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Short communication

Two novel male-associated peroxinectin genes are downregulated by exposure to delousing drugs in *Caligus rogercresseyi*

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ABSTRACT

Peroxinectin (PX) is a protein involved in cell adhesion, peroxidase activities, and the encapsulation of invaders in diverse species, including parasitic copepods. Recently, a transcript denoted *peroxinectin-like* was identified in the salmon louse *Lepeophtheirus salmonis*, and this was significantly correlated with the immune response of host fish. Thus, the PX gene is a potential candidate to evaluate host–parasite interactions, as well as to analyze responses to delousing drugs used in the salmon aquaculture industry worldwide. The objective of this study was to identify *Peroxinectin* transcripts in the Chilean salmon louse *Caligus rogercresseyi*, and to determine expression levels after exposition to the antiparasitics deltamethrin and azamethiphos. Two novel transcript homologs to peroxinectins were identified from a transcriptomic library of *C. rogercresseyi*. Moreover, in silico gene transcription levels were evaluated through RNA-seq analyses based on unique gene read levels in transcriptomic libraries that were constructed from sea lice exposed to delousing drugs. The identified transcripts were named *Peroxinectin-Cr1* and *Peroxinectin-Cr2*, which, respectively, had lengths of 2543 and 2555 base pairs. Both PX transcripts were highly associated with male adults, and transcription levels were significantly reduced by deltamethrin and azamethiphos. This result suggests a modulation of peroxinectin in response to delousing drugs.

1. Introduction

Peroxinectin (PX) is a protein with diverse biological functions in the immune system and prophenoloxidase activities, especially in crustacean species (Du et al., 2013). The first described biological processes for PX in crustaceans were cell adhesion and the degranulation of crayfish hemocytes (Johansson and Soderhall, 1988, 1989). Later, other functions were identified, such as the promotion of hemocyte encapsulation (Kobayashi et al., 1990), opsonization (Thornqvist et al., 1994), and peroxidation to control infectious agents (Johansson, 1999). Of the different homologs found in crustacean species, each PX contains at least one putative integrin-binding motif and a peroxidase domain (Liu et al., 2004, 2005). These structural features confer the biological processes mentioned and also allow PX to participate in the peroxidase activity of other antioxidant proteins such as superoxide dismutase (Holmblad and Soderhall, 1999). Furthermore, PX is an inducible gene in response to specific pathogen-associated molecular patterns and bacteria, therefore suggesting a role in the innate immune response of crustaceans (Liu et al., 2005; Dong et al., 2009, 2011).

The importance of studying PX genes in different sea lice species is based on a recently published work, which found a correlation between the gene transcription of a peroxinectin-like transcript of *Lepeophtheirus salmonis* and the immune innate response of the host species (*Salmo salar*) (Wotton et al., 2014). Herein, a correlation between the expression patterns of parasite PX and fish chemokines was found, suggesting that peroxinectin has a role in host–parasite interactions. In addition to this, there is concern that sea lice have developed resistance to commonly used antiparasitic drugs (Lees et al., 2008). As sea lice species cause great economic loses in the salmon industry (Costello, 2009), novel control strategies have to be developed, and in this context, understanding the host–parasite interaction is a promising possibility (Ingvarsdóttir et al., 2002).

The main objective of this study was to evaluate the response of PX transcripts in the Chilean salmon louse *Caligus rogercresseyi* to treatment with two delousing drugs currently applied in salmon farming, deltamethrin and azamethiphos. Subsequently, the expression levels of PX were measured in exposed sea lice. The results provided novel insights into the regulation of this gene, thus contributing to discussions regarding the effects that these chemicals have for PX gene transcription, including sex-specific bias.

Abbreviations: PX, peroxinectin; UTR, untranslated region; bp, base pairs; mRNA, messenger RNA; RNA-seq, RNA sequencing; ORF, open reading frame; RACE, rapid amplification of cDNA ends; GO, Gene Ontology.

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Table 1
Primers used in this study to obtain full sequences of two peroxinectin mRNAs in *Caligus rogercresseyi*.

Primer name	Sequence (5′ to 3′)	Specification
PX-Cr1_5i	TTCACATCTCCAAAGGCCAA	5′-RACE
PX-Cr1_5o	CTAATTCGTTCTTCTATGCTC	5′-RACE
PX-Cr1_3i	ATGTCACTGGAGGTTTCAT	3′-RACE
PX-Cr1_3o	GACCAATTCGCTAGGCTTAAGA	3′-RACE
PX-Cr2_5i	GTGTCCCTAAATAGATTACTC	5′-RACE
PX-Cr2_5o	AGTTTCACATCATTTTGG	5′-RACE
PX-Cr2_3i	GTATCATCAGCGATCAATTCG	3′-RACE
PX-Cr2_3o	AGTTCTTTTACACCTGATCAAT	3′-RACE

2. Materials and methods

2.1. Peroxinectin gene discovery

Two transcripts annotated as *Peroxinectin* (*PX*) were obtained from a RNA-seq library constructed for *C. rogercresseyi* (Gallardo-Escárate et al., 2014). Briefly, a RNA-seq library was generated through high-throughput sequencing of the developmental stage of the *C. rogercresseyi* lifecycle in the MiSeq platform (Illumina®, San Diego, CA, USA). Then, *PX* sequences from marine invertebrates were downloaded from the GenBank database in order to perform a MultiBlast analysis against all contigs using the CLC Genomic Workbench software (version 6.0, CLC Bio, Aarhus, Denmark). Two contigs annotated as *peroxinectin* genes were extracted from the transcriptomic library in order to obtain the partial sequences.

2.2. Full PX cDNA characterization

For each contig, a pair of primers was designed to amplify a sequence fragment of the PCR (Table 1) by using the Geneious 6.0.4 software (Biomatters, Auckland, New Zealand). PCR amplification was conducted using 200 ng of cDNA obtained from adult sea lice, 10 μM of each primer, 1.5 mM $MgCl_2$, and 0.06 U taq DNA polymerase (Thermo Scientific). PCR cycles consisted of initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 60 s, annealing at 60 °C for 30 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min. PCR products were visualized through electrophoresis in agarose gels stained with GelRed™ (Biotium, Hayward, CA, USA) and then directly sequenced in an ABI 3730xl capillary sequencer.

Full-length cDNA of each *PX* transcript was obtained by the rapid amplification of cDNA ends (RACE) technique using the SMARTer™ RACE cDNA Amplification kit (Clontech Laboratories Inc., Mountain View, CA, USA). Primers for amplifying the 5′UTR and 3′UTR ends were designed according to the manufacturer's instructions (Table 1). Amplicons were purified and cloned using the TOPO TA cloning kit (Invitrogen, Life Technologies), followed by transformation into *Escherichia coli* JM109 in LB/amp/IPTG/Xgal. Positive clones were selected by galactosidase reaction visualization, and resulting plasmids were purified using the E.Z.N.A.® Plasmid DNA Mini kit II (Omega Bio-tek, Doraville, GA, USA). Purified plasmids were sequenced in both forward and reverse directions using M13 universal primers. Sequence analyses were carried out in the Geneious software and consisted of quality visualizations, assembly, and BLASTn against the GenBank non-redundant database.

2.3. PX gene transcription analysis in sea lice developmental stages

Total RNA extractions were performed from the following developmental stages of *C. rogercresseyi* (N = 10): nauplius I, nauplius II, copepodid, chalimus, and female and male adults. Extractions were performed using the Trizol Reagent® (Invitrogen, USA) following the manufacturer's instructions.

Primers for qPCR analysis were designed by the Geneious software (Table 1). Dynamic range analysis was conducted to obtain qPCR efficiencies and optimal conditions for qPCR runs. For this, five serial dilutions of cDNA were prepared starting with 80 ng and with a serial factor of 1:5 in order to generate an amplification curve with both pairs of primers designed for qPCR analysis. The qPCR runs were assayed in an ABI StepOnePlus™ Mastercycler (Applied Biosystems®, Life Technologies) with the Maxima Kit® SYBR Green/ROX qPCR Master Mix (Thermo Scientific®) following manufacturer's instructions. The run method comprised of a holding stage at 95 °C for 10 min to activate the enzyme, then 40 cycles of 95 °C for 15 s, 57 °C for 30 s, and 72 °C for 30 s. After the amplification stage, a melting curve was performed from 57 °C to 95 °C, and data was collected every 0.3 °C to determine the existence of a sole amplification product and to verify the inexistence of contaminations and primer dimers. Efficiencies were calculated

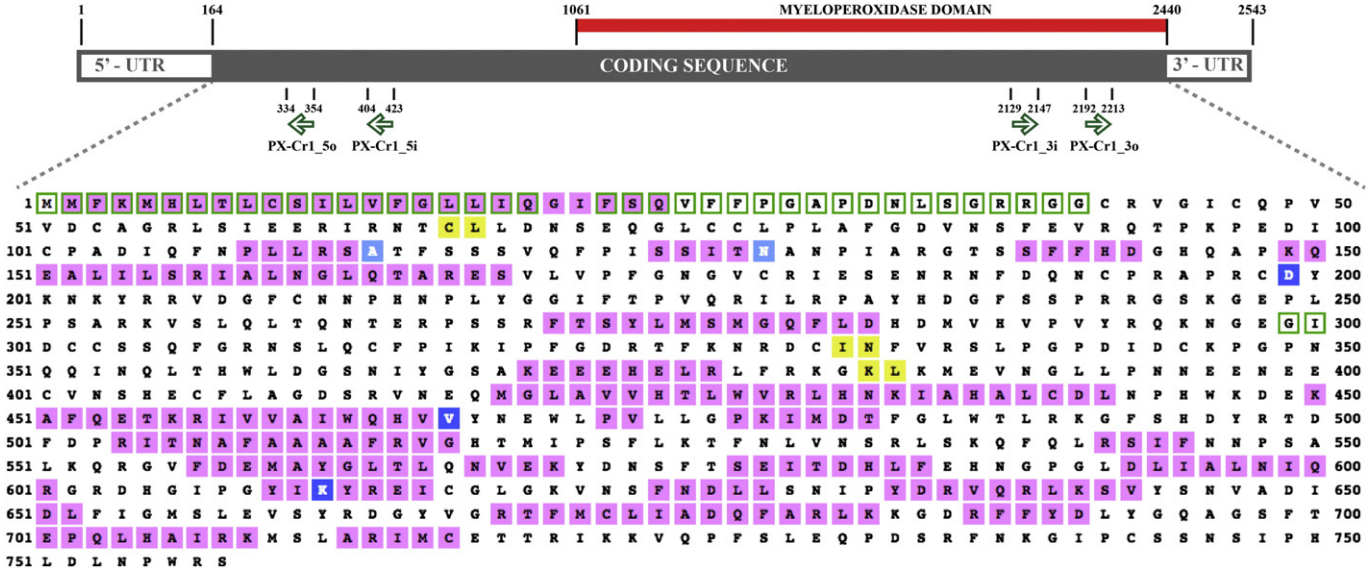

Fig. 1. Full mRNA sequence and amino acid prediction of *Peroxinectin-Cr1*. The schematic structure of mRNA is shown in the gray rectangle, and a characteristic myeloperoxidase domain is shown in red. The secondary structure of the predicted amino acid sequence is shown as pink = helix; yellow = sheet; blue = boundaries; and green = disordered protein binding sites. Additionally, green arrows show the position of primers designed for RACE.

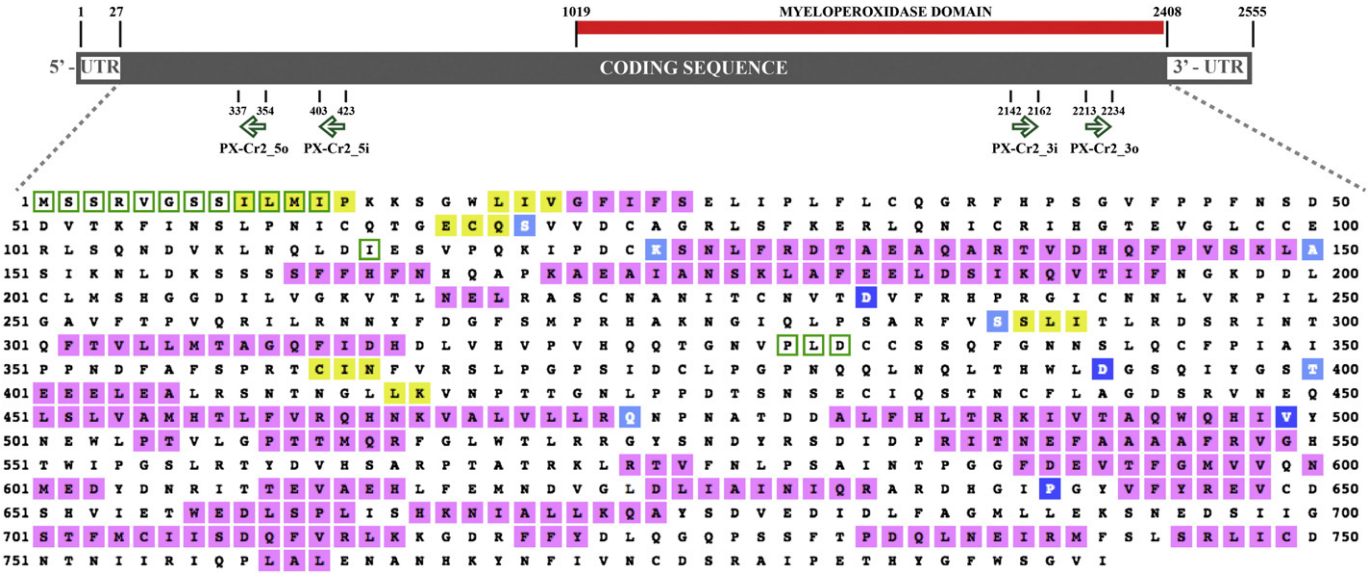

Fig. 2. Full mRNA sequence and amino acid prediction of *Peroxinectin-Cr2*. The schematic structure of mRNA is shown in the gray rectangle, and a characteristic myeloperoxidase domain is shown in red. The secondary structure of the predicted amino acid sequence is shown as pink = helix; yellow = sheet; blue = boundaries; and green = disordered protein binding sites. Additionally, green arrows show the position of primers designed for RACE.

in the StepOnePlus™ Software (version 2.2, Applied Biosystems®). To quantify mRNA relative levels, the $\Delta\Delta C_T$ method was used adding the β-tubulin gene as an endogenous control. Finally, statistical analyses were performed in Microsoft Excel and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA).

2.4. PX gene transcription analyses in sea lice exposed to delousing drugs

Bioassays with delousing drugs were performed following the methodology proposed in Chavez-Mardones and Gallardo-Escárate (2014). Samples were collected from a farming facility located in Puerto Montt, Chile. The concentrations of drugs used in bioassays were 2 ppb of deltamethrin and 3 ppb of azamethiphos.

Gene transcription analyses were performed using the same conditions mentioned in the previous section for qPCR reactions and quantification methods. Ten adult males and females (N = 10) were used for qPCR analyses in both bioassays.

3. Results and discussion

3.1. Molecular characterization of peroxinectin transcripts in C. rogercresseyi

The complete mRNA sequences of the peroxinectin transcripts *PX-Cr1* and *PX-Cr2* contained 2453 base pairs (bp) (Fig. 1) and 2555 bp (Fig. 2), respectively. These are available in the GenBank database (Accession numbers KM667938 and KM667939, respectively). The predicted protein sequences consisted of 758 amino acids for *PX-Cr1* and 792 amino acids for *PX-Cr2*. The PX sequences had short UTR regions, especially *PX-Cr2*, in which the 5′-UTR was composed by 27 nucleotides. The deduced protein sequence of *PX-Cr1* was similar to peroxinectin in *Pacifastacus leniusculus* (Blast hit with e-value = 1.4E−164), while *PX-Cr2* was similar to peroxinectin from *Eriocheir sinensis* (Blast hit with e-value = 2.07E−146). Additionally, both sequences contained a myeloperoxidase domain. For *PX-Cr1*, this contained 458 amino acids and had a best p-value of 3E−08 in Uniprot database. In turn, *PX-Cr2* consisted of 460 amino acids, but with the same p-value as *PX-Cr1*.

One of the strongest pieces of evidence supporting the hypothesis that these transcripts correspond to peroxinectin genes is the presence of the myeloperoxidase domain in both *PX-Cr1* and *PX-Cr2*. Previous research has shown that this domain confers its function to a diverse family of proteins, including different peroxidases and peroxinectin in invertebrates (Daiyasu and Toh, 2000). In general, this domain has a length around 500 bp, which is similar to the length obtained for *C. rogercresseyi*. Furthermore, p-values and e-values were robust enough to consider these transcripts as peroxinectins, taking into account that both were similar to other sequences from vertebrate and invertebrate taxa, including insects and humans.

Regarding Gene Ontology (GO) analysis, some relevant GO terms related to peroxinectins were found for both transcripts. First, the GO term known as “cell surface receptor signaling pathway” (GO:0007166) was found with one of the highest probability values for *PX-Cr1* and *PX-Cr2* in the biological process category (Table 2). Additionally, in the molecular function category, calcium ion binding was the most relevant GO term found for both sequences. It is suggested that this molecular function is involved in the cell adhesion process of peroxinectins. Therefore,

Table 2
Gene Ontology (GO) terms found in two peroxinectins in *Caligus rogercresseyi*.

GO term	Name	Probability in PX_Cr1	Probability in PX_Cr2
Biological process predictions			
GO:0009101	Glycoprotein biosynthetic process	0.87	–
GO:0007166	Cell surface receptor signaling pathway	0.84	0.57
GO:0010468	Regulation of gene expression	0.703	0.804
GO:0006810	Transport	0.667	—
GO:0009888	Tissue development	0.594	–
GO:0005975	Carbohydrate metabolic process	0.583	–
GO:0006952	Defense response	0.572	–
GO:0042592	Homeostatic process	0.507	–
GO:0006508	Proteolysis	0.506	0.648
GO:0070887	Cellular response to chemical stimulus	0.504	–
GO:0006355	Regulation of transcription, DNA-dependent	–	0.615
Molecular function prediction			
GO:0005509	Calcium ion binding	0.569	0.524
GO:0016491	Oxidoreductase activity	0.523	–
GO:0008270	Zinc ion binding	–	0.665
GO:0008233	Peptidase activity	–	0.546
GO:0003677	DNA binding	–	0.516

finding this GO term supports the hypothesis that these proteins are related to cell-surface superoxide dismutase in crustaceans (Johansson et al., 1999). This hypothesis is also supported by the GO term "oxidoreductase activity" being found in *Cr-PX1*.

3.2. Expression patterns of PX-Cr1 and PX-Cr2

Gene transcription analyses of peroxinectin transcripts were carried out for the developmental stages of the sea lice lifecycle and in adult parasites exposed to delousing drugs. On the one hand, both transcripts evidenced differential expression levels in different stages, with notable mRNA levels beginning from the copepodid or chalimus stages (Fig. 3A–B). Larval stages had insignificant expression levels of *PX-Cr2*, and only *PX-Cr1* was observed in the copepodid stage. In both cases, greater transcription levels were found in male adults, with significant differences in comparison to other stages, especially with female adults ($p < 0.01$). Sexual differences were even more notable for *PX-Cr2*, with practically no detectable mRNA levels in females (Fig. 3B). This is the first time that sexual differences have been found in the expression levels of a peroxinectin gene in any species. One of the possible explanations for these differences could be in relation to the sexual behavior of this parasitic species. It is known that virgin *C. rogercresseyi* females attract adult males through semiochemical cues, allowing the displacement of males to adult females with reproductive purposes (Pino-Marambio et al., 2007). This different biological process is one of the causes for transcriptomic expression between sexes in sea lice, which are especially related to the completion of female development, including egg-string formation, a process that demands high energy requirements (Eichner et al., 2008).

On the other hand, transcription levels of both peroxinectins were down-regulated after exposure to delousing drugs (Fig. 3C–D). Expression levels were, as expected, more associated with male adults. Interestingly, the effects of down-regulation were greater for both transcripts in the azamethiphos assay than in the deltamethrin assay. This is also the first time that the expression levels of peroxinectins were measured after exposure to delousing drugs. However, there is strong evidence that deltamethrin has greater performance as a delousing chemical in the mobile stages of the *C. rogercresseyi* lifecycle, including mobile adult males (Arriagada et al., 2014). This evidence supports the hypothesis that expression levels of *peroxinectins* would be more affected by this drug in male adults, taking into consideration its mobility when looking for virgin females and its higher expression levels in this stage.

Furthermore, it is relevant to look into the differential effect of deltamethrin and azamethiphos on PX expression levels. First, both delousing

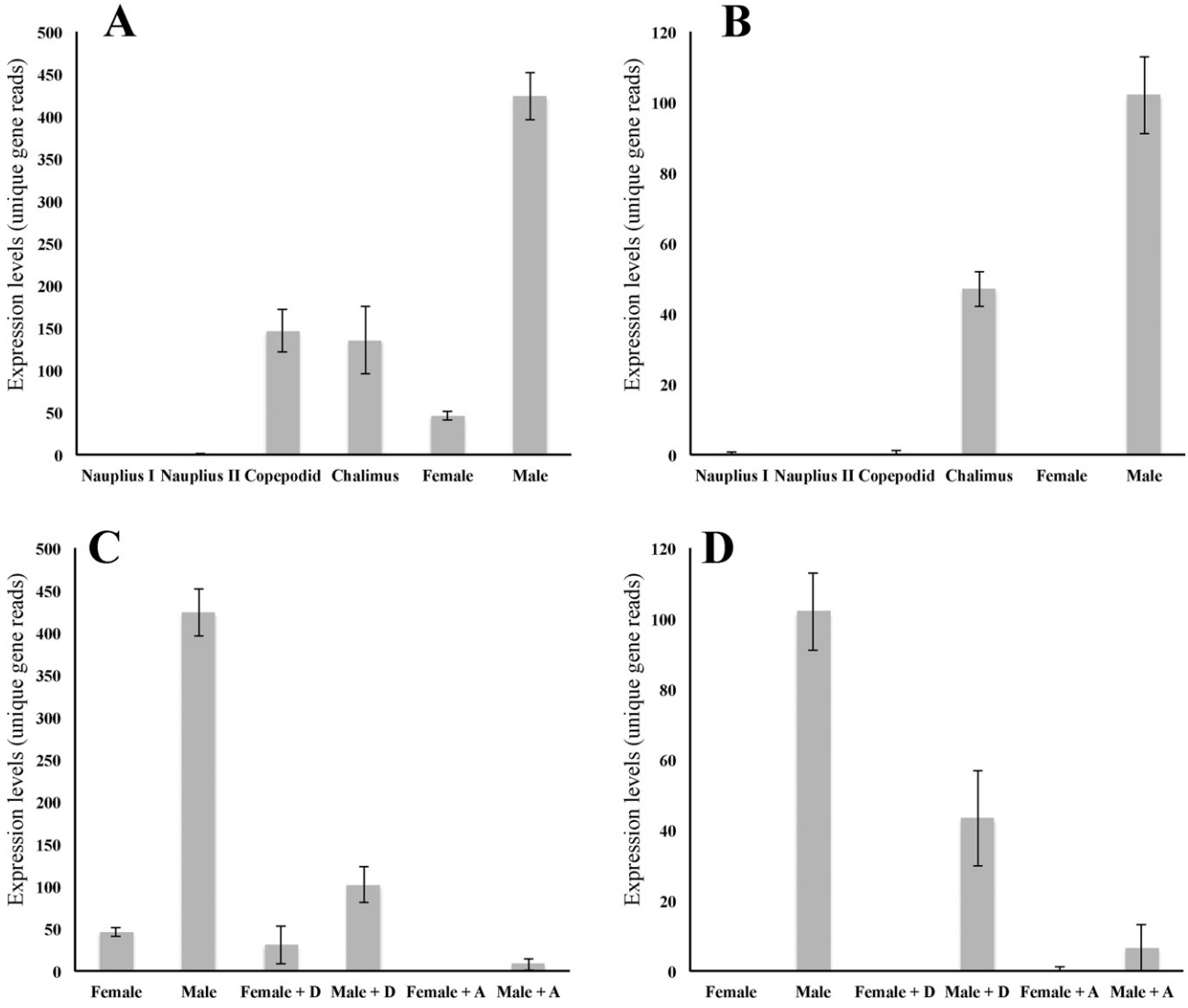

Fig. 3. Expression levels of two peroxinectin transcripts in *Caligus rogercresseyi*. In silico RNA-seq analyses were performed to measure the expression levels of each transcript by calculating the unique gene reads. Expression levels were evaluated during different developmental stages for *Peroxinectin-Cr1* (A) and *Peroxinectin-Cr2* (B). Expression levels were also evaluated after male and female adults were exposed to deltamethrin (+D) and azamethiphos (+A) drugs for *Peroxinectin-Cr1* (A) and *Peroxinectin-Cr2* (B).

drugs showed a decreasing effect in the expression levels of PX, suggesting that these chemicals directly or indirectly inhibit biological processes regarding cell adhesion and/or peroxidase activity in the parasite. Secondly, it is also notable that azamethiphos had a greater effect on PX transcription. Until now, there was no direct information comparing these two drugs in relation to their effects at a molecular level in *C. rogercresseyi*. Nonetheless, there is some evidence in fish that deltamethrin has greater effects on the global transcriptomic response of *S. salar* than in combination with azamethiphos (Olsvik et al., 2014). Furthermore, azamethiphos has lower lethal effects in other species, such as lobsters, than the synthetic pyrethroid cypermethrin, which is similar to deltamethrin (Burridge et al., 2000). The present results showed an opposite trend regarding the effect of PX genes, however, further studies are needed to address the effects of delousing drugs in *C. rogercresseyi* at the transcriptomic level.

4. Conclusions

For the first time, two novel PX genes were identified in *C. rogercresseyi*. The full mRNA sequences of these transcripts were dissimilar, but these were similar to peroxinectin genes identified in other crustacean species. On the other hand, the expression levels of both transcripts were comparable, showing an association with male adults and, interestingly, down-regulation when exposed to two delousing drugs currently used in Chilean and worldwide salmon farms to control sea lice. The main contribution of this study is in suggesting use of this gene in the process of evaluating delousing drug application in salmon farms, as well as in assessing the sex-specific effects of some chemical treatments in salmon lice. With the contributions of this work, further studies might evaluate the physiological implications of decreased PX levels at the cellular level post-exposure to different chemical compounds.

Acknowledgments

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References

Arriagada, G.A., Stryhn, H., Campistó, J.L., Rees, E.E., Sanchez, J., Ibarra, R., Medina, M., St-Hilaire, S., 2014. Evaluation of the performance of pyrethroids on different life stages of *Caligus rogercresseyi* in southern Chile. Aquaculture 426–427, 231–237.

Burridge, L.E., Haya, K., Waddy, S.L., Wade, J., 2000. The lethality of anti-sea lice formulations Salmosan® (Azamethiphos) and Excis® (Cypermethrin) to stage IV and adult lobsters (*Homarus americanus*) during repeated short-term exposures. Aquaculture 182, 27–35.

Chavez-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.

Costello, M.J., 2009. The global economic cost of sea lice to the salmonid farming industry. J. Fish Dis. 32, 115–118.

Daiyasu, H., Toh, H., 2000. Molecular evolution of the myeloperoxidase family. J. Mol. Evol. 51, 433–445.

Dong, B., Liu, F., Gao, H., Wang, B., Xiang, J., 2009. cDNA cloning and gene expression pattern following bacterial challenge of peroxinectin in Chinese shrimp *Fenneropenaeus chinensis*. Mol. Biol. Rep. 36, 2333–2339.

Dong, C., Wei, Z., Yang, G., 2011. Involvement of peroxinectin in the defence of red swamp crayfish *Procambarus clarkii* against pathogenic *Aeromonas hydrophila*. Fish Shellfish Immunol. 30, 1223–1229.

Du, Z.-Q., Ren, Q., Huang, A.-M., Fang, W.-H., Zhou, J.-F., Gao, L.-J., Li, X.-C., 2013. A novel peroxinectin involved in antiviral and antibacterial immunity of mud crab, *Scylla paramamosain*. Mol. Biol. Rep. 40, 6873–6881.

Eichner, C., Frost, P., Dysvik, B., Jonassen, I., Kristiansen, B., Nilsen, F., 2008. Salmon louse (*Lepeophtheirus salmonis*) transcriptomes during post molting maturation and egg production, revealed using EST-sequencing and microarray analysis. BMC Genomics 9, 126.

Gallardo-Escárate, C., Valenzuela-Muñoz, V., Nuñez-Acuña, G., 2014. RNA-Seq analysis using de novo transcriptome assembly as a reference for the salmon louse *Caligus rogercresseyi*. PLoS One 9, e92239.

Holmblad, T., Soderhall, K., 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. Aquaculture 172, 111–123.

Ingvarsdóttir, A., Birkett, M.A., Duce, I., Genna, R.L., Mordue, W., Pickett, J.A., Wadhams, L.J., Mordue, A.J., 2002. Semiochemical strategies for sea louse control: host location cues. Pest Manag. Sci. 58, 537–545.

Johansson, M.W., 1999. Cell adhesion molecules in invertebrate immunity. Dev. Comp. Immunol. 23, 303–315.

Johansson, M.W., Soderhall, K., 1988. Isolation and purification of a cell-adhesion factor crayfish blood-cells. J. Cell Biol. 106, 1795–1803.

Johansson, M.W., Soderhall, K., 1989. A cell-adhesion factor from crayfish hemocytes has degranulating activity towards crayfish granular cells. Insect Biochem. 19, 183–190.

Johansson, M.W., Holmblad, T., Thornqvist, P.-O., Cammarata, M., Parrinello, N., Soderhall, K., 1999. A cell-surface superoxide dismutase is a binding protein for peroxinectin, a cell-adhesive peroxidase in crayfish. J. Cell Sci. 112, 917–925.

Kobayashi, M., Johansson, M., Söderhäll, K., 1990. The 76 kD cell-adhesion factor from crayfish haemocytes promotes encapsulation in vitro. Cell Tissue Res. 260, 13–18.

Lees, F., Baillie, M., Gettinby, G., Revie, C.W., 2008. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar*) in Scotland, 2002–2006. PLoS One 3, e1549.

Liu, C.H., Cheng, W., Kuo, C.M., Chen, J.C., 2004. Molecular cloning and characterisation of a cell adhesion molecule, peroxinectin from the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 17, 13–26.

Liu, C.H., Cheng, W., Chen, J.C., 2005. The peroxinectin of white shrimp *Litopenaeus vannamei* is synthesised in the semi-granular and granular cells, and its transcription is up-regulated with *Vibrio alginolyticus* infection. Fish Shellfish Immunol. 18, 431–444.

Olsvik, P.A., Ørnsrud, R., Lunestad, B.T., Steine, N., Fredriksen, B.N., 2014. Transcriptional responses in Atlantic salmon (*Salmo salar*) exposed to deltamethrin, alone or in combination with azamethiphos. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 162, 23–33.

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

Thornqvist, P.O., Johansson, M.W., Soderhall, K., 1994. Opsonic activity of cell-adhesion proteins and beta-1,3-glucan binding-proteins from two crustaceans. Dev. Comp. Immunol. 18, 3–12.

Wotton, H., Purcell, S., Covello, J., Koop, B., Fast, M., 2014. Comparison of host selection and gene expression of adult *Lepeophtheirus salmonis* and *Salmo salar* during a cohabitation of initially infected and uninfected fish. J. Aquac. Res. Dev. 5.