Antimicrobial peptides from *Salmon salar* skin induce frontal filament development and olfactory/cuticle-related genes in the sea louse *Caligus rogercresseyi*

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ABSTRACT

The discovery of key molecules involved in host-recognition has the potential to develop novel tools against the most prevalent pathogens in aquaculture. However, how mucosal surface compounds of teleost fish can modulate the attraction mechanisms of ectoparasites remains unknown. This study evaluated the effect of antimicrobial peptides (AMPs) highly expressed in *Salmo salar* and *Oncorhynchus kisutch* during the *Caligus rogercresseyi* infection. RNA-seq analysis from infested skin tissue revealed that Cathelicidin (CATHL) was the most AMPs expressed in comparison to other AMPs. To evidence morphological and transcriptional modulation of sea lice exposed to AMPs, copepodids were incubated with agar medium containing salmon mucus and CATHL1 or 2, or a combination of these. Interestingly, exposure to CATHL promoted the development of the frontal filament of sea lice, mainly CATHL2 peptide. Significant variations in transcript expressions were observed in chemosensory reception- and cuticle formation-related genes. Thus, copepodids exposed to CATHL2 showed significant increases in the mRNA abundance of cuticle formation genes and chemosensory receptors, mainly ionotropic kainate receptors. These results suggest that CATHL can trigger transcriptional responses in sea lice that are not directly linked with the effects of AMPs. The AMP-mediated activation of ionotropic kainate receptors in *C. rogercresseyi* raises novel questions regarding the molecular aspects of olfactory signal transduction in the host–parasite interactions.
Statement of relevance: Understanding of molecules that promote the frontal filament during the sea lice infection can be used to develop novel control tools and to explore nutritional additives able to modulate the AMPs fish mucosal.

1. Introduction

Sea lice infestations cause significant economic losses and social consequences for the salmonid aquaculture industry worldwide (Costello, 2009). In the Southern Hemisphere, the *Caligus rogercresseyi* sea lice species has the greatest impact. This species has three larval stages, four juvenile stages, and one adult instar stage marked by molting (Bravo, 2010). Understanding the copepodid larval stage is of particular importance to identify chemical molecules related to host recognition mechanisms (Núñez-Acuña et al., 2014; Pino-Marambio et al., 2007). Also in this stage, sea lice acquire a frontal filament that attaches to fish skin (Pike and Wadsworth, 1999). Cuticle formation genes were recently described in this species, and these may play a pivotal role in molting and cuticle formation between each developmental stage (Chávez-Mardones et al., 2016). While these processes are critical for

successful sea lice development and infestation, it is unknown if host fish possess a defense response that could affect these processes.

The deployment of antimicrobial peptides (AMPs) is among the most studied defense mechanism against different pathogens (Bulet et al., 2004). Fish produce all of the major AMP classes, including defensins and cathelicidins, in addition to fish-specific peptides termed piscidins (Masso-Silva and Diamond, 2014). Regarding salmonids, AMP expressions, especially of cathelicidins, defensins, hepcidins, and histone-derived AMPs, form the basis for defense mechanisms against various pathogens (Valero et al., 2013). Of these AMPs, cathelicidins might be implicated in long-term antimicrobial response against pathogens in salmonid species, due to its response associated to continuous exposure to peptidoglycan in salmon trout (Casadei et al., 2015). On the other hand, some antimicrobial peptides, such as piscidins-3 have suggested a putative role against the ectoparasite *Ergasilus* infestations (Dezfuli et al., 2011). Regarding cathelicidins, these peptides correspond to a widely studied group of AMPs in vertebrate species, but with specific distinctive features in salmonid fishes, such as differences in their

sequences and tridimensional structures, that could derive in novel functions in these species (Zhang et al., 2015). This class of AMPs was also highly associated to the response of Atlantic salmon to the bacterial infectious diseases known as Yersiniosis (Bridle et al., 2012). Furthermore, a previous study in Atlantic salmon showed that cathelicidin peptides are activated after sea lice infestation (*Lepeophtheirus salmonis*), while other AMPs such as defensin were suppressed (Krasnov et al., 2015). Nonetheless, there is no evidence regarding the effect that this class of AMPs could trigger in sea lice infesting salmon fishes.

The aim of this study was to assess if cathelicidins derived from salmonid species have the potential to interact with sea lice causing effects in its morphological development and attraction mechanisms. From *in silico* transcription analyses, highly expressed AMPs in infested salmons were identified. The amino acid sequences were used to synthesize AMPs, evaluating their effects on assays including sea lice at the copepodid stage. Evaluation on parasites was conducted through direct observation of frontal filament development by microscopy and expression analyses of selected genes in lice exposed to salmon AMPs. A group of genes related to cuticle formation was also used due to its relation to developmental process in this species (Chávez-Mardones et al., 2016). Furthermore, due to the involvement of the frontal filament structure in host recognition, particularly at the attachment event (between copepodid and chalimus stages), a group of genes related to the chemosensory system of sea lice, was also selected for this study (Núñez-Acuña et al., 2014). Interestingly, the cathelicidins exhibited a noticeable modulation on *C. rogercresseyi*, giving novel information of molecules present on fish mucosal with putative role in host-ectoparasite interaction.

2. Materials and methods

2.1. Gene transcription analyses of AMPs in fish skin

From NCBI Genbank database a group of salmon's AMPs sequences were obtained: cathelicidin (AY728057), defensin beta 4 (NM_001195169), defensin beta 3 (NM_001195183), hepcidin (XM_014170058) and NK-lysin (NM_001141110). The expression patterns of these AMPs were evaluated in transcriptome data obtained from salmonids species infested with sea lice *C. rogercresseyi* (unpublished data). Briefly, specimens of *Salmo salar* and *Oncorhynchus kisutch* species were used for a sea lice challenge, consisting in 35 copepodids/fish of *C. rogercresseyi*. Skin tissues samples were collected from both infested species at different sampling points: 0 (control), 7 and 14 days after infestation. Samples were fixed with RNA Later solution (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −80 °C until RNA isolation, which was performed with the RNAeasy Mini Kit protocol (Qiagen, Hilden, Germany). From purified RNA, libraries for transcriptomic sequencing were made using the TruSeq RNA Library Preparation kit (Illumina, San Diego, CA, USA). Sequencing runs were conducted in a MISEQ platform (Illumina, San Diego, CA, USA).

To estimate AMP transcription levels, RNA-seq analyses were performed using default settings to calculate Transcripts per million reads (TPM) in the CLC Genomic Workbench software (version 9.0, CLCBio, Qiagen, Germany). Hierarchical clustering of TPM values was calculated in the same software and a heatmap was constructed based on Manhattan distances and a complete linkage to visualize expression changes.

2.2. AMP synthesis

From the expression analyses described in the previous point it was observed that cathelicidin peptide has the higher abundance and transcriptional differences among experimental groups. This peptide corresponded to cathelicidin previously reported in *S. salar*. For further analyses this gene was selected along with cathelicidin-2, which was also previously reported in the same species, for peptide synthesis (Chang et al., 2006). Both peptides were named CATHL1 and CATHL2 respectively and were chemically synthesized at GenicBio service

(GenicBio Limited, http://www.genicbio.com). Sequences of peptides corresponded to RRSQARKCSRGNGGKIGSIRCRGGGTRL GGGSLIGRLRVALLLGVAPFLLDLSQINVMEIAFA for CATHL1 and RRGKPSGGSRGSKMGSKDSKGGWRGRPGSGSRPGFGSSIAGA-SGRDQGGTRNA for CATHL2 peptide. Purity and molecular weight of both peptides were determined by HPLC and mass spectrometry in the GenicBio facilities.

2.3. Copepodids and exposure assays

Ovigerous *C. rogercresseyi* females kept under laboratory conditions produced the 1400 copepodids used in the exposure assays. The copepodids were grouped ($n = 100$) and exposed to one of the following treatments: (1) CATHL1, (2) CATHL2, (3) both CATHL1 and 2, or (4) control group with no AMPs exposure. Three replicates of each group were used. Each group was incubated in a glass bottle with 400 mL of seawater and a Petri dish containing 30 g of agar with 1:9 of fish mucus as a source of stimulus for host recognition. To the CATHL1 and CATHL2 groups, 7 ppm of the respective AMPs was added. For the CATHL1&2 group, 3.5 ppm of each CATHL was added. All copepodids were incubated with constant aeration and 14 °C for 48 h. Then, the water was filtered; live copepodids were collected, and dead copepodids were counted and discarded.

2.4. Evaluation of frontal filament development

Frontal filament development in the collected copepodids was observed under a microscope. The following score of three categories were established: (A) lack of frontal filament, (B) internally formed frontal filament, and (C) fully developed and visible frontal filament. The percentage of sea lice in each category was calculated for each group. Two-way ANOVA analysis was conducted to evaluate statistical significant differences according to two variables: AMPs in each treatment and frontal filament stage. A multiple comparison of groups was performed against the control group (without AMPs) to identify where the differences occurred. *P*-values lower than 0.05 were established as cut-off to determine statistical significant differences.

2.5. RNA extraction and qPCR analyses

A group of cuticle formation-related genes and chemosensory transduction signal-related genes were obtained from previous publications in *C. rogercresseyi* (Chávez-Mardones et al., 2016; Núñez-Acuña et al., 2014). The selected cuticle formation genes were *Prolyl 4-hydroxlase* (P4H) and *Cuticle protein 1, 2, and 3* (Cut1, Cut2, Cut3). The selected chemosensory transduction genes were *Ionotropic kainate receptor 2* (KAR2), *Ionotropic kainate receptor 2-like b* (KAR2b), and *metabotropic glutamate receptor A and B* (mGluR-A, mGluR-B). Primer list can be found in Table 1.

To evaluate gene transcriptions, after 48 h exposure with the corresponding peptides total RNA were isolated from each group using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and manufacturer protocol. Sea lice within the same group were pooled to obtain high quality RNA. A total of 50 copepodids were pooled for each group to have enough RNA for further analyses. One pool was used for each experimental replicate (three replicates). Purity was measured using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and integrity was determined by agarose gel under denaturant conditions obtained by adding the MOPS running buffer. From 200 ng/μL of total RNA, cDNA was synthetized using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). For qPCR reactions, specific primer pairs were tested in 80 ng of cDNA using a 1:5 ratio to establish dynamic range and efficiency (efficiency in Table 1). The qPCR runs were performed with the StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using the comparative ΔCt method. *β-tubulin*

Table 1
Primers list and characteristics for qPCR reactions.

Gene	Primer sequence (5′ to 3′)	T° annealing (°C)	qPCR efficiency (%)
KAR2	TTTCCAAAGGGAGGGCTTTC TTCACTGATAAGTCCTGCGC	60	92.1
KAR2b	TTGTCAAGTTCACAGGAGCC CTCAATGTCCAACGTCGGAT	60	92.27
mGluR-A	CCCTCAAACATCCACCCAAA GGAGTGGCTCATTTCCAGAG	60	100
mGluR-B	GTGCGATCTCTATGCTGGTC GGCATTCCTCTTGTTGTCCA	60	99.98
Cut1	GCCTACAAGGAGGCACCCGCCGT TTGAATGCGGCCGTCGGGGAGGG	62	100
Cut2	TGCCTACAAGGAGGCACCCGCCG GAATGCGACGCTCGGGGAGGGCA	66	100
Cut3	AGGCTTGTATGGGGCAGGGGCGG ACCGTTGCCCTTCCCGACGGACG	66	108.67
P4H	AGAATGCTCCACCACGACCTCAG GGCGTTGAAGTCTGGACTCTTCCT	63	100

was selected as the endogenous reference gene due to its stability, as determined by previous publications (Gallardo-Escárate et al., 2014; Núñez-Acuña et al., 2014). Each reaction was conducted in a final volume of 20 μL using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The amplification cycle was as follows: 95 °C for 10 min, 40 cycles at 95 °C for 30 s, and 60 °C for 1 min, followed by a melting curve from 60 to 95 °C (annealing temperatures in Table 1). Statistical analysis was conducted through one-way ANOVA using the GraphPad Prism v6.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Significant differences were established at $p < 0.05$.

2.6. Correlation and statistical analyses

A principal component analysis (PCA) of correlations was conducted using the relative mRNA abundance of each transcript (variables). Stages of frontal filament formation were used as supplementary variables. As these are categorical variables, the numbers of copepodids in each stage were used as numerical variables. The grouping variable was exposure to different CATHLs. Principal components were included with an eigenvalue >1 (Kaiser criterion). These analyses were performed in JMP v9.0 software (Statistical Discovery, SAS, Cary, NC, USA). Pearson's correlations of gene transcription levels and the number of copepodids in each frontal filament stage were estimated using JMP v9.0 software (Statistical Discovery, SAS, Cary, NC, USA). The correlation matrix was plotted using the Corrplot package (https://github.com/taiyun/corrplot) included in the R software (Tem, 2015).
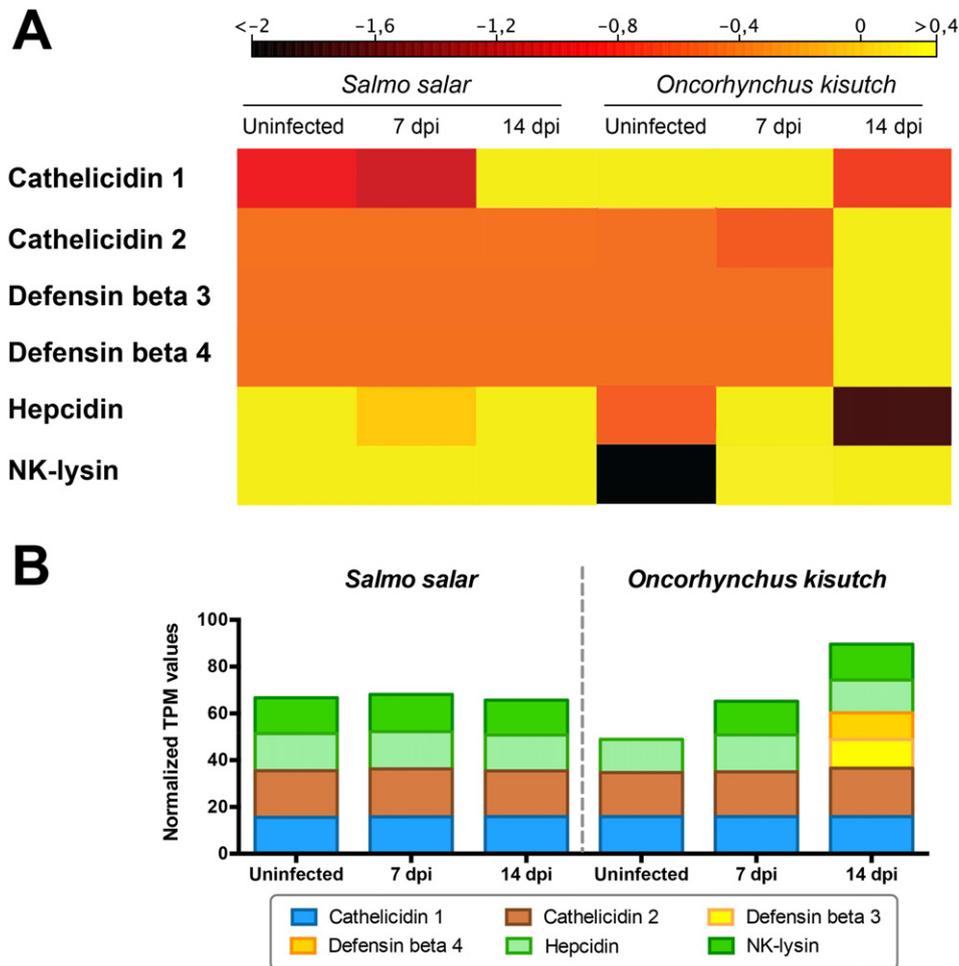

Fig. 1. Relative abundance of antimicrobial peptides messenger RNA in skin tissue of *Salmo salar* and *Oncorhynchus kisutch*. Gene transcription levels were calculated by measuring the TPM values through bioinformatic analyses on transcriptomic data. (A) Heatmap based on hierarchical clustering of TPM values throughout infestation times. Clustering was performed on Manhattan distances using a complete linkage. Color scaling ranges from lowest expression levels (black), to medium levels (red) and to highest expression levels (yellow). (B) Proportion of normalized TPM values of AMPs in each species at different sampling points. Normalization was done by scaling method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. AMP expression patterns in infested skin tissue of salmonid species

To verify if AMPs are being produced in salmon infested tissues, an evaluation of the expression patterns of six selected antimicrobial peptides was performed in transcriptome data obtained from Atlantic (*S. salar*) and Coho salmon (*O. kisutch*) infested with *C. rogercresseyi*. Differential expression results were obtained for each AMP during the 14 day-infestation trials. From 0 to 14 days, an increasing trend in the relative abundance of *Cathelicidin 1* was observed in Atlantic salmon, but a down-regulation in Coho salmon. In contrast, *cathelicidin 2* maintained its expression levels in Atlantic salmon, but is up-regulated at 14 days in Coho salmon. *Defensin beta 4* and *defensin beta 3* exhibited a similar tendency than CATHL2, while other AMPs exhibit a different pattern including *hepcidin* and *NK-lysin*, which presented stable expression levels in Atlantic salmon, but an increasing trend in Coho salmon at 7 and 14 days after infestation (Fig. 1A). Overall, the relative abundance of all AMPs in Atlantic salmon maintained a similar expression pattern after infestation. Meanwhile, AMP expression pattern in Coho salmon while highly divergent, observing an increase in the expression levels after infestation (Fig. 1B).

3.2. Frontal filament development after AMP exposure

Cathelicidin-1 (CATHL1) and *cathelicidin-2* (CATHL2) peptides were selected for chemical synthesis. Purity was 96.66% for CATHL1 and 95.46% for CATHL2. These peptides were used to evaluate their effect on sea lice frontal filament formation. Compared to the control group, the experimental copepodid groups exhibited differentiated frontal filament formation following exposure to CATHLs (Fig. 2). Exposure to these AMPs triggered frontal filament development in copepodids, increasing the number of maturing larvae in the slightly visible and fully developed stages. CATHL1 exposure increased the proportion of individual (copepodids) on score A from 10 to 38.9%, decreased the proportions of individuals on score B from 70 to 27.8% and increased the lice with score C from 20 to 33%. Meanwhile, CATHL2 exposure decreased lice with score A to 5%, with score B to 30%, and increased copepodids with score C to 65%. Overall, both peptides affected frontal filament formation in the copepodids, but this effect was the opposite between each other. Apparently, CATHL1 tends to inhibit or perhaps delay in frontal filament formation, but CATHL2 tends to promote its development. Combination of both peptides applied at the same time produced similar results than control group: 11.1% of copepodids with score A, 55.6% with score B and 33.3% with score C.

3.3. Gene expressions of olfactory and cuticle-related genes in C. rogercresseyi

Group differences for the assessed gene expressions were more notable than the differences recorded for the frontal filament stages (Fig. 3). Among the chemosensory signal transduction genes, ionotropic KAR2 and KAR2b showed strong up-regulations in larvae exposed to CATHL2. In turn, the chemosensory-related mGluR-A was less expressed in the CATHL2 group, whereas mGluR-B had a higher expression in the CATHL1&2 group, followed by CATHL2. Regarding the
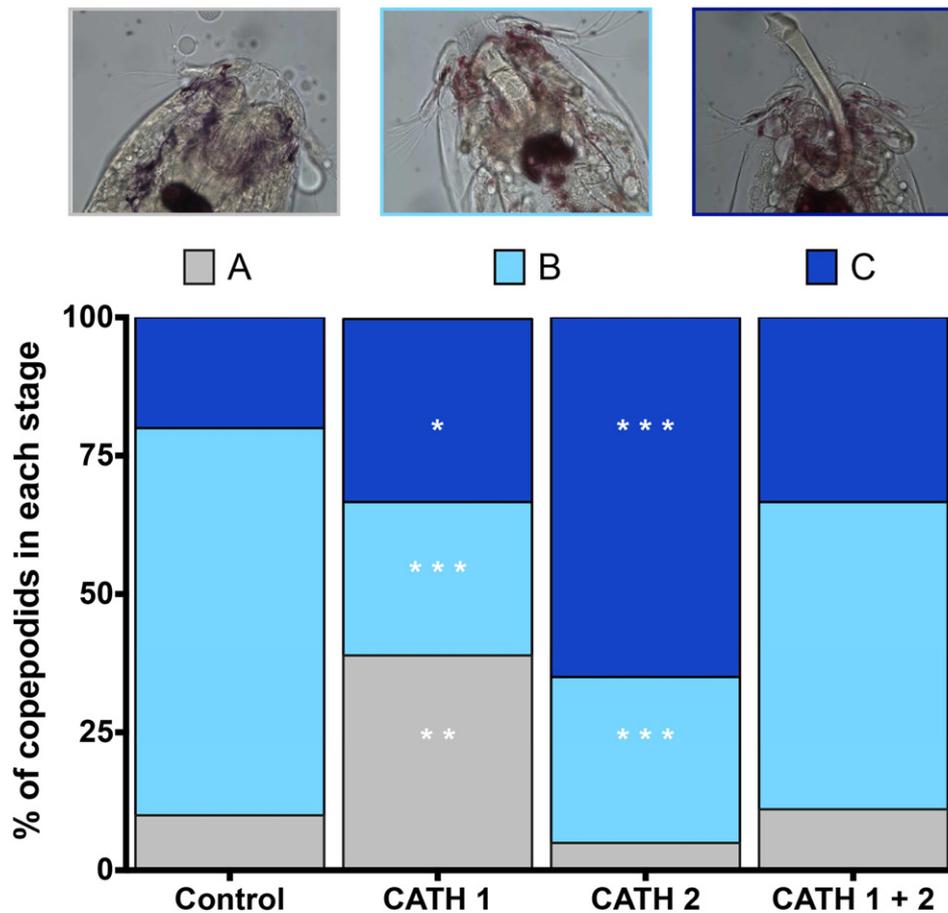

Fig. 2. Frontal filament formation in copepodids exposed to *Salmo salar* cathelicidins. Pictures show copepodid samples microscopically observed during four stages of frontal filament development. The graph corresponds to the number of copepodids at each stage of frontal filament development. (A) lack of frontal filament, (B) internally formed frontal filament, and (C) fully developed and exposed frontal filament. One-way ANOVA was applied to determine statistical significant differences, which are indicated by asterisks (*P*-value <0.05*, <0.01**, <0.001***).
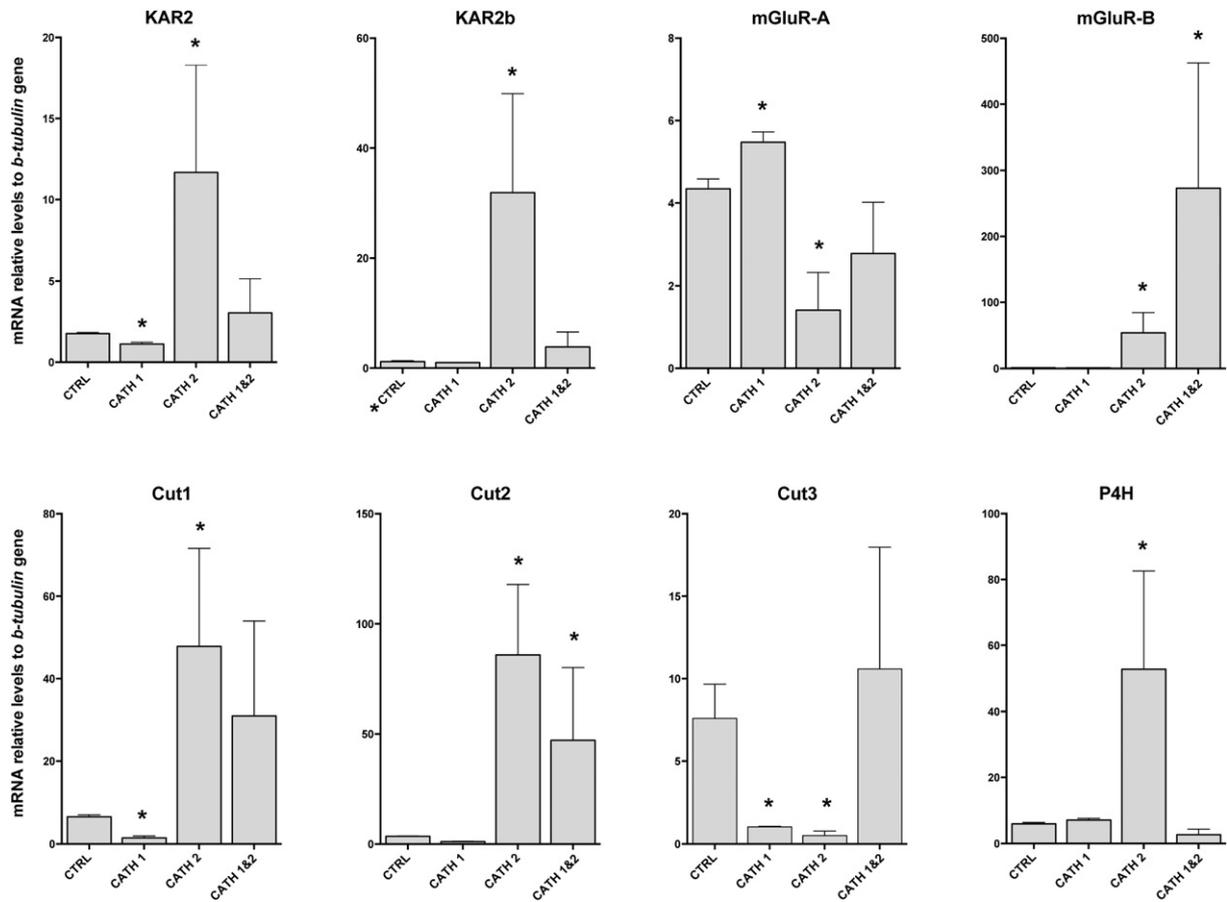
Fig. 3. Gene transcription levels for chemosensory receptors and cuticle formation proteins in copepodids exposed to *Salmo salar* cathelicidin. Expression levels were measured by qPCR reactions normalized by β-tubulin as an endogenous control. The Y-axis represents expression values quantified by the $\Delta\Delta$Ct method. Chemosensory-related receptors included ionotropic kainate receptor 2 (KAR2), ionotropic kainate receptor-like 2 (KAR2b), and metabotropic glutamate receptors A and B (mGluR-A, mGlur-B). Cuticle synthesis-related transcripts included cuticle proteins 1, 2, and 3 (Cut1, Cut2, Cut3) and prolyl 4-hydroxylase (P4H). Significant differences in respect to control group were set up at $p < 0.05$, and are marked with * symbols. Error bars represent standard deviation.

cuticle-related genes, most exhibited an expression pattern similar to the KAR receptors. The Cut1 and 2 genes exhibited higher expression in sea lice exposed to CATHL2 and CATHL1&2. The P4H gene presented a similar pattern, but expression was lower in the CATHL1&2 group. On the other hand, Cut3 was most expressed in CATHL1&2 and control group copepodids, while expression levels were significantly lower in sea lice exposed to CATHL2. In summary, most of the cuticle- and chemosensory transduction-related genes were associated with exposure to the CATHL2 peptide.

3.4. Correlations between variables

Principal component analysis explained 82.5% of correlation between gene expression and frontal filament stages (Fig. 4A). Exposure to CATHL2 was more related to greater frontal filament formation (slightly visible and fully developed) and to transcription for most of the assessed genes. Specifically, CATHL2 exposure was more related to KAR2, KAR2b, P4H, Cut1, and Cut2 transcripts. On the other hand, CATHL1 exposure was more related to a lack of frontal filament formation, and CATHL1&2 exposure was related to the expression of Cut3 and mGluR-B.

The constructed Pearson's correlation plot graphically depicted associations between each variable (Fig. 4B). The most significant positive correlations were found between fully developed frontal filaments and the transcriptional expressions of KAR2, KAR2b, and P4H. The most significant negative correlations were observed between fully developed frontal filaments and Cut3 expression, as well as between slightly visible frontal filaments and mGluR-A gene transcription. In summary, both the PCA and Pearson's correlation were consistent in associating the expression of most chemosensory reception- and cuticle-related genes with frontal filament formation and CATHL2.

4. Discussion

Over the last decade, the development of AMPs and polypeptides by fish species has been described as major defense mechanisms against pathogens. These molecules are expressed in secretions present in mucus produced in salmon skin (Noga et al., 2011; Rajanbabu and Chen, 2011). Relevant to this, *C. rogercresseyi* is a marine ectoparasite that feeds on fish skin and mucus (González and Carvajal, 2003), suggesting that this sea lice species is exposed to fish AMPs. However, little is known about the effects that AMPs might provoke in *C. rogercresseyi*.

The transcriptional expression of AMPs has exhibited differential responses in both host fish species (Fig. 1). Overall, temporal changes in gene expression were found with an increasing trend in Coho salmon. These findings are consistent with the hypothesis that explains resistance of Coho salmon to sea lice due to an early immune response (Johnson et al., 1992; Sutherland et al., 2014). Focusing in the parasite perspective, this is also related to an earlier defense response associated to sea lice that are infesting the susceptible species Atlantic salmon (Vera-Bizama et al., 2015).

This study was aimed on describing the effects of *S. salar* AMPs on chemosensory transduction and cuticle formation, two crucial processes in sea lice lifecycle development. These processes are also related to frontal filament formation, which allows salmon lice to attach to fish skin. Species from the *Caligus* genus have a consistent pattern of frontal
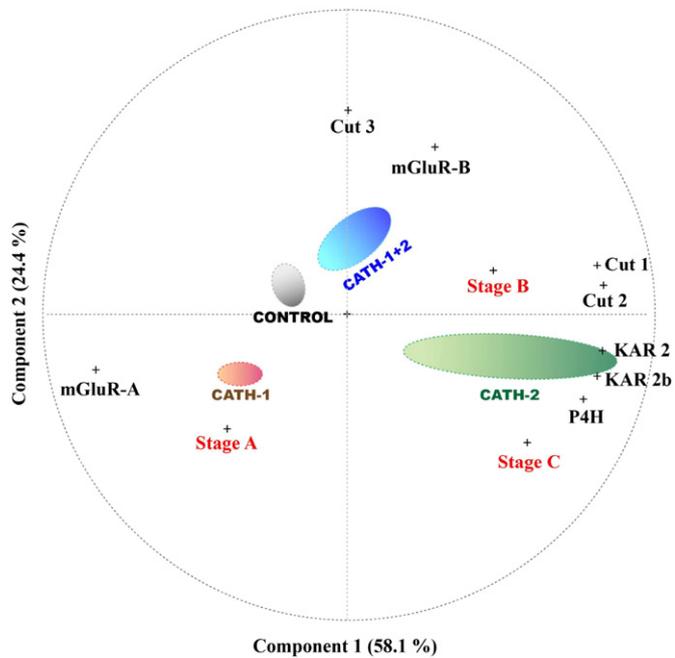
Fig. 4. Principal component analysis (PCA) of correlations between gene transcription levels, stage of frontal filament formation, and peptide exposure. Colored labels with a peptide name correspond to centroids of replicates in each treatment. The brown arrows correspond to gene expression levels, while green arrows indicate the frontal filament stages. Components 1 and 2 explained 82.5% of total variability. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

filament origin, formation, and function (Piasecki and MacKinnon, 1993; Pike and Wadsworth, 1999; Pike et al., 1993). Specifically, the frontal filament is preformed inside the copepodid body, posteriorly extruded, and finally attached to the skin of the fish host. Therefore, frontal filament formation in *C. rogercresseyi* is a good measurement of whether the sea louse is attempting host attachment. The present results suggest that exposure to fish AMPs, especially CATHL2, promotes frontal filament development. To further evaluate the effects of CATHLs in sea lice, gene transcription analyses were conducted.

Among the assessed genes, ionotropic kainate receptors were selected based on association with the chemosensory system (Núñez-Acuña et al., 2014). However, the relation of these receptors with chemosensory transduction in invertebrates remains under debate (Croset et al., 2010). The ionotropic kainate receptors belong to the G-protein-coupled receptor family and play a pivotal role in the neurotransmission of synaptic signals in various species (Krishnan and Schioth, 2015). Notably, a recent study described a novel class of ionotropic chemoreceptors able to bind semiochemicals (Benton et al., 2009). This class was first discovered in insects and corresponds to olfactory receptors that evolutionarily appeared before insect-exclusive olfactory and gustatory receptors (Benton, 2015).

A group of receptors from this novel class were found expressed in the olfactory organs of crustaceans (Corey et al., 2013). This finding opened the possibility of finding ionotropic receptors with olfactory functions in a number of marine species. Prior studies assessed a group of these genes and ionotropic kainate receptors in sea lice, finding expression in the presence of semiochemical-blocking compounds (Núñez-Acuña et al., 2014; Núñez-Acuña et al., 2016). Interestingly, the expression pattern of ionotropic receptors and kainate receptors in this current work was similar to the aforementioned studies. Overall, these evidences provide novel insights towards the understanding of the receptors implicated in the chemosensory systems of this marine invertebrate species.

In the present study, the kainate receptor expressions were highly associated with the presence of CATHL2, the same peptide that

increased the levels of frontal filament formation (Fig. 4A–B). This suggests that a neurotransmission process might have been stimulated in response to the presence of specific molecules, such as salmon's AMPs. Unfortunately, the current study could not establish a direct link to the molecular mechanisms associated on this interaction. Future studies will be conducted to determine if binding proteins are involved in host–parasite chemosensory reception or, on the other hand, it is triggered as an immunological response during the infection process against AMP skin exposure.

Metabotropic receptors are also G-protein-coupled receptors and are some of the main neurotransmission mediators in the eukaryotic nervous system (Krishnan and Schioth, 2015). These receptors can mediate chemoreception in olfaction and gustation, but this is a slow response process that normally requires key enzymes to produce glutamate as a neurotransmitter, the presence of a second messenger, and the activation of G-proteins (Wicher, 2012). This might explain the different patterns of gene expression found between the assessed metabotropic and ionotropic receptors. Likewise, further studies are needed to determine the key enzymes and binding proteins involved in the molecular functions of G-protein-coupled receptors.

The evaluated cuticle formation genes had expression patterns similar to the ionotropic receptors (Fig. 3). This group of genes has been found in different arthropod species. In insects, there is great concern about cuticle formation-related proteins as these are associated with insecticide sensitivity or resistance (Vannini et al., 2014). Critically, cuticle thickness is related to pesticide resistance in insects (Lin et al., 2012). In relation to sea lice species, some cuticle formation-related genes were differentially expressed in resistant strains of *L. salmonis* when exposed to the insecticide emamectin benzoate (Carmichael et al., 2013). Interestingly, a wide set of cuticle-related genes have been found in *C. rogercresseyi*, and some are related to drug exposure (Chávez-Mardones et al., 2016). Nevertheless, the present study is the first to measure the abundance of cuticle-related transcripts in parasites exposed to host AMPs. Given the importance of cuticle formation in the subsequent molting stages of the sea lice lifecycle, expanding upon the present results and fully understanding the effects of the CATHLs produced by salmon hosts are important for the management of sea lice infestations.

Overall, both salmon's CATHL peptides have an opposite effect on infesting sea lice. While CATHL1 seems to inhibit the development of the frontal filament, CATHL2 might be promoting its formation. These trends are also supported by gene expression analyses. This is interesting considering that the general mechanism of AMPs molecular function consists in the defense against pathogens. The finding of an AMP with potential beneficial molecular interactions on an ectoparasite such as *C. rogercresseyi* opens the question about unraveled molecular functions of these compounds, or a molecular adaptation by the parasite. To answer this questions further studies relying on the molecular mechanisms of AMP in the host–parasite interactions context should be conducted. The cumulative knowledge in this field would be helpful to develop novel strategies for parasitic disease in salmon farming industry.

5. Conclusion

The obtained results suggest a strong relation between AMP exposure and the regulation of key transcripts involved in sea lice chemosensory and cuticle processes. Despite this advancement in knowledge, there is an urgent need to characterize binding proteins and downstream signaling pathways involved in these sea lice mechanisms. This information would aid in fully comprehending and possibly utilizing AMP production in fish as a strategy for defending against sea lice infestations.

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The following are the supplementary data related to this article.

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