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Catching the complexity of salmon-louse interactions

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A B S T R A C T

The study of host-parasite relationships is an integral part of the immunology of aquatic species, where the complexity of both organisms has to be overlayed with the lifecycle stages of the parasite and immunological status of the host. A deep understanding of how the parasite survives in its host and how they display molecular mechanisms to face the immune system can be applied for novel parasite control strategies. This review highlights current knowledge about salmon and sea louse, two key aquatic animals for aquaculture research worldwide. With the aim to catch the complexity of the salmon-louse interactions, molecular information gleaned through genomic studies are presented. The host recognition system and the chemosensory receptors found in sea lice reveal complex molecular components, that in turn, can be disrupted through specific molecules such as non-coding RNAs.

1. Introduction

Host-parasite interactions are a complex relationship where one organism benefits the other and often where there is a negative impact on the host [1,2]. In the presence of a parasite, the host displays changes in its behavior, reproduction, regulation of immune response, among other processes [2]. Meanwhile, the parasite to establish itself in the host must counteract its defense mechanisms [1]. This explains why parasites have evolved to develop multiple strategies to evade the defense mechanisms of their hosts, and in parallel, the latter have had to develop strategies to evade the parasites [3]. In parasites , there are several different evasion mechanisms of the host response. For example, the plant parasite *Meloidogynei* secretes proteins such as proteases, superoxide dismutase and calreticulin [3]. In the case of ectoparasites, proteases, protease inhibitors, cathepsins, and other molecules are released. These secreted proteins can immuno-suppress the host, facilitating ectoparasite penetration in unbroken skin tissues where feeding can occur [4,5]. A large part of the life cycle of ectoparasites occurs when they are established in their hosts with different developmental stages often involving different molecular mechanism driving host-parasite interactions [6,7]. The molecular basis of host-parasite interactions has been studied in the context of different environments but there is limited information from marine systems given the emerging landscape of these interactions associated with environmental change.

Sea lice is a marine ectoparasite that mainly infests salmonid species. Given their high impact on the production of salmonid fish worldwide the most studied sea lice species are *Lepeophtheirus salmonis* and *Caligus rogercresseyi* [8–10]. Annually the salmon industry presents estimated losses of US $ 480 million, representing between 4 and 10% of production costs [10]. The presence of sea lice on the fish causes skin damage, immunosuppression, and facilitate the co-infection with bacterial pathogens [11–13]. Like all ectoparasites, lice spend a large part of its life cycle on a host, which makes it necessary for this ectoparasite to generate mechanisms for evading the immune response of its host.

Recently, genomic tools have allowed increasing knowledge about the molecular mechanisms associated with the parasite-host interaction process [14–17]. As lice are organisms with a significant economic impact on aquaculture, numerous studies have been aimed to understand the mechanisms of immune responses of salmonids as well as the evasion mechanisms used by lice for a successful infection. In this, several hypotheses have been proposed to explain the clear differences that exist in host with different susceptibility to lice infections. Some of these hypotheses have been associated with host skin thickness [18], mucus composition [19], rapid and effective innate immune response [20], homeostasis and iron regulation [21], adaptive immunity [22] and regulation of responses measured by non-coding RNA [23]. In this review, different mechanisms sea lice use for recognition and response to salmonid defenses are described. In addition, immune response mechanisms that have been identified in salmonid species are discussed.

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2. Recognition of host and chemosensory receptors in sea lice

Chemical or "semiochemical" signals have been crucial for the evolution of various species of animals in the marine ecosystem [24–26]. For instance, copepods are a class of marine species that communicate through various intra- or inter-specific chemical signals [27,28]. This group of aquatic crustaceans has developed various receptor structures that allow the identification of various chemical signals. Among them, the most described are the *sensilla*, which are neurons located in the main chemosensory organs where the most common are known as unimodal sensilla [29,30]. However, the molecular basis for the recognition of semiochemicals from the environment remains virtually unknown in these marine invertebrates.

The life cycle of the sea louse species is complex and consist of several instars divided by molts. For instance, *L. salmonis* has eight stages, comprising five larval stages, two preadult stages and the adult stage [157]. Meanwhile, *C. rogercresseyi* comprises two larval stages (nauplius I, nauplius II and copepodite), four juvenile stages (chalimus I - IV) and one adult stage (male or female) [138]. During the copepodite stage, the process of host identification occurs, preparing the lice for infestation and settlement. From this point, the juvenile stages can initialize the infective process. The successful infestation process on the host allows the parasites the intake of nutrients for reproduction and adult development [31]. Previous studies have shown that lice species, such as *L. salmonis* that infects fish in the northern hemisphere, have developed physical mechanisms of host recognition. Among these, lice can identify the temperature of the water in which white fish species live, salinity changes, and detect small variations in water movement generated swimming fish [28,32,33]. Host identification via detection of semiochemicals has also been reported [34,35]. The presence of advanced chemoreceptors in *L. salmonis* has been described, which are capable of identifying specific molecules of different host species [29,32,35,36]. The main chemosensory receptors described in lice have been found in antennae located in the parasite's head. Neuronal activity measured through electrophysiological probes increases in the antennae exposed to water containing fish mucus [29,37]. Likewise, there is evidence that the ablation of antennae generates a reduction in the capacity to seek the host [35]. Low molecular weight compounds such as isophorone and 6-methyl-5-hepten-2-one, produced by Atlantic salmon can generate attractancy on *L. salmonis* [34]. With respect to *C. rogercresseyi*, it has been described that there is a preference for water conditioned with fish mucus, suggesting that there is a similar chemical recognition phenomenon [38]. Receptors involved in host recognition have been identified in the sea louse *C. rogercresseyi*, suggesting that the gene family of ionotropic receptors (IRs) are key molecular components for the salmon-louse interaction [39]. Notably, these genes have differential expression depending on the host species, and the use of in-feed ingredients such as odor-masking compounds [40]. The functional role related to the chemosensory system has been based on the structure highly similar to those reported for insects, with a known olfactory function [41]. Additionally, these genes have an expression profile highly linked to the infective stage of copepodid (IR25a), or to adult males (kainate receptors), which have a role in mating behavior [42]. These receptors generally act in conjunction with binding proteins, which in the case of insects are known as odorant binding proteins (OBP), which have not been identified yet in sea lice [43]. However, this does not exclude all types of binding proteins, since there are other types that could interact with chemosensory receptors, such as chemosensory proteins (CSP), which are found in various arthropod species [44]. On the other hand, in *L. salmonis* it was identified that chemosensory genes were mainly associated to the gene expression of the parasite's antennules, which are structures with chemosensory function [45]. A recent study in *L. salmonis* showed that the silencing of an ionotropic receptor (IR25a) decreases parasite activity related to host recognition, evidencing a key role related to host recognition [46]. The IR genes have significant sequence similarity for *L. salmonis* and *C. rogercresseyi*.

3. Effect of masking compounds on sea lice infections *C. rogercresseyi*

It has been shown that there is a differential expression of genes related to the chemosensory system of *C. rogercresseyi* depending on the host species. Through a temporal scale, there is a relationship between the expression of IRs in the parasite and the dynamics of parasite load in infected salmonids [39,40]. In these studies, the Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*) have been identified as susceptible and resistant species, respectively, for lice infestation. Salmon gene expression patterns have been correlated with in-feed additives to disrupt the parasite host-recognition, and with immunostimulants to strengthen the fish immune defense [47]. The decrease in the expression of IRs in parasites infecting Atlantic salmon fed with masked diets indicates that the infestation success is reduced by disruption of chemosensory capacity.

4. Role of host salmon antimicrobial peptides on the chemosensory system of sea lice

In salmonids, antimicrobial peptides (AMPs) have been described as important defense mechanisms that are expressed in tissues that are generally exposed to infections. These molecules are secreted by the mucus and in the case of salmon through the skin [48,49]. Fish produce various types of AMP, including defensins and cathelicidins, as well as others specific to the group such as piscidins [50]. As for salmonid fish, they produce AMPs such as those mentioned above, as well as hepcidins and histone-derived AMPs. Together these AMPs form a main basis of defense against pathogens [51]. Of these AMPs, the cathelicidin group could also be involved in long-term defense, as described in trout [52]. Cathelicidins have been widely studied in several vertebrate species, but in salmonids, they have their own characteristics at a structural level, which could confer new functions [53]. This class of AMP is highly associated with the response of Atlantic salmon to bacterial infections such as yersiniosis [54]. Cathelicidins were also found among the genes that were activated in salmon infected with the sea lice ectoparasite *L. salmonis*, while other AMPs such as defensins decreased their expression levels [55].

It is important to point out that *C. rogercresseyi* begins to feed on host fish mucus when the copepodid is attached to the skin [31], so direct contact between AMPs and lice is suggested. It has been reported in other fish species that their AMPs may come into contact with ectoparasites that infect them in target tissues [56]. The expression profiles of AMPs in *C. rogercresseyi* hosts have been shown to be expressed in early stages of infection in *O. kisutch*, which is congruent with the observation that this species is resistant given a rapid and effective activation of the defensive mechanisms [57]. One of the AMPs produced by salmonids, cathelicidin-2 (Cath-2), promotes the development of the frontal filament of *C. rogercresseyi* and activates the expression of IRs, as well as genes related to the formation of the cuticle [58]. The frontal filament is a structure highly associated with the success of infection by this parasite so that the stimulus of its development suggests the host favors the adhesion of the louse on the fish skin [59].

Regarding the antimicrobial peptide Cath-2, it has an attractant activity on lice, especially in its infective stage. Characteristics of Cath-2 including its molecular weight, three-dimensional structure, chemical nature and concentration in the water meet all criteria to be detectable by sea lice [29]. Moreover, it has been established that the movement of copepodids when stimulated by Cath2 is directed, similar to that observed in response to stimuli with light [60]. This is consistent with that reported in another crustacean, *Daphnia pulex*, which has swimming behavior addressed by the presence of stimuli that generate attraction [61]. In addition, there is a global transcriptome activation in response to Cath-2 [58,60], which is consistent with other species of arthropods

that show a broad activation at the RNA level in the presence of specific attractant molecules [62]. Previous studies in *C. rogercresseyi* has revealed that genes modulated in copepodids match with chemosensory genes and synaptic-related genes [58], which is consistent with studies in crustaceans [63,64]. With all this evidence it is inferred that the antimicrobial peptide Cath-2 acts as a kairomone that attracts sea lice, favoring the infection process.

5. Modulation of the host's immune response during the lice infestation

The development of aquaculture worldwide has made it necessary to increase efforts to understand the defense mechanisms of fish against infections. Due to the explosive advance of the sequencing technologies, the amount of genomic information of aquatic organisms, as well as the knowledge of the immune response pathways of fish has substantially increased [65]. In general, teleost organisms present an immune system similar to that of other vertebrates with elements of the innate and adaptive immune response. Studies on the immune response mechanisms of fish in the presence of copepods are scarce. In Atlantic salmon infested with *L. salmonis*, the immune response has been described and is associated with the expression of pro-inflammatory cytokines [66], secretion of proteases [5], and humoral response [22,66]. Specifically, increasing levels of IL4/13 and IL10 have been reported in families of Atlantic salmon resistant to *L. salmonis*, as well as in pink and Coho salmon that are less susceptible, suggesting a $CD4^+$ T helper 2 (Th2) type response to cope with lice infestation [9,22,67] (Fig. 1). The response mechanisms of salmonids infested by *C. rogercresseyi* differ from those described for salmon infested with *L. salmonis* [20,66–69] (Table 1). Notably, Atlantic salmon has higher levels of susceptibility to *C. rogercresseyi* compared to Coho salmon, which also modulates the types of the immune response during the infestation process. Atlantic salmon responds to the presence of *C. rogercresseyi* by activating a toll-like receptor (TLR), specifically tlr22a2, which has been reported exclusively in fish [70]. This TLR has also been associated in other species of fish infested by ectoparasites, as one of the first mechanisms to activate the immune response. For example, upregulation of tlr22a2 in *L. rohita* infested by the ectoparasite *A. siamensis* has been described [71,72] (Table 1). In L. *rohita*, a direct relationship was observed between expression levels of tlr22a2 and the amount of *A. siamensis* in fish [73]. Notably, Coho salmon does not have a significant increase in the expression of trl22a2, contrary to the increase in the expression of tlr13, which in fish has a common ancestor with TLR22 [74] (Fig. 1). Thus, overregulation of tlr13 in Coho salmon suggests that this salmon species could respond via TLR to the infestation of *C. rogercresseyi*, similarly to Atlantic salmon with tlr22a2 [75] (Table 1). Furthermore, studies conducted on salmon infected with *L. salmonis* suggest a role for MHC II in response to skin damage in fish [22,67]. This has also been reported in fish with the presence of *Paramoeba perurans* [76]. The inflammatory response is the first reaction associated with the coping against sea lice, generating variations in the transcriptional activity of genes such as interleukin 1α, MMP13, MMP19, TNF-α, prostaglandins E2 and IL-8 [9,20,22,66,67,77,78]. Fish that have lower susceptibility to infestation with *L. salmonis* display a Th2 type immune response [22,66,67,77] (Table 1). Coho salmon, tolerant to infection with *C. rogercresseyi*, displays a low expression of genes associated with the Th2 response. However, an early response to the presence of *C. rogercresseyi* in Coho salmon shows an increase in the expression levels of genes associated with Th1 response [75] (Fig. 1). We hypothesize that the low expression of Th2 genes in Coho salmon could be a strategy to avoid the overproduction of mucus in fish, the main food source of *C. rogercresseyi*, since it has been described that the Th2 response increases secretion of mucus through the production of mucin [66,79,80] (Table 1).

6. Role of iron homeostasis mechanisms in the Salmon-louse interaction

Parasites depend on host nutrients, thus it is crucial to select those that have the greatest amount of nutritional resources for their development, survival, and reproduction [81,82]. A type of immunity called nutritional immunity has been proposed and consists of the limitation of nutrients by the host [81], generating a hostile environment for the development of parasites. Here, the availability of ions such as Fe, Mn, Zn is mainly modulated through molecular mechanisms that involve key signaling pathways and regulatory components [81]. Among the most important nutrients in this immune strategy is iron, due to its role in different physiological processes such as respiration, and enzymatic activity [81,83]. It has also been observed that iron can modulate proinflammatory cytokines and acute phase proteins [84]. Evidence of nutritional immunity has been reported in fish infested by *L. salmonis*, observing a correlation between resistance to lice infection and heme regulation [69]. In parallel, fish infested with lice display low expression levels of hepcidin, a protein responsible for the transport of iron into the cell [22].

Transcriptome studies in infected-fish with *C. rogercresseyi* have shown a large number of transcripts associated with ion binding [85] (Table 1). For instance, an increase in hepcidin has been associated with the secretion of IL-6 after the activation of pattern recognition receptors (PPRs) during bacterial infections [86]. In macrophages, it has been observed that iron limitation reduces the expression of MHCII, iNOs, and also inhibits the IFN-g signaling pathway [84]. Likewise, in fish with low susceptibility to the ectoparasite *L. salmonis*, an increase in regulatory molecules of the heme group has been observed, reflected in the increase in the expression levels of heme oxygenase [69]. From transcriptomic data of the skin and head kidney of Atlantic and Coho salmon infested *C. rogercresseyi*, over-regulation of hemoglobin subunit in both species have been reported [85]. In contrast, Coho salmon infested with *C. rogercresseyi* a decrease in expression of heme oxygenase is associated with the heme degradation, and also an increasing level of the biosynthesis pathway comprising the aminolevulinic acid synthetase and coproporphyrinogenase genes [85]. For Atlantic salmon, there has been reported an increase in the expression of BLVr, a gene associated with the degradation of the heme group at seven days post-infestation [85]. This result, similar to that reported by Gonzáles et al. [87], where a reduction of hemoglobin in Atlantic salmon infested by *C. rogercresseyi* was observed, suggests a reduction of the heme group as an iron source, as a strategy to cope with the lice infestation. Iron homeostasis is not only given by the regulation of the heme group, but there are also proteins responsible for reducing circulating iron as a consequence of the degradation of the heme group [83]. Among this, hepcidin, haptoglobin, ferritin, transferrin proteins have been reported to play a role in iron regulation. Atlantic salmon infected with *C. rogercresseyi* increases of BLVr levels has been reported in conjunction with the transferrin gene. It has been also observed high levels of hepcidin, a protein that has also been associated with anemic states in fish [88,89]. The modulation of the availability to store iron through biosynthesis or degradation of the heme group, and also iron transporter genes, suggests that this mechanism is a key molecular component exploited by fish during the lice infestation [85] (Table 1).

The nutritional strategy seems to be a unique response to lice infestation in Atlantic salmon. A comparative study in skin and head-kidney in infested fish with *L. salmonis* and *C. rogercresseyi* showed significant differences in genes associated with biosynthesis/degradation of the heme group as well as iron transporter genes and oxidative stress response [21]. Among the differences observed, it can be noted that fish infested with *C. rogercresseyi* showed high expression of hepcidin, such as the one observed in salmon resistant to *L. salmonis* [22]. In addition, fish infested with *C. rogercresseyi* showed high modulation of genes associated with heme group degradation. While the genes associated with biosynthesis pathway such as ferritin and transferrin

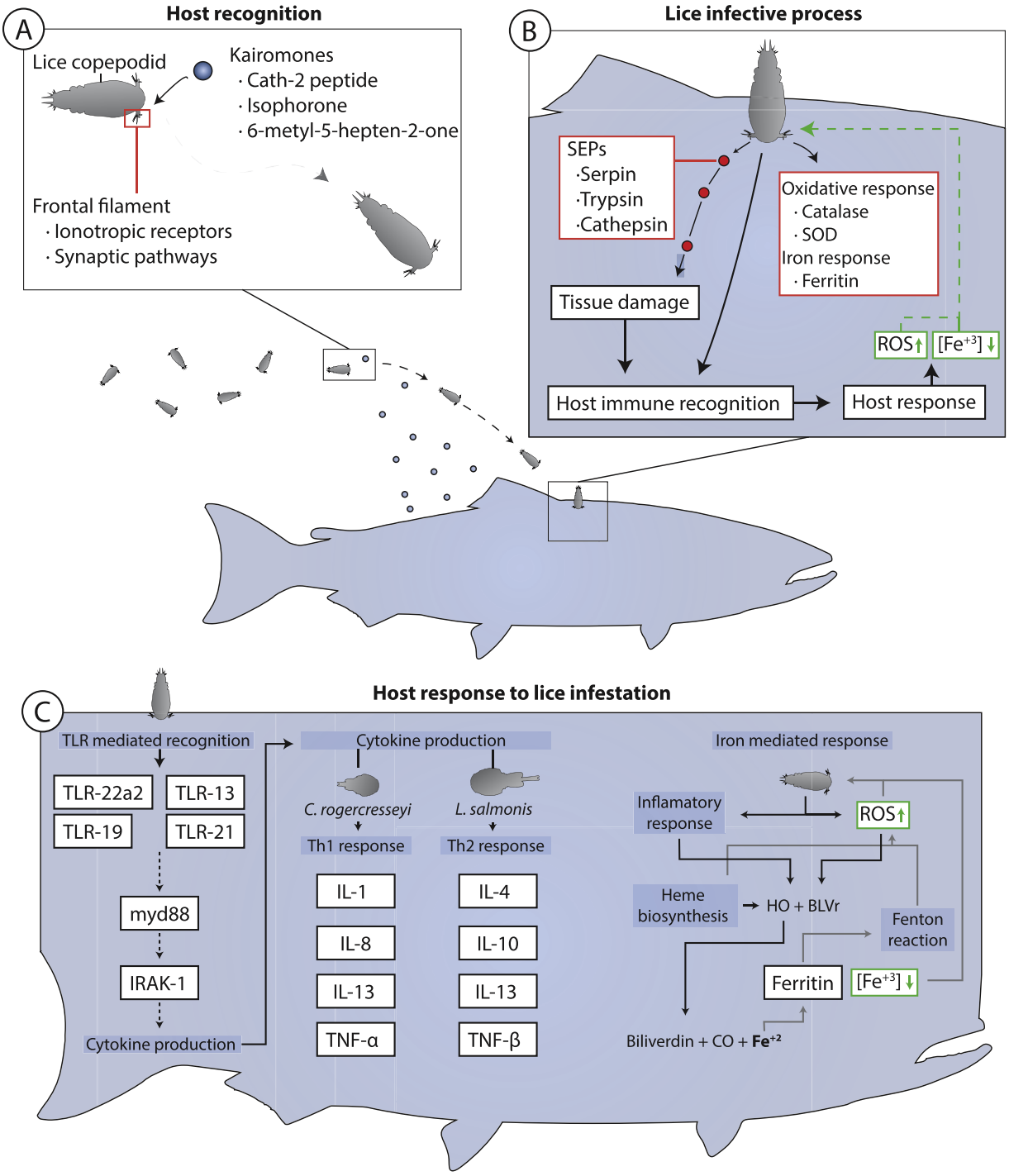

Fig. 1. Schematic model of salmon-louse interactions. (a) Description of the molecular mechanism of sea lice for host recognition. (b) Sea lice molecular response for avoid ing host response and (c) Host innate and adaptive immune mechanism and iron homeostasis modulation in response to sea lice infestation.

receptor were highly expressed in fish infested with *L. salmonis* [21]. In addition, the increase in heme biosynthesis is associated with an increase in oxidative stress levels [90], which is reflected in fish infested with *L. salmonis* through the increasing of glutathione S-transferase (GST) and superoxide dismutase (SOD) transcription expression. With this information, it is possible to confirm that there is a nutritional response mechanism in Atlantic salmon infested by sea lice. According to the results, the nutritional strategy to respond to *L. salmonis* and *C. rogercresseyi* varies in the same species of salmonids, being of greater relevance in the response to *L. salmonis*, which is a hematophagous organism [90], whereas *C rogercresseyi* is a facultative hematophagous organism (Fig. 1).

7. The immune system of sea lice

From the recognition process through infestation and the development of different life stages, lice must cope with the rejection mechanisms displayed by the fish. In order to understand the molecular mechanisms that lice use for its defense, transcriptomic data from different stages of development of *C. rogercresseyi* [7] have been characterized. Innate immune response pathways IMD and TLR [91], genes associated with the antioxidant system (ROS) [92] and secretory

Table 1
Transcriptome studies associated with the host's immune response during sea lice infestation.

Host species	Sea lice specie s	Molecular mechanism	Experimental design	Reference
O. gorbuscha	*L. salmonis*	Up-regulation of MHII, COX-2, CRP and IL-8	24-48 hpi (juvenile)	Braden et al. (2015)
O. gorbuscha	*L. salmonis*	Down-regulation of MHII, C-reactive protein, IL-1b, IL-8, COX-2	24-48 hpi (mature)	Braden et al. (2015)
O. gorbuscha	*L. salmonis*	Expression of MH class II, IL-6, CRP, MMP13, IL-1β and COX-2 genes	24-48 hpi	Braden et al. (2012)
O. gorbuscha	*L. salmonis*	Increase expression of TNF-alpha	24-48 hpi	Braden et al. (2015)
O.s gorbuscha	*L. salmonis*	Pro-inflammatory response, PRRs and iron homeostasis	6 dpi	Sutherland et al. (2014)
O. gorbuscha	*L. salmonis*	Oxidative stress	6 dpi	Sutherland et al. (2014)
O. gorbuscha	*L. salmonis*	Suppressed antiviral immunity	6 dpi	Sutherland et al. (2014)
O. keta	*L. salmonis*	Suppressed antiviral immunity	6 dpi	Sutherland et al. (2014)
O. keta	*L. salmonis*	Pro-inflammatory response, PRRs and iron homeostasis	6 dpi	Sutherland et al. (2014)
O. kisutch	*L. salmonis*	Early pro-inflammatory TH1-type pathway and regulatory TH2-type processes	24-48 hpi	Braden et al. (2015)
O. kisutch	*C. rogercresseyi*	Oxidative stress	7 dpi	Vera-Bizama et al. (2015)
O. kisutch	*C. rogercresseyi*	TLR pathway (up-regulation of tlr13)	14 dpi	Valenzuela-Muñoz et al. (2016)
O. kisutch	*C. rogercresseyi*	Increase in Th1 gene related	14 dpi	Valenzuela-Muñoz et al. (2016)
O. kisutch	*C. rogercresseyi*	Pro-inflammatory response	7-14 dpi	Valenzuela-Muñoz et al. (2016)
O. kisutch	*C. rogercresseyi*	Up-regulation of Heme synthesis	7-14 dpi	Valenzuela-Muñoz et al. (2017)
S. salar	*L. salmonis*	Suppressive effect in pro-inflammatory mediators	24 h dpi	Braden et al. (2012)
S. salar	*L. salmonis*	Innate immune response reactions	1 dpi	Tadiso et al. (2011)
S. salar	*L. salmonis*	Decreases of values of immune-related genes	4 dpi	Holm et al. (2017)
S. salar	*L. salmonis*	Suppressed antiviral immunity	6 dpi	Sutherland et al. (2014)
S. salar	*L. salmonis*	TLR/IMD signaling Pathway	7 dpi	Valenzuela-Muñoz et al. (2016)
S. salar	*L. salmonis*	Up-regulation of Heme synthesis	8 dpi	Valenzuela-Muñoz et al. (2017)
S. salar	*L. salmonis*	Increase of Th1, Th17 and Th2 pathways	8 dpi	Holm et al. (2017)
S. salar	*L. salmonis*	Increase expression of IL-1b, TNFa, MHII, TGFb-like cytokine and COX-2	9 dpi	Fast et al. (2006)
S. salar	*L. salmonis*	Adaptative Humoral Immune Response	5-10 dpi	Tadiso et al. (2011)
S. salar	*L. salmonis*	Up-regulation of alternatively activated macrophages	3-33 dpi	Skugor et al. (2008)
S. salar	*L. salmonis*	Pro-inflammatory response (Th2-like pathway)	3-33 dpi	Skugor et al. (2008)
S. salar	*L. salmonis*	Increase expression of the MH class I gene	21 dpi	Fast et al. (2006)
S. salar	*L. salmonis*	Down-regulation of alternatively activated macrophages	22 dpi	Skugor et al. (2008)
S. salar	*L. salmonis*	Increase expression of MH class II and TGFβ-like genes	33 dpi	Fast et al. (2006)
S. salar	*L. salmonis*	Increase expression of Interleukin-1b and TNFα-like genes	40 dpi	Fast et al. (2006)
S. salar	*L. salmonis*	Th2 immune response	3 weeks dpi	Holm et al. (2015)
S. salar	*C. rogercresseyi*	Early responses of TLR/IMD signaling pathway	3 dpi	Vera-Bizama et al. (2015)
S. salar	*C. rogercresseyi*	Oxidative stress	7 dpi	Vera-Bizama et al. (2015)
S. salar	*C. rogercresseyi*	TLR pathway (up-regulation of TLR22 and MHCII)	14 dpi	Valenzuela-Muñoz et al. (2016)
S. salar	*C. rogercresseyi*	Inflammatory and oxidative stress response (Th1 pathway)	14 dpi	Boltaña et al. (2016)
S. salar	*C. rogercresseyi*	Up-regulation of Heme degradation and iron transport	7-14 dpi	Valenzuela-Muñoz et al. (2017)
S. salar	*C. rogercresseyi*	Up-regulation of various innate immune and detoxication genes	15 dpi - in-feed additives	Núñez-Acuña et al. (2016)

products such as serpins and trypsins [93] have been described. In addition, variations in the response of *C. rogercresseyi* infesting Atlantic and Coho salmon have been observed [94]. Here, both fish species increase the transcription activity of genes associated with oxidative stress, suggesting that lice respond to a large number of reactive oxygen species (ROS) generated by infected fish. Notably, high expression of antioxidant genes in lice has been correlated with low susceptibility to *L. salmonis* infestation [69]. In addition, the modulation of ROS-related genes in fish has been linked to increased expression of cytokines [11] and metalloproteases [68] released during an infestation. In parallel, *C. rogercresseyi* infecting Atlantic salmon showed a significant increase in the expression of ferritin, suggesting a compensatory response against the ROS that in turn, can be controlled by the Fenton reaction catalyzed by ferritin [95]. It is important to note that ferritin is also highly expressed in Atlantic salmon during the infestation, so it can be suggested that both the host and the ectoparasite compete for free iron [94]. However, the iron competition will differ from the host and the parasite proposes, while the molecular mechanisms are common and highly conserved.

During the infestation process, the ectoparasites feed on the host's blood by penetrating the skin, and in parallel, release molecules such as proteases and immunosuppressive molecules. The excretory/secretory proteins (SEPs) of the pathogens change the environment of the host cell by modulation of the host's immune system, facilizing the infection and its proliferation [96]. SEPs participate in the parasite-host interaction, modulating the timing related to the life-stages development [97]. Among the main components of parasite, secretions are proteolytic enzymes including cathepsin L, cathepsin B, serine proteases (serpins) and carboxypeptidases or trypsin-like [98].

Serine proteases inhibitors (serpins) regulate the innate immunity of insects through the inhibition of cascades of serine proteinases that initiate immune responses such as melanization and the production of antimicrobial peptides [99,100]. The serpins are a superfamily of proteins widely distributed with a SERPIN domain and participate in several immune responses. From the transcriptomic database of *C. rogercresseyi* [7] several isoforms have been identified. Among them, Cr-serpin 10 is outstanding, expressed during the different stages of ectoparasite development [93] (Table 2). In one study, high levels of expression of Cr-serpin 10 and Cr-serpin 3 were observed in copepodids, suggesting a role of these serpins in the evasion process of the immune response of *C. rogercresseyi* [93]. Other proteins associated with the secretome of sea lice are the cathepsins. In L. *salmonis* the cathepsin-L has been observed within the secretory/excretory products, indicating a putative role of these proteases in immune-evasion and parasite survivor [101]. The function of these proteins appears to be essential in a variety of important parasite biological processes, such as molting, cuticle remodeling, embryogenesis, and feeding, making them an attractive target for vaccine development [102]. There are different groups of cathepsins, the cathepsins of the L group being the most abundant in arthropods [103]. In L. *salmonis*, three highly expressed cathepsins have been identified during the infective stage, including cathepsin L, suggesting a putative role for feeding and immune defense [104]. In *C. rogercresseyi*, a comprehensive study has identified 56 cathepsin-like sequences distributed in five groups of cysteine proteases (B, F, L, Z, and S) and one for the aspartic protease group (D) [105]. As in L. *salmonis*, cathepsin L is one of the most abundant cathepsins in *C. rogercresseyi* [105]. In *C. rogercresseyi*, high levels of Cr-CatL1 expression have been reported in females, associating their function with

Table 2
Studies associate to transcriptome analysis of sea lice secretome.

Host species	Sea lice species	Molecular mechanisms	Experimental design	Reference
S. salar	*C. rogercresseyi*	Prostaglandin Enolase Cathepsin D1, B1 PhoC Trypsin 2, 5, 11, 12, 13, 17 Serpin 3, 4, 10	3 and 7 dpi	Vera-Bizama et al. (2015)
O. kisutch	*C. rogercresseyi*	PTGSE Enolase Cathepsin D1, B1 PhoC Tryp 2, 5, 11, 12, 13, 17 Serpin 3, 4, 10	3 and dpi	Vera-Bizama et al. (2015)
S. salar	*C. rogercresseyi*	Serpin 1, 2, 3, 4, 5, 6, 7, 8, 10, 11	Ontogenic development	Maldonado-Aguayo and Gallardo-Escárate (2014)
S. salar	*C. rogercresseyi*	chymotrypsin 1, trypsin 1, 6, 10, 36, 41, 42	Delousing drugs treatments	Valenzuela-Miranda and Gallardo-Escárate (2016)
S. salar	*L. salmonis*	serine-type endopeptidases, 2 cysteine type endopeptidases, 1 phosphatase, 1 acetylcholine receptor	Collection of E/S material from adult stages of the parasite	Hamilton et al. (2018)
S. salar O. gorbuscha and O. keta	*L. salmonis*	secretory products	E/S products evaluation from primary culture of salmon	Lewis et al. (2014)
S. salar head kidney-isolated macrophage	*L. salmonis*	secretory/excretory products	SHK-1 exposition	Fast et al. (2007)
O. kisutch, S. salar, O. nerka	*L. salmonis*	Trypsin 1, trypsin-like serine protease, collagenase, carboxypeptidase B, Chymotrypsin BI, Chymotrypsin A chain C	24 and 48 h post-infection	Braden et al. (2017)
S. salar	*C. rogercresseyi*	Serine proteases inhibitor, alpha 1 antiproteinase-like, antithrombin protein, heat shock protein 47	15 dpi	Núñez-Acuña et al. (2015)
S. salar	*C. rogercresseyi*	Cathepsin B1 Cathepsin D1, D2 Cathepsin L1, L26, L27 Cathepsin Z10	Distribution and abundance of cathepsin-like transcript identified in sea lice developmental stages.	Maldonado-Aguayo et al. (2015)
Salmon	*L. salmonis*	Cathepsin L1 Cathepsin L2	Library construction of various life stages (chalimus I–IV, preadult male I–II, preadult female I–II, adult male, adult female and egg strings)	McCarthy et al. (2012)
Salmon	*L. salmonis*	Cathepsin B cysteine protease	Adult *L. salmonis* were collected from infected salmon found	Cunningham et al. (2010)
O. mykiss, O. kisutch, S. salar, P. americanus	*L. salmonis*	Proteases and alkaline phosphatase	Adult and preadult sea lice	Fast et al. (2003)

louse embryonic development. In addition, Cr-CatL1, Cr-CatL26, and Cr-CatL27, together with Cr-CatB1, are highly expressed during the larval stages, nauplius, suggesting their possible participation in the processes of molting or cuticle remodeling [105].

Among the proteins of greater abundance within the SEPs, trypsins are found [106]. Trypsin is a protease stored as zymogen, which is activated during feeding, being deposited in the saliva through autolysis or the action of proteases provided by the host [107]. In L. *salmonis*, LsTryp10 has been identified and characterized. This trypsin is distributed uniformly in the ovaries and oocytes of the ectoparasite, suggesting the regulation of the degradome of the yolk [108] (Table 2). In *C. rogercresseyi*, 44 trypsin and seven chymotrypsin were identified [109]. Notably, these proteins seem to be also modulated in adults of *C. rogercresseyi* exposed to delousing drugs [109]. Here, the authors report sex-specific modulation of Cr-Tryp1-6-10-36 and 41 transcripts differentially expressed in response to pesticides, suggesting a putative role in drug metabolism.

8. Role of non-coding transcripts during C. rogercresseyi infestation

The increasing availability of transcriptome data has revealed the importance of the non-coding RNAs as key regulators of the mRNA transcription. Herein, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been studied [110,111]. The lncRNAs are long sequences greater than 200 nucleotides, transcribed in a similar way to the coding RNAs [112,113]. The study of lncRNAs is relatively new. Genomic studies have shown that in mammals as well as other vertebrates the number of RNAs that are translated into protein is close to 20,000 and due to differences in the genomic sizes of organisms, it has been suggested that numbers of lncRNAs have been increasing during the evolutionary process, so that organisms with more complex mechanisms have acquired more lncRNAs, as a mechanism to control several biological processes [114]. lncRNAs studies report that there is a proportion that is expressed in a cell-specific manner, and even in cells of the immune system [114,115]. For instance, in zebrafish 342 lncRNAs are transcribed in more than one tissue and only 77 lncRNAs are expressed specifically in a single tissue [116]. Another example is in rainbow trout where it has been observed that of 2935 lncRNAs, about 5.2% of lncRNAs identified in this species, are expressed in a specific tissue manner [117]. In Atlantic and Coho salmon lncRNAs were identified from the skin and head-kidney tissues, observing a greater abundance of exclusive lncRNAs in the head-kidney for both fish species [23]. In addition, exclusive lncRNAs were identified in the skin, which is similar to that reported for rainbow trout [117].

LncRNAs play important roles in the modulation of biological processes, being involving in regulatory processes of the immune response associated with Toll [118] and NF-kB [119] signaling pathways. In fish, the role of lncRNAs as regulators of the immune response has been poorly studied. However, some studies conducted in *Larimichthys polyactis* after exposure to *Vibrio anguillarum*, reveal a high number of lncRNAs upregulated in response to infection. Here, the authors suggest that the immune system can be modulated through lncRNAs closely localized to immune-related genes [120]. Another transcriptome study conducted in Atlantic salmon infected with the ISA virus identified 4967 lncRNAs differentially expressed and positively correlated with the viral burden [121]. LncRNAs have also been reported in salmon infected with the pathogenic bacteria *P. salmonis*, showing that the lncRNAs are modulated during infection. Here, lncRNAs were directly correlated with genes involved in the response to this bacterium, such as endocytosis and iron homeostasis [122]. Furthermore, a comparative study in Atlantic salmon exposed to virus, bacteria, and ectoparasites demonstrated that lncRNAs are specifically regulated in response to each type of infection [123]. With respect to the modulation of lncRNAs in Atlantic and Coho salmon exposed to *C. rogercresseyi*, it was observed that the abundance of lncRNAs expressed during the time of infestation is similar to that observed for coding transcripts. In both salmon species, a high number of lncRNAs were expressed at 7 dpi and 14 dpi in skin and head-kidney, respectively [23]. The lncRNAs expressed in Coho salmon infested with *C. rogercresseyi* were closely located to genes associated with the response to external stimuli, tissue repair, and cell communication [23]. On the other hand, the lncRNAs identified in Atlantic salmon expressed during the infestation by *C. rogercresseyi* were associated with biological processes such as stress, immune and inflammatory response [23]. Additionally, 439 lncRNAs expressed in the head-kidney of Atlantic salmon after 15 days post-infestation with *C. rogercresseyi* were correlated with genes associated with transcriptional regulation processes [123]. From the identification and expression analysis of the lncRNAs in salmon, it is possible to establish that the lncRNAs play an important role during the lice infestation. However, it is necessary to design and conduct functional studies, uncovering the molecular mechanisms that drive the fish immune response.

Another class of ncRNAs is the microRNAs (miRNAs), which are small RNA molecules of ~22 nucleotides that bind to a messenger RNA, mainly at its 3 ′end UTR and block the gene translation [124,125]. Like the lncRNAs, miRNAs are expressed in a tissue-specific manner and are capable of regulating biological processes such as development, growth, metabolism, apoptosis, etc. [126–128]. In regard to the immune response, miRNAs participate in the regulation of both innate and adaptive immune responses, as well as differentiators of T cells, modulators of inflammatory response and activation of Toll-like receptors [129–132]. In Atlantic salmon, miRNAs have been identified and differentially expressed in response to lice infection [133]. Here, 1718 miRNAs from skin and head-kidney were annotated, while miRNA families named as mir-21, mir-181 and let-7 were the most enriched [133]. These families have been found to be abundant in other species of fish such as trout and flatfish [134–136]. Due to the role of miRNAs to interfere the transcriptional activity [124,125], it can be inferred that the abundance of miRNAs post-lice infestation in key tissue as head kidney and skin can modulate genes related to the immune response in Atlantic salmon. Among the highly expressed miRNAs in Atlantic salmon, mir-140 was highly expressed in the head-kidney at 7 dpi, which have as target gene the toll-like receptor *tlr22a2*, which is also negatively regulated. Another miRNA that seems to influence the response of Atlantic salmon to *C. rogercresseyi* is mir-21, which targets the C–C Motif Chemokine Receptor 3 gene (CCR3) associated with Th2 response. This receptor is highly relevant during the response of salmon to *L. salmonis* [22]. Additionally, and associated with the regulation of iron, mir-181a-2-5 was also identified with a putative regulation on ALAs. This gene has been associated with the biosynthesis of the heme group, a process which was also found to be inhibited in Atlantic salmon infested with *C. rogercresseyi*.

There are studies that show that miRNAs are not only regulators of biological processes, but also that they can participate in the parasite-host interaction processes. In insects affected by viruses, it has been observed that viruses are capable of releasing miRNAs that can regulate the expression of their host genes in order to successfully establish the infection [137]. For *C. rogercresseyi*, several miRNAs expressed during the different stages of development have been characterized [138,139]. Within the profile of miRNAs characterized in *C. rogercresseyi*, the miRNA annotated as Bantam is highly expressed in the infective stage of copepodid [138]. This suggests that Bantam has a key role in the success of the infection. Predictions of Bantam target genes have shown that this miRNA could have an important role in the parasite-host interaction process by regulating genes associated with the secretome of *C. rogercresseyi* such as trypsin and cathepsin [138]. Notably, Bantam seems to be a putative regulator of genes associated with the immune response of Atlantic salmon such as *trl22a2*, *cd83* and *IFNg* [138].

9. Host-parasite microbiome modulation in sea lice infection

The microbiota community of an organism actively participates in vital functions with a leading role. It is associated mainly with the homeostatic, nutritional, metabolic and defensive performance [140–142]. In fish, mucosa associated microbiota have been studied thoroughly [140,141,143] and host-specific signatures have been found, especially in the skin [143]. However, several factors can disrupt mucosal microbiome stability, such as stress, water quality, and diet [144]. Since commensal microbiota constitute a resistance barrier to opportunistic pathogens colonization [145], if dysbiosis occurs, diseases can easily emerge. Sea lice impact fish health in several ways, and are a strong stressor regardless of infestation pressure [146,147], changing mucus production and composition [148,149]. However, the impact of sea lice infection in the fish epidermal mucosal microbiome has only recently been investigated [150]. Atlantic salmon's skin microbial community changed identity as infection with *L. salmonis* progressed, and the richness and beta-diversity of this community were also impacted [150]. During infection, an increase in Rhizobiales and NS10_marine_group, a member of Cryomorphacae family, was observed together with a decrease in *Arthrobacter* genus. The biological significance of these changes is yet to be uncovered, but it indicates the modulatory effect of lice in the fish skin microbiome.

In copepods, mucosal microbiome research is not developed as in fish, but it is known that microorganism communities are more abundant in the mouth, digestive tract and egg sacs [151], and it is possible to observe a central or “core” natural microbiome that is stable, as well as host-specific associations, symbiosis, and other associations that are environment and diet-dependent [152]. In the case of *C. rogercresseyi*, diet is mainly composed by fish mucus, skin and muscle and this might influence the parasite's microbiome, especially it the host is suffering a mucosal dysbiosis. Minniti et al. [153] found that 24 h after handling, a significant effect on Atlantic salmon's microbial composition is observed, with the rising of the genus *Burkholderia* – a potentially pathogenic taxon for several animal species – whereas the abundance of *Methylobacterium* affiliated phylotypes – a poly-B-hydroxybutyrate producing beneficial bacteria - decreased. Data on the interaction on host mucus-lice microbiota is rather scarce, although Llewellyn et al. [150] found some similarities reflected mainly by the abundance of the genera *Vibrio* and NS10_marine_group. More research is necessary to properly infer interactions and host-parasite microbiome associations, and this takes special relevance in parasitic copepods. It is known that in copepods the interaction with bacteria includes permanent and transient endo and epibiotic associations that might have a significant role in copepod's health [152]. In the case of parasitic copepods such as *C. rogercresseyi*, it might have an effect on their infectious capacity and resistance and be directly related to disease outbreaks.

In addition, sea lice associated microbiota can play a role as a reservoir of secondary pathogens as seen in several studies. Sepulveda et al. [154] found that *Caligus lalandei* – an ectoparasite common in *Seriola lalandi* – cultured microbiota has a high abundance of *Vibrio alginolyticus*, a known fish pathogen, whereas Nese and Enger [155] and Novak et al. [156] reported that *L. salmonis* is a potential vector for *Aeromonas salmonicida*. The potential role of *C. rogercresseyi* as a vector for relevant pathogens such as *P. salmonis* is presently under debate, but so far it has been only reported that it is a reservoir of ISA (Infectious salmon anaemia) virus and can act as a vector for spreading this disease [13]. Research on salmon-louse microbiome associations and interactions is underway for *C. rogercresseyi*, and information that is being generated will allow for a better understanding of possible modulations for better control of Caligidosis. Moreover, considering the impact of *C. rogercresseyi* infestations in aquaculture in Chile, the constant usage of delousing drugs and increasing observed resistance in lice the study of the effect of microbiome variations on drug disposition, action, and toxicity (pharmacomicrobiomics) will be an important field going forward.

10. Conclusion and perspectives

The understanding of how the parasite survives in its host and how they display molecular mechanisms to face with the immune system. The detection of semiochemicals has been associated to the ionotropic receptors (IRs) gene family, involving key molecular components for the salmon-louse interaction. For instance, a relevant finding is the parasite's ability to detect the antimicrobial peptide Cath-2 expressed from infected-fish, allowing the subsequent infection process. Moreover, the inflammatory response is the first reaction associated with the coping against sea lice, generating strong modulation of immune-related genes and signaling pathways (Table 1). From the recognition process through infestation and the development of different life stages, lice must cope with the rejection mechanisms displayed by the fish. Innate immune response pathways IMD and TLR, antioxidant system and secretory products are pivotal molecular components utilized by lice species to infect. The increasing availability of transcriptome data has revealed the importance of the non-coding RNAs as key regulators of the mRNA transcription. Notably, salmon-louse interactions are significantly modulated by lncRNAs and miRNAs during the infestation process. The understanding of how these molecules interact with pivotal signaling pathways involved in the immune response can be applied to develop novel control strategies.

Conflicts of interest

The authors declare no conflict of interest.

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