REVIEWS IN Aquaculture

Reviews in Aquaculture, 1–15 doi: 10.1111/raq.12334

The race between host and sea lice in the Chilean salmon farming: a genomic approach

Cristian Gallardo-Escárate, Gabriel Arriagada, Crisleri Carrera, Ana Teresa Gonçalves, Gustavo Nuñez-Acuña, Diego Valenzuela-Miranda and Valentina Valenzuela-Muñoz

Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile

Correspondence
Cristian Gallardo-Escárate, Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile. Email: crisgallardo@udec.cl

Received 20 June 2018; accepted 11 January 2019.

Abstract

Sea lice are a group of ectoparasite copepods negatively affecting fish health in the salmon farming industry worldwide. Due to their biology, including several stages of development with different sensitivities to chemotherapeutants and their complex host–parasite interactions, the control of sea lice represents one of the major obstacles for sustainable aquaculture. Interdisciplinary approaches are required to avoid the environmental impacts of antiparasites commercially used during the fish production cycle and the increasing emergence of drug resistance in lice populations. Herein, control methods based on genomic analyses will allow for the development of novel tools such as vaccines, immune-modulators, in-feed masking compounds and non-pharmacological therapies. This review highlights the genomic knowledge on the race between hosts and sea lice, with emphasis on *Salmo salar* and *Oncorhynchus kisutch* as host fish species and *Caligus rogercresseyi* as the main threat affecting the Chilean salmon industry.

Key words: gene modulation, parasite resistance, pharmacological treatments, sea lice, transmission.

Introduction

Sea louse is the common name given to a group of parasitic copepods that affect both farmed and wild salmonids in its marine phase and can cause severe skin damage. *Currently*, sea lice are considered one of the most economically significant parasites to salmon industries around the world (Costello 2006, 2009). The most important sea lice species in Chile is *Caligus rogercresseyi*, affecting farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Gonzalez *et al.* 2000; González & Carvajal 2003). In other relevant salmon farming countries such as Norway, Canada, Ireland and Scotland, the major concern is *Lepeophtheirus salmonis* (Costello 2006; Burka *et al.* 2012; Torrissen *et al.* 2013). This parasite is also considered as a threat to wild salmon populations in British Columbia (Canada's Pacific coast) and in some regions in Europe (Costello 2009). Sea lice infections generate severe skin damage leading to chronic stress, reduced growth and reduced feed-conversion efficiency (González & Carvajal 2003; Johnson *et al.* 2004; Rozas & Asencio 2007; Revie *et al.* 2009; Gonzalez *et al.* 2015). Thus, this can cause increased costs due to sea lice infections, together with the cost of chemotherapeutant treatments, and reduced marketability due to skin lesions (Costello 2009; Liu & Bjelland 2014). The economic impact of sea lice infestations at the industry level has been estimated at USD$480 million, which is equivalent to 4 to 10% of production value, depending on the country (Costello 2009). However, a more recent study estimated that lice parasitism produced US$436 million in damages to the Norwegian industry alone in 2011 (Abolofia *et al.* 2017).

The Chilean salmon industry is an important economic sector at the provincial and national levels. In 2016, this industry produced 727 812 tons of farmed salmonids of which 73% corresponded to Atlantic salmon, 15% to Coho salmon and 12% to rainbow trout (SERNAPESCA, 2016). Production of Chinook salmon was marginal. Moreover, projections for 2017 indicate that the total production will surpass 800 thousand tons. In 2016, this industry accounted for 5.8% of the country's total exports (DIRECON, 2017). The Chilean salmon industry consists of about 500 companies that employ more than 50 000 people, including salmon growing, salmon processing, and service companies

(CONICYT, 2012). During 2016, more than 490 fish farms (fresh or seawater facilities) reported commercial activity (SERNAPESCA, unpublished data). Internationally, Chile accounts for about 30% of the world's production of farmed salmon and second largest producer in the world (FAO, 2017; SERNAPESCA, 2016).

Infectious diseases have been major constraints to the sustainable development of the Chilean salmon industry (Alvial *et al.* 2012). In particular, sea lice have been considered one of the most challenging fish health problems for the following reasons: (i) complex control, as the sea lice stages have different sensitivities to chemotherapeutants, and, more recently, cases of low sensitivity and resistance of sea lice populations to particular agents have been reported. Consequently, performance of delousing drugs is not always as expected; (ii) in areas where fish farming activity is intense, sea lice are ubiquitous in sea water, therefore, despite the control measures taken at the farm, new cohorts from neighbouring farms will reinfect the fish; and (iii) treatments may also affect biosecurity and health of wildlife and ecosystem processes. Thus, a deep understanding of sea lice biology and their interaction with salmon hosts is pivotal to improve and develop novel control methods. Furthermore, critical genomic information is required to implement strategies based on pharmacological treatments and to avoid delousing drug resistance in salmon aquaculture. The present review will highlight the current knowledge of the race between the salmon host and sea lice in the Chilean aquaculture. Our approach will focus on genomics as a powerful tool to uncover complex biological processes involved in the host–parasite interaction. The concern about delousing drug resistance will be analysed and possible solutions to overcome this threat will be discussed from a molecular perspective.

Sea louse: a "hard to kill" parasite

Sea louse is the common name given to a group of ectoparasite copepods, from the family Caligidae, which affect fish in salt water (Burka *et al.* 2012; Torrissen *et al.* 2013). This family of parasites accounts for more than 60% of reported parasites affecting fish in marine environments (Johnson *et al.* 2004). The salmon louse *L. salmonis*, which is found only in the northern hemisphere, affects predominantly salmonids in the genera *Salmo*, *Oncorhynchus* and *Salvelinus*, including farmed Atlantic salmon (Pike & Wadsworth 1999). Notably, *O. kisutch* and *O. gorbuscha* have been described to be more resistant to *L. salmonis* infections than other salmonids species (Fast *et al.* 2003; Johnson *et al.* 2004; Jones *et al.* 2007; Wagner *et al.* 2008). Furthermore, *C. elongatus* can also be found on farmed salmonids in the Northern Hemisphere but affecting the host to a lesser degree compared to *L. salmonis* (Costello 2006). In turn, *L. salmonis* has been the responsible agent for important salmon disease outbreaks in Canada, Faroe Islands, Ireland, Maine (USA), Norway and Scotland (Costello *2006*; Burka *et al.* 2012; Torrissen *et al.* 2013).

C. rogercresseyi is the most important species of sea louse in Chile affecting the salmon industry. In contrast to *L. salmonis*, *C. rogercresseyi* has a lower host specificity, as it has been found in local wild species, such as the native rock cod *Eleginops maclovinus* and the sea silverside smelt *Odontesthes regia*, as well as the introduced *S. salar* and rainbow trout (Carvajal *et al.* 1998). Coho salmon (*O. kisutch*) has been reported to be less susceptible than other salmonid species (Bravo 2003). *C. rogercresseyi* was described by Boxshall and Bravo in 2000, though it was originally reported as *Caligus flexispina* (Carvajal *et al.* 1998; Boxshall & Bravo 2000). Because the first introduced salmonids in Chile were grown-up from eggs, it is believed that *C. rogercresseyi* was transmitted from native species naturally infected by this parasite and commonly found near salmon farms (Carvajal *et al.* 1998). *C. rogercresseyi* can be found along the southern Pacific coast of Chile (41°S) and was recently reported in the far south (51°S) (Arriagada *et al.* 2018, unpublished).

Transmission of sea lice occurs during the copepod planktonic stages (larvae). The dispersal range of their larvae will depend mostly on water currents (McKibben & Hay 2004; Brooks 2005; Costello 2006; Penston *et al.* 2008; Amundrud & Murray 2009; Krkosek *et al.* 2010; Molinet *et al.* 2011), although temperature and salinity could also play a role, as these factors affect development and survival of the parasite (Genna *et al.* 2005; Bravo *et al.* 2008). Several studies have found that planktonic stages can be transported tens of kilometres from their source by currents (McKibben & Hay 2004; Costello 2006). More recently, an observational study by Kristoffersen *et al.* (2013) suggests that *C. rogercresseyi* can be transported up to 30 km. In intensive salmon farming regions like Chile, where farms are closely located, this implies the transmission of sea lice among farms. This situation can lead to impaired treatment efficacy as the treated fish may be colonized shortly after treatment by new lice originating from neighbouring farms (Tully & Nolan 2002; Todd *et al.* 2004). In parallel, sea lice transmission between infected fish on the same farm is strongly influenced by host density. When the host density is high, the chances of sea lice exchange between fish are greater, as hosts are closer together. Moreover, the increased transmission at this level increases the reproductive rate of lice (Keeling & Rohani 2008). Further, it has been described that farms with a large number of fish contribute more to the density of copepodids in the water column than farms with fewer fish (Penston & Davies 2009). Kristoffersen *et al.* (2013) found a significant association between the number of gravid females at neighbouring

farms and the level of juvenile lice on farms. In Chile, *C. rogercresseyi* has been found on wild native fish and on free-living salmonids close to salmon farms (Carvajal *et al.* 1998; Bravo 2003), thus it is possible that wild fish play a role in sea lice transmission as well.

Economic impacts of sea lice treatments at the farm level have been estimated between US$ 0.18 and 0.45 per kg of produced salmon in Scotland, between US$ 0.08 and 0.11 per kg of produced salmon in New Brunswick, Canada (Johnson *et al.* 2004) and US$ 0.46 (Abolofia *et al.* 2017) per kg of harvested biomass, equivalent to 9% of farm revenues in Norway, producing US$436 millions in damages in 2011. In Chile in the late 1990s, losses were calculated at US$ 0.3 per kg of produced salmon (Carvajal *et al.* 1998), estimating direct and indirect costs associated with the infestation of about US$320 million per year (INTESAL, 2014). Costello (2009) estimated that the sea lice costs to the world salmonid farming industry in 2006 was USD$480 million, which was 6% of the worldwide production value that year but this figure needs to be updated.

Life cycle: more than developmental stages

The life cycle of sea lice comprises both free-swimming planktonic and parasitic phases. The planktonic phase consists of two nauplii stages and one copepodid stage, the latter being the first infective stage. The planktonic stages of *L. salmonis* have around 7 days to find a host, although this time is temperature and species dependent (Stucchi *et al.* 2011). The copepodid finds its host by following behavioural traits, including gradients of host-derived chemicals (e.g. kairomones), and vibrations of host origin (Heuch *et al.* 1995; Heuch & Karlsen 1997; Bailey *et al.* 2006). The copepodid attaches to the fish skin using its hooked pair of antennae; this process is mediated by chemoreceptors in the copepodid's antennules (Gresty *et al.* 1993). During its developmental process, the parasite gradually increases in size and is attached at all times to the fish. Once on the fish, the copepodid moults into chalimus I and develops a frontal filament to attach itself to the fish (Johnson & Albright 1991; González & Carvajal 2003). The *C. rogercresseyi* parasite moults into three more chalimus stages (II, III and IV) while attached to the fish, while in the case of *L. salmonis* (González & Carvajal 2003), recent research has reported only two chalimus stages (I and II) before the louse develops to the pre-adult stage (Hamre *et al.* 2013). Finally, the individual matures into an adult stage (González & Carvajal 2003). Sexual dimorphism can be seen at the chalimus III stage; however, it is clearer at chalimus IV. In the case of *L. salmonis*, two pre-adult stages have been described after the chalimus stages and before the adult stage (Johnson & Albright 1991; Boxshall & Bravo 2000; González & Carvajal 2003). The mean lengths for the developmental stages of *C. rogercresseyi* are nauplii 0.45 mm, copepodid 0.66 mm, chalimii 1.85 mm, and adult 4.81 mm (González & Carvajal 2003).

The reproductive potential of sea lice is significant. For both sea lice species, one female louse can produce up to 11 generations of egg strings after a single copula which can take 74 days in the case of *C. rogercresseyi* (Heuch *et al.* 2000; Bravo 2010). Each egg string can hatch around 30 eggs in the case *of C. rogercresseyi*, while in *L. salmonis* this number can reach 285 (Heuch *et al.* 2000; Bravo 2010). Multiple copulation and polyandry have been described for *L. salmonis* (Todd *et al.* 2005; Costello 2006), but not for *C. rogercresseyi*. Moreover, female *C. rogercresseyi* have demonstrated survival up to 79 days (Bravo 2010), while female *L. salmonis* reach 191 days (Heuch *et al.* 2000).

Environmental conditions directly influence the life cycle. Indeed, González and Carvajal (2003) determined that the duration of the life cycle of *C. rogercresseyi* is dependent on the water temperature, and found that at 16.7°C the cycle is completed in 18 days, and in 45 days at 10.3°C. In the same study, it was determined that the minimum temperature at which the parasite can develop is 4.2°C. Later, Bravo (2010) estimated the duration of each stage in degree-days and found that the first egg string is produced at 389 degree-days (°D) after egg incubation, and the periodicity at which egg strings are produced depends on water temperature. This latter feature has also been described for *L. salmonis* (Heuch *et al.* 2000). Additionally, sea lice are organisms whose optimal development occurs in high-salinity waters. In particular, it has been described that larval stages are the most sensitive to low salinity, which impacts their attachment success rates. There is evidence that salinity values below 20% seriously impair both *L. salmonis* and *C. rogercresseyi* development (Pike & Wadsworth 1999; Brooks 2005; Genna *et al.* 2005; Costello 2006; Bravo *et al.* 2008). However, there is also evidence that in some cases sea lice have adapted to low-salinity waters (Bravo *et al.* 2008). In addition, it has been described that *C. rogercresseyi* females better tolerate lower salinity levels compared to males (Bravo *et al.* 2008).

Host recognition system: unravelling the molecular mechanisms in sea lice

The larval infective stage of *C. rogercresseyi*, the copepodid stage, is responsible for attachment to the host target organ (skin). In this developmental stage, sea lice identify the host in the sea to infect a proper fish and continue their lifecycle in optimal conditions (González & Carvajal 2003). Host recognition behaviour in this species was first suggested by inferring chemical attraction of sea lice to salmon conditioned water, as it was probed using Y-maze assays (Pino-Marambio *et al.* 2007). This is consistent with the sea lice

L. salmonis species, where host-seeking behaviour has been well described by detecting physical and or chemical cues from the environment to identify a correct host (Ingvarsdóttir *et al.* 2002; Genna *et al.* 2005; Fields *et al.* 2007; Heuch *et al.* 2007; Mordue & Birkett 2009).

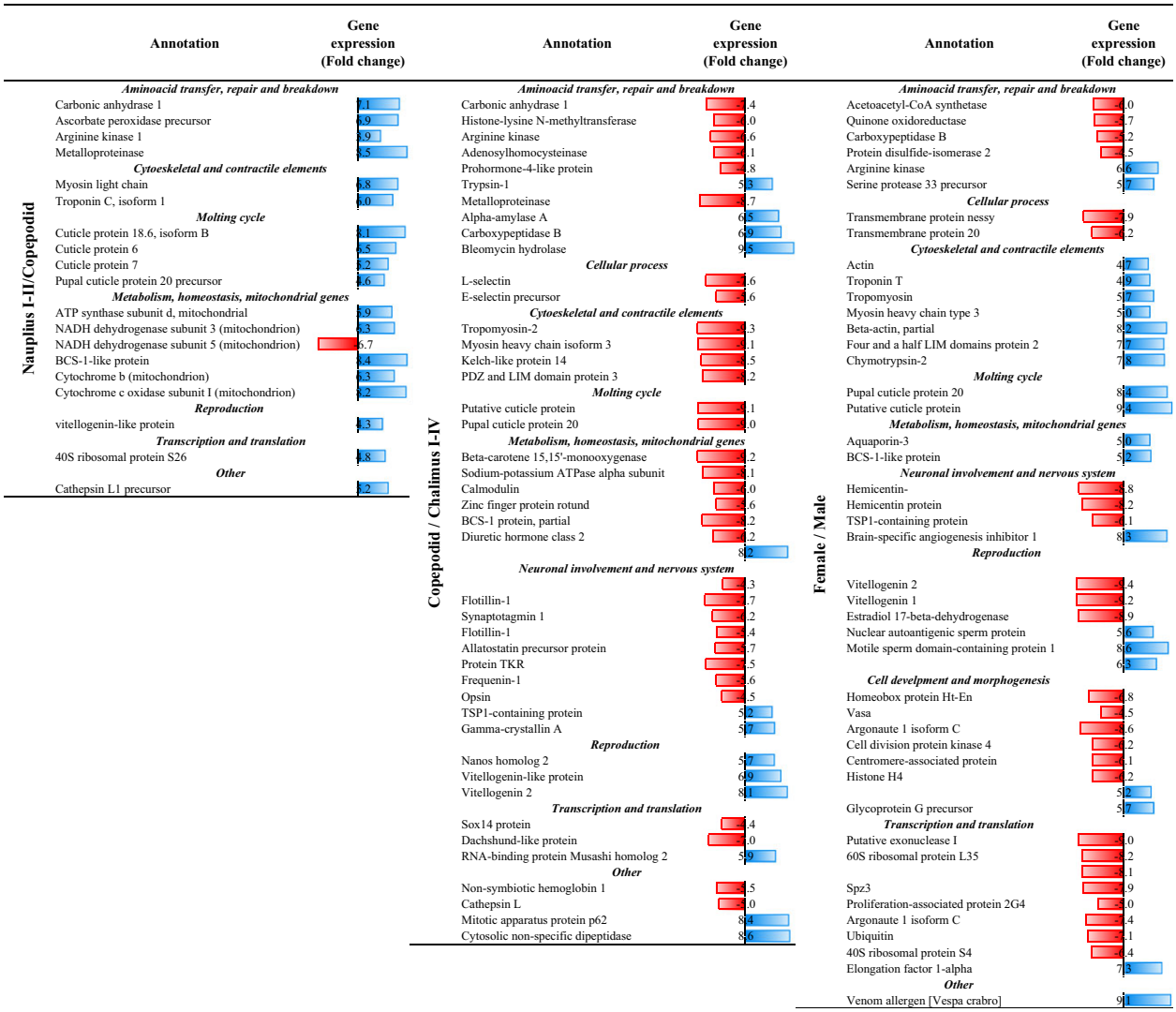
C. rogercresseyi is part of the marine copepod group that responds to different chemical cues and signals in the marine environment (semiochemicals), including cues derived from host fish (kairomones) that give information about host location (Heuschele & Selander 2014). These kairomones are detected through the development of specialized chemosensory systems including various chemosensory receptors. Among these, olfactory and gustatory receptors are the main proteins involved in chemosensory perception and are mainly described in non-marine arthropods (Touhara & Vosshall 2009; Benton 2015). But in marine arthropods, such as sea lice, there is no evidence of these receptors. However, an orthologous group of genes was described in the marine crustacean *Homarus americanus*, which were highly expressed in olfactory organs of this species (Hollins *et al.* 2003). These were named "olfactory-enriched transcripts", but further research determined that they were similar to ionotropic glutamate receptor genes found in insects (Benton *et al.* 2009). Furthermore, in another crustacean, *Panulirus argus*, a group of genes named *ionotropic receptors* (IRs), were found and described as the olfactory receptors for this crustacean group (Corey *et al.* 2013). This group is a subset derived from the ionotropic glutamate receptors, which are similar to IR genes in insects, and with suggested chemosensory functions due to its molecular 3D structure and expression patterns (Rytz *et al.* 2013).

A group of ionotropic receptor genes was found in the *Caligus rogercresseyi* sea lice species (Nuñez-Acuña *et al.* 2014a,b). Among these genes, the *IR25a* gene was highly associated with the copepodid stage, and the whole group responded to the presence of semiochemical-blocking compounds, which also suggests a role in chemosensory perception for this species. These genes present specific expression profiles in sea lice depending on the host species they infect (Nuñez-Acuña *et al.* 2016c), with higher expression levels when the animals are infecting susceptible host species, such as the Atlantic salmon *Salmo salar*. In sea lice, these receptors are tightly linked to synapse gene cascades and neurotransmission pathways, which are also responsible for the presence of other chemicals present in the environment, such as antiparasitic drugs (Nuñez-Acuña *et al.* 2016a). These results demonstrate that *C. rogercresseyi* deploys a specific group of genes as part of chemosensory systems that are critical for semiochemical detection in the environment. A similar chemosensory system, including the same group of genes, was also found in the *L. salmonis* sea lice species where, through RNA interference methods, an association of IR genes to the olfactory perception of the parasites was inferred (Komisarczuk *et al.* 2017). Furthermore, novel studies have deeply described the relationship between IR genes and semiochemical detection in the sea lice. The chemosensory system-related genes of this parasite are activated in the presence of the peptide cathelicidin-2 diluted in seawater, and there is an association with the development of copepodids' frontal filament (Nuñez-Acuña *et al.* 2016b). This suggests that this peptide is the first specific kairomone that *C. rogercresseyi* can detect in the marine environment. Furthermore, it was recently described that this same peptide triggers host-seeking behaviour in the sea lice *L. salmonis*, strengthening this hypothesis, along with an overexpression of a wide group of chemosensory and synapse transduction-related genes (Núñez-Acuña *et al.* 2018a). Herein, *IR25a* plays a pivotal role in host recognition and semiochemical detection in sea lice, as was previously described by RNA interference of this gene and reduction of host-seeking behaviour (Núñez-Acuña *et al.* 2018b).

Sea lice genomics

Knowledge of the *C. rogercresseyi* life cycle and epidemiology has increased greatly since the first report on the species (Boxshall & Bravo 2000). However, information regarding the molecular basis of the parasite's biological functioning, development, and responses to life cycle challenges is recent. In 2012, Yasuike *et al.* (2012) reported a compilation of genomic information on different sea lice genera, including *C. rogercresseyi*, that could be used to understand the parasite's biology. However, it was not until 2014 that Gallardo-Escárate *et al.* (2014a) revealed the complete transcriptome modulation occurring in the copepod during ontogenetic development, highlighting several key features of the *C. rogercresseyi* life cycle that was previously unknown (Table 1). The same study also revealed for the first time fundamental molecular differences between male and female adults, information that was only available at the time with EST collections for *L. salmonis*. Using high-throughput transcriptomics, more than 132 million reads were assembled using a *de novo* (reference-free) approach in an unprecedented data set that served as a basis to reveal new candidate genes as putative participants in processes such as moulting, cuticle formation, myogenesis, metabolism, the immune response, nervous system development and reproduction, and increasing the integration of a disease control strategy based on biotechnology. As an example, Gallardo-Escárate *et al.* (2014b) identified the gene that codes for couch potato protein (*CrCPO*), which plays a pivotal role in the development and functioning of the peripheral and central nervous systems in arthropods, and found an upregulation from the nauplius to

Table 1 Differential expressed genes identified in the lifecycle of *Caligus rogercresseyi* according Gallardo-Escárate *et al.* (2014a)



Fold-change expression values were estimated between lice stages where up- and downregulated genes are indicated in blue and red respectively. ■ Upregulated genes; ■ Downregulated genes.

copepodid stages probably associated with a functional role in the development of the nervous system during the transition from the nauplius stage. Farlora *et al.* (2015) characterized the gene *CrPhb2* that codes for prohibitin 2 – conserved proteins that are involved in a variety of cellular processes and also play an important role in sex differentiation – and observed an overexpression in females. Another example of a characterized gene that was mostly expressed in females and could be used as part of a biotechnology tool for *C. rogercresseyi* control is the *Pgp* gene that codes for P-glycoprotein, a highly conserved membrane-bound protein that functions as an ATP-dependent efflux pump regulating drug concentration in cells and conferring resistance (Valenzuela-Muñoz *et al.* 2014). Moreover, in-depth evaluations of the molecular underpinnings of the copepod's key biological processes were performed to better understand the ectoparasite.

Genomic information regarding *C. rogercresseyi* metabolism and the nutrient acquisition was highlighted by Gallardo-Escárate *et al.* (2014a) with the identification of genes coding for proteins involved in pathways such as the biosynthesis of amino acids, oxidative phosphorylation,

glycolysis, the citrate cycle and lipid metabolism. Goncalves *et al.* (2014) reported the transcriptional response of metabolic pathways such as fatty acid degradation, ketone body synthesis and degradation, as well as steroid and ecdysteroid synthesis. Differential expression analysis showed differences from copepodid and chalimus stage stressed metabolic adjustments to a transition of exogenous feeding at the molecular level. Chalimus and adult females evidenced upregulation of genes coding for proteins participating in lipid digestion and metabolism, as well as for key enzymes such as cholesterol esterase. This enzyme increases cholesterol availability for the synthesis of ecdysteroids that in females have a regulatory role in vitellogenesis (Farlora *et al.* 2014), and also play a key role in ecdysis or moulting (Goncalves *et al.* 2014). Regarding moulting, a comprehensive set of genes coding for cuticle forming proteins was identified by Chávez-Mardones *et al.* (2016), reporting higher regulation of *cuticle precursors* in larval stages, whereas in adults the *cuticle proteins* were also more expressed and with some degree of sex-dependency.

Understanding the mechanisms by which the ectoparasite evades the host defensive strategy and assures survival during parasitic phases is key for the development of control measures from the host–parasite interaction perspective. Valenzuela-Muñoz and Gallardo-Escárate (2014) characterized the Toll and IMD (immune deficiency) pathways in lice during the infective process on Atlantic salmon. IMD pathways include proteins that recognize lipopolysaccharides from Gram-negative bacteria and genes such as *DREDD* (a *caspase8* homologue) and *Akirin* were identified. The latter is an essential protein for embryo development and the anti-inflammatory response, and the gene has been previously reported in *C. rogercresseyi* (Carpio *et al.* 2011) and proposed as a target for a potential vaccine. On the other hand, the Toll pathway responds by the presence of fungi and Gram-positive bacteria and plays a role in cellular immune response processes such as phagocytosis and encapsulation, and its genes, such as *MyD88* and *Tollip,* were also identified. In copepodids, the genes *Toll3*, *Dorsal* and *Caspase1* presented higher regulation, whereas in the chalimus stage this was observed in *Tollip* and *Toll-like*. Adults, on the other hand, presented higher expression in practically all genes, indicating a more active and perhaps mature immune response mechanism for protection and defence from host responses. However, by using next-generation sequencing technology other *C. rogercresseyi* defensive and infectious mechanisms were unravelled. Maldonado-Aguayo and Gallardo-Escárate (2014) reported 11 *serpin* – serine protease inhibitor – sequences with robust similarities with serpin superfamily leucocyte elastase inhibitor members, but also with B serpins. These proteins play essential roles in parasite strategies to evade host exogenous proteases and the immune response, assuring survival, and together with secreted proteases make a strategic part of the defensive secretome of the parasite. Valenzuela-Miranda and Gallardo-Escárate (2016) identified the presence of several genes coding for serine proteases (trypsins and chymotrypsins) associated with possible drug resistance mechanisms (discussed later in this review) showing a complex arrangement of these proteases functioning beyond protein digestion or the immune response in the parasite. In addition, Maldonado-Aguayo *et al.* (2015) described the presence of 56 putative *cathepsin-like* genes with differential expression along ontogenic development. Proteases from the cathepsin family participate in protein degradation in lysosomes or endosomes at low pH but also in the cytosol and nucleus, and a variety of groups were identified in *C. rogercresseyi* (cysteine protease groups B, F, L, Z and S and aspartic protease group D), also highlighting a diversity of biological processes that are regulated by their functioning, such as moulting, cuticle remodelling, embryogenesis, feeding and immune evasion. Interestingly, the genes participating in the parasite strategy to defend and attack the host are not only differentially expressed along ontogenic development but also present different orchestration depending on the host. Vera-Bizama *et al.* (2015) studied the transcriptional modulation of some of the above mentioned molecular markers of the *C. rogercresseyi* immune response, secretome and antioxidant system during Atlantic and Coho salmon infestation – two species with noticeable different resistance to the parasitosis – and revealed an overall higher regulation of the transcripts when infecting Atlantic salmon, especially in the earlier infection stages. Another group of proteins that may play a role in *C. rogercresseyi's* infectious capacity is the Aquaporins. These are a family of small integral membrane proteins that are hydrophobic and facilitate water movement through the lipid bilayer that encloses cells and in addition to a putative role in spermatogenesis reported by Farlora *et al.* (2014) due to an overexpression of *Cr-Aquaporin3* in adult males. The expression analysis of 10 encountered aquaporin-related genes showed not only a modulation along ontogenic development but also an overall lower regulation in *C. rogercresseyi* infecting Atlantic salmon when compared with coho salmon (Farlora *et al.* 2016).

Several genes were identified in response to drugs (Valenzuela-Muñoz *et al.* 2015a; Valenzuela-Muñoz & Gallardo-Escárate 2016) and were reported as potential markers for drug resistance. The ATP-binding cassette (ABC) protein family (Valenzuela-Muñoz *et al.* 2015b), cuticle formation (Chávez-Mardones *et al.* 2016), the NOTCH signalling pathway (Boltaña *et al.* 2016a,b), the antioxidant system (Chávez-Mardones & Gallardo-Escárate 2014; Chavez-Mardones *et al.* 2017) and for several processes of reproduction and development of the parasite, such as *vitellogenin 1* and *2* and *vasa* (Farlora *et al.* 2017) are

examples of modulated genes by delousing drugs such as Azamethiphos, Deltamethrin or Hydrogen peroxide. Lice genomic response to delousing drugs and drug resistance patterns are further detailed in the following section.

Nuñez-Acuña *et al.* (2014a) identified thousands of SNPs in genes associated with relevant biological processes of *C. rogercresseyi*. For instance, a strong association of SNPs linked to *vitellogenins* in chalimus and adult stages was observed, suggesting putative roles in reproduction. The presence of SNPs associated with *vitellogenins* was further confirmed by Farlora *et al.* (2014). On the other hand, genes related to immune response and post-moulting processes such as *C-type lectin* and *cuticle protein* presented SNPs and were highly expressed at the copepodid stage (Nuñez-Acuña *et al.* 2014a), and since the expression of these genes is modulated by delousing drugs (Chávez-Mardones *et al.* 2016), this could be associated with drug resistance patterns. SNPs were identified in practically all of the studied *C. rogercresseyi* biological processes suggesting associations with nervous system development (Gallardo-Escárate *et al.* 2014b), sex differentiation (Farlora *et al.* 2014, 2015), phenotypes of susceptible or resistant to host defensive and immune responses (Maldonado-Aguayo & Gallardo-Escárate 2014; Valenzuela-Muñoz & Gallardo-Escárate 2014; Maldonado-Aguayo *et al.* 2015), but also with susceptibility or resistance to delousing drugs (Valenzuela-Miranda & Gallardo-Escárate 2016; Valenzuela-Muñoz *et al.* 2015a, 2014; Valenzuela-Muñoz *et al.* 2015b).

The regulation of the genes participating in lice key biological features at the post-transcriptional level has been under scrutiny in our research group since Valenzuela-Miranda *et al.* (2015) reported the transcriptome of genes involved in the miRNA biogenesis pathway of the parasite. The authors identified the genes *Drosha* and *Pasha* associated with processing, *Exportin-5* with exporting, *Dicer* and *PACT* with maturation and *Argonaute*, *GW*, *PABP*, *Decapping*, *Tudor*, *CCR4-Not* and *Mov-10* associated with miRNA's function, with considerable modulation during ontogenic development as well as during exposure to delousing drugs. These findings evidenced that the molecular machinery for miRNA biosynthesis is indeed present in *C. rogercresseyi*, and shows a modulation that could be correlated with miRNAs transcriptional response upon exposure to drugs or during its life cycle. In fact, Gallardo-Escárate *et al.* (2017) revealed the ectoparasite's miRNome with 663 unique miRNA sequences. These miRNAs presented differential expression along ontogenic development where bantam, mir-8, mir-9, mir-996 and mir-184 were mostly expressed during early stages, and these have been reported to have functions in the regulation of neural development, ontogenic development and chemoreception among others. Also, the miRNAs bantam, mir-8, mir-100 and mir-2765 were found to putatively bind transcripts related to *cuticle proteins*, *metalloproteinases* and *carbonic anhydrases*, suggesting a potential role of these ncRNAs during moulting. Furthermore, the same bantam, mir-8 and mir-100 but also the mir-81 and let-7 were found to potentially target *peptidases*, whereas mir-124 and mir-996-4 had a sex-biased expression, and the latter was determined to be potentially able to knockdown lice *vitellogenin I-II* and *vasa* genes in adult males. From this comprehensive study, it was also highlighted that the miRNA bantam has a high number of variants with 215 isomiRs. This is of special interest since apart from the already known key role in growth regulation, here a novel function was suggested, possibly related to immune evasion of the parasite during the infestation of Atlantic salmon. Another type of non-coding RNA, the long ncRNAs were also identified in *C. rogercresseyi* (Valenzuela-Miranda *et al.* 2017) and showed differential expression after exposure to delousing drugs. A set of lncRNAs were strongly correlated with genes commonly associated with drug responses such as *ABC transporters*, *cytochrome P450* and *glutathione S-transferase*.

Catching the complexity of salmon louse interactions

Sea lice infections produce a generalized chronic stress response. The areas of the fish where sea lice are commonly found are those with less hydrodynamic disturbance, such as the base of fins (Genna *et al.* 2005). Damage is caused by attachment to the host and feeding on the epidermal mucus, skin and blood (Gonzalez *et al.* 2000; Johnson *et al.* 2004). Once settled on the host, the parasite secretes enzymes for the digestion of mucus, which helps the louse to feed and suppress the salmon's immune response at the attachment site (Ross *et al.* 2000). Pathological changes in the skin include loss of epithelium, bleeding, increase in mucus secretion, change in the chemical composition of mucus, tissue necrosis and the consequent loss of function of the skin as a physical and microbiological barrier (Costello 2006).

During the lice infestation, salmon display different response mechanisms, which change between salmon species. From transcriptomic analyses of two salmon species infected with *C. rogercresseyi,* Atlantic salmon and Coho salmon, tissue-specific transcript profiles were observed, with high abundance of transcripts at 7 dpi in the skin and 14 dpi in the head kidney. From GO enrichment it was possible to identify immunological processes such as NF-Kβ signalling, lymphocyte activation, cytokines receptor activity and cell chemotaxis (Valenzuela-Muñoz *et al.* 2016). Coho salmon, a species resistant to *C. rogercresseyi* infestation, exhibit a proinflammatory response associated with upregulation of Th1 response genes (Valenzuela-Muñoz *et al.* 2016), whereas *L. salmonis* -resistant salmon species

present a Th2-type immune response (Braden *et al.* 2015). However, after 14 days of infestation with *C. rogercresseyi*, the susceptible species, Atlantic salmon showed an increased expression of genes, such as *il10* and *il4,* associated with the Th2 immune response (Valenzuela-Muñoz *et al.* 2016). Furthermore, *C. rogercresseyi* infestation triggers the activation of the innate immune system in Atlantic salmon. This is associated with *tlr22a2* gene expression (Valenzuela-Muñoz *et al.* 2016), which shows a putative role of this TRL in the immune response associated with ectoparasites. This is similar to that observed in fish infested with *A. siamensis*, where a high abundance of *A. siamensis* induces the over-regulation of *tlr22a2* (Panda *et al.* 2014; Kar *et al.* 2015). Moreover, in Atlantic salmon infected with *C. rogercresseyi,* a direct correlation between density and immune system activation has been reported, with high expression of inflammatory and oxidative stress response genes in salmon with high abundance after 14 days of *C. rogercresseyi* infestation (Boltaña *et al.* 2016b). Another mechanism of salmon response to sea lice infestation is the nutritional immune response. This mechanism is a strategy where the host reduces iron availability to avoid the uptake of these ions by the parasite (Toh *et al.* 2010; Hood & Skaar 2012). For instance, in resistant salmon infected with *L. salmonis*, a high regulation of *haem oxygenase* and *hepcidin* genes was observed (Sutherland *et al.* 2014; Braden *et al.* 2015). From the transcriptome of Atlantic and Coho salmon infected with *C. rogercresseyi*, a high number of transcripts associated with iron modulation was observed from a GO enrichment analysis (Valenzuela-Muñoz *et al.* 2017a,b). Furthermore, Atlantic salmon exhibit a high number of transcripts with functions including ion binding, ferric ion binding and ferrous ion binding, processes that were not observed in Coho salmon after infestation (Valenzuela-Muñoz *et al.* 2017a,b). Moreover, RT-qPCR analysis showed high expression levels of *aminolevulinic acid synthase* (ALAs) and *coproporphyrinogenase*, genes involved in the haem biosynthesis process. In contrast, for Atlantic salmon, high regulation of genes involved in haem degradation was observed, such as *biliverdin reductase* (BLVr). Additionally, iron transport genes such as ferritin, transferrin and *IRP* were upregulated in Coho salmon skin at early infestation times, while in Atlantic salmon they were overexpressed at 14 dpi (Valenzuela-Muñoz *et al.* 2017a,b). Also, differences in iron homeostasis genes were observed between Atlantic salmon infested with *C. rogercresseyi* and *L. salmonis*. From PCA analysis, a strong modulation of iron-modulating genes was observed in salmon infested with *L. salmonis* (Valenzuela-Muñoz & Gallardo-Escárate 2017). Furthermore, in the skin of salmon infested with *L. salmonis*, upregulation of *ferritin, transferrin receptor, aminolevulinic acid dehydrogenase* (ALAd), ALAs, and *coproporphyrinogenase was observed*. This is different to salmon infested with *C. rogercresseyi,* which shows overexpression of the *hepcidin gene, haem oxygenase and BLVr* (Valenzuela-Muñoz & Gallardo-Escárate 2017).

Additionally, to understand the modulation of the salmon response mechanism to *C. rogercresseyi* infestation, the role of non-coding RNA has been studied. Non-coding RNA are transcripts without coding potential that regulate the expression of mRNA. Among them are long non-coding RNA (lncRNA) and micro RNA (miRNA) (Johnsson *et al.* 2014). A comparative analysis of lncRNA in Atlantic salmon infected with bacteria, virus and *C. rogercresseyi* showed a difference in parasite-dependent lncRNA modulation, with 438 lncRNA modulated exclusively in response to *C. rogercresseyi* (Tarifeño-Saldivia *et al.* 2017). Other studies performed in Atlantic and Coho salmon infested with *C. rogercresseyi* reported 3678 and 2123 lncRNAs modulated during infestation in Atlantic and Coho salmon respectively (Valenzuela-Muñoz *et al.* 2018). Furthermore, both salmon species showed a tissue-specific expression pattern with a high abundance of lncRNAs, observed at 7 and 14 dpi in the skin and head kidney tissue respectively. Moreover, neighbour genes' GO enrichment of the 100 more regulated lncRNAs in Atlantic salmon showed that putative genes regulated by lncRNAs are associated with the immune response. On the other hand, the most regulated Coho salmon lncRNAs are near genes associated with tissue repair processes (Valenzuela-Muñoz *et al.* 2018). In turn, this evidence suggests that lncRNAs in salmon could be involved in tolerance-resistance interplays, reducing the damage caused by lice (tolerance) or by limiting lice burden (resistance). Notably, this genomic information could be explored in salmon for selective breeding programmes as putative DNA markers. Regarding salmon miRNA roles in *C. rogercresseyi* infestation, from small RNA sequencing of Atlantic salmon skin and head kidney, 1718 miRNAs were reported. Tissue-specific miRNAs with 1404 and 529 annotated miRNAs in head kidney and skin, respectively, were reported with high abundance of miRNA families mir-10, mir-21, mir-30, mir-181 and let7. Furthermore, miRNAs target prediction showed that mir-140-4, mir-181a-2-5 and let-7c-1 modulate the expression of *tlr22a2*, *ALAs* and *ferritin* respectively. The emerging information regarding the role of non-coding RNA, including lncRNAs and miRNAs during *C. rogercresseyi* infestation in salmon, opens up new perspectives to understand the salmon response mechanism to sea lice infestation.

Combating sea lice outbreaks

Strategies for controlling sea lice levels in salmon aquaculture used currently or in studies, are varied and include

management practices, biologic control, vaccination, selective breeding, use of immune-modulators and chemotherapeutants (Jones 2009; Burka *et al.* 2012; Torrissen *et al.* 2013). Whatever the control method used, in most salmon-producing regions, local authorities encourage the adoption of integrated pest management (IPM) programmes with the objective of applying control methods that impact sea lice levels through different mechanisms, and in this way, provide an integrated approach to sea lice control (Grist 2002; Rosie & Singleton 2002; Health Canada, 2003; Heuch *et al.* 2005; BC Ministry of Agriculture and Lands, 2008). In Chile, a specific programme for sea lice named "Health Program for the Surveillance and Control of Caligids" has been established and continually improved from 2007. However, the complexity of the salmon industry and several environmental and biological factors that impact the life cycle of sea lice requires the development of interdisciplinary research from socioeconomic aspects to the deep understanding of the *C. rogercresseyi* biology.

Delousing drug resistance

Evidence for the resistance of sea lice to chemotherapeutants is abundant around the world. The first incidence of this phenomenon was reported in Norway, where tolerance to organophosphates, in particular, azamethiphos, increased to the point of having totally lost their effect by the mid-1990s (Jones *et al.* 1992; Roth *et al.* 1996; Fallang *et al.* 2004). Later, treatment failures associated with pyrethroids were reported in Norway, Scotland and Ireland (Sevatdal *et al.* 2005). Subsequent analysis, based on bioassays, confirmed reduced sensitivity to deltamethrin and cypermethrin (Sevatdal *et al.* 2005). A recent study based on bioassays confirmed the low sensitivity of *C. rogercresseyi* to pyrethroids (Helgesen *et al.* 2014). Furthermore, this investigation found the level of sensitivity of the parasite in Chile to be practically equal to that of deltamethrin-resistant lice in Norway, which constitutes strong evidence that pyrethroid-specific resistance genes are present in the Chilean sea lice genetic pool.

Antiparasitic agents can be broadly classified into two groups, based on their administration method: in-feed additives or immersion treatments, the latter also known as bath treatments. Avermectins and chitin synthesis inhibitors are administered as in-feed additives, while synthetic pyrethroids, organophosphate and oxidizing agents are delivered through bath treatments. However, recent research has shown a decrease in the effectiveness of these treatments (Aaen *et al.* 2015). This resistance to insecticides in invertebrates is generally linked to changes in gene regulation, which is in turn connected to ion channels dependent on voltage-gates, enzymes involved in esterase activity and detoxifying molecules, among others (Ffrench-Constant *et al.* 2004). Recently, studies in *C. rogercresseyi* exposed to delousing drugs demonstrate changes in different molecular mechanisms associated with azamethiphos, deltamethrin and hydrogen peroxide treatments (Chávez-Mardones & Gallardo-Escárate 2014; Chavez-Mardones *et al.* 2017). The detoxification mechanisms employed by resistant organisms are the ABC transporters, which transport the drugs along the membrane and which participate in processes of detoxifying pyrethroids, organophosphates and avermectins, among other chemicals, generally used in the control of invertebrates (Pouliot *et al.* 1997; James & Davey 2009; Pohl *et al.* 2012). In *C. rogercresseyi,* an RNA-Seq analysis of ABC transporter families showed high expression of ABCB and ABCC genes in females and males of *C. rogercresseyi* after treatment with deltamethrin and azamethiphos (Valenzuela-Muñoz *et al.* 2015b). Furthermore, NOTCH signalling pathway genes, positive regulators of ABC transporters, also are affected by exposure to delousing drugs, showing downregulation of these pathways in adult *C. rogercresseyi* after deltamethrin and azamethiphos treatments, suggesting a putative mechanism of resistance against delousing drugs (Boltaña *et al.* 2016a). Among the mechanisms that influence a reduction in the sensitivity to xenobiotics are reduced capacities of the chemical to penetrate the cuticle of the organism. As such, the main barrier against the absorption of insecticides is the cuticle (Vontas *et al.* 2007). RNA-Seq studies performed in *C. rogercresseyi* exposed to deltamethrin and azamethiphos showed changes in cuticle-related gene expression in females and males as treatment consequences (Chávez-Mardones *et al.* 2016). Furthermore, RNA-Seq analysis of *C. rogercresseyi* after exposure to delousing drugs also showed effects in processes such as reproduction, oxidative stress and protease regulation. For instance, adults of *C. rogercresseyi* exposed to deltamethrin and hydrogen peroxide exhibit differences in gene expression profiles between females and males showing a sex-specific response to the delousing drug in genes such as *vitellogenin*, *vasa, estradiol 17-beta-dehydrogenase, glicerolporin* and *the ecdysone receptor* (Chávez-Mardones & Gallardo-Escárate 2015; Farlora *et al.* 2017). Genes associated with oxidative stress control are also regulated differentially between *C. rogercresseyi* females and males exposed to hydrogen peroxide (Chavez-Mardones *et al.* 2017). Effects in oxidative stress genes have been reported in *C. rogercresseyi* exposed to 2 ppb of deltamethrin showing upregulation of genes such as *superoxide dismutase*, *catalase*, *peroxiredoxin* and *phospholipid-hydroperoxide glutathione peroxidase* (Chávez-Mardones & Gallardo-Escárate 2014). Moreover, enzymes involved in esterase

activity have been reported as players in the metabolism of xenobiotics (Villani *et al.* 1983). In *C. rogercresseyi,* trypsin and chymotrypsin have been identified and the transcriptional modulation of these after exposure to deltamethrin and azamethiphos has been evaluated. High-throughput transcriptome sequencing identified 44 putative trypsin-like and 7 putative chymotrypsin-like transcripts in *C. rogercresseyi* that showed differentiated transcriptional modulation after drug exposure. Taken together, these results suggest that trypsin-like transcripts in *C. rogercresseyi* might play a role in metabolizing delousing drugs (Valenzuela-Miranda & Gallardo-Escárate 2016).

Additionally, gene expression changes in delousing drug target genes may be a consequence of the presence of single nucleotide polymorphisms (SNPs). Such mutations have been observed in *A. gambiae* and *A. coluzzii*, species in which a variation of G119S in the acetylcholinesterase gene generates a change in the amino acid Ser for Gly, resulting in a resistance response to the insecticides used in the control of this parasite, among which are organophosphates (Essandoh *et al.* 2013). In sea lice, notable evidence has been reported for *L. salmonis*, where a standard TaqMan assay for rapid and high-throughput screening of resistance markers has been developed. The pyrethroid-marker test targets a resistance-associated SNP in the cytochrome b gene in the mitochondrial genome (Jensen *et al.* 2017). On this basis, the parasites can be classified as either carrying the resistance marker (R) or not (S). Furthermore, the same research group proposed an organophosphate-marker test that targets the mutation Phe362Tyr in the AChE1a gene of *L. salmonis* described by Kaur *et al.* (2016). Based on this assay, each parasite could be classified as homozygote wild-type (SS; Phe362/Phe362), heterozygote (RS; 362Tyr/Phe362) or homozygote mutated (RR; 362Tyr/362Tyr). For *C. rogercresseyi*, 3439 SNP variants were identified from *de novo* assembly on *the C. rogercresseyi* transcriptome (Nuñez-Acuña *et al.* 2014b). Around 63.5% of total SNPs were mainly identified as transitions (A/G or C/T), while less than 36.5% were found to be transversions (A/C, G/T and C/G). Following this, 43 SNPs were present in the ATP-binding cassette subfamily C member 2 gene, which corresponds to an ABC transporter, and the calmodulin gene, which is present in the Dopaminergic synapse, long-term potentiation and the neurotrophic signalling pathway (Nuñez-Acuña *et al.* 2014b). However, the SNPs identified in *C. rogercresseyi* are not associated with resistance or susceptibility strains in contrast to the SNP reported at the AChE1a gene of *L. salmonis.* It is necessary to increase efforts to identify resistant and susceptibility *C. rogercresseyi* strains to associate the gene expression or SNP variations to be implemented as a tool for decision-making regarding the treatments to be used for *C. rogercresseyi* control.

Reverse vaccinology: a genomic tool to develop novel vaccines against sea lice

The reverse vaccinology approach was first described by Rappuoli (2000) as a result of the advantages made in genome technologies. This was a new paradigm in vaccine discovery as the pathogen itself did not need to be cultivated or isolated. The approach involves the screening of all of the protein antigens that the pathogen could express at any time with screening for protective immunity. The reverse vaccinology approach also can identify several key components of beyond proteins. Here, the emergent role of non-coding RNAs, such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) add to the original *in silico* reverse vaccinology pipeline, a complement to genome mining for vaccine discovery. Notably, according to the Rappuoli (2000), the time estimated to develop vaccines using the reverse vaccinology approach will be between 1 and 2 years from the *in silico* antigens selection to immunogenicity testing in animal models. This research effort, comparatively less to the conventional vaccine development considering 5-15 years, opens novel opportunities to develop vaccines against sea lice. Unfortunately, none published information is available for vaccines developed using reverse vaccinology approach against sea lice. Herein, our research group has identified relevant candidate genes, mainly upregulated in the infective stages (for candidate antigens see Table 1) and tested as recombinant vaccines in challenge trials. Furthermore, transcriptome sequencing in lice and fish exposed to the vaccine prototypes have been also analysed. The results obtained from the experimental trials revealed that individuals of Atlantic salmon immunized with specific antigens displayed a protection up to 90% at 25 days post infestation, evidencing the reduction of adult juveniles, females and males. Notably, the transcription analysis of key genes related to ontogeny and reproduction in lice were highly downregulated in response to the immunized fish. Furthermore, the gene profiling of immune-related genes in vaccinated fish revealed that relevant transcripts were modulated. In turn, the current experimental evidence suggests that at least one prototype could be assayed in field or under commercial aquaculture conditions.

Conclusions

Sea lice are considered one of the main health challenges for the salmon industry worldwide and are considered a major threat in Chile as well. Pharmacological treatments are currently the most used strategy for controlling sea lice in farmed salmonids around the world. As a consequence of this, sea lice resistance to chemical agents has become an emerging problem. Proactive monitoring of resistance

should consider evaluating drug performance in the field by routinely assessing antiparasitic treatments results, periodical evaluation of the sensitivity level of sea lice populations through bioassays. Ongoing diagnosis of resistance based on molecular makers has been focused on direct drug targets, such as ion channels for pyrethroids or Acetylcholine esterase enzyme for organophosphates. For *L. salmonis* from Canada and Norway, point mutations on those direct targets have been identified as being responsible for resistance/low sensitivity to either pyrethroids or organophosphates. However, a full screening of gene expression associated with resistance that provides evidence of complex biological processes and additive effects, such as polygenic resistance, is still lacking. In this area, studies interrogating global gene expression dynamics have been performed through bioassays in *C. rogercresseyi*, indicating several biological processes that are modulated by pesticides. However, the development of functional analyses to confirm the molecular mechanisms underlying resistance is needed. Among novel control methods, vaccination and immunomodulators are the most promising. As for vaccines, the use of transcriptomics and the development of molecular tools to perform functional studies on resistance will allow for the fast identification of antigens for new vaccine formulations while parasites evolve to bypass drug treatments. Immunomodulators and in-feed ingredients are versatile stimulators of the immune system which could be administrated during restricted periods as an immune boost. More research on the field of functional nutrition must be performed to extend the list of functional additives, as well as to evaluate secondary effects of the use of these compounds.

Acknowledgements

This study was funded by projects FONDAP #15110027, FONDECYT #1180867 and FONDECYT #3170152 awarded by CONICYT-Chile.

References

Aaen SM, Helgesen KO, Bakke MJ, Kaur K, Horsberg TE (2015) Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology* **31**: 72–81.

Abolofia J, Wilen JE, Asche F (2017) The cost of lice: quantifying the impacts of parasitic sea lice on farmed salmon. *Marine Resource Economics* **32**: 329–349.

Alvial A, Forster J, Burgos JM, Ibarra R, St-Hilaire S (2012) *The recovery of the Chilean salmon industry. The ISA Crisis and Its Consequences and Lessons. Study for the Global Aquaculture Alliance co-sponsored by the World Bank*. the Undersecretariat of Fisheries-Chile and the Chilean Salmon Industry Association, Puerto Montt, Chile.

Amundrud TL, Murray AG (2009) Modelling sea lice dispersion under varying environmental forcing in a Scottish sea loch. *Journal of Fish Diseases* **32**: 27–44.

Bailey RJE, Birkett MA, Ingvarsdóttir A, Mordue AJ, Mordue W, O'Shea B *et al.* (2006) The role of semiochemicals in host location and non-host avoidance by salmon louse (*Lepeophtheirus salmonis*) copepodids. *Canadian Journal of Fisheries and Aquatic Sciences* **63**: 448–456.

BCMAL (2008) Sea Lice Management Strategy 2007/2008. British Columbia Ministry of Agriculture and Lands.

Benton R (2015) Multigene family evolution: perspectives from insect chemoreceptors. *Trends in Ecology & Evolution* **30**: 590–600.

Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB (2009) Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. *Cell* **136**: 149–162.

Boltaña S, Chavez-Mardones J, Valenzuela-Muñoz V, Gallardo-Escárate C (2016a) Evidence for the induction of key components of the NOTCH signaling pathway via deltamethrin and azamethiphos treatment in the sea louse *Caligus rogercresseyi*. *International Journal of Molecular Sciences* **17**: 304.

Boltaña S, Sanchez M, Valenzuela-Muñoz V, Gallardo-Escárate C (2016b) Density-dependent effects of *Caligus rogercresseyi* infestation on the immune responses of *Salmo salar*. *Fish & Shellfish Immunology* **59**: 365–374.

Boxshall GA, Bravo S (2000) On the identity of the common *Caligus* (Copepoda : Siphonostomatoida : Caligidae) from salmonid netpen systems in southern Chile. *Contributions to Zoology* **69**: 137–146.

Braden LM, Koop BF, Jones SR (2015) Signatures of resistance to *Lepeophtheirus salmonis* include a T H 2-type response at the louse-salmon interface. *Developmental & Comparative Immunology* **48**: 178–191.

Bravo S (2003) Sea lice in Chilean salmon farms. *Bulletin of the European Association of Fish Pathologists* **23**: 197–200.

Bravo S (2010) The reproductive output of sea lice *Caligus rogercresseyi* under controlled conditions. *Experimental Parasitology* **125**: 51–54.

Bravo S, Pozo V, Silva MT (2008) The tolerance of Caligus rogercresseyito salinity reduced in southern Chile. *Bulletin of the European Association of Fish Pathologists* **28**: 198–206.

Brooks KM (2005) The effects of water temperature, salinity, and currents on the survival and distribution of the infective copepodid stage of sea lice (*Lepeophtheirus salmonis*) originating on Atlantic salmon farms in the Broughton Archipelago of British Columbia, Canada. *Reviews in Fisheries Science* **13**: 177–204.

Burka JF, Fast MD, Revie CW (2012) *Lepeophtheirus salmonis and Caligus rogercresseyi*. CABI, Wallingford, Oxfordshire, Cambridge, MA.

Carpio Y, Basabe L, Acosta J, Rodríguez A, Mendoza A, Lisperger A *et al.* (2011) Novel gene isolated from *Caligus rogercresseyi*: a promising target for vaccine development against sea lice. *Vaccine* **29**: 2810–2820.

Carvajal J, González L, George-Nascimento M (1998) Native sea lice (Copepoda: Caligidae) infestation of salmonids reared in netpen systems in southern Chile. *Aquaculture* **166**: 241–246.

Chávez-Mardones J, Gallardo-Escárate C (2014) Deltamethrin (AlphaMax™) reveals modulation of genes related to oxidative stress in the ectoparasite *Caligus rogercresseyi*: implications on delousing drug effectiveness. *Aquaculture* **433**: 421–429.

Chávez-Mardones J, Gallardo-Escárate C (2015) Next-generation transcriptome profiling of the salmon louse *caligus rogercresseyi* exposed to deltamethrin (AlphaMax™): discovery of relevant genes and sex-related differences. *Marine Biotechnology* **17**: 793–810.

Chavez-Mardones J, Asencio G, Latuz S, Gallardo-Escárate C (2017) Hydrogen peroxide modulates antioxidant system transcription, evidencing sex-dependent responses in *Caligus rogercresseyi*. *Aquaculture Research* **48**: 969–978.

Chávez-Mardones J, Valenzuela-Muños V, Gallardo-Escárate C (2016) *In silico* transcriptome analysis of cuticle-related genes associated with delousing drug responses in the sea louse *Caligus rogercresseyi*. *Aquaculture* **450**: 123–135.

CONICYT (2012) *The Fishery and Aquaculture Sectors in Chile: Research Capabilities and Science & Technology Development Areas*. In: Government of Chile MoE, National Commission for Scientific and Technological Research (CONICYT), Department of International Relations – European Union Program (eds), pp. 58. Salviat Impresores S.A., Santiago, Chile.

Corey E, Bobkov Y, Ukhanov K, Ache BW (2013) Ionotropic crustacean olfactory receptors. *PLoS ONE* **8**: e60551.

Costello MJ (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**: 475–483.

Costello MJ (2009) The global economic cost of sea lice to the salmonid farming industry. *Journal of Fish Diseases* **32**: 115–118.

DIRECON (2017) *Chile International Trade trimestral report January-December 2016*. In: Government of Chile MoFA, General Directorate of Foreign Economic Relations (DIRECON), Department of Studies (eds), pp. 27. DIRECON, Santiago, Chile.

Essandoh J, Yawson AE, Weetman D (2013) Acetylcholinesterase (Ace-1) target site mutation 119S is strongly diagnostic of carbamate and organophosphate resistance in *Anopheles gambiae*ss and *Anopheles coluzzii*across southern Ghana. *Malaria Journal* **12**: 404.

Fallang A, Ramsay JM, Sevatdal S, Burka JF, Jewess P, Hammell KL *et al.* (2004) Evidence for occurrence of an organophosphate-resistant type of acetylcholinesterase in strains of sea lice (*Lepeophtheirus salmonis*Kroyer). *Pest Management Science* **60**: 1163–1170.

FAO (2017) *Global aquaculture production dataset 1950–2015* (FishstatJ). Available at: www.fao.org/fishery/statistics/software/fishstatj/e

Farlora R, Araya-Garay J, Gallardo-Escárate C (2014) Discovery of sex-related genes through high-throughput transcriptome sequencing from the salmon louse *Caligus rogercresseyi*. *Marine Genomics* **15**: 85–93.

Farlora R, Nuñez-Acuña G, Gallardo-Escárate C (2015) Prohibitin-2 gene reveals sex-related differences in the salmon louse *Caligus rogercresseyi*. *Gene* **564**: 73–80.

Farlora R, Valenzuela-Muñoz V, Chavez-Mardones J, Gallardo-Escárate C (2016) Aquaporin family genes exhibit developmentally-regulated and host-dependent transcription patterns in the sea louse *Caligus rogercresseyi*. *Gene* **585**: 119–127.

Farlora R, Valdebenito-Aguayo F, Valenzuela-Muñoz V, Gallardo-Escárate C (2017) Hydrogen peroxide treatment modulates the transcription of sex-related genes in the sea lice *Caligus rogercresseyi*. *Journal of Fish Diseases* **41**(6): 921–926.

Fast MD, Burka JF, Johnson SC, Ross NW (2003) Enzymes released from *Lepeophtheirus salmonis* in response to mucus from different salmonids. *Journal of Parasitology* **89**: 7–13.

Ffrench-Constant RH, Daborn PJ, Le Goff G (2004) The genetics and genomics of insecticide resistance. *Trends in Genetics* **20**: 163–170.

Fields DM, Weissburg MJ, Browman H (2007) Chemoreception in the salmon louse *Lepeophtheirus salmonis*: an electrophysiology approach. *Diseases of Aquatic Organisms* **78**: 161–168.

Gallardo-Escárate C, Valenzuela-Muñoz V, Núñez-Acuña G (2014a) RNA-seq analysis using *de novo* transcriptome assembly as a reference for the salmon louse *Caligus rogercresseyi*. *PLoS ONE* **9**: e92239.

Gallardo-Escárate C, Valenzuela-Muñoz V, Nuñez-Acuña G, Chavez-Mardones J, Maldonado-Aguayo W (2014b) Transcriptome analysis of the couch potato (CPO) protein reveals an expression pattern associated with early development in the salmon louse *Caligus rogercresseyi*. *Gene* **536**: 1–8.

Gallardo-Escárate C, Valenzuela-Muñoz V, Boltaña S, Nuñez-Acuña G, Valenzuela-Miranda D, Gonçalves A *et al.* (2017) The *Caligus rogercresseyi* miRNome: discovery and transcriptome profiling during the sea lice ontogeny. *Agri Gene* **4**: 8–22.

Genna RL, Mordue W, Pike AW, Mordue AJ (2005) Light intensity, salinity, and host velocity influence presettlement intensity and distribution on hosts by copepodids of sea lice, *Lepeophtheirus salmonis*. *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 2675–2682.

Goncalves AT, Farlora R, Gallardo-Escárate C (2014) Transcriptome survey of the lipid metabolic pathways involved in energy production and ecdysteroid synthesis in the salmon louse *Caligus rogercresseyi* (Crustacea: Copepoda). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* **176**: 9–17.

González L, Carvajal J (2003) Life cycle of *Caligus rogercresseyi*, (Copepoda: Caligidae) parasite of Chilean reared salmonids. *Aquaculture* **220**: 101–117.

Gonzalez L, Carvajal J, George-Nascimento M (2000) Differential infectivity of *Caligus flexispina* (Copepoda, Caligidae) in three farmed salmonids in Chile. *Aquaculture* **183**: 13–23.

Gonzalez MP, Marin SL, Vargas-Chacoff L (2015) Effects of *Caligus rogercresseyi*(Boxshall and Bravo, 2000) infestation on physiological response of host *Salmo salar* (Linnaeus 1758): establishing physiological thresholds. *Aquaculture* **438**: 47–54.

Gresty KA, Boxshall GA, Nagasawa K (1993) *Antennulary Sensors of the Infective Copepod Larva of the Salmon Louse Lepeophtheirus salmonis (Copepoda: Caligidae). Pathogens of Wild and Farmed Fish: sea Lice*, pp. 83–98. Ellis Horwood, Chichester.

Grist B (2002) The regulatory system for aquaculture in the Republic of Ireland. *Pest Management Science* **58**: 609–615.

Hamre LA, Eichner C, Caipang CMA, Dalvin ST, Bron JE, Nilsen F *et al.* (2013) The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. *PLoS ONE* **8**: e73539.

Health-Canada (2003) Integrated pest management of sea lice in salmon aquaculture: overview, Monograph Available at http://publications.gc.ca/collections/Collection/H114-9-2003E.pdf. 37pp. Government of Canada Publisher.

Helgesen KO, Bravo S, Sevatdal S, Mendoza J, Horsberg TE (2014) Deltamethrin resistance in the sea louse *Caligus rogercresseyi* (Boxhall and Bravo) in Chile: bioassay results and usage data for antiparasitic agents with references to Norwegian conditions. *Journal of Fish Diseases* **37**: 877–890.

Heuch PA, Karlsen E (1997) Detection of infrasonic water oscillations by copepodids of *Lepeophtheirus salmonis* (Copepoda Caligida). *Journal of Plankton Research* **19**: 735–747.

Heuch PA, Parsons A, Boxaspen K (1995) Diel vertical migration: a possible host finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 681–689.

Heuch PA, Nordhagen JR, Schram TA (2000) Egg production in the salmon louse *Lepeophtheirus salmonis* (Kroyer) in relation to origin and water temperature. *Aquaculture Research* **31**: 805–814.

Heuch PA, Bjorn PA, Finstad B, Holst JC, Asplin L, Nilsen F (2005) A review of the Norwegian 'National Action Plan Against Salmon Lice on Salmonids': the effect on wild salmonids. *Aquaculture* **246**: 79–92.

Heuch PA, Doall MH, Yen J (2007) Water flow around a fish mimic attracts a parasitic and deters a planktonic copepod. *Journal of Plankton Research* **29**: i3–i16.

Heuschele J, Selander E (2014) The chemical ecology of copepods. *Journal of Plankton Research* **2014**: 1–19.

Hollins B, Hardin D, Gimelbrant AA, McClintock TS (2003) Olfactory-enriched transcripts are cell-specific markers in the lobster olfactory organ. *The Journal of comparative neurology* **455**: 125–138.

Hood MI, Skaar EP (2012) Nutritional immunity: transition metals at the pathogen–host interface. *Nature Reviews Microbiology* **10**: 525–537.

Ingvarsdóttir A, Birkett MA, Duce I, Genna RL, Mordue W, Pickett JA *et al.* (2002) Semiochemical strategies for sea louse control: host location cues. *Pest Management Science* **58**: 537–545.

James CE, Davey MW (2009) Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode *Caenorhabditis elegans*. *International Journal for Parasitology* **39**: 213–220.

Jensen EM, Sevatdal S, Bakke MJ, Kaur K, Horsberg TE (2017) A selection study on a laboratory-designed population of salmon lice (*Lepeophtheirus salmonis*) using organophosphate and pyrethroid pesticides. *PLoS ONE* **12**: e0181388.

Johnson SC, Albright LJ (1991) The developmental stages of *Lepeophtheirus salmonis*(Krøyer, 1837) (Copepoda: Caligidae). *Canadian Journal of Zoology* **69**: 929–950.

Johnson SC, Treasurer JW, Bravo S, Nagasawa K, Kabata Z (2004) A review of the impact of parasitic copepods on marine aquaculture. *Zoological Studies* **43**: 229–243.

Johnsson P, Lipovich L, Grandér D, Morris KV (2014) Evolutionary conservation of long non-coding RNAs; sequence, structure, function. *Biochimica et Biophysica Acta (BBA)-General Subjects*, **1840**, 1063–1071.

Jones S (2009) Controlling salmon lice on farmed salmon and implications for wild salmon. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **4**: 1–13.

Jones M, Sommerville C, Wootten R (1992) Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis,* to the organophosphate dichlorvos. *Journal of Fish Diseases* **15**: 197–202.

Jones SRM, Fast MD, Johnson SC, Groman DB (2007) Differential rejection of salmon lice by pink and chum salmon: disease consequences and expression of proinflammatory genes. *Diseases of Aquatic Organisms* **75**: 229–238.

Kar B, Mohanty J, Hemaprasanth K, Sahoo PK (2015) The immune response in rohu, *Labeo rohita* (Actinopterygii: Cyprinidae) to *Argulus siamensis* (Branchiura: Argulidae) infection: kinetics of immune gene expression and innate immune response. *Aquaculture Research* **46**: 1292–1308.

Kaur K, Jansen PA, Aspehaug VT, Horsberg TE (2016) Phe362-Tyr in AChE: a major factor responsible for azamethiphos resistance in *Lepeophtheirus salmonis* in Norway. *PLoS ONE* **11**: e0149264.

Keeling M, Rohani P (2008) *Modelling Infectious Diseases in Humans and Animals*, p. 368. Princeton University Press, Princenton.

Komisarczuk AZ, Grotmol S, Nilsen F (2017) Ionotropic receptors signal host recognition in the salmon louse (*Lepeophtheirus salmonis*, Copepoda). *PLoS ONE* **12**: e0178812.

Kristoffersen AB, Rees EE, Stryhn H, Ibarra R, Campisto JL, Revie CW *et al.* (2013) Understanding sources of sea lice for salmon farms in Chile. *Preventive Veterinary Medicine* **111**: 165–175.

Krkosek M, Bateman A, Proboszcz S, Orr C (2010) Dynamics of outbreak and control of salmon lice on two salmon farms in the Broughton Archipelago, British Columbia. *Aquaculture Environment Interactions* **1**: 137–146.

Liu YJ, Bjelland HV (2014) Estimating costs of sea lice control strategy in Norway. *Preventive Veterinary Medicine* **117**: 469–477.

Maldonado-Aguayo W, Gallardo-Escárate C (2014) Increasing transcriptome response of serpins during the ontogenetic stages in the salmon louse *Caligus rogercresseyi* (Copepoda: Caligidae). *Marine Genomics* **15**: 55–64.

Maldonado-Aguayo W, Chavez-Mardones J, Gonçalves AT, Gallardo-Escárate C (2015) Cathepsin gene family reveals transcriptome patterns related to the infective stages of the salmon louse C*aligus rogercresseyi*. *PLoS ONE* **10**: e0123954.

McKibben MA, Hay DW (2004) Distributions of planktonic sea lice larvae *Lepeophtheirus salmonis* in the inter-tidal zone in Loch Torridon, Western Scotland in relation to salmon farm production cycles. *Aquaculture Research* **35**: 742–750.

Molinet C, Caceres M, Gonzalez MT, Carvajal J, Asencio G, Diaz M *et al.* (2011) Population dynamic of early stages of *Caligus rogercresseyi* in an embayment used for intensive salmon farms in Chilean inland seas. *Aquaculture* **312**: 62–71.

Mordue AJ, Birkett MA (2009) A review of host finding behaviour in the parasitic sea louse, *Lepeophtheirus salmonis* (Caligidae: Copepoda). *Journal of Fish Diseases* **32**: 3–13.

Nuñez-Acuña G, Valenzuela-Muñoz V, Gallardo-Escárate C (2014a) High-throughput SNP discovery and transcriptome expression profiles from the salmon louse C*aligus rogercresseyi* (Copepoda: Caligidae). *Comparative Biochemistry and Physiology D-Genomics & Proteomics* **10**: 9–21.

Nuñez-Acuña G, Valenzuela-Muñoz V, Marambio JP, Wadsworth S, Gallardo-Escárate C (2014b) Insights into the olfactory system of the ectoparasite *Caligus rogercresseyi*: molecular characterization and gene transcription analysis of novel ionotropic receptors. *Experimental Parasitology* **145**: 99–109.

Nuñez-Acuña G, Boltaña S, Gallardo-Escárate C (2016a) Pesticides drive stochastic changes in the chemoreception and neurotransmission system of marine ectoparasites. *International Journal of Molecular Sciences* **17**: 700.

Nuñez-Acuña G, Marambio JP, Valenzuela T, Wadsworth S, Gallardo-Escárate C (2016b) Antimicrobial peptides from *Salmon salar* skin induce frontal filament development and olfactory/cuticle-related genes in the sea louse C*aligus rogercresseyi*. *Aquaculture* **464**: 171–177.

Nuñez-Acuña G, Vera-Bizama F, Boltaña S, Hawes C, Marambio JP, Wadsworth S *et al.* (2016c) In-feed additives modulate ionotropic receptor genes from the sea louse *Caligus rogercresseyi*: a comparative analysis in two host salmonid species. *Aquaculture* **451**: 99–105.

Panda RP, Chakrapani V, Patra SK, Saha JN, Jayasankar P, Kar B *et al.* (2014) First evidence of comparative responses of Toll-like receptor 22 (TLR22) to relatively resistant and susceptible Indian farmed carps to *Argulus siamensis* infection. *Developmental & Comparative Immunology* **47**: 25–35.

Penston MJ, Davies IM (2009) An assessment of salmon farms and wild salmonids as sources of *Lepeophtheirus salmonis* (Kroyer) copepodids in the water column in Loch Torridon, Scotland. *Journal of Fish Diseases* **32**: 75–88.

Penston MJ, Millar CP, Zuur A, Davies IM (2008) Spatial and temporal distribution of *Lepeophtheirus salmonis* (Kroyer) larvae in a sea loch containing Atlantic salmon, *Salmo salar* farms on the north-west coast of Scotland. *Journal of Fish Diseases* **31**: 361–371.

Pike A, Wadsworth S (1999) Sealice on salmonids: their biology and control. *Advances in parasitology* **44**: 233–337.

Pino-Marambio J, Mordue Luntz AJ, Birkett M, Carvajal J, Asencio G, Mellado A *et al.* (2007) Behavioural studies of host, non-host and mate location by the Sea Louse, *Caligus rogercresseyi* Boxshall & Bravo, 2000 (Copepoda: Caligidae). *Aquaculture* **271**: 70–76.

Pohl PC, Klafke GM, Reck J, Martins JR, Vaz ID, Masuda A (2012) ABC transporters as a multidrug detoxification mechanism in *Rhipicephalus (Boophilus) microplus*. *Parasitology Research* **111**: 2345–2351.

Pouliot JF, Lheureux F, Liu Z, Prichard RK, Georges E (1997) Reversal of P-glycoprotein-associated multidrug resistance by ivermectin. *Biochemical Pharmacology* **53**: 17–25.

Rappuoli R (2000) Reverse vaccinology. *Current Opinion in Microbiology* **3**: 445–450.

Revie C, Dill L, Finstad B, Todd CD (2009) Sea Lice Working Group Report: Report from the Technical Group on Sea Lice (A sub-group of the Working Group on Salmon Disease) of the Salmon Aquaculture Dialogue. (39 ed.) WWF. Available at http://www.nina.no/archive/nina/PppBasePdf/temahefte/039.pdf

Rosie AJ, Singleton PTR (2002) Discharge consents in Scotland. *Pest Management Science* **58**: 616–621.

Ross NW, Firth KJ, Wang AP, Burka JF, Johnson SC (2000) Changes in hydrolytic enzyme activities of naive Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. *Diseases of Aquatic Organisms* **41**: 43–51.

Roth M, Richards RH, Dobson DP, Rae GH (1996) Field trials on the efficacy of the organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquaculture* **140**: 217–239.

Rozas M, Asencio G (2007) Evaluación de la Situación Epidemiológica de la Caligiasis en Chile: Hacia una estrategia de control efectiva. *Salmociencia* **2**: 43–59.

Rytz R, Croset V, Benton R (2013) Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. *Insect biochemistry and molecular biology* **43**: 888–897.

SERNAPESCA (2016) *Anuario estadístico de pesca 2016*. Ministerio de Economía, Fomento y Turismo, Gobierno de Chile, Chile.

Sevatdal S, Copley L, Wallace C, Jackson D, Horsberg TE (2005) Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modelling. *Aquaculture* **244**: 19–27.

Stucchi DJ, Guo M, Foreman MG, Czajko P, Galbraith M, Mackas DL *et al.* (2011) Modeling sea lice production and concentrations in the Broughton Archipelago, British Columbia. In: Jones S, Beamish R (eds) *A Salmon Lice: An Integrated Approach to Understanding Parasite Abundance and Distribution*, pp. 117–150. John Wiley & Sons, Ltd., Chichester, UK.

Sutherland BJ, Koczka KW, Yasuike M, Jantzen SG, Yazawa R, Koop BF *et al.* (2014) Comparative transcriptomics of

Atlantic *Salmo salar*, chum *Oncorhynchus keta*and pink salmon *O. gorbuscha* during infections with salmon lice *Lepeophtheirus salmonis*: a review of the impact of parasitic copepods on marine aquaculture. *Bmc Genomics* **15**: 1–17.

Tarifeño-Saldivia E, Valenzuela-Miranda D, Gallardo-Escárate C (2017) In the shadow: the emerging role of long non-coding RNAs in the immune response of Atlantic salmon. *Developmental and Comparative Immunology* **73**: 193–205.

Todd CD, Walker AM, Ritchie MG, Graves JA, Walker AF (2004) Population genetic differentiation of sea lice (*Lepeophtheirus salmonis)* parasitic on Atlantic and Pacific salmonids: analyses of microsatellite DNA variation among wild and farmed hosts. *Canadian Journal of Fisheries and Aquatic Sciences* **61**: 1176–1190.

Todd CD, Stevenson RJ, Reinardy H, Ritchie MG (2005) Polyandry in the ectoparasitic copepod *Lepeophtheirus salmonis* despite complex precopulatory and postcopulatory mate-guarding. *Marine Ecology Progress Series* **303**: 225–234.

Toh S, Glanfield A, Gobert G, Jones M (2010) Heme and blood-feeding parasites: friends or foes? *Parasites and Vectors* **3**: 108.

Torrissen O, Jones S, Asche F, Guttormsen A, Skilbrei OT, Nilsen F *et al.* (2013) Salmon lice - impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases* **36**: 171–194.

Touhara K, Vosshall LB (2009) Sensing odorants and pheromones with chemosensory receptors. *Annual Review of Physiology* **71**: 307–332.

Tully O, Nolan D (2002) A review of the population biology and host–parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Parasitology* **124**: 165–182.

Valenzuela-Miranda D, Gallardo-Escárate C (2016) *Caligus rogercresseyi* serine proteases: transcriptomic analysis in response to delousing drugs treatments. *Aquaculture* **465**: 65–77.

Valenzuela-Miranda D, Nuñez-Acuña G, Valenzuela-Muñoz V, Asgari S, Gallardo-Escárate C (2015) MicroRNA biogenesis pathway from the salmon louse (*Caligus rogercresseyi*): emerging role in delousing drug response. *Gene* **555**: 231–241.

Valenzuela-Miranda D, Etebari K, Asgari S, Gallardo-Escárate C (2017) Long noncoding RNAs: unexplored players in the drug response of the sea louse C*aligus rogercresseyi*. *Agri Gene* **4**: 1–7.

Valenzuela-Muñoz V, Gallardo-Escárate C (2014) TLR and IMD signaling pathways from*Caligus rogercresseyi* (Crustacea: Copepoda): *in silico* gene expression and SNPs discovery. *Fish & Shellfish Immunology* **36**: 428–434.

Valenzuela-Muñoz V, Gallardo-Escárate C (2016) Transcriptome mining: multigene panel to test delousing drug response in the sea louse C*aligus rogercresseyi*. *Marine Genomics* **25**: 103–113.

Valenzuela-Muñoz V, Gallardo-Escárate C (2017) Iron metabolism modulation in Atlantic salmon infested with the sea lice *Lepeophtheirus salmonis* and *Caligus rogercresseyi*: a matter of nutritional immunity? *Fish & Shellfish Immunology* **60**: 97–102.

Valenzuela-Muñoz V, Nuñez-Acuña G, Gallardo-Escárate C (2014) Molecular characterization and transcription analysis of*P-glycoprotein*gene from the salmon louse C*aligus rogercresseyi*. *Journal of Aquaculture Research & Development* **5**: 236.

Valenzuela-Muñoz V, Chavez-Mardones J, Gallardo-Escárate C (2015a) RNA-seq analysis evidences multiple gene responses in *Caligus rogercresseyi* exposed to the anti-salmon lice drug azamethiphos. *Aquaculture* **446**: 156–166.

Valenzuela-Muñoz V, Sturm A, Gallardo-Escárate C (2015b) Transcriptomic insights on the ABC transporter gene family in the salmon louse C*aligus rogercresseyi*. *Parasites & Vectors* **8**: 209.

Valenzuela-Muñoz V, Boltaña S, Gallardo-Escárate C (2016) Comparative immunity of *Salmo salar* and *Oncorhynchus kisutch* during infestation with the sea louse C*aligus rogercresseyi*: an enrichment transcriptome analysis. *Fish & Shellfish Immunology* **59**: 276–287.

Valenzuela-Muñoz V, Boltaña S, Gallardo-Escárate C (2017a) Uncovering iron regulation with species-specific transcriptome patterns in Atlantic and coho salmon during a *Caligus rogercresseyi* infestation. *Journal of Fish Diseases* **40**: 1169–1184.

Valenzuela-Muñoz V, Novoa B, Figueras A, Gallardo-Escárate C (2017b) Modulation of Atlantic salmon miRNome response to sea louse infestation. *Developmental & Comparative Immunology* **76**: 380–391.

Valenzuela-Muñoz V, Valenzuela-Miranda D, Gallardo-Escárate C (2018) Comparative analysis of long non-coding RNAs in Atlantic and Coho salmon reveals divergent transcriptome responses associated with immunity and tissue repair during sea lice infestation. *Developmental & Comparative Immunology* **87**: 36–50.

Vera-Bizama F, Valenzuela-Muñoz V, Gonçalves AT, Marambio JP, Hawes C, Wadsworth S *et al.* (2015) Transcription expression of immune-related genes from *Caligus rogercresseyi* evidences host-dependent patterns on Atlantic and coho salmon. *Fish & Shellfish Immunology* **47**: 725–731.

Villani F, White GB, Curtis CF, Miles SJ (1983) Inheritance and activity of some esterases associated with organophosphate resistance in mosquitoes of the complex of *Culex pipiens* (Diptera: Cnlicidae). *Bulletin of Entomological Research* **73**: 153–170.

Vontas J, David JP, Nikou D, Hemingway J, Christophides G, Louis C *et al.* (2007) Transcriptional analysis of insecticide resistance in Anopheles stephensi using cross-species microarray hybridization. *Insect molecular biology* **16**: 315–324.

Wagner GN, Fast MD, Johnson SC (2008) Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids. *Trends in Parasitology* **24**: 176–183.

Yasuike M, Leong J, Jantzen SG, von Schalburg KR, Nilsen F, Jones SRM *et al.* (2012) Genomic resources for sea lice: analysis of ESTs and mitochondrial genomes. *Marine Biotechnology* **14**: 155–166.