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**Genetic drivers for resistance and susceptibility traits in Atlantic salmon
(*Salmo salar*) towards salmon lice (*Lepeophtheirus salmonis*)**

Systematic literature review

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Abstract

Salmon lice (*Lepeophtheirus salmonis*), a common parasite on salmonids is one of the biggest problem the aquaculture industry faces today. The current methods used to combat salmon lice in farmed Atlantic salmon (*Salmo salar*) industry are often considered to affect the ecosystem negatively; and inefficient in a long run long run due to development of resistance in the parasite. The potential of methods involving epigenetic modification of farmed fish to develop a lice resistant progeny of farmed salmon is not widely explored. A systematic literature review was used to collect and analyse data from peer reviewed scientific articles, science journals and industrial reports. Data were extracted from 55 peer reviewed articles, 2 science journals and 3 industrial reports based on the inclusion criteria. The summary results lice resistance in Atlantic salmon is described to be a polygenic trait. The differential expression of immune related genes have a significant role in variation in resistance and susceptibility of Atlantic salmon towards salmon lice. Vaccines and immune-modulatory in-feed additives could induce differential gene expressions leading to increased lice resistance in salmon. The heritability of lice resistance trait in salmon is moderate to low, but could be improved with epigenetic methods including selective breeding. The epigenetic memory in salmon is reported to be preserved in the form of DNA methylation. Taking this into account, the epigenetic memories of previous lice infection and differential gene expression could be stored in fish DNA and potentially inheritable. Further research on epigenetic memory in Atlantic salmon on the perspective of the trait for lice resistance would be a great step towards developing a lice resistant progeny of salmon. However, the impacts of epigenetic modification on farmed salmon and ecosystem have to be considered in further studies.

1 Introduction

1.1 Background

Salmon lice, (*Lepeophtheirus salmonis*) are ectoparasitic copepod parasites affecting the salmonids, a family of fish that include Atlantic salmon, Arctic charr (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*), thereby; causing great cutbacks in the aquaculture industry. Their life cycle includes eight stages separated by moulting; two nauplius stages, one copepodite stage, two chalimus stages, two pre-adult stages and the adult stage, respectively (Hamre et al., 2013). According to Prof. Geoff Boxshall, a researcher of copepod crustaceans “The infective larvae of salmon lice are less than a millimetre long, so in the wild finding a host is a difficult part of their life cycle; While in aquaculture facilities, fish are kept at unnaturally high densities, so the parasites will exploit that, and their lives become easy” (Osterloff, n.d.). The treatment methods to limit salmon lice infection in the in the Atlantic salmon aquaculture requires a large economic investments from the industry. As per the yearly reports of industries and peer reviewed articles, methods currently deployed for delousing includes medicinal and non- medicinal approaches.

The Sustainability report by Nova sea (2019) mentions that, non-medicinal treatments make up to 87% of treatments to reduce lice in fish cages. Non-medical treatments may be mechanical and non-mechanical methods. The mechanical treatments that are proven to be effective include use of sea lice skirts around fish cages (Stien et al., 2018); snorkel cage technology (Stien et al., 2016); and laser treatment (Bui et al., 2020).

Non mechanical treatments are chemical treatments which can be medical or non-medical (Hannisdal et al., 2020; Helgesen et al., 2015; Overton et al., 2019), thermal treatment (Andrews et al., 2021), salinity treatment (Andrews et al., 2020; Sievers et al., 2019), use of cleaner fish such as wrasse species (*Ctenolabrus rupestris*, *Symphodus melops* or *Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) (Cerbule et al., 2020; Overton et al., 2020), and use of in feed additives, which could be medical (Covello et al., 2012) or non-medical substances (Jodaa Holm et al., 2016; Refstie et al., 2010). The chemical methods are proven to reduce efficiency in a long run as the lice have developed resistances against them (Aaen et al., 2015). Thermal treatment of infected fish, is presumed to be a sustainable method (Grøntvedt et al., 2015), discovery of heat shock proteins in *L.salmonis* proved that the nauplii acclimated to 10 °C can survive heat shocks up to 30 °C and are capable of hardening by a sublethal heat shock (A. Borchel et al., 2018). In other words, the chemical method and thermal treatment are capable of inducing coevolution of the parasites (Andreas Borchel et al., 2018; Coates et al.,

2021). Hypo-saline water causes mortality in early stages of salmon lice (Sievers et al., 2019), however there are concerns that it might create a selection pressure leading to co-evolution of lice to develop resistance towards this method (Groner et al., 2019). Although using cleaner fish is considered more sustainable method compared to chemical delousing methods, it raises concerns related to fish welfare and transmission of pathogens from one species to the other (Erkinharju et al., 2021).

Taking into account the associated challenges by using the mentioned methods, the genetic modification of farmed Atlantic salmon and the methods to improve genetic resistance of the host to the parasite requires to be explored. The European Union (EU) has in place a comprehensive and strict regulation on *genetically modified* organisms (GMOs). GMOs are officially defined in the EU legislation as "organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination" (Plan et al, 2010). This implies that the epigenetic modifications in the Atlantic salmon can be done to incorporate the trait for lice resistance. The methods such as selective breeding are being currently practiced by the industry to raise progenies resistant to lice and pancreatic disease (Mowi, 2020;Nova sea, 2019). Figure 1 depicts the life cycle of salmon lice, which include larval to adult stages. The infective stages are chalimus stages, were they attach themselves to the host i.e., Atlantic salmon (Armstrong, 2001).

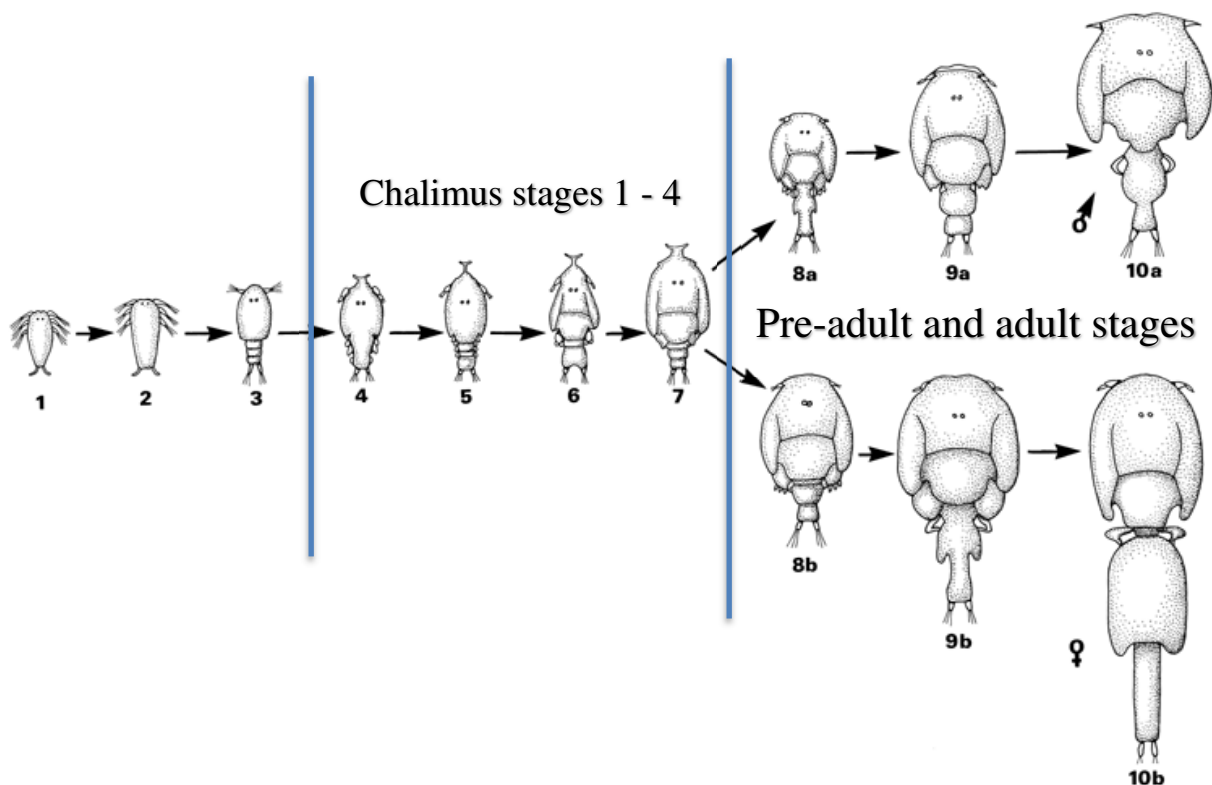


Figure 1 : Different stages in lifecycle of *L.salmonis* (Armstrong, 2001)

1.2 Scope of the study

The aim of this study is to explore the genes responsible variation in resistance and susceptibility of Atlantic salmon towards the salmon lice . The Pacific salmon are more resistant to salmon lice as compared to the Atlantic salmon (Sutherland et al., 2014; Valenzuela-Muñoz et al., 2016). The gene expressions that make Atlantic salmon susceptible to salmon lice are explored in this systematic literature review aims to answer the following research questions:

1. What are the significant gene expressions that impact the resistance and susceptibility of Atlantic salmon towards salmon lice?
2. To what extent are these genes related to lice resistance and susceptance heritability?

Figure 2 shows Atlantic salmon infected by salmon lice (*L.salmonis*). The parasites attached themselves around the anal fins. The parasite usually gets attached on the host, from the edge of the eyes to the caudal pendula and with a few lice around the anal and pelvic fins (Torrissen et al., 2013).



Figure 2 : Atlantic salmon infected with salmon lice; Image retrieved from: Fraser (2019)

The thesis follows an IMRaD format, including introduction, methodology , results, discussion and conclusion. Methodology section in this study consists of the protocols and criteria followed for the systematic literature review. Results, discussion and conclusions are summarised as separate sections in this systematic literature review

2 Methodology

This study is a systematic literature review. A systematic literature review is a secondary study, by identifying, evaluating and interpreting all available research relevant to a particular research question(s), topic area, or phenomenon of interest. The individual studies contributing to a systematic review could be referred as primary studies (Keele, 2007). A systematic review helps to explain differences among studies on the same topic area or research question(s) by summarizing large bodies of evidence from the previous research (Cook et al., 1997); Systematic reviews and meta-analyses are being increasingly used in healthcare (Moher et al., 2009), to inform medical decision making, plan future research agendas, and establish clinical policy (Cook et al., 1997).

2.1 Search strategy

The search for the primary data were conducted in the databases including PubMed, Science direct, mdpi and ProQuest. The selected databases were searched in 15th - 22nd December 2020 and 18 - 25th January 2021. The final search for newly published articles and journals was done in 9th May 2021. The following keywords were used: ('lice' and 'salmon' and 'resistance') , ('lice' and 'salmon' and 'susceptibility') , ('lice' and 'salmon' and 'genetic' and 'resistance') and ('lice' and 'salmon' and 'genetic' and 'susceptibility').The keywords were connected with the Boolean operator 'and', to obtain the publications addressing host resistance and susceptibility to parasite in the genetic perspective. The scientific publications in languages other than English are excluded. However, the industrial reports published in Norwegian are included in the review to have an overview on the current delousing methods practiced in the aquaculture industry.

2.2 Defining the inclusion criteria

The salmon and the lice species addressed in this study are Atlantic salmon (*S.salar*) and salmon lice (*L.salmonis*). The genes upregulated or downregulated in the Atlantic salmon throughout the period of infection by the lice are pointed out in the light of available scientific literature. The methods to stimulate these genetic drivers and the efficiency of those are studied in relation to the publications reviewed.

The search results are sorted by the title, abstract and the results of the publications. The main inclusion criteria for the study were: 1. The publication that include either Atlantic salmon (*S.salar*) Figure 3 or salmon lice (*L. salmonis*) Figure 1 in the respective study; 2. The publication is related to the study of immune genes and their expressions; 3. The publication is assessing the heritability of lice resistance or susceptibility of the host; 4. The publications

involving experimental study with the host and parasite. The inclusion criteria and the reasons to include the specific criteria is shown in Table 1.

Table 1: The criteria followed in the search strategy to identify the relevant articles

Inclusion Criteria	Reason to include the criteria
Published in English or Norwegian	English is the most common language for the scientific publication, while many official reports and studies from the industry are in Norwegian
Addresses the genetics and immunity of Atlantic salmon	The articles dealing with the studies of genes associated with immunity of Atlantic salmon
Publications on genes related to lice susceptibility and immunity	The articles with detailed study on genetic traits associated with lice resistance and susceptibility
Inclusion of either Atlantic salmon (<i>S. salar</i>) or salmon lice (<i>L. salmonis</i>) in the study	<i>S. salar</i> is the most commercially farmed fish species (FAO 2007) and <i>L. salmonis</i> are the most prevalent parasite in Atlantic salmon aquaculture perspective.



Figure 3: Atlantic salmon (*Salmo salar*) Image from: Studer (2018).

2.3 Data extraction

The data was extracted and analysed based on a data extraction form (Table 2) as proposed in the literature by Petticrew et al. (2008).

Table 2: Data extraction table used for this study (Petticrew et al., 2008)

Data to be extracted	Notes of the reviewer
Title of study	
Author	
Year of publication	
Setting	
Time	
Study objective clearly stated?	
Study objective as stated by authors	
Study methodology (or methodologies) used	
Inclusion of sufficient data to assess validity of conclusions?	
Data source	
Experimental results	
Lice	
Lice reduction	
Number of lice initially (/fish)	
Number of lice at the end of experiment (/fish)	
Control	

Up regulated genes	
Downregulated genes	
Heritability	

The aim of data extraction table was to identify the genes upregulated and downregulated and their role in effecting the resistance and susceptibility of Atlantic salmon to salmon lice. The data on upregulated genes; downregulated genes; and lice species and heritability was crucial for answering the research questions. Hence they were categorized as primary outcomes (Vetter et al., 2017). While the secondary outcomes include the data on lice density, lice species and control. In case of unavailability of a specific primary data, the respective column was left blank. The publications that did not provide the data on either genes, heritability or lice resistance were excluded since they are irrelevant for the review.

2.4 Data synthesis

The data regarding the number of publications found, screened, excluded and included in this review is represented as a flowchart in Figure 6. The data regarding individual genes which are differentially expressed to affect lice resistance and susceptibility was summarised in a tabulated form. The data on the respective function of individual genes in connection to lice resistance trait were collected from various scientific publications. The immune modulatory feed ingredients capable of stimulating genes in favour of lice resistance is also summarised in the same manner. This table summarises the genes stimulated by the respective immune-modulatory compound. The methods to induce differential gene expression in Atlantic salmon against salmon lice; the heritability of such epigenetic changes are also discussed.

3 Results

3.1 Data collection and extraction:

Most of the publications including peer reviewed articles, journals, industrial yearly reports and master thesis used in the study; (i.e. 79% of them) were published in the time period between 2011-2021 in all the databases (Figure 4). The first search on PubMed was done on 12 December 2020. PubMed initially provided 248 results based on the keywords used on the search strategy of this study. This database had most of the publications relevant for this study, out of which 34 articles were included in the review. The last search was done on 15 March 2021, which provided 2 more articles to be included in the study.

Science Direct provided a hit of 994 articles, among which 20 articles were included in the review. The rest were excluded since, they generally addressed coevolution and genetics of the parasite, which indeed has a great significance on research to combat salmon lice but is beyond the scope of this review.

Up to 55 articles were chosen from the databases including ProQuest, MDPI, and science daily. 2 scientific reviews and 3 journals included in the study appeared in search results of more than one databases.

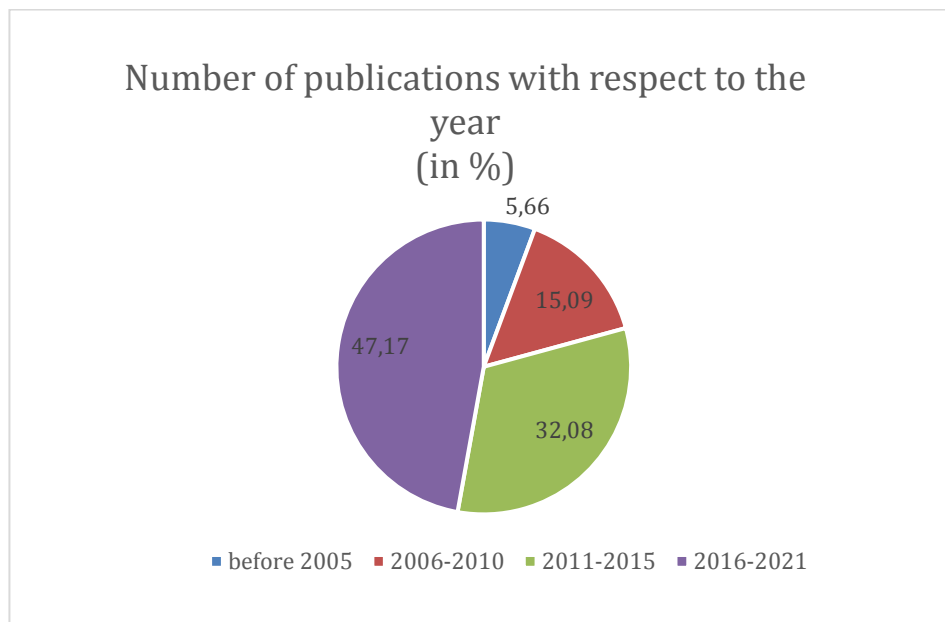


Figure 4: Publications in respective time periods (in percentage).

Out of 1342 search results, only 55 articles were included in the review after examination of abstract and titles of the respective publications (4% of the results). 1287 publications had to be excluded after analysing the title and abstracts based on the criteria set for this review. Figure 5 gives an overview regarding the number of articles included in this review and the databases where the publications are extracted from. The yearly sustainability reports from 3 aquaculture companies (1 international and 2 Norwegian salmon producers), although not peer reviewed, were included in this study for the knowledge about current methods of salmon lice treatments. The articles that did not include Atlantic salmon (*S. salar*) or lice species (*L. salmonis*) were excluded.

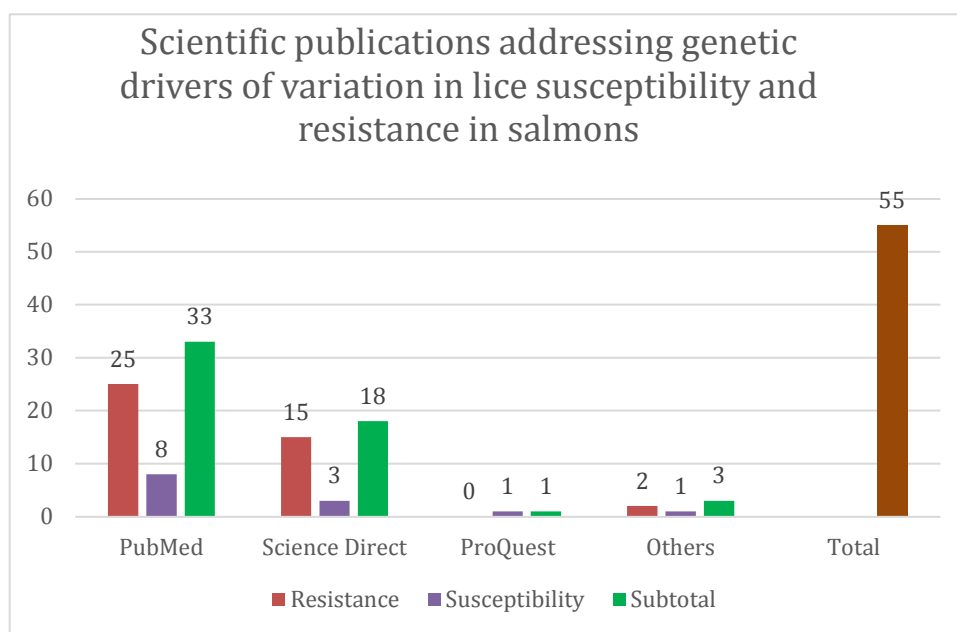


Figure 5: The number of scientific publications (peer reviewed) included in the study.

'PubMed' had most of the scientific publications addressing the genetic drivers of fish immunity, The quorum chart provided below (Figure 6) is a summary of the search results from PubMed, Science Direct, MDPI and ProQuest. Although 67 articles apart from the industrial reports were initially chosen to be included in the study, full text analysis of the publications resulted in excluding 15 articles due to insufficient primary data. The primary data for this study included addressing genes linked to salmon lice resistance, heritability of lice resistance, prediction of heritability, methods that induced epigenetic changes resulting in host resistance or susceptibility and most importantly include either *S. salar* (host) or *L. salmonis* (parasite) in the study.



PRISMA 2009 Flow Diagram

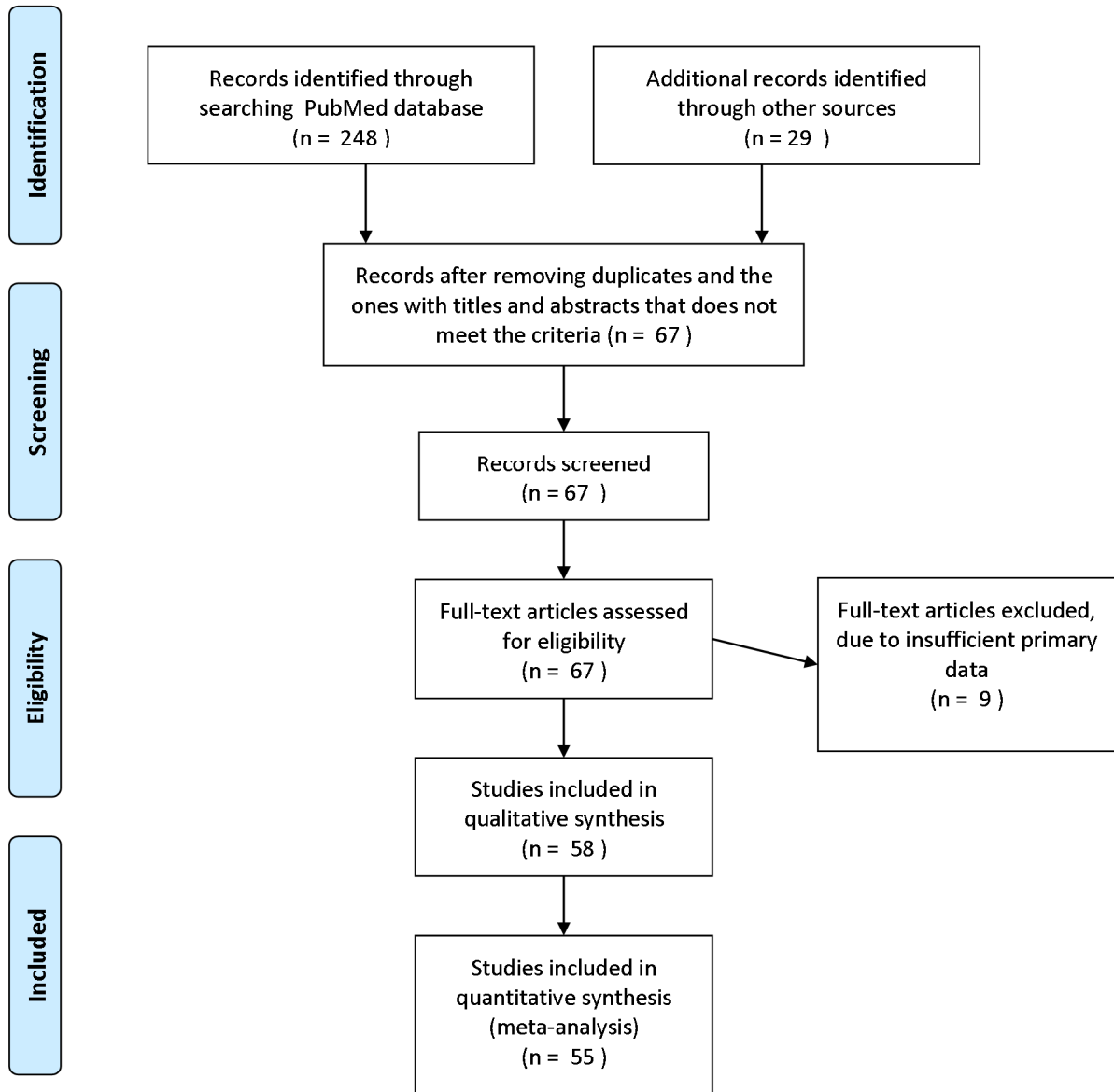


Figure 6: QUORUM chart prepared for this study (Moher et al., 2009). 3 articles were excluded on the basis of data quality.

3.2 Data extraction and analysis

Lice resistance in salmonids is basically a function of immune system mainly connected to Th1 and Th2 type immune response (Krasnov et al., 2015; Sutherland et al., 2014). Epigenetic changes i.e. differential expression of genes among the individuals have a significant role in determining the magnitude of host resistance towards salmon lice (Holm et al., 2015; Jones et al., 2007; Valenzuela-Muñoz et al., 2016).

3.2.1 Genetic drivers for variation in lice resistance and susceptibility:

The genes, especially the immunity related ones, expressed differently in a host could impact the susceptibility to parasites and pathogens to a great extent (Reyes-López et al., 2015). Lice resistance in salmon is a polygenic trait (Robledo et al., 2019; Tsai et al., 2016), and variations in resistance and susceptibility are observed among the individuals of similar species and families (Holm et al., 2015). The studies on host resistance and susceptibility are generally associated with the immune related genes of the organism. Table 3 gives an overview of genes studied due to their significant role in determining the host resistance and susceptibility towards salmon lice.

Table 3: Genes and their respective function(s) in relation with immunity

Interleukin-6 (IL-6)	A pleiotropic (i.e. gene with multiple effects), multifunctional cytokine secreted by T cells and macrophages. This plays a central role in host defence to parasites due to its wide range of functions including acute phase response, chronic inflammation, autoimmunity, endothelial cell dysfunction and fibrogenesis (Tanaka et al., 2014; Velazquez-Salinas et al., 2019).
Major histocompatibility complex (MHC)	These are a series of genes that code for cell surface proteins that control adaptive immunity (Wieczorek et al., 2017). This includes MHC class 1 and MHC class 2, sharing the function of antigen presentation to be recognised by the T cells. Their role on host resistance to parasites are being studied in many organisms (Froeschke et al., 2012; Rodrigues et al., 2009).

Interleukin-10 (IL-10)	An anti-inflammatory cytokine, controlling and limiting the host response to pathogens and parasites, thereby minimising the damage to the host by immune responses and maintaining normal tissue homeostasis (Iyer et al., 2012).
Matrix metalloproteinases (MMPs)	They are zinc dependant proteolytic enzymes with function of degrading extracellular matrix and non-matrix proteins (Jabłońska-Trypuć et al., 2016; Rodríguez et al., 2010).
Tumour Necrosis Factor alpha (TNF alpha)	An inflammatory cytokine, produced by macrophages during acute inflammation. Function of these genes include wide range of signalling events within the cells that lead to necrosis (i.e. cell death triggered by external factors) and apoptosis i.e. normal cell death in a healthy body (Idriss et al., 2000).
Transforming growth factor beta (TGF- β)	A multifunctional set of peptides controlling proliferation, differentiation and immunosuppressive function (Valluru et al., 2011). They suppress immune reactions by promoting the activity of regulatory T (Treg) cells.
Cluster of differentiation 8 (CD8)	They serve as co receptor for T cell receptor and is a transmembrane glycoprotein. Studies show CD 8 T cell mediated response can limit the parasite replication (Jordan et al., 2010).
Interleukin-8 (IL-8)	A chemoattractant chemokine produced mainly by macrophages and is one of the mediators of inflammatory response (Wang et al., 2016).
Interleukin-1 β	A key regulator of inflammation and innate immunity (Gov et al., 2013).

The recent studies on immunomodulatory in-feed additives and impacts of lice on microbiome in salmon skin by Bergh (2019) reported the change in microbiome in the skin cells of fish fed with modulated feed but the processes triggered by the microbiota to effect immunity towards the parasite is not known. However, Parra et al. (2020) studied the role of microbiota in modulating the efficiency of filifolinone, an immunomodulatory compound. Some immune modulatory effects of filifolinone was observed to require a microbial component from the gastro-intestinal tract of the fish.

The Pacific salmon species such as Pink salmon (*Oncorhynchus gorbuscha*) and Coho salmon (*Oncorhynchus kisutch*) are known to resist lice more as compared to Atlantic salmon. The comparative studies on transcriptomics and immunity, showed the immune related genes being expressed differently in the more susceptible Atlantic salmon than in resistant species of Pacific salmon (Sutherland et al., 2014; Valenzuela-Muñoz et al., 2016). The immune response towards the parasites and pathogens are associated with inflammatory, anti-inflammatory, wound healing and immune genes. In results in high susceptibility to parasites and secondary infection by pathogens (Jodaa Holm, 2016).

A comparative infection model study by Braden et al. (2015) reported histochemistry and transcriptomics in a comparative infection model with susceptible (*S. salar*, *Oncorhynchus nerka*) and resistant (*O. kisutch*) salmon. The study reported high upregulation of Interleukin 6 (IL-6) in resistant Coho salmon while weakly upregulated in Atlantic salmon. Another notable gene expression was the differential expression of Major histocompatibility complex (MHC II Beta). A decrease in this gene expression was observed in Atlantic and sockeye salmon (*O. nerka*) which are susceptible to lice after 24 hours post infection and locally suppressed after 72 hours. MHC is a series of genes that code for the proteins, generally the cell surface proteins that control adaptive immunity. MHC has been reported to impact the parasite resistance and innate immunity in many fish species (Gharbi et al., 2009; Glover et al., 2007). Increased homozygosity at MHC-linked loci resulted in fewer salmon lice (*L. salmonis*) abundance particularly for 13-month-old post-smolts (Pawluk et al., 2019).

Interleukin 10 (IL-10) was observed to be weakly upregulated in resistant Coho salmon, while the gene was down regulated in susceptible species i.e. Atlantic and sockeye salmon (Braden et al., 2015). IL-10 is an important immunosuppressive cytokine that, in addition to dampening potentially harmful inflammatory responses during chronic infection, can contribute to pathogen persistence. IL-10 can both impede pathogen clearance and ameliorate immunopathology (Couper et al., 2008). This could be a result of upregulation of inflammatory cytokines including Tumour Necrosis Factor alpha (TNF α), Interleukin 1 Beta, Cluster of

differentiation 8 (CD8) and Cyclooxygenase 2 (cox2) (Couper et al., 2008). Here, we observe a pattern of differential gene expression and the role of this particular gene in the pathway for lice resistance by Coho salmon.

Matrix metalloproteinases (MMPs) are extracellular zinc-dependent endopeptidases involved in the degradation and remodelling of extracellular matrix in physiological and pathological processes (Kudo et al., 2012). Although MMP 9 and MMP 13 were initially upregulated in Atlantic salmon along with resistant Coho salmon, MMP 13 decreased in Atlantic salmon after 48 hours (Braden et al., 2015). In Atlantic salmon the immune responses decrease in 22 days after infection and are activated again at 33 days after infection resulting in higher susceptibility to the parasite as compared to the other species (Fast, 2014).

Pro-inflammatory genes including Tumour necrosis factor (TNF alpha-1), interleukin 8 and interleukin-1beta (IL-1 β) are highly upregulated in skin and kidneys of pink salmon at the early stage of lice infection, indeed enabling a mechanism of rapid lice rejection (Jones et al., 2007). However, the pathway followed by the immune system of different salmon species to combat lice varies. For example, when Coho salmon are concerned, the resistance to *L. salmonis* may be associated with ability to regulate inflammation, limit pathological effects and switch to a tolerant response as observed in other host–parasite relationships (Braden et al., 2015). Transducer of erbB-2 1 (*tob1*) is a protein coding gene located on chromosome 3 of Atlantic salmon. It is a transcription factor that negatively regulates cell proliferation, specifically T lymphocytes, and weakly expressed in the skin with attached lice at their chalimus stage. The overexpression of *tob1* has been described during the response of very small juvenile pink salmon prior to achieving natural resistance (Braden et al., 2020).

Interferons (IFN) and interferon related genes are generally known for their ability to confer protection against viral infections (Ank et al., 2006; Jodaa Holm et al., 2016; McNab et al., 2015). The stimulation of interferon related genes in Atlantic salmon using in-feed additives has been reported to reduce lice loads. The lice loads on fish with higher expression of IFN related genes showed up to 25% decrease as compared to the control, the fish with lower gene expression (Jodaa Holm et al., 2016). Apart from increasing pro inflammatory gene and cell responses, interferons have a significant role in prevention of secondary infections especially by pathogens as well by enhancing extracellular and intra cellular microbial defence and inducing anti-viral state in the cells (Levy et al., 2001).

3.2.2 Heritability

Estimating heritability of the trait to resist lice would be crucial to raise healthy progeny of salmon. Scientific publications address lice resistance as an epigenetic trait (Glover et al., 2007), i.e., the trait that is effected by the changes in gene expression rather than alteration of the genetic code itself. The literature addressing the heritability of the trait included the experimental studies with individuals from the same family and sub-families. The parasite used for the study purposes were *L. salmonis* and the variation in susceptibility are observed among individuals in the same family.

The trait of lice resistance is linked to the immune system (Holm et al., 2015; Jodaa Holm et al., 2016; Sutherland et al., 2014; Valenzuela-Muñoz et al., 2016), which indeed is governed by genes, therefore the resistance is inheritable from the brood stock to offspring. As the trait is governed by more than one gene, estimating the heritability of this trait from a brood stock is complex. In Norwegian aquaculture, common methods deployed to control salmon lice is selective breeding and use of cleaner fish in reference to the industry's yearly report (Mowi, 2020; Nova Sea, 2018). To be selected efficiently, a trait must exhibit significant genetic variation (Correa et al., 2017). Studies by infecting Atlantic salmon with *L. salmonis* showed substantial additive genetic variation in the resistance to the salmon lice in Atlantic salmon and that the resistance measured at different life stages of the fish and the lice may be regarded as the same genetic trait (Gjerde et al., 2010). In 2019, QTL affecting the salmon lice resistance were discovered, and showed 7-13% heritability of the trait for lice resistance (Robledo et al., 2019). QTL is a particular region in a chromosome containing the genes that govern phenotypic traits (Paudel et al., 2020).

Accuracy in prediction of phenotypes for the host resistance has a significant role in ensuring the inheritance of the trait. Genomic selection is reported to be the most efficient method as compared to the traditional 'pedigree based' methods (Tsai et al., 2016). In the study to compare the breeding value prediction of lice resistance with pedigree based and genomic based prediction approaches, the accuracy of genomic predictions increased with increasing single-nucleotide polymorphism (SNP) density and was observed to be up to 22% higher than pedigree-based best linear unbiased prediction (BLUP) predictions. However, both Bayesian and Genomic Best Linear Prediction (G-BLUP) methods can predict breeding values with higher accuracies than pedigree-based BLUP (Correa et al., 2017). SNPs are single genetic code variations and is considered as most common form of nucleotide modification (Vallejos-Vidal et al., 2020).

The heritability observed by natural infection of salmon in natural conditions are much lower than estimated in laboratory conditions (Kolstad et al., 2005). This is a result of stable conditions in the laboratory and high infection during the tests unlike natural set up with dynamic condition and low intensity of infection. In the study about genetic variation of *S.salar* to salmon lice *L.salmonis* by Kolstad et al. (2005), it was suggested to use the challenge tests in selective breeding to increase the resistance to salmon lice since natural infection by lice is highly variable in time depending on the parasite density. Selective breeding is a technique with a good potential to increase the resistance in salmon towards the lice, due to the substantial additive genetic variance in lice resistance (Gharbi et al., 2015; Gjerde et al., 2010).

3.2.3 Methods to stimulate genetic expressions for lice resistance:

3.2.3.1 In feed additives:

Today, the aquaculture industry is exploring ways to supplement fish feed with nutritionally acceptable plant protein sources for Atlantic salmon (Jodaa Holm et al., 2016). Some of these plant products, which could be incorporated to fish feed have immunomodulatory properties. Table 4 gives an overview on some immunomodulatory compounds that could improve lice resistance in farmed Atlantic salmon.

Table 4: In feed additives that enhances lice resistance in salmon

<p>Glucosinolates (plant extract from family Brassicaceae)</p>	<p>This ingredient is capable of upregulating immune genes including Matrix metalloproteinases (MMPs), antiviral genes , mainly Interferon related genes (Jodaa Holm et al., 2016).</p>
<p>17b-estradiol and testosterone (hormones)</p>	<p>They are hormones concerned with sexual maturation of the fish. However, multiple genes involved in wound healing, cell differentiation and remodelling were stimulated along with sexual maturation (Krasnov et al., 2015).</p>

6-gingerol (plant extracts)	6-gingerol induces upregulation of inflammatory and anti-bacterial genes including cytokines (Smith et al., 2018).
Lipopolysaccharides (extracted from <i>Escherichia coli</i> bacteria)	They could stimulate inflammatory genes including interleukin beta 1, cyclooxygenase 2 (cox2), soluble toll like receptor 5 (sTLR5) and interleukin 8 by 6-gingerol (Smith et al., 2018).
Resveratrol (plant extract)	These additives could reduce the action of inflammatory genes (Smith et al., 2018).
Filifolinone (plant extract from <i>Heliotropum sclerocarpum</i>)	This compound stimulates early expression of genes including IFN- α 1, TGF- β , TNF- α , IL-1 β , and IFN- γ (Parra et al., 2020).

The use of functional feed incorporated with dietary phytochemicals capable of modulating epigenetic mechanisms to resist salmon lice is a delousing method used in the industry (Tacchi et al., 2011). The functional feed to improve the resistance towards salmon lice is commercially available e.g. Shield, Skretting; Robust, EWOS/Cargill (Barrett et al., 2020). The change in genetic expression and the gene stimulated varies based on the respective phytochemical. The addition of glucosinolates to the feed resulted in 25% reduction in the lice loads in the *S. salar* infested with *L. salmonis* (Jodaa Holm et al., 2016). Glucosinolates are the compounds extracted from plant belonging to family Brassicaceae, and is known for effecting cell proliferation and growth besides antioxidant and detoxifying properties (Jodaa Holm et al., 2016). The genes upregulated included type 1 inflammatory genes including cytokines, IFN related genes and matrix metalloproteinases (Jodaa Holm et al., 2016).

Administering feed additives including 17 β -estradiol and testosterone induced sexual maturation in smolts Atlantic salmon simultaneously resulted in up to 2-fold reduction of lice load (Krasnov et al., 2015). Antibacterial proteins such as cathelicidin was upregulated while defensin was being downregulated; In short, many interleukins which are strong inhibitors were down regulated as skin immune responses are concerned (Krasnov et al., 2015). Sexual

maturation of salmon although effective in improving host resistance to lice, could result in development of sex organs. This could cause reduction in weight and therefore would be undesirable for the aquaculture industry.

The study on effect of β -glucans and mannan oligosaccharide rich product (MOS) in improving the effectiveness of fish feed containing sunflower oils and soyabean meal by reducing side effects to the fish by the feed additives by the latter, explores the methods to simultaneously increase growth rate and lice resistance (Refstie et al., 2010). The compounds such as 6-gingerol was observed to increase the activation of cytokines (Refstie et al., 2010); Although anti-bacterial in nature these genes were observed to have a significant role in enhancing host resistance against salmon lice (Smith et al., 2018), the adverse effects due to increased activation of inflammatory genes by 6-gingerol is controlled by addition of resveratrol (Smith et al., 2018).

Histopathological and differential gene expression analyses indicate that localized and systemic inflammatory mechanisms may be transiently altered by immunostimulatory feeds and may result in increased host resistance to salmon lice (Covello et al., 2012). The role of microbiota in the host skin was often not studied in connection with the lice resistance. The immunomodulatory effect of Filifolinone upregulating cytokines including TNF-alpha, IL-1Beta, and IFN gamma, involved in Th1-type are reported to be dependent on microbiota on the host in salmonids (Parra et al., 2020). The change in host microbiota has been reported in the earlier studies on feeds as well (Bergh, 2019).

3.2.3.2 Breeding techniques

Considering the fact that the resistance to salmon lice varies from one individual to another (Holm et al., 2015), a proportion of individuals among the population of Atlantic salmon have increased resistance to salmon lice compared to others. Selective breeding is one of the methods practiced in the aquaculture industry to improve resistance to diseases (Mowi, 2020). Developing screens to identify the genes conferring resistance in salmon is a method to increase the efficiency of selective breeding (Jones et al., 2002). Identifying the novel genes could help to select brood stocks that are more effective in resisting lice.

Scientific research are being carried out to explore the potential of CRISPR cas9 to make Atlantic salmon resistant to salmon lice and pathogens by deletion or editing respective genes (Nofima, 2021). CRISPR cas9 is a tool that could enable to make the changes in genetic code of targeted species. 'CRISPR-Cas9 could be used to delete a few base sequences of the code to disrupt a gene's function. But an intense research effort is needed, first to determine which

genes could be edited to have the desired effect, and secondly to be able to successfully make the desired edits' (Kraugerud, 2020). Ross Houston of the Roslin Institute UK addresses the use of CRISPR cas9 in aquaculture research as a relatively new technology, with a potential of allowing very precise and targeted changes at specific genes in the salmon genome known to be involved in cross-species variation in resistance to lice, while the success of its use depends on the type of change that is needed and on the position and code of the gene to be edited (Kraugerud, 2020). Genome editing via CRISPR CAS9 has been successfully used in plants (Wada et al., 2020). However, genome editing in animals for human consumption raises ethical issues as well as environmental concerns.

3.2.3.3 Vaccines

Vaccines are reported to have an impact on gene expression linked to resistance towards salmon lice (Contreras et al., 2020). Contreras et al. (2020) also discovered the new candidate protective antigens, putative Toll-like receptor 6 (P30), potassium chloride, and amino acid transporter (P33). The study on impact of a vaccine in *Atlantic salmon* infected with the lice species *L. salmonis* showed highly upregulated cluster of proinflammatory cytokines genes in spleen, highly upregulated regulatory cytokine genes in head kidney and mixed upregulated gene expression of Th1, Th2, T reg, IgM and IL-8 in skin (Swain et al., 2020); The vaccinated fish had a reduced lice load as compared to the control and gravid lice on the vaccinated fish lost the fecundity of their eggs (Contreras et al., 2020; Swain et al., 2020).

4 Discussion

The significance of gene expressions and the methods stimulating epigenetic changes leading to increased host resistance towards salmon lice have been explored in this review. Lice resistance in salmon is widely studied in connection with immune system and as a polygenic trait over time. Marker-assisted selection can be used to select favourable genes and QTL alleles conferring host resistance towards salmon lice (Odegård et al., 2014), especially while selecting brood stocks to develop a healthy progeny at the absence of any phenotypic traits. The selection of necessary genes would require the precise knowledge of the role of genes in making the host resistant towards salmon lice. The variation in MHC in salmon is observed to have a great impact on its resistance to the salmon lice and is suggested to be considered in selective breeding (Pawluk et al., 2019). The selection of brood stock based on individuals with increased homozygosity of MHC linked loci indeed yielded promising results by significantly reducing lice loads on infected fish. This could be an important step towards breeding lice resistant progenies of salmon.

IL-6 is an inflammatory gene, unlike MHC not studied in detail in relation with salmon and salmon lice. Research on mice reported that IL-6 is necessary for parasite specific response in hosts. IL-6 mediates anti-parasite protective responses in the vertebrates (Gao and Pereira 2002), and for example, IL-6 deficient mice are highly susceptible to parasite Infection though exhibited normal intestinal immunoglobulin A responses against the parasite (Bienz et al., 2003). The other inflammatory genes with significant role in lice resistance include IL 8, IL-1 β and MMP 9 and MMP 13. The excessive activity by inflammatory genes such as MMP9 and MMP13 may contribute to the development of chronic wounds (Skugor et al., 2008), delay in healing these wounds, which in turn leads to secondary infection by pathogens. Immunosuppressive and anti-inflammatory genes including IL 10 and TGF- β must not be ignored since wound healing and dampening of harmful inflammation has equivalent significance in conferring protection to the host against salmon lice and secondary infections by salmon lice or pathogens (Skugor, Glover et al. 2008).

In other words, the marker based selection of genes based on their role in determining the host resistance and susceptibility towards salmon lice while selecting the brood stock may be crucial step to raise progeny with an enhanced immune response against salmon lice.

The phenotypic variation on the trait for lice resistance in Atlantic salmon based on the observed phenotypes (lice number, lice density, initial weight, initial length and weight and length gain during infestation) proved that the trait is polygenic in nature (Robledo et al., 2019;

Tsai et al., 2016). Three QTL regions were identified with genes including that by *tob1*, that negatively regulates cell proliferation including T cells; serine / threonine-protein kinase 17 B (STK 17B), a gene connected to apoptosis and T-cell regulation; Heme binding protein 2 (HEBP2), a gene that regulate iron (Robledo et al., 2019). The research on other vertebrates such as mice shows that, the T cells of mice lacking STK 7B are hyper sensitive to stimulation (Honey, 2005), which implies its role in immune system in vertebrates.

4.1 Heritability

The heritability of the trait for salmon lice resistance in Atlantic salmon is studied to be low to moderate (Lhorente et al., 2012; Robledo et al., 2019). These QTL regions observed in Atlantic salmon contained large number of genes (Robledo et al., 2019). The number of mutations that are likely to occur in these genes as predicted by genome wide association study (GWAS) could have a moderate or large functional effect on lice resistance (Robledo et al., 2019). This implies that, the potential of increasing this trait among the Atlantic salmon population bred for farming purposes are high.

Unlike the common myth that fish have a poor DNA memory as compared to humans (Ortega-Recalde et al., 2019), new studies by researchers in the University of Otago report that memory in fish is preserved in the form of 'DNA methylation' between generations of fish (Ortega-Recalde et al., 2019). "Methylation sits on top of DNA and is used to control which genes are turned on and off. It also helps to define cellular identity and function. In humans and other mammals, DNA methylation is erased at each generation; however, we found that global erasure of DNA methylation memory does not occur at all in the fish we studied." (Ortega-Recalde et al., 2019). Since fish does not experience the erasure of DNA methylation, they could transmit life experience and epigenetic memory through the germline through their DNA in the form of methylation (Ortega-Recalde et al., 2019).

DNA methylation represents a stable, flexible gene expression control system that is critical for formation of cell identity during development. In contrast to mammalian species, indirect evidence suggests that in at least some fish species, epigenetic marks are not erased and can be inherited from one generation to the next (Ortega-Recalde et al., 2019).

In Atlantic salmon, differed DNA methylated regions were observed in sperms of wild salmon and salmon produced in hatchery; the epigenetic and phenotypic changes due to methylation are transferrable between generations (Rodriguez Barreto et al., 2019). However, the significance of DNA methylation and inheritance of epigenetic memories in relation with salmon lice resistance is not explored. Further research on DNA methylated regions in Atlantic

salmon, linked to trait of increased salmon lice resistance could provide results on the extent to which these epigenetic changes can be inherited.

The discovery of DNA methylation in salmon raises concerns related to escapees from the sea cages interbreeding with the wild salmon. Epigenetic introgression that could occur as escapees are being interbred with the wild salmon populations could compromise locally adapted fish populations, causing reduced fitness and even extinction of wild species (Rodriguez Barreto et al., 2019). In other words, epigenetic modifications on farmed salmon to induce lice resistance could result in introducing undesirable traits too. Sterilizing the farmed salmon by CRISPR cas9 method or raising triploid salmon generations are a solution for the problem. The other sustainable solution would be land based salmon farming with RAS (recirculating aquaculture system) technology, however this demands high investments from the industry (Martins et al., 2010). “Raising fish in RAS is very different from traditional systems such as sea-cages or land-based flow-through systems. Because the water is recirculated, bacteria, viruses, and fish metabolites can accumulate. Therefore, water treatment is a key part in these systems” (Johansen, 2020).

4.2 Vaccines against lice

Vaccines are generally administered to farmed salmon for the protection against bacterial and viral diseases (Sommerset et al., 2005). Although a vaccine against salmon lice is commercially unavailable at the moment, the research to develop a vaccine against the parasite *L. salmonis* shows promising results (Contreras et al., 2020; Swain et al., 2020). The research showed that vaccine against lice are not only capable of inducing differential expression of genes against salmon lice, but also reduces fecundity of the eggs of the parasites on the vaccinated fish (Swain et al., 2020). The Norwegian veterinary institute is currently working on a project Louse off 2 (LO2) to develop a vaccine against salmon lice which is expected to have 30% to 50% efficiency (Veterinærinstituttet, 2020). The candidate vaccine studied by (Swain et al., 2020) although had a slight impact on fish weight, showed an efficiency of 56% against salmon lice, including loss of fecundity of eggs in gravid lice. Figure 7 illustrates the methods capable of inducing epigenetic changes in farmed Atlantic salmon and the scope of selecting DNA methylated brood stock from the population.

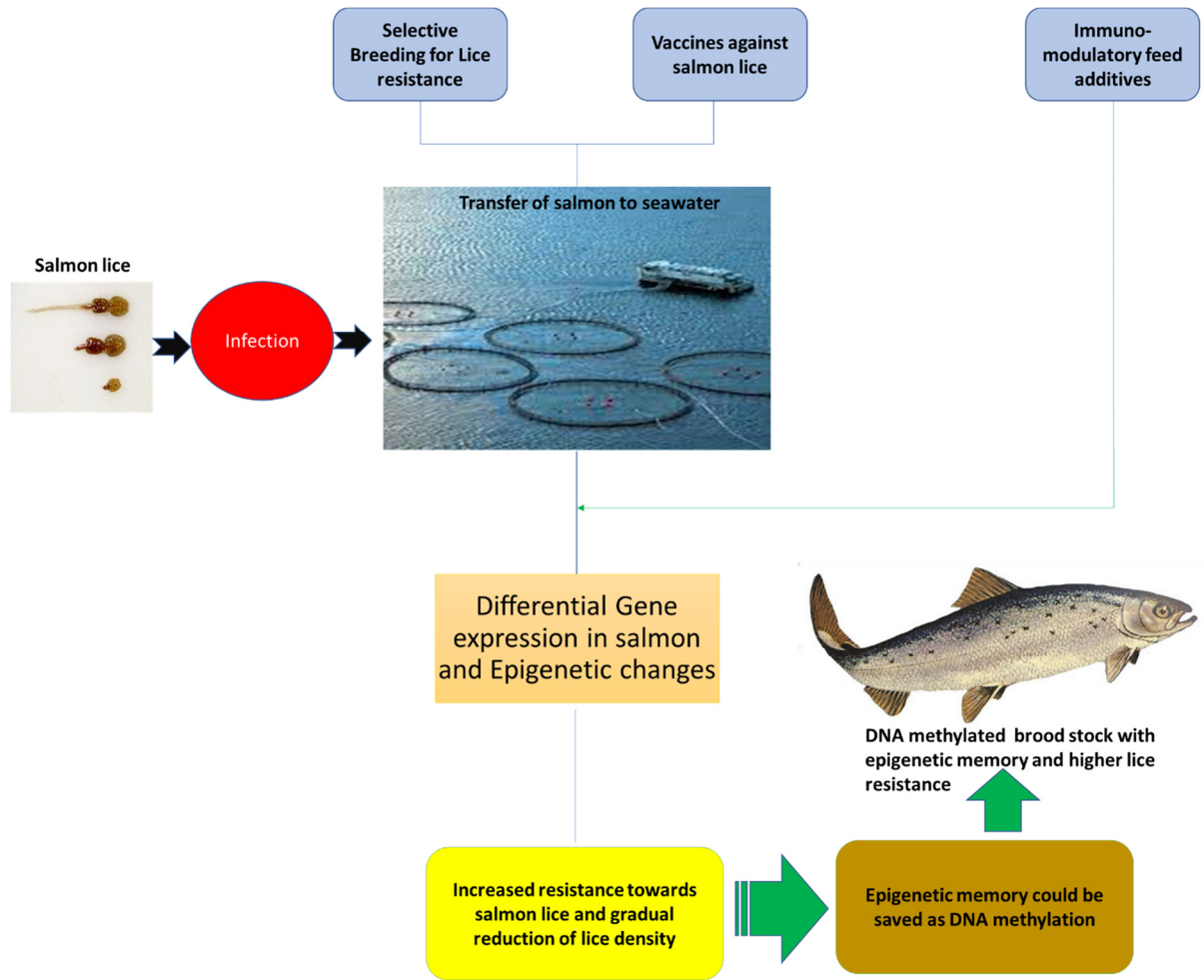


Figure 7: Model to demonstrate differential gene expression to selection of brood stock from a population with an epigenetic memory of previous infection

5 Conclusion

The current delousing practices include mechanical and non-mechanical methods; Mechanical methods include, use of sea lice skirts around fish cages, snorkel cage technology and laser treatment while the non-mechanical methods include the use of chemicals, thermal treatment, salinity treatment and the use of cleaner fish. Previous studies have highlighted that in many instances, these methods lack a long term effectiveness against salmon lice as they could result in the coevolution of the parasite (Coates et al., 2021). The potential of genetic modification to develop more lice resistant salmon population have been explored in this decade.

The lice resistance in Atlantic salmon is described to be a polygenic trait (Robledo et al., 2019; Tsai et al., 2016). The expertise on the function of individual genes linked to lice resistance is crucial for selection for the trait of lice resistance. Genome based selection is described to be more accurate as compared to the pedigree based prediction of phenotypes for the host resistance to lice (Correa et al., 2017; Vallejos-Vidal et al., 2020). The heritability of lice resistance trait is studied to be low to moderate but could be improved with epigenetic methods including selective breeding. In feed additives and vaccines were studied to be inducing differential expression of genes resulting significant reduction in lice load in farmed salmon. The vaccine inducing differential gene expression was reported to be 56% efficiency against salmon lice (Swain et al., 2020).

Therefore, the discussed methods involving the introduction of epigenetic changes in the farmed salmon is comparatively efficient in developing lice resistance as a trait in the fish stock. Animals with epigenetic modification are not considered as GMOs by the EU legislation. Studies report that, up to 58% of the European consumers have negative perception on genetically modified food (Costa-Font et al., 2008). This would mean that the consumer acceptance for the epigenetically modified farmed salmon could still be uncertain.

The efficiency of epigenetic modification of farmed salmon and the genes to be selected to induce lice resistance are still under research. Although the vaccines are reported to be effective against the salmon lice by stimulating immune genes (Contreras et al., 2020; Swain et al., 2020), potential side effects have to be considered.

The epigenetic memory in fish is reported to be preserved in the form of DNA methylation, that could be inherited to the offspring (Rodriguez Barreto et al., 2019). DNA methylation is already being studied in Atlantic salmon in the perspective of epigenetic introgression on wild salmon interbreeding with escapees from aquaculture facilities. Based on the conclusions of this study, the research on epigenetic modifications and DNA methylation

in connection with the inheritance of salmon lice resistance is suggested. The reviewed sources show that selecting the brood stocks with high resistance in selective breeding method could breed a progeny of highly lice resistant salmon. The research on DNA methylation in connection with the inheritance of salmon lice resistance would potentially contribute to selection of highly resistant brood stocks.

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