2018) were also included. However, the number of genotyped individuals from the 2011year class was low due to the degradation of tissue because of ethanol evaporation in the fin clip tubes resulting in low yields of DNA.

SNPs were exported from Axiom Analysis Suite (version 1.1.1.66) if they presented two (NoMinorHom) or three (PolyHighResolution) well-separated clusters (FLD ≥5). Only SNPs that were clearly in a diploid region of the Atlantic salmon genome were used.

The dataset was filtered for Mendelian errors using PLINK to ensure no difference in allele states between parent and offspring at the same SNP. Quality control thresholds were set at a SNP call rate of >0.95, a sample call rate of >0.9, a minor allele frequency (MAF) of >0.01, and for SNPs in Hardy-Weinberg equilibrium (p value < 0.001). The QFAM test was performed

using 100,000 permutations. P values from the within family test were visualized using the qqman package in R (version 3.3.2) using the *manhattan* function (Turner, 2014).

2.4 Association Analysis

Historical admixture can be problematic in association analyses as allele frequencies between populations can differ substantially (Bourret et al., 2013). We used STRUCTURE (version 2.3.4) to estimate individual ancestry coefficients (K = 2) based on the method described in Liu et al. (2017). Individuals with 90% or greater North American ancestry were to be included in the GWA analysis as described in Holborn et al. (2018). However, standard GWA methods such as GenABEL were not equipped for the high level of admixture within this multiple year class dataset, and based on the STRUCTURE analysis many fish were to be removed due to historical admixture which would greatly reduce the statistical power. Therefore, other association analysis methods were investigated that were more robust to population stratification so that all available genotypes could be included from all challenged families which would also be more genetically representative of the population of study.

For the association analysis between phenotypes and genotypes we used the family-based association test for quantitative traits (QFAM) function in PLINK (version 1.9), which performs a linear regression of phenotype on genotype method followed by permutation at the family level (Purcell et al., 2007). This method is suitable for this dataset as the QFAM-within procedure is robust to population stratification because the test is family-based (within-sibling) and the permutation procedure corrects for relatedness between families (Purcell et al., 2007; Duijvesteijn et al., 2010). Therefore, genotypes were not removed from the dataset due to population stratification. The QFAM procedure has been used successfully in aquaculture populations to detect SNPs associated with disease resistance in channel catfish and blue catfish hybrids (Geng et al., 2016; Geng et al., 2017) and SNPs associated with early maturity in post-smolt Atlantic salmon (Boulding et al., 2018).

As the QFAM association analysis does not allow for the inclusion of covariates, the lice count phenotype needed to first be adjusted for contemporary group. A linear transformation was applied where the residual mean for each contemporary group was subtracted from the lice

counts of fish within that contemporary group. To avoid negative phenotypic values, a value of 50 was then added to each louse count as this shifted the smallest phenotypic value to a positive value. As QFAM uses a permutation procedure no further transformation was applied to make the data normally distributed (Purcell et al., 2007).

Some families had a low number of genotyped fish represented which is problematic with a family-based association test when parent genotypes are not available (Abecasis et al., 2000). The within family component of association is calculated using parent genotypes, or when parent genotypes are unavailable the parent genotypes are estimated using the genotypes of siblings within a family which may be inaccurate due to small sampling size (Abecasis et al., 2000; Purcell et al., 2007). Therefore, as many parent genotypes as possible were included within the analysis to include the small families. This is important as the higher number of families in the analysis the more statistical power to detect SNP associations as sample size will be greater (Spencer et al., 2009).

As this GWA method involved individual SNP testing, we accounted for multiple testing using Bonferroni correction (Korte and Farlow, 2013; Armstrong, 2014). Bonferroni correction is known to be overly conservative, therefore a less conservative Bonferroni correction threshold (p value $< 0.10/N$, where N is the total number of SNPs tested), also referred to as a suggestive threshold, was used. A suggestive threshold has been used in previous studies for initial discovery of regions of the genome associated with a trait (Geng et al., 2017). A suggestive chromosome wide threshold was also considered (p value $< 0.10/C$, where C is the total number of SNPs tested for each respective chromosome).

Using the resource SalmoBase, an integrated molecular data resource for salmonid species (Samy et al., 2017), and the locations of the most suggestive SNPs, we predicted candidate genes and identified gene products.

3. Results

The narrow sense heritability of salmon lice resistance for these five year classes of data was estimated to be 0.21 ± 0.03. This estimate was based on non-transformed lice counts.

A total of 1756 fish from 248 families from five year classes had both salmon lice count phenotypes and had been genotyped on a high density SNP array were used for the GWA analysis. The number of genotyped fish per family ranged from 1 individual to 14, with an average of 7.1 fish per family (SD = 2.6). After adjusting the raw phenotypes for contemporary group, the average lice count was 50.8 lice (SD = 21.1) with a louse count range of 4.7 to 183.6 lice per fish (Figure 1). After removing families with less than three phenotyped individuals, the average family lice load ranged from 31.26 to 99.12 lice per fish (Figure 2).

A total of 317 parent genotypes were available for inclusion in the analysis. However, 35 families had no parental genotypes available as earlier year classes have gaps in parental genotyping. The families that have no parental genotypes however have a minimum of 7 fish per family genotyped up to 12 fish per family, which provides sufficient SNP allele information for proper execution of the QFAM test in PLINK (Abecasis et al., 2000; Purcell et al., 2007). Additionally, 36 families had at least one parent genotyped, and 177 families had both parents genotyped.

A total of 46,538 SNP markers passed initial screening and were exported from Axiom Analysis Suite. Subsequent quality control through PLINK resulted in 37,118 SNPs and all 1756 offspring and 317 parent samples passed all quality control measures. No SNPs surpassed the suggestive genome wide level, however, several SNPs surpassed the suggestive chromosome wide level (Figure 3). Seven SNPs from three chromosomes were suggestive at the chromosome wide level (Table 2).

The chromosome wide suggestive SNPs on chromosome *Ssa*04 are located relatively close together. SNPs 4a, 4c, and 4d are located consecutively on the SNP array, and therefore form a peak on the resulting Manhattan plot (Figure 3). All five suggestive SNPs on *Ssa*04 are within a 220 SNP range on the SNP array, which represents a 9.4 Mb span of the chromosome.

The most suggestive SNP, 4a, was located at 46.6 Mb, and the tag SNPs, 4c and 4d, are adjacent. Two potential gene products are within the region: ceramide-1-phophate transfer protein and acetylcholine receptor subunit gamma-like isoform. The suggestive SNP on chromosome *Ssa*14 is located at 31.3 Mb had one gene located within 31.2 and 31.4 Mb, with a gene product of protein Wnt-9a. The suggestive SNP on chromosome *Ssa*20 located at 78.6 Mb had two genes located within 78.5 and 78.7 Mb, with gene products of sialidase-4-like protein and P2Y purinoceptor 13-like protein.

4. Discussion

The results of the salmon lice challenges confirm significant variation for lice counts among families in this population of Atlantic salmon. This is demonstrated with the moderate estimated heritability of 0.21 ± 0.03, which is within the range of previous heritability estimates for salmon lice resistance in Atlantic salmon (0.17-0.33; Kolstad et al., 2005; Gjerde et al., 2011; Houston et al., 2014b; Ødegård et al., 2014; Tsai et al., 2016; Rochus et al., 2018). The large range in average family lice loads indicated genetic variation for lice resistance in the SJR strain. Heritable variation in lice counts among families has already been determined in this population by Rochus et al. (2018), but in this study three more year classes were included. Other studies have found heritable variation for lice resistance in different populations of Atlantic salmon (Glover et al., 2005; Kolstad et al., 2005; Gjerde et al., 2011; Tsai et al., 2016). Pre-adjusting the phenotypes for contemporary group before the QFAM analysis narrowed the range in lice counts. However, this adjustment accounted for both variation in lice challenges among different tanks, including any variation in lice number and viability/activity, as well as variation among infection years.

The results of the QFAM association analysis indicated several SNPs suggestive at the chromosome level on *Ssa*04. Five SNPs surpassed a suggestive chromosome wide threshold on *Ssa*04, and these SNPs where located within a relatively small range on the chromosome. Three SNPs (4a, 4c, and 4d) within a small region of the genome indicated the presence of a QTL in this region of the genome, as multiple SNPs in the area were associated with lice count. This 0.2 Mb range of the genome has two predicted genes, LOC106603603 (+ strand) and LOC106603604 (- strand). LOC106603603 codes for the protein acetylcholine receptor subunit gamma-like isoform which is part of the neuroactive ligand reception interaction pathway. LOC106603604 codes for ceramide-1-phophate transfer protein which is important in physiological processes associated with disease such as cell survival and inflammatory signaling (Gómez-Muñoz et al., 2005).

No SNP from the association analysis surpassed the suggestive genome wide threshold, therefore we conclude that there was no evidence in this study for a major locus that accounts for

a substantial proportion of the phenotypic variation in lice count (Figure 3). This is consistent with other studies from both the European subspecies (Tsai et al., 2016) and the North American subspecies (Rochus et al., 2018).

Four hypotheses might explain why Rochus et al. (2018) detected two experiment wide significant SNPs located on *Ssa*01 and *Ssa*23 using 2010 and 2011 data whereas the current study detected SNPs associated with lice counts on chromosomes *Ssa*04, *Ssa*14, and *Ssa*20. The first is that Rochus et al. (2018) did not genotype lice challenged offspring, as was done here, but instead used salmon lice counts and seawater weights from the offspring along with parental freshwater weights to estimate EBVs for lice counts for the parents and other relatives. They then used GenABEL (Price et al., 2006; Aulchenko et al., 2007) to find associations between the EBVs for lice counts and their SNP genotypes. Their analysis used the *egscore* function after correcting for population stratification using the first two principal components calculated from the genomic relationship matrix. This method resulted in considerably more genomic inflation than was seen in the current study (their Fig. 2b) although they attempted to correct by adjusting the p value by dividing the test statistic by the genomic inflation factor, lambda. The second hypothesis is that Rochus et al. (2018) used genotypes at 3034 SNP loci for a total of 299 fish of which only 155 were parents of salmon lice challenged fish from 90 full-sibling families whereas the current study used 37,118 SNP genotypes of 1756 offspring and 317 of their parents from 248 families. The almost three-fold increase in the number of families increased the statistical power to detect associations and the ten-fold increase in the number of SNPs covered a higher proportion of the genome. The third hypothesis is that generation interval within this breeding nucleus has historically been four years (Liu et al. 2017) and the GWA by Rochus et al. (2018) used two isolated breeding lines or populations, whereas this study used five year classes including two overlapping lines/populations (2010 and 2014 as well as 2011 and 2015) and therefore had more related individuals. A fourth hypothesis, however, is the reason that the location of SNPs between these two studies does not overlap is the presence of false positives in one or both studies. It has been shown that using EBVs as phenotypes in GWA studies, as was done in the Rochus et al. (2018) analysis, can inflate the number of false positives (Ekine et al., 2014).

The merging of data from all five lice challenges, with different fish sizes, means that the trait analyzed in this GWA analysis was lice resistance at all fish sizes within the range in this

study. Separating each year class would also introduce year class effects into the results, where a SNP may be falsely suggestive or significant, but will also only test for associations at that life stage. Phenotypes recorded at different life stages would be considered different traits, although potentially genetically correlated (Falconer, 1952). Therefore, combining the datasets allows for QTL to be detected that are associated with salmon lice resistance across a size range, which is more comparable with production fish at sea cages during saltwater grow out.

SNPs from three different chromosomes were suggestive at the chromosome wide level indicating that salmon louse resistance in this population of North American Atlantic salmon is a polygenic trait, which was also concluded by Rochus et al. (2018) for this same commercial population. The salmon lice resistance trait has also been shown to be polygenic in other commercial populations (Tsai et al., 2016; Robledo et al., 2019). Therefore, rather than including only SNPs that are significantly associated with salmon lice load into a breeding program, genomic selection which integrates either all available SNPs or a large subset should be used to add salmon lice resistance into the breeding program. The suggestive SNPs found to be associated with salmon lice resistance in this GWA study can be added to the subset of SNPs currently being used for genomic breeding values. Genomic selection approaches have been recommended by studies with European Atlantic salmon subspecies for the salmon louse (Tsai et al., 2016) and *C. rogercresseyi*, a louse species common in Chile (Correa et al., 2017).

Genomic selection for salmon louse resistance is becoming an integral part of an integrated pest management program. This modern approach includes a variety of traditional methodologies such as existing treatments for lice removal to novel ideas of salmon lice maintenance like the use of cleaner fish such as cunners and lumpfish, potential vaccination, and other developing innovations (Aaen et al., 2015; Yossa and Dumas, 2016). Utilizing genomic selection to enhance salmon louse resistance in this population of Atlantic salmon has already been implemented in breeding programs, and results from this study support its continued use. However, noticeable differences in salmon lice counts may take many years as inbreeding needs to be avoided while strongly selecting for increased resistance (Ødegård et al., 2014) and the long generational interval of commercial Atlantic salmon of four years (Liu et al., 2017). Norwegian aquaculture has already seen a measurable increase in resistance within lice resistance strains, but application to Canadian aquaculture could differ depend on breeding objectives. The lice count phenotypes were transformed, making the resulting estimated SNP

effects more difficult to interpret, and lice counts being higher than those encountered in sea cages due to higher lice infection pressure in the study. The genetic correlations between other economically important traits such as growth and age at sexual maturity and salmon louse resistance should be investigated before full scale integration into the breeding program. If two important traits have a negative correlation a weight needs to be placed on each trait based on the level of importance to the company's specific breeding strategy (Gjedrem, 2000; Quinton et al., 2005).

5. Conclusion

This was the first GWA analysis for salmon louse resistance in North American Atlantic salmon performed using high density genotypes of the challenged fish. This GWA analysis detected seven suggestive SNPs at a Bonferroni threshold at the chromosome wide level located on three chromosomes. Chromosome *Ssa*04 had five of the suggestive SNPs within a relatively small region of the chromosome, which suggests at least one QTL for the trait of salmon lice load is present nearby. The most suggestive SNP on *Ssa*04 is near a predicted gene which codes for ceramide-1-phophate transfer protein which is involved with pathophysiological functions such as cell survival and inflammation. Further investigation into the roles of these genes and gene products is required to make any concrete conclusions about the effects of these genes on salmon lice resistance or susceptibility, but should be cautioned as the QTL within this region is only suggestive. As salmon lice resistance is a polygenic trait, these seven SNPs should be integrated into a larger subset of SNPs used for generating genomic estimated breeding values to increase salmon lice resistance in this population.

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Author Contributions

CMR and SL assisted with the assisted with the design and execution of the salmon lice challenge (2010 and 2011 year classes). MKH and SL assisted with the design and execution of the salmon lice challenge (2013, 2014, 2015 year classes). MKH led and performed DNA extractions (all year classes), performed the genome wide association analysis, and prepared the manuscript. FP reared pedigreed fish. KPA, JAKE, and FP provided genetic material from their pedigreed year-classes for DNA extractions. EGB contributed with the filtering of SNPs, the translation of parental SNP genotypes, statistical analysis discussion, manuscript discussion, and overall supervision of the work of MKH. All authors have read and provided comments on the manuscript.

Ethics Statement

Fish handling and infection procedures were approved by the Fisheries and Oceans Canada Animal Care Committee. All fish handlers had the required animal care training. Fish were anesthetized with tricaine methanesulfonate (TMS) (0.1 g/L dosage) prior to any handling event. Fish were euthanized with a lethal dose of TMS prior to lice counting.

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Table 1. The average fish weight (± standard deviation) of number of fish and families challenged in salmon lice challenges and the subsequent number of fish and families genotyped with the NA Ssa50K SNP array for each year class of Atlantic salmon.

Year Class	Average fish weight in grams (± SD)	Number of challenged fish	Number of challenged families	Number of genotyped fish from the challenge	Number of genotyped families
2010	207.9 ± 96.8	790	41	377	41
2011	515.5 ± 198.5	942	47	82	39
2013	163.5 ± 56.3	563	56	386	52
2014	652.7 ± 119.1	732	53	421	53
2015	153.2 ± 46.1	1684	63	490	63
Total	___	4711	260	1756	248

Table 2. Suggestive SNPs associated with salmon lice resistance at the suggestive chromosome wide level (p-value < 0.10/C, where C is the total number of SNPs tested for each respective chromosome) from three chromosomes.

SNP	Chromosome	Position	MAF	Beta*	P value
4a	*Ssa*04	46.4 Mb	0.12	0.3764	0.00002
14	*Ssa*14	31.3 Mb	0.070	-2.18	0.00002
4b	*Ssa*04	41.9 Mb	0.17	1.036	0.00005
20	*Ssa*20	78.6 Mb	0.079	-1.268	0.00005
4c	*Ssa*04	46.6 Mb	0.18	0.3741	0.00006
4d	*Ssa*04	46.6 Mb	0.13	0.116	0.00006
4e	*Ssa*04	51.4 Mb	0.24	-0.06071	0.00006

*Beta represents the regression co-efficient and the direction of the SNP effect.

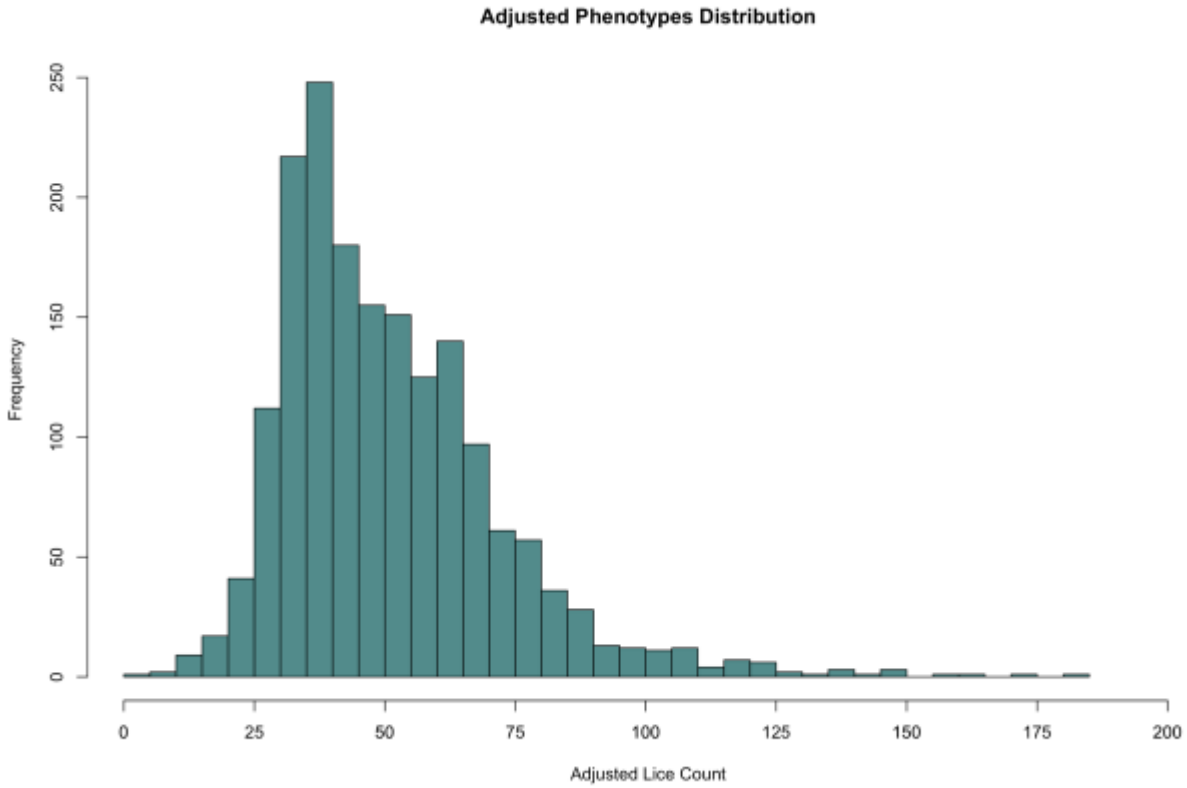

Figure 1. Distribution of adjusted lice count for all genotyped Atlantic salmon. The average lice load was 50.8 (SD = 21.1), with a range of 4.7 to 183.6 lice per fish. Although the range is large, most fish had lice load between 25 and 35 (median lice count of 28).

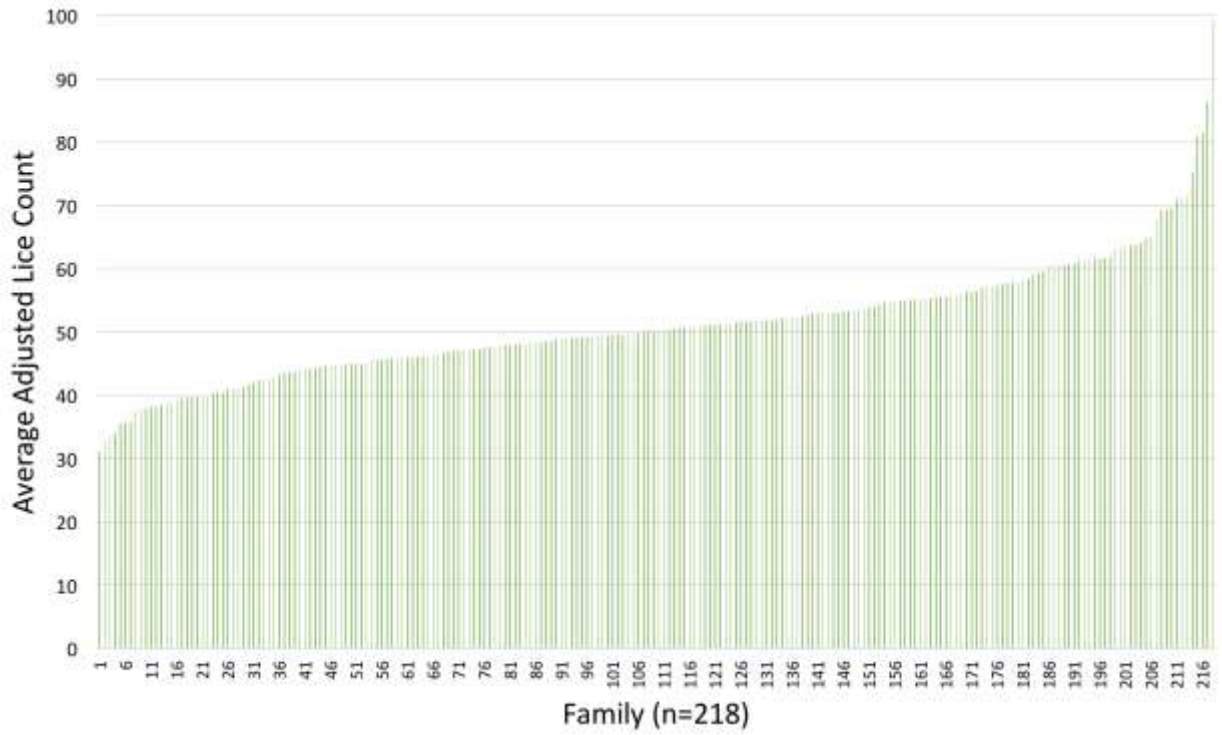

Figure 2. Average adjusted lice count per family, only considering families with three or more individuals (218 families out of 248).

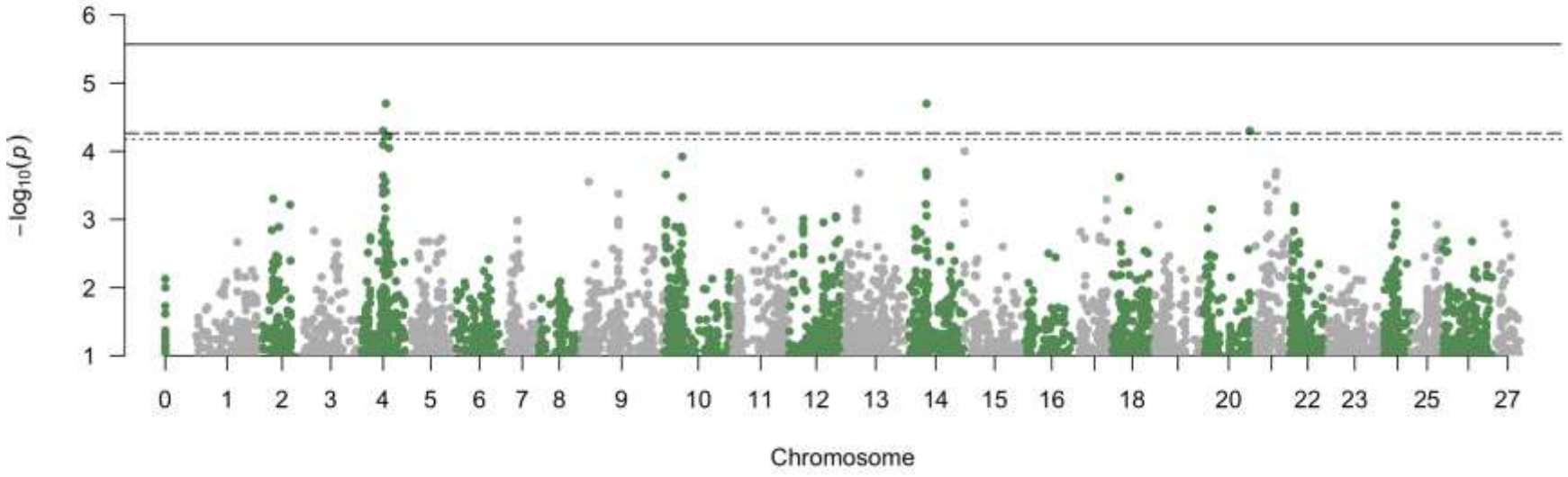

Figure 3. Genome wide association study for salmon louse resistance measured as total lice count. The solid line represents the suggestive genome wide Bonferroni threshold, the dashed line represents the suggestive chromosome wide Bonferroni threshold for *Ssa*14 ($-\log_{10}(4.26)$), and the dotted line represents the suggestive chromosome wide Bonferroni thresholds for *Ssa*04 and *Ssa*20 ($-\log_{10}(4.18)$ and $-\log_{10}(4.16)$, respectively).