Full length article

Transcriptomic and targeted immune transcript analyses confirm localized skin immune responses in Atlantic salmon towards the salmon louse

Aina-Cathrine Øvergård [a,*], Christiane Eichner [a], Noelia Nuñez-Ortiz [b,1], Heidi Kongshaug [a], Andreas Borchel [a], Sussie Dalvin [b]

[a] SLCR-Sea Lice Research Centre, Department of Biological Sciences, University of Bergen, Pb. 7803, Bergen, NO-5020, Norway
[b] SLCR-Sea Lice Research Centre, Disease and Pathogen Transmission, Institute of Marine Research, Pb. 1870 Nordnes, Bergen, NO-5817, Norway

ARTICLE INFO

ABSTRACT

Atlantic salmon (*Salmo salar*) are highly susceptible to infestations with the ectoparasite *Lepeophtheirus salmonis*, the salmon louse. Infestations elicit an immune response in the fish, but the response does not lead to parasite clearance, nor does it protect against subsequent infestations. It is, however, not known why the immune response is not adequate, possibly because the local response directly underneath the louse has been poorly evaluated. The present study describes the transcriptomic response by RNA sequencing of skin at the site of copepodid attachment. Analysing differentially expressed genes, 2864 were higher and 1357 were lower expressed at the louse attachment site compared to uninfested sites in the louse infested fish, while gene expression at uninfested sites were similar to uninfested control fish. The transcriptional patterns of selected immune genes were further detailed in three skin compartments/types: Whole skin, scales only and fin tissue. The elevation of pro-inflammatory cytokines and immune cell marker transcripts observed in whole skin and scale samples were not induced in fin, and a higher cytokine transcript level in scale samples suggest it can be used as a nonlethal sampling method to enhance selective breeding trials. Furthermore, the immune response was followed in both skin and anterior kidney as the infestation developed. Here, newly moulted preadult 1 stage lice induced a higher immune response than chalimi and adult lice. Overall, infestation with salmon louse induce a modest but early immune response with an elevation of mainly innate immune transcripts, with the response primarily localized to the site of attachment.

1. Introduction

The salmon louse (*Lepeophtheirus salmonis*) is an ectoparasitic copepod on salmonid fish species of the Northern hemisphere. As of today, the salmon louse represents a significant challenge to sustainable Atlantic salmon (*Salmo salar*) aquaculture. Even though milder infestations are typically not detrimental to the host, high parasite burdens are associated with more intense grazing of the skin, mucus and later in development also blood. This creates wounds that disturb the osmotic balance of the fish and induce stress that directly and indirectly increases the susceptibility to other pathogen [1–6]. Mortality can be seen in smaller fish with high louse burdens, and infestations can represent a problem for e.g. migrating post-smolt salmon [2,5]. Chemotherapeutants have therefore been used in salmon farming to limit the

amount of salmon louse on farmed fish and to minimize the production of new parasites that can spread to wild salmonids. The louse has, however, developed resistance against the majority of these chemotherapeutants [7], promoting the development of non-medical treatment methods that unfortunately have a negative effects on fish welfare [8]. Thus, there is an immediate need for new control measures, and an increased knowledge of the host-parasite interaction is of importance to help such development.

Consequently, many studies have addressed the damage and associated immune response in salmonids upon salmon louse infestation. The infective stage of the salmon louse, the copepodid, inflicts some damage to the host skin already at the initial attachment phase as it buries its hooked-shaped second antennae into the epidermis [9–11]. As moulting approaches, the copepodid extrudes a frontal filament that

---

anchors the louse to the surface of the fish and restricts the movement of the two next louse stages, chalimus 1 and 2, to the site of filament attachment. The filament is attached underneath the epithelium, but still, only a limited tissue response is induced [9,12]. Further, when the salmon louse proceeds through two pre-adult stages, it is no longer attached by a filament between moults, but can move freely over the host surface and continues to be mobile after the final moult to the adult stage [11,13]. Adherence to the host is now mediated by a suction cup shaped cephalothorax in addition to the grasping action of the second antennae, creating a depressed oval ring in the epidermis underneath the lice [14].

Although lice attachment itself damages the host skin, the more severe lesions are caused when the louse feeds on the host skin and blood [10,12,14–16]. The small juvenile louse stages, the copepodid and the chalimus, mainly feed on the fish mucus and epidermis causing only superficial wounds. Underneath the juvenile louse stages, influx of immune cells is accordingly minor and pro-inflammatory cytokine transcripts as *IL1β*, *IL6* and *IL8* are only moderately elevated in both Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) [9,10,15,17]. When the lice reach the mobile pre-adult and adult stages, they inflict deeper wounds reaching the dermal blood supply [14,15,18]. The mobile louse stages are thus more virulent, and a higher number of immune cells are typically seen attracted to skin immediately below preadult and adult lice [14,15]. Nevertheless, adult lice seem to efficiently dampen immune responses [15,19–21], likely as a result of immune dampening factors secreted by salmon louse exocrine glands [22,23]. Thus, infestations are long lived where the interaction between the fish and the parasite changes over time [15,17,24,25], as the morphology of the lice and also its mode of attachment and feeding changes [9–11,14].

While the gene expression in unaffected skin of salmon louse infested fish has been carefully studied in Atlantic salmon [24,26–29], the local immune response directly underneath the lice has received less attention. Gene expression studies that have compared the expression of selected immune genes between infested and unaffected sites indicates, however; that the transcriptional response is mainly localized to a small area directly underneath the louse in both Atlantic salmon and rainbow trout, while nearby unaffected skin closely resembles that of untreated fish [15,17,19,23,30–32].

The present study aimed to explore the Atlantis salmon local immune response against the salmon louse more thoroughly. Initially we applied a transcriptomic approach to investigate the skin response directly underneath the salmon louse copepodid. Further, we compared the level of selected immune transcripts in skin and fins in addition to scale samples at this early stage of settlement. Finally, the knowledge generated by these initial studies was utilized to investigate the skin immune response towards older developmental stages of salmon louse, comparing it to the systemic head kidney response at transitional time points right after moulting to chalimus 1, preadult 1 and adult stages.

2. Material and method

2.1. Animals and infection studies

The LsGulen laboratory strain of *L. salmonis salmonis* [33] was maintained on farmed Atlantic salmon according to Hamre et al. [34]. The fish were hand fed on a commercial diet and reared in sea water with a salinity of 34.5 ppt and a temperature of 8–10 °C unless otherwise specified. Louse eggs, nauplii and copepodids were kept in seawater from the same supply. Nauplia were obtained from hatching eggs and kept in single wells in a flow through system [34].

All experiments were carried out in strict accordance with relevant guidelines and regulations. The study was carried out with approval granted from the Ethic committee of Norwegian Food Safety Authority (approval numbers 8589) following ARRIVE guidelines. Three infestation experiments were conducted. In each experiment, the fish were allowed to acclimatize to the experimental tank for 14 days prior to

infestation. The infestation was carried out by lowering the water level in the tanks to a minimum whilst maintaining normal water supply and adding copepodids to the tanks. Tank levels returned to normal levels within approximately 5–10 min after which non-attached copepodids were slowly flushed out of the system. All control fish went through procedural infestation where no copepodites were added. Immediately before all samplings, fish were sedated in a bucket containing seawater with 100 mg/l benzocaine and 10 mg/l metomidate and subsequently euthanized with a sharp blow to the head. Blood was drawn prior to tissue sampling to avoid bleeding, which would contaminate samples with immune cells from the blood. Skin was obtained only from areas with scales since distinct differences in expression of Atlantic salmon immune genes after salmon louse infestation has been observed in scaled compared to scaleless skin [28]. Moreover, the skin samples did not include subcutaneous tissue or lateral line. From infested fish, two different sample types were collected: one immediately under a louse (infested site), and one from nearby and thus similar but non-parasitized skin (unaffected site). Skin was also sampled from uninfested control fish and were always taken from the same region as from the infested fish at a given time point. All skin sampling was performed immediately after euthanasia and all fish were processed sequentially to avoid degradation of RNA.

In the first two experiments (Exp. 1 and 2), Atlantic salmon were placed in a single fish tank system [35], and kept at 12 °C in a salinity of 34.5 ppt. In both experiments, the fish were divided in an infested and uninfested group, where samples were taken ventrally to the lateral line, between the pectoral and pelvic fins in both groups. Moreover, as the dissected samples were only around 3 × 3 mm, samples from three attachment sites from individual fish were pooled to obtain sufficient tissue for RNA purification. The lice were not removed before tissue preparation. In Exp. 1, six fish weighing 104 ± 8 g were infested with 80 copepodids fish^{-1}, while six fish were kept uninfested. Skin samples were taken at 3 days post infestation (dpi) when lice were still copepodids (Fig. 1). In Exp. 2, eight fish weighing 148 ± 17 g were infested with 150 copepodids fish^{-1}, while eight fish were kept non-infested. The higher number of copepodids per fish was given to enable sampling of separate scale and fin samples in addition to skin samples. Samples were also in this experiment taken at 3 dpi, and all sample types were collected from each tissue type: unaffected and infested sites on infested fish and unaffected sites on uninfested fish. As for the skin samples, three fin samples were pooled from each fish. In the scale samples, a total of seven scales with or without lice had to be pooled for RNA extraction. This because each scale sample contains much less RNA than each skin sample.

In a third experiment (Exp. 3), 60 Atlantic salmon (200 ± 28 g) kept at 8.6 °C and a salinity of 34.5 ppt were equally divided into four 200 l tanks, where two tanks were infested with 80 copepodids fish^{-1}. Skin and head kidney samples were collected from five fish from each tank at 7, 23 and 43 dpi, corresponding to the time when the majority of lice had just moulted into the chalimus 1, preadult 1 and adult stage, respectively (Fig. 1). The preferred site of attachment varies during louse development, and sampling sites were adjusted accordingly. When lice were at the chalimus 1 stages, samples were taken ventral to the lateral line, between the pectoral and pelvic fins including the lice in further tissue preparation. At the preadult and adult stages, the lice were removed, and samples were taken from areas where many lice were feeding, caudal to the dorsal or adipose fin.

2.2. Total RNA purification

All samples for RNA isolation were collected in RNA later (Life-Technologies), kept at 4 °C overnight and stored at −20 °C. Total RNA was isolated with a combined Tri reagent (Sigma Aldrich) and RNeasy (Qiagen) method, as previously described [36], with DNase treatment performed on column. Extracted RNA was kept at −80 °C until use.
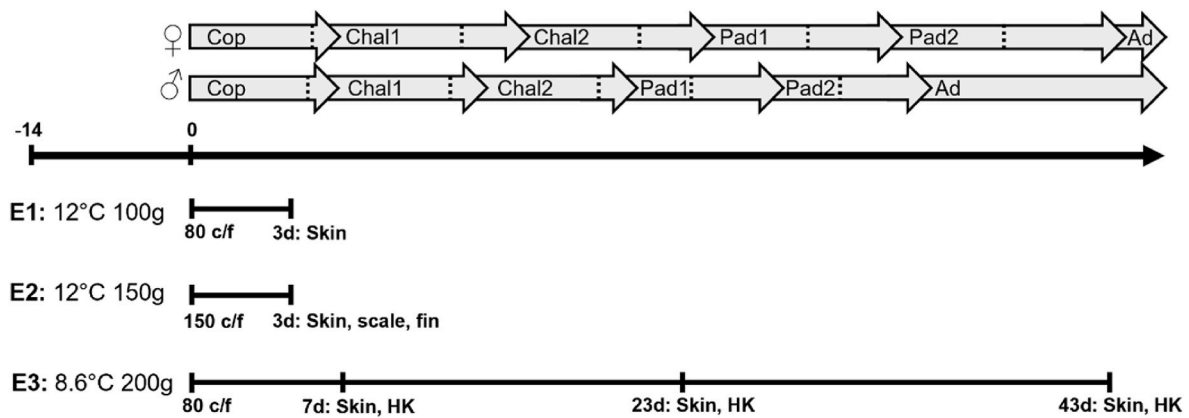
Fig. 1. Diagram of the experimental setup of the three experiments (E) 1–3. In all experiments, fish were placed in experimental tanks 14 days prior to infestation (−14), with the rearing temperature and average weight of the fish indicated below. At day 0, the fish were infested with varying number of copepodids per fish (c/f). The type of tissue collected is indicated at the time of sampling (days post infestation, d) and is related to the lice stage shown with arrows above. The arrows indicate the relative age meaning the time it takes for the majority of the cohort to reach a certain stage, and the dotted line in each arrow indicates the time when the first lice begin molting into the next stage. Cop – copepodids, chal – chalimus, pad – pre-adult, ad – adult.

2.3. Transcriptomic and bioinformatic analysis

From both Exp. 1 and 2, five samples from uninfested fish and ten samples from infested fish, five from unaffected and five from infested sites, were randomly chosen for further transcriptomic analyses by Illumina sequencing at the Genomics Core Facility, University of Bergen. Libraries were prepared with Illumina® TruSeq® mRNA Stranded Sample Preparation kit from 400 ng total RNA. Samples were barcoded, randomized and sequenced by Illumina HiSeq4000 (Illumina, Inc., San Diego, CA, USA) producing 2×75 base pair (bp) paired end reads. Illumina's RTA software version 2.4.11 was used for image analysis and base calling. Conversion of data to FastQ format was done using bcl2fastq version 2.1.7.1.14.

All analyses were performed in Galaxy [37]. Alignment of sequences was performed by HISAT [38] (version 2.0.3.2) against the Atlantic salmon genome (ICSASG_v2_with_refseq_genes) as reference and default alignment options. Feature counts (v1.6.4) was used to count aligned reads [39]. Differential expression analysis and normalization of counts were done in DEseq2 version: 1.22.1 [40]. Gene Ontology enrichment was executed with GOenrichment Version 2.0.1 (https://github.com/DanFaria/GOEnrichment) and Benjamin-Hochberg multiple test correction (adjusted p-value <0.01). For KEGG pathway enrichment investigation, the web-based platform KOBAS 3.0 was used [41].

2.4. cDNA synthesis and real time RT-PCR

Total RNA was synthesised to cDNA using the AffinityScript qPCR cDNA Synthesis Kit (Stratagene) according to supplier's recommendations adding 1000 ng of DNase treated total RNA. Samples were diluted 5 times and stored at −20 °C. No template control (NTC) and RT negative samples were created to test for non-specific amplification and contamination. None were detected.

Real time RT-PCR was performed with 1x PowerUp™ SYBR Green Master Mix (Thermo Fisher Scientific), 500 nM forward and reverse primers (Table 1) and 2 µl diluted cDNA in 10 µl reactions. Samples were run in duplicate on the Applied Biosystems QuantStudio™ 3 Real-Time

Table 1
Primers used for real time RT-PCR. C3a – complement factor 3a, IL – interleukin, IFNγ – interferon gamma, MMP – matrix metalloproteinase, CATH2 – cathelicidin 2, TCRα – T-cell receptor alpha, CD – cluster of differentiation, NCCRP1 – non-specific cytotoxic cell receptor P1, MHC – major histocompatibility complex, GATA3 – GATA binding protein 3, Ig – immunoglobulin, MSL – mannose specific lectin, pIgRL – polymorphic immunoglobulin receptor like, EF1α – elongation factor 1 alpha, TRIM16 – tripartite motif 16.

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')	Accession no.
C3a	ATTCTTCCCCTCCACTCCCTCG	CGATTTGGTCGTCAAGCCAGG	XM_014186867
IL1β	GCTGGAGAGTGCTGTGGAAGA	TGCTTCCCTCCTGCTCGTAG	XM_014170479
IL4/13a	CGTACCGGCAGCATAAAAATCACCATTCC	CCTTGCATTTTGTGGTGGTCCCA	NM_001204895
IL6	ACCAACAGTTTGTGGAGGAGTTTCAGAAGC	CCTGCAGACATGCCTCCTTGTTG	KJ425513
IL8	GCATCAGAATGTCAGCCAGCC	ACGCCTCTCAGACTCATCCC	NM_001140710
IFNγ	ATGGATGTGTTATCAAGGGCTGTGATGTG	CAGCTGGTCCTTGGAGATCTTATAGTGGAC	AY795563, XM_045698695
MMP13	ACTCTTTGCCAATATCGCCACCCA	TGGGCCCTCGTTTGAACGCA	BT058668
CATH2	GGTGACTGTAAGGTTGAGCTTCCCC	CCTTGCTGCCCCTGTGCCTT	AY542961.1
TCRα	ATGAGCCATCCTACTACACGTTGAACTCAA	CACTCTGGTGGCCTCTGTATTGTTGAAGAC	BT057540
CD4	GAGTACACCTGCGCTGTGGAAT	GGTTGACCTCCTGACCTACAAAGG	EU585750
CD8α	TAGAGTGCAAGACAACGCTGGAATGGA	TCTCGAGCCTTTTTGAAAGCCTTCAG	AY693393, AY701521
NCCRP1	AATCCTGCGCCTCACGGTGTGAGTC	GCGAGGAGGTCCTTCTGGTGGAAAC	NM_001166257
MHC1	CAAGACCAACTGGAATGACCCCAA	GGAAGTGCTGGCCGGAACAAA	AF508864.2
MHC2	GGACGTGAGGTGAAGTCTGATGTGACC	CTGATGTGCTCCACCATGCAGGA	BT058598
GATA3	ATATCGACTCACAGGGCAACCACG	GCAGAGAGCCGTGCAGTAGAGAGG	NM_001171800.1
IgD	CACCAGGAGGAAAGTTTGGCATCA	CCCCAAGGAGCTCTGGTTTGGA	AF141606
IgM	TGAGGAGAACTGTGGGCTACACT	TGTTAATGACCACTGAATGTGCAT	BT058539
IgT	GGTGGTCATGGACGTACTATTT	CCTGTGCAGGCTCATATCTT	GQ907004
MSL	ATCTTTCAGACCGATGGCAACT	GTGTGGTGTAGATGACCAGGTT	XM_014201873.2
pIgRL	TCTTTGTCCATGGACCATCAGG	CACAGTACATAAGGGCCAGGTT	HM452379.1
EF1α	CACCACCGGCCATCTGATCTACAA	TCAGCAGCCTCCTTCTCGAACTTC	BT043567
TRIM16	TTACTGTAGGAGCTGTATTGAGGGCTGCTG	TTCTCCACCAGCTCAGCCAACATG	XM_014170167

PCR System under standard conditions (50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by a melt curve analysis at 60–95 °C). The relative differences in threshold cycle between the target gene and the geometric mean of the reference genes, *EF1α* and *TRIM16*, (ΔCT), and expression relative to uninfested control fish ($\Delta\Delta$CT) were calculated, transformed by the equation $2^{-\Delta\Delta CT}$ [42].

Statistical analyses on qPCR data were performed in GraphPad Prism 9.4.1 (GraphStats). As the data sets did not meet the assumption of normal distribution and homogeneity of variance, a nonparametric Kruskal-Wallis test with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli correction for multiple comparison by controlling the false discovery rate was done [43]. Threshold p-value was set to 0.05 for differently regulated genes.

3. Results

3.1. Transcriptomic response in skin towards salmon louse copepodids

Skin samples from Exp.1 and 2 were analysed for global transcription by sequencing of RNA. On average 46 million reads was obtained from each sample. On average 84% mapped to the Atlantic salmon reference genome (78% one time, 7% to multiple loci). Data are available at the NCBI SRA read archive (BioProject: PRJNA927139). The transcriptome of salmon skin from uninfested control fish was compared to samples taken from infested fish, both from louse attachment site and unaffected

sites. Additionally, skin from louse attachment sites was compared to that of unaffected sites.

Skin samples from the louse attachment sites showed a distinctively different overall gene expression profile compared to samples from unaffected sites and that of uninfested fish (Fig. 2a and b). The respective groups also exhibited an overall similar gene expression pattern in both experiments, however, looking at the number of differently expressed genes (DEGs) between the different groups, some distinctions are seen between the experiments (Fig. 2c). Whilst a similar number of DEGs were identified in both experiments comparing the louse attachment site to uninfested fish (11505 and 13155 in exp 1 and 2, respectively, Fig. 2c), twice as many genes were found differentially expressed in Exp.1 (10253 genes) than in Exp.2 (5091 genes) when skin from attachment site was compared to that of unaffected sites taking individual fish into account (paired analysis). As an increased number of DEGs between unaffected sites and uninfested control fish were seen in Exp 2 (1926 genes). For further analysis, we therefore focused on genes that were differently expressed in both experiments (Supplementary Table 1), to exclude unspecific batch effects. Of these DEGs, 2864 had a higher and 1357 had a lower expression at the louse attachment site compared to uninfested sites. Overall, transcripts displaying an increased expression were more regulated than transcripts with a decreased expression (Fig. 2b). Moreover, only a limited number of genes were found to be regulated at unaffected sites in the louse infested fish compared to uninfested fish in both experiments; only one gene was
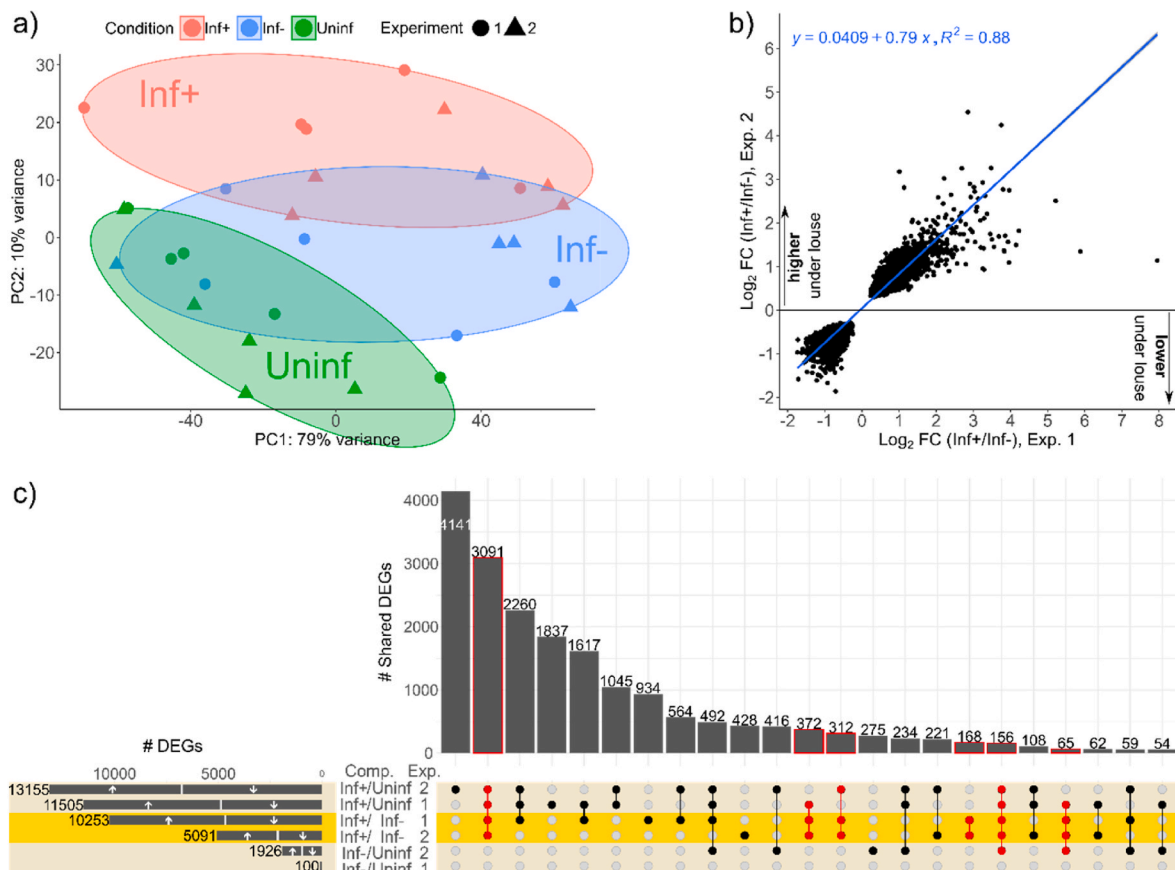

Fig. 2. RNA-Seq analysis. Skin retrieved from salmon uninfested (Uninf) or infested (Inf) with salmon lice (*Lepeophtheirus salmonis*). From infested fish, samples were taken directly under the louse (Inf+) or at nearby sites without lice (Inf-). a) Principal component analysis of transformed variance-stabilized- expression data. b) Correlation of fold change (Log$_2$ FC) rates for differently expressed genes (DEGs) in experiment 1 and 2. c) UpSet plot showing the number of differentially expressed genes (DEGs) and their overlap in the experiments. Horizontal bars (left side) depict total number of DEGs per comparison, including the proportion of up- and down-regulated genes (arrows). Vertical bars show the number of DEGs exclusively shared by the experiments and skin sites, marked with dots below. Only genes marked with a yellow background and red dots were selected for further analysis, representing genes differently expressed between Inf+ and Inf-in both experiments. Moreover, the respective vertical bars showing the numbers of genes are marked with a red frame. Only intersections with at least 50 DEGs are shown, excluding 258 DEGs from this figure.

found to be down-regulated in infested fish, annotated as *midkine-B-like*, and four genes were found to be higher expressed in infested fish; *rap1 GTPase-activating protein 1-like, C5a anaphylatoxin chemotactic receptor 1-like, eyes absent homolog 2-like, and interleukin 4/13A*.

Next, a gene ontology (GO) analysis was performed for the genes differently expressed between unaffected and affected sites on infested fish (Supplementary Table 2). Analysing the genes showing a higher expression at the attachment site, 180, 93 and 53 GO categories for biological processes (BP), cell compartment (CC) and molecular processes (MP), respectively, were found to be significantly enriched. Most
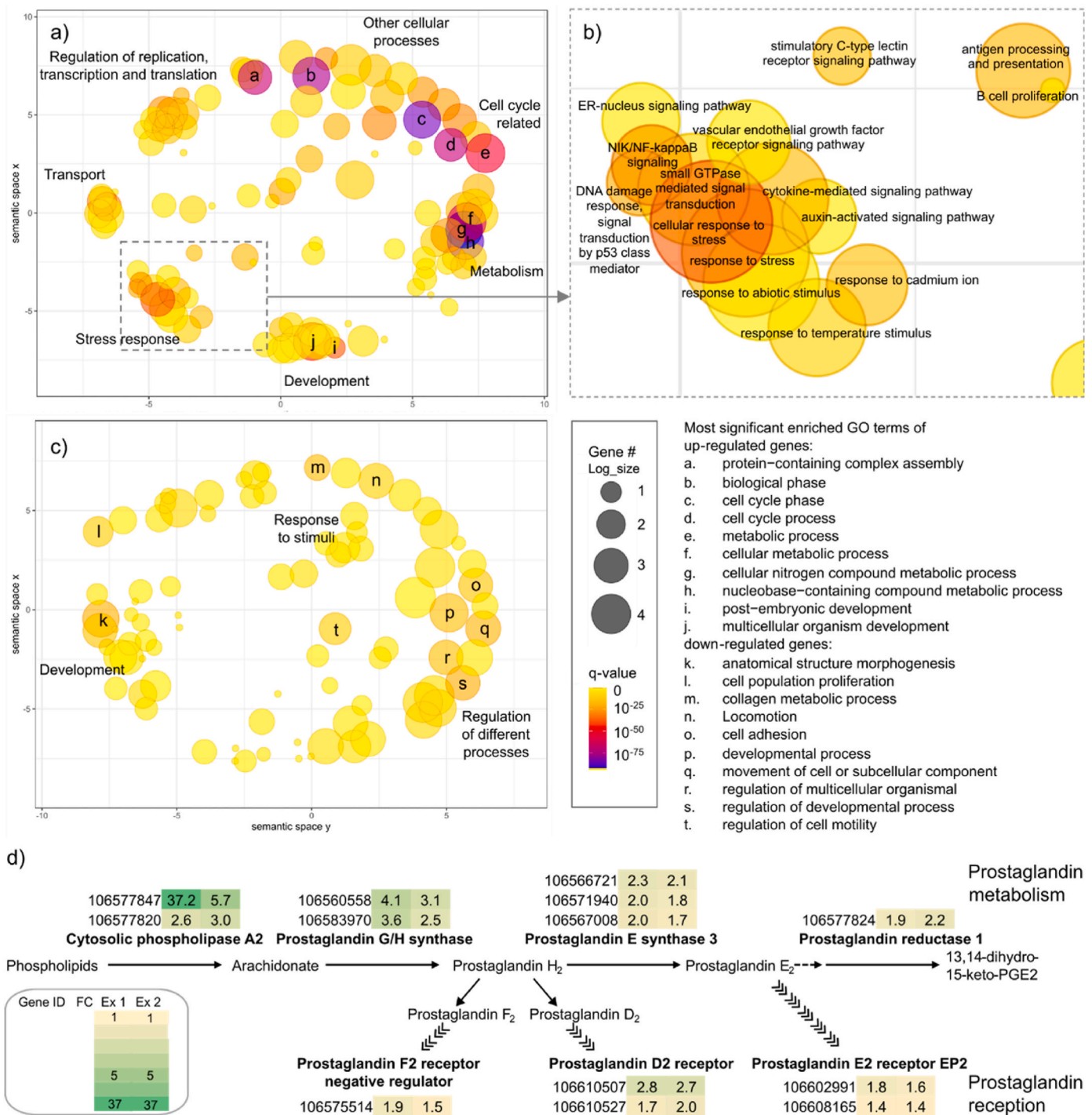

Fig. 3. Putative function of differently expressed genes (DEGs). Genes differently expressed between attachment and unaffected sites on infested fish were analysed. a) – c) Significantly enriched Gene Ontology (GO) terms of the DEGs were summarized and clustered after semantic relationships by Revigo [44]. Significance of GO term enrichment is marked by different colours, while the number of genes belonging to the different GO terms are indicated by the size of the circles. a) Enriched GO terms of the genes with significantly higher expression at attachment site compared to unaffected site in both experiments. b) Magnification of the cluster marked with dashed rectangle in (a), c) Enriched GO terms of the genes showing a significantly lower expression at attachment site compared to unaffected site in both experiments. The ten most significantly enriched GO terms of up and down regulated genes are marked with letters from a to j (a) or k to t (b), respectively, with GO terms listed at lower right. d) Fold change (FC) of 13 DEGs involved in prostaglandin metabolism with increased expression at the louse attachment site.

GO terms identified included categories involved in cell proliferation (Fig. 3a), including the most enriched GO terms "PeBoW complex", "positive regulation of transcription of nucleolar large rRNA by RNA polymerase I", and "DNA polymerase processivity factor activity". Also, both the "epidermal growth factor receptor signalling pathway" and "fibroblast growth factor receptor signalling pathway" categories were enriched in BP (Supplementary Table 2). Categories involved in cell stress as "cellular response to stress" and "detoxification", and processes related to immune regulation as "NIK/NF-kappaB signalling", "antigen processing and presentation", "cytokine-mediated signalling pathway", "stimulatory C-type lectin receptor signalling pathway", "Fc receptor signalling pathway", "activation of MAPKK activity", "B cell proliferation", "transformation of host cell by virus", "virion part", and "viral nucleocapsid" were also found within the significant enriched processes (Fig. 3b).

Analysing the genes with a decreased mRNA level at the lice attachment site, 139, 33 and 25 GO-terms were found to be enriched in BP, MP and CC, respectively. The most significant BP categories were mainly related to cellular components and development (Fig. 3c), as "regulation of developmental process", "anatomical structure morphogenesis" and "movement of cell or subcellular component", while the only process mainly related to immune functions was "positive regulation of tumor necrosis factor production". Moreover, in categories for CC and MF, "extracellular matrix" and "extracellular matrix structural constituent" were detected under the genes with a decreased expression.

A Kegg pathway analysis was performed to investigate which pathways the regulated genes could be assigned to and to identify highly enriched pathways. Genes with increased expression at the louse attachment site could be assigned to 157 different Kegg pathways, 51 of these significantly enriched (p-value <0.05) (Supplementary Table 3). Also here, pathways associated with cell proliferation were identified, with "spliceosome", nucleocytoplasmictransport", "ribosome biogenesis in eukaryotes", "proteasome", "DNA replication" and "cell cycle" as the most significantly enriched. Interestingly, components of the prostanoid pathway, particularly prostaglandin E_2 synthesis, were enriched with upregulated genes (Fig. 3d). Genes with a decreased transcript level at the louse attachment site could be assigned to 118 different KEGG pathways, where 16 were significantly enriched (p-value <0.05). The most significantly enriched pathways were involved in adhesion between cells and the extracellular matrix with "ECM-receptor interaction" and "Focal adhesion" as the two most enriched pathways identified.

Furthermore, immune related genes were manually identified by name from the significantly regulated genes at the louse attachment site. A total of 315 immune related genes were identified, where 231 were found to be significantly higher and 84 were found to be significantly lower expressed at the attachment site compared to unaffected site (Supplementary Table 4). Among these genes, the most regulated transcripts in response to salmon louse copepodids were a mannose-specific lectin (MSL) and a cytosolic phospholipase A_2 gamma-like (cPLA2G) gene (Table 2, Fig. 4). Also, pro-inflammatory genes like *tumor necrosis factor alpha* (TNFα) and *TNFα* related genes, *IL1 beta* (*IL1β*), *IL1β-like* (*IL1βL*), *IL8, IL8L, IL11, cathelicidin 1* (CATH1) and *matrix metalloproteinase* (MMP) *9* and *13* were found to be up-regulated. In addition, cytokine receptors were elevated, as were the chemokines *CXCL2, CCL20L, CCL13L,* and *leukocyte cell-derived chemotaxin-2-like* (*LECT2L*) and the chemokine receptors *CCR9L, CXCR1L* and *CXCR2L*. Specific markers for some immune cells lack in teleost, making the evaluation of immune cell influx based on transcriptomic data difficult. Though, a modest elevation of many T-cell related genes and the *non-specific cytotoxic cells receptor P1* (NCCRP1) was seen. An elevation in B-cell marker transcripts was, however, not detected, though increased levels of *IL4/13A* and a *polymeric immunoglobulin receptor-like* (pIgRL) gene were found. Analysing the identified immune related genes that showed a significantly decreased transcript level at the louse attachment site, all genes showed a rather moderate decrease (Fig. 4q-t, Table 2). Of the most down modulated ones, *complement C1q tumor necrosis factor-related protein 7-like* (C1QTNF7L), *cluster of differentiation* (CD) 55, IL17D and *IL11 receptor subunit alpha-like* (IL11RAL) were identified.

3.2. Differential expression of selected immune genes in skin, scale and fin

After the initial transcriptomic analysis of the response in whole skin sample with scales, we therefor wanted to compare the immune response here with that induced in fin and samples where only the scale was taken. Immune genes were selected based on the transcriptomic data and previous reports [31], and were analysed by qPCR.

As seen in whole skin samples, the transcript level of the selected immune genes in scale and fin samples at unaffected sites were in general like that of uninfested fish (Fig. 5). Moreover, a significant increase of *IL1β, IL8, MMP13, NCCRP1,* and *IL4/13A* transcripts were detected at the louse attachment site compared to that of untreated fish and unaffected sites in both skin and scales samples. When analysing the fin samples, however, similar, or even decreased transcript levels were detected when comparing louse attachment site with those sampled from unaffected sites and untreated fish. Comparing the expression underneath lice from the different samples, the highest median transcript level of *IL1β, IL8, MMP13, NCCRP1,* and *IL4/13A* was seen in scales, though with a high variation between individuals. On the opposite, a lower median transcript level was seen in fin as compared to skin and scales of *IL1β, IL8, MMP13, NCCRP1, IL4/13A, CD4, CD8α* and *IgT*, with a significant lower expression level detected for *NCCRP1* and *CD8α*.

A correlation analysis was also conducted to see if the transcript levels in scales and whole skin correlated to each other on a given fish. The expression of either *IL1β, IL8, MMP13, NCCRP1,* and *IL4/13A* did, however, not show any correlation (results not shown), indicating that the level of response is not fish specific.

3.3. Local cutaneous and systemic transcript level of immune genes in fish infested with older louse stages

After the thorough analysis of the newly settled copepodids, we extended our analysis to older developmental stages looking at immune gene expression at transition time points just after moulting to chalimus 1, preadult 1 and adult stage. This to enable an analysis of responses induced by the morphological changes the lice go through just prior to these stages. Selected immune genes were analysed in both skin and head kidney at 7, 23, and 43 days post infestation (dpi).

Upon sampling, the infested fish were found to have a significant decrease in lice numbers (p = 0.01), holding on average 19.9 ± 3.6 and 12.1 ± 5.3 preadult 1 and adult lice respectively, per fish. Macroscopic examination revealed limited signs of inflammation at the site of infestation as swelling, though more pronounced signs of wounding were seen at the adult stage. When analysing the transcript level of selected immune genes in skin from louse attachment sites, signs of an inflammatory response were detected as a significantly higher transcript levels of *IL1β, IL8, IL6, MMP13, CATH2, NCCRP1, MSL, pIgRL, IL4/13A,* and *C3A* associated with one or more time points (Fig. 6). Generally, the highest level of induction was seen at 23 dpi when the lice had just developed to the preadult 1 stage and differences were only significant at attachment sites. A relatively large variation between individual fish was, however, observed, possibly due to the mobility of the lice at this time point. Underneath newly moulted adults, the increase in the inflammatory transcripts were again more dampened, and a significant decrease in *IL4/13A* was detected in skin underneath adult lice as compared to the chalimus 1 stage (Fig. 6i). A decrease in the expression of *GATA3* was also seen, though both below preadult 1 and adult lice. Fish carrying adult lice also displayed a decreased level of *GATA3* at unaffected sites compared to uninfested fish. A significant decreased expression was also detected in the transcript level of *MHC2* in skin below lice in the preadult 1 stage, while a decreasing trend (albeit not

significant) were observed in the *IgM, IgT* and *IgD* mRNA levels. No regulation of T-cell markers was detected.

Opposite to skin, little gene regulation in response to louse infestation were detected in the head kidney (Fig. 7), with only *MMP13, CATH2, NCCRP1, and pIgRL* exhibiting significantly higher transcriptional levels in infested fish compared to uninfested fish. This regulation was only seen after the lice had molted into the preadult I stage but continued to be significant at the adult stage for all genes. The levels of remaining transcripts were not significantly affected by infestation.

Table 2
Expression of selected immune genes analysed by RNA seq. Log2(FC) of genes significantly regulated at the louse attachment site, with significant regulated genes in bold (p adjusted value > 0.05). Uninf – uninfected, Inf+ – louse attachment site, inf- – unaffected site on infested fish, exp - experiment. Green denotes increased expression, yellow no difference and orange decreased expression.

Gene info		Uninf/Inf-		Inf+/Uninf		Inf+/Inf-	
GeneID	product	Exp1	Exp2	Exp1	Exp2	Exp1	Exp2
LOC100195957	Mannose-specific lectin	0,348	0,443	**5,975**	**6,313**	**7,952**	**1,138**
LOC106577847	Cytosolic phospholipase A2 gamma-like	0,578	1,068	**5,735**	**6,381**	**5,218**	**2,505**
LOC100196393	CXCL2	0,163	0,046	**2,966**	**4,246**	**3,800**	**2,770**
LOC106579494	C-C motif chemokine 20-like	-0,170	-0,354	**3,012**	**2,241**	**3,101**	**2,367**
LOC106587939	C-C motif chemokine 13-like	-0,004	0,328	**2,482**	**2,152**	**2,365**	**0,927**
LOC106611589	Leukocyte cell-derived chemotaxin-2-like	0,285	**1,123**	**2,930**	**3,232**	**2,323**	**1,926**
LOC106597311	C-C chemokine receptor type 9-like	-0,202	0,623	**1,151**	**1,830**	**1,279**	**1,071**
LOC106575635	C-X-C chemokine receptor type 1-like	0,217	0,409	**3,026**	**3,361**	**2,059**	**2,132**
LOC106575644	C-X-C chemokine receptor type 2-like	0,002	0,050	**2,109**	**3,318**	**2,028**	**1,969**
LOC100136453	Cathelicidin	**0,856**	0,630	**3,831**	**3,666**	**2,054**	**2,586**
LOC100136449	Interleukin-1 beta	0,044	0,086	**2,718**	**1,165**	**2,563**	**1,072**
LOC106570815	Interleukin-1 beta-like	0,544	0,456	**3,073**	**3,555**	**2,399**	**2,646**
LOC100270808	Interleukin-1 receptor type II	0,265	0,760	**1,914**	**2,308**	**1,466**	**1,352**
LOC100534607	Interleukin 4/13A	**0,896**	**0,827**	**2,001**	**1,724**	**0,970**	**0,742**
LOC100195064	Interleukin-4 receptor alpha chain	0,221	0,040	**1,105**	**0,590**	**0,844**	**0,531**
LOC106563436	IL13 receptor subunit alpha-2-like	0,258	0,607	**1,817**	**1,256**	**1,447**	**0,465**
LOC106586015	Interleukin-7 receptor subunit alpha-like	-0,042	0,810	**2,210**	**2,543**	**1,893**	**0,719**
LOC106577833	Interleukin-8-like	-0,158	0,167	**1,505**	**1,171**	**1,710**	**0,534**
LOC100195681	Interleukin 8	0,192	0,072	**1,470**	**1,071**	**0,603**	**0,519**
LOC106605846	Interleukin-11-like	-0,214	0,391	**1,798**	**2,211**	**1,808**	**1,092**
LOC106575827	Interleukin 11	-0,058	0,177	**1,918**	**1,484**	**1,928**	**0,798**
LOC106607975	Interleukin-12 subunit beta-like	0,067	0,645	**0,819**	**1,554**	**0,696**	**0,750**
LOC100380871	Interleukin-31 receptor A	0,297	0,140	**1,367**	**0,735**	**0,989**	**0,575**
LOC106569884	Interleukin enhancer-binding factor 2	-0,004	-0,248	**0,868**	**0,904**	**0,856**	**1,048**
LOC106589795	Interferon regulatory factor 1-like	0,307	**1,097**	**2,036**	**2,435**	**1,813**	**0,927**
LOC106586842	Interferon alpha/beta receptor 1a-like	0,200	0,347	**2,325**	**2,173**	**2,046**	**1,593**
LOC106562284	Interferon regulatory factor 8-like	0,129	0,445	**1,443**	**1,495**	**1,231**	**0,986**
LOC106600963	Interferon a3-like	0,184	-0,034	**1,179**	**1,343**	**0,852**	**1,088**
LOC106578554	T-cell activation inhibitor 1-like	0,043	0,363	**1,575**	**2,041**	**1,528**	**1,441**
LOC100196291	T-cell differentiation protein	-0,215	0,333	**0,736**	**1,486**	**0,986**	**1,080**
LOC100196484	T$_C$-cell-originated protein kinase	-0,029	-0,284	**0,772**	**1,023**	**0,796**	**1,103**
LOC106565782	Granzyme K-like	0,094	0,457	**0,838**	**1,147**	**0,703**	**0,536**
LOC100136515	CD3gammadelta-A	0,064	0,584	**0,759**	**1,085**	**0,654**	**0,379**
LOC100136450	CD8 alpha	-0,123	0,831	0,487	**1,107**	**0,630**	0,119
LOC100136451	CD8 beta	-0,217	0,743	**0,719**	**1,112**	**0,906**	0,182
LOC106578585	LCK	0,220	0,493	**0,846**	**0,934**	**0,537**	**0,318**
LOC100194878	LCK	0,154	0,663	**0,896**	**1,141**	**0,679**	**0,336**
LOC106613845	ZAP-70-like	0,053	**0,870**	**0,992**	**1,507**	**0,901**	**0,430**

LOC106567924	Collagenase 3-like	0,066	1,062	3,015	4,291	2,907	2,899
LOC100195428	Matrix metalloproteinase 9	0,392	1,114	2,694	2,837	2,083	1,594
LOC100195495	Collagenase 3	0,666	1,324	4,664	4,664	2,214	3,023
LOC106579980	TNF ligand superfamily member 6-like	-0,468	0,394	1,904	2,045	2,289	1,552
LOC106582339	TNF-inducible gene 6 protein-like	0,358	0,076	2,438	2,711	1,916	2,061
LOC106580524	TNF receptor superfamily member 10B-like	-0,026	0,668	1,552	1,756	1,496	0,910
LOC106572390	TNF receptor superfamily member 9-like	0,110	-0,010	1,517	1,299	1,339	1,258
LOC106564305	LPS-induced TNF-alpha factor	0,197	0,510	1,384	1,875	1,132	1,237
LOC100286415	NCCRP1	0,074	0,126	1,193	1,399	1,097	1,170
LOC106606429	Suppressor of cytokine signaling 3-like	0,168	0,275	1,261	0,873	1,033	0,564
LOC100196188	B-cell translocation gene 3	-0,050	-0,188	1,708	1,324	1,711	1,361
LOC106605635	B-cell receptor CD22-like	0,202	0,794	1,204	1,407	0,866	0,471
LOC106577470	B-cell lymphoma 3 protein-like	0,235	0,020	2,000	0,803	1,476	0,774
LOC106584298	Polymeric immunoglobulin receptor-like	-0,168	0,669	1,186	1,587	1,224	0,747
LOC106584223	C1q TNF-related protein 7-like	0,200	0,119	-1,073	-1,256	-1,286	-0,959
LOC106560251	C1q TNF-related protein 7-like	-0,083	0,246	-1,390	-1,109	-1,300	-0,727
LOC106612242	C1q and TNF related protein 2	0,190	0,344	-0,794	-1,067	-1,071	-0,674
LOC106564378	C1q TNF-related protein 1-like	-0,391	0,446	-1,517	-0,941	-1,090	-0,565
LOC106583003	CD55	0,239	0,298	-0,970	-1,291	-1,313	-0,903
LOC106601645	TNF receptor superfamily member 16-like	-0,024	0,816	-0,855	-1,490	-0,862	-0,437
LOC106568611	IL11 receptor subunit alpha-like	0,092	0,129	-0,859	-1,460	-0,979	-1,341
LOC100195864	Interleukin 17D	0,262	0,251	-0,820	-1,003	-1,172	-0,665
LOC106588859	TLR4 interactor with LRR-like	0,127	0,737	-0,998	-1,914	-1,264	-1,174
LOC106570527	TLR4 interactor with LRR-like	0,113	0,314	-0,629	-1,202	-0,758	-0,784
LOC106560332	Pre-B-cell leukemia transcription factor 1	-0,338	0,458	-1,082	-1,199	-0,741	-0,723
LOC106586652	CD200 molecule	0,176	0,219	-0,865	-0,991	-1,157	-0,757

4. Discussion

In the present study, the immune response towards salmon louse was analysed in three separate experiments addressing key aspects of louse infestations: Early transcriptomic response in the skin, differential early immune response in diverse skin compartments, and finally, local and systemic immune responses at transitional time points as the lice matured towards the adult stage. Overall, the experiments demonstrated an early but modest, long-lasting innate immune response locally in the skin underneath the lice, likely caused by louse attachment and feeding, repetitively wounding the skin around the site of attachment.

The skin response towards salmon louse copepodids has been poorly evaluated, likely as it is challenging to dissect a small enough tissue sample underneath the approximately 0.6 mm long copepodid [25]. In the present study, small skin pieces including the louse attachment sites were pooled from each fish to accomplish an adequate sample preparation, enabling a more thorough transcriptomics investigation of this early phase. The transcriptomic analysis indicates that early infestations lead to regulation of genes involved in cell proliferation and innate immune responses, while only some enrichment was seen for e.g. wound healing processes. Healing of deep wounds in fish involves conserved processes, namely re-epithelialization, inflammation, formation of granulation tissue and tissue remodelling, and time dependent expression of genes involved in these processes has been investigated in Atlantic salmon [45,46]. Salmon louse copepodids however induce mainly superficial wounds [12,15]. In line with that, the transcriptomic analysis identified an elevation of many genes involved in the cell cycle,

metabolism and regulation of replication, transcription and translation, in accordance with processes expected to be activated during skin re-epithelialization. Moreover, a moderate inflammatory response can be suggested based on the transcriptomic data, as a slight increase of pro-inflammatory transcripts as *IL1β, IL6, IL11, Cath2, MMP13* and *MMP9* were detected, though without any macroscopic signs of inflammation seen in the skin below the copepodid at the time of sampling. Moreover, the moderate elevation of inflammatory transcripts continued throughout louse development, as previously reported [15, 31]. Interestingly, as all enzymes of the PGE_2 pathway were found to be upregulated, a local increase of salmon derived PGE_2 appears to be part of the inflammatory response, at least in response to copepodids. PGE_2 is a multifunctional signalling molecule, where down-stream effects are elicited by four PGE_2 receptors (EP1-4) that have the potential of inducing both pro-inflammatory and immunosuppressive responses in different cell types [47]. There is, however, not much known about salmon EP receptors and how they respond to PGE_2 in various salmon cell types. Atlantic salmon epithelial and immune cells have been found to express EP4, and different concentrations of PGE_2 were found to regulate IL1β expression in a salmon macrophage cell line [48]. Moreover, PGE_2 has been detected in secretory/excretory products (SEPs) of dopamine stimulated salmon louse, and is tentatively secreted by the louse as an immune dampening molecule [49,50], similar to what is observed in tick [51,52]. Though, expression of salmon louse *prostaglandin E2 synthase* (*LsPGES*) genes are not detected in salmon louse exocrine glands that secretes immune dampening substance, and knock-down of *LsPGES* does not appear to affect the host-parasite
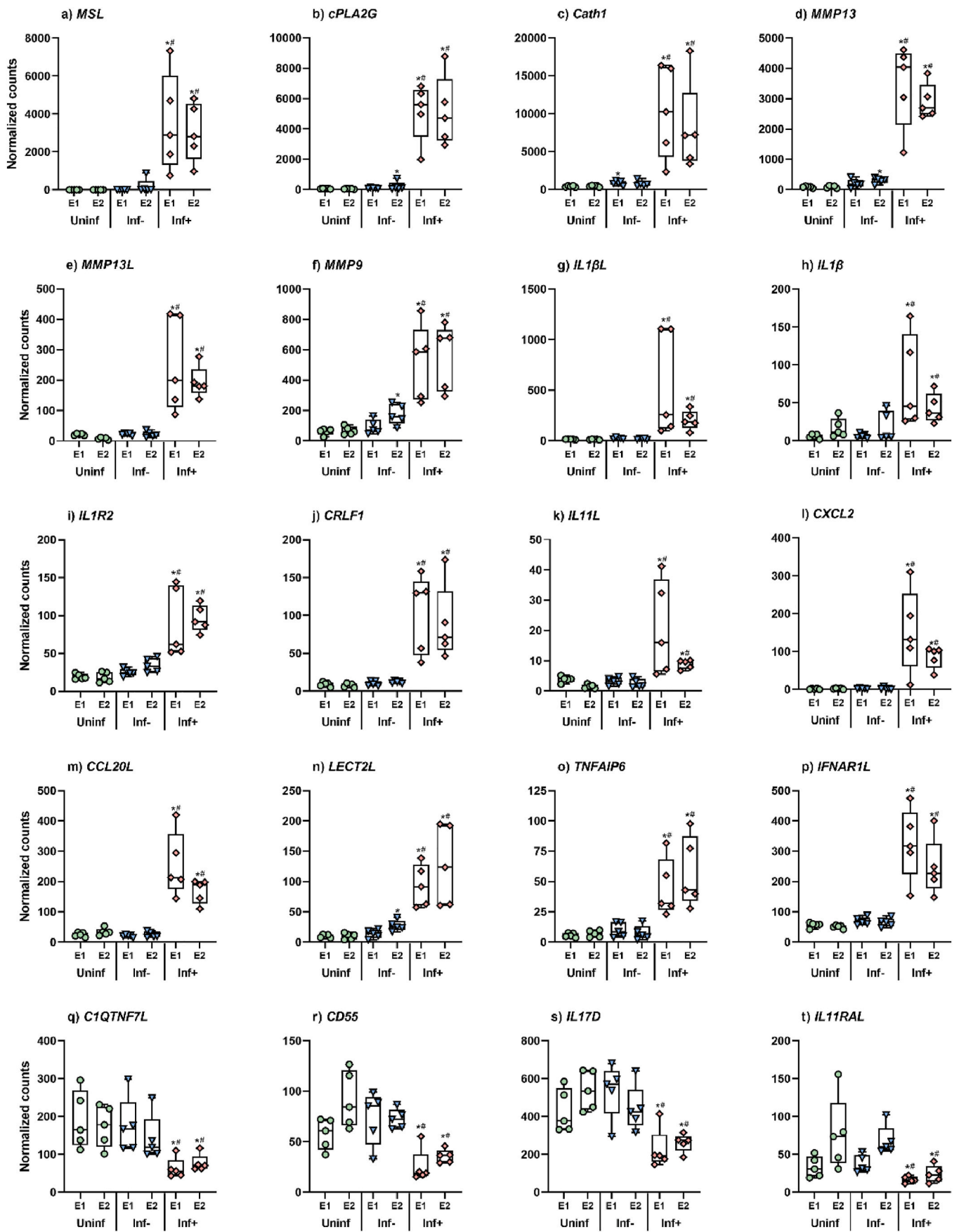
(caption on next page)

Fig. 4. Expression of selected immune genes in skin analysed by RNA seq. The genes showing the highest induction (a–p) or reduction (q–t) in gene expression in response to salmon louse (*Lepeophtheirus salmonis*) copepodids from Experiment 1 and 2 (E1, E2). Normalized counts for each sample are plotted above a boxplot of median ± interquartile range (N = 5) in uninfested control fish (Uninf, green dots) and infested fish, both unaffected (Inf-, blue dots) and affected (Inf+, red dots) sites. Statistical significance from uninfested fish is denoted with an * and between unaffected and infested sites with #. Mannose-specific lectin (MSL), cytosolic phospholipase A2 gamma-like (cPLA2G), cath - cathelicidin, MMP - matrix metalloproteinase, IL - Interleukin, IL1R2 – interleukin 1 receptor type II, CRLF1 – cytokine receptor like factor 1, CXCL2 – C-X-C motif chemokine 2, CCL20L – C–C motif chemokine 20-like – LECT2L – Leukocyte cell derived chemotaxin 2, TNFAIP6 – tumor necrosis factor alpha induced protein 6, IFNAR1L – interferon alpha and beta receptor subunit 1-like, C1QTNF7L – complement C1q tumor necrosis factor-related protein 7-like, CD55 – cluster of differentiation 55, IL11RAL – IL11 receptor subunit alpha-like.
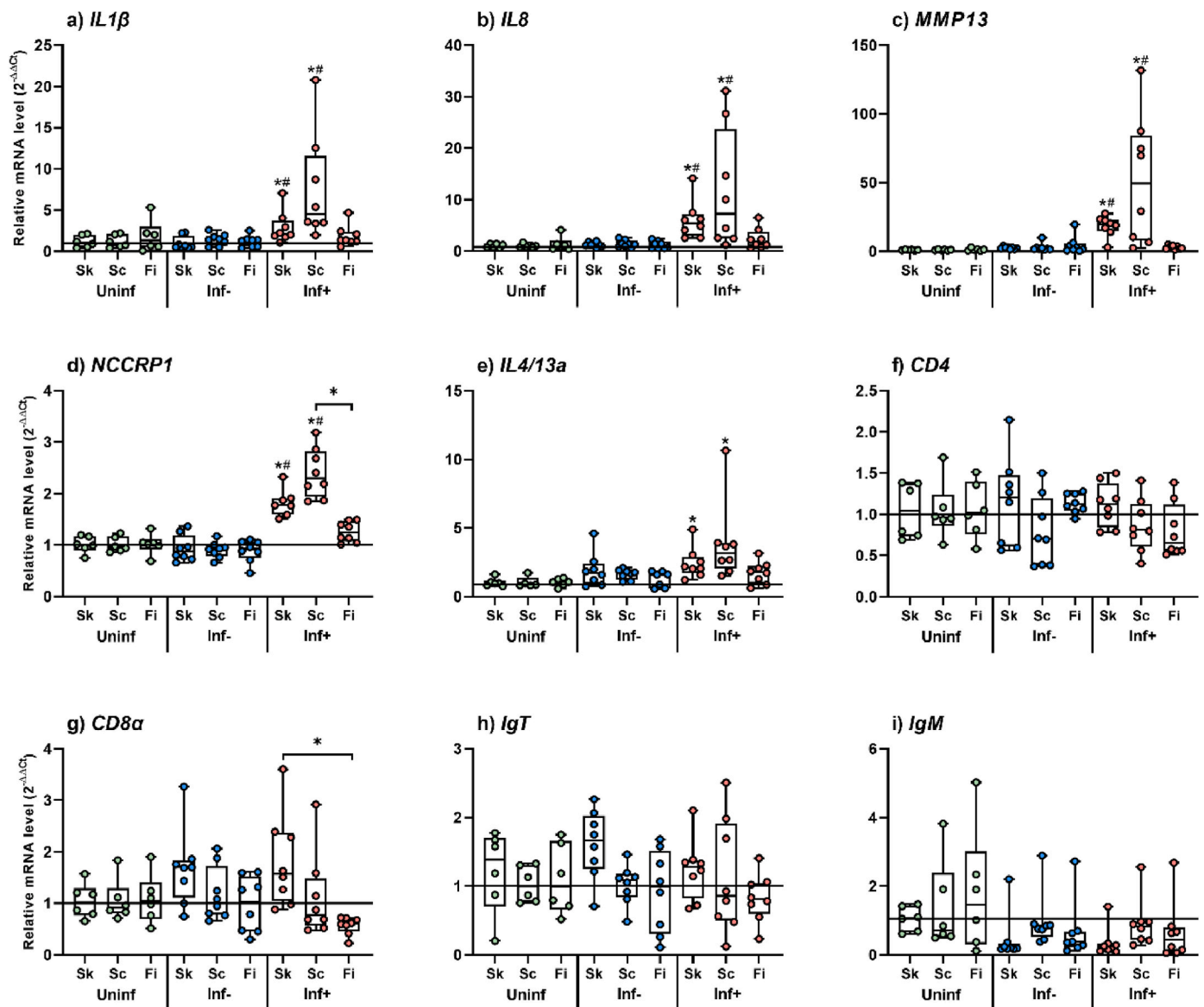

Fig. 5. Expression of selected immune genes in different skin sites analysed by qPCR. Relative transcript level of nine selected immune genes in Atlantic salmon (*Salmo salar*) skin (Sk), scales (Sc) and fin (Fi) infested with salmon lice (*Lepeophtheirus salmonis*). Samples were collected at 3 days past infection (Exp 2), in infested fish at the site of lice attachment (inf+, red dots) and in unaffected sites (inf-, blue dots), and from similar sites in untreated control fish (uninf, green dots). The expression level was calculated as $2^{-\Delta\Delta Ct}$, related to the reference genes, EF1α and TRIM, using uninfested fish as calibrator. Each value is plotted in a boxplot of median ± interquartile range (N = 8). Statistical significance from uninfested fish is denoted with an * and between unaffected and infested sites with #. * denotes significant difference between skin, scale and fin samples in infested sites. IL – interleukin, MMP13 – matrix metalloproteinase 13, NCCRP1 – non-specific cytotoxic cell receptor P1, Ig – immunoglobulin.

interaction [23,31,53]. Nevertheless, further studies are of importance to evaluate the possible collective effect of salmon and louse derived PGE$_2$, maybe including a further analysis of the salmon EP2 receptor, as this was the only receptor transcript found to be elevated at the copepodid attachment site.

Additional genes of the prostanoid pathway were also upregulated,

as leukotriene A4 hydrolase (*LTA4H*) (Supplementary Table 4). In mammals, *LTA4H* catalyse the formation of leukotriene B4 (LTB4), that when signalling through the LTB4 receptor 1 is a chemoattractant for neutrophils [54]. Other chemo attractants as the chemokines *CXCL2*, *CCL20L*, *CCL13L*, leukocyte cell-derived chemotaxin-2-like (*LECT2L*), *IL8* and *IL8-like* were also elevated in skin underneath the copepodids, and
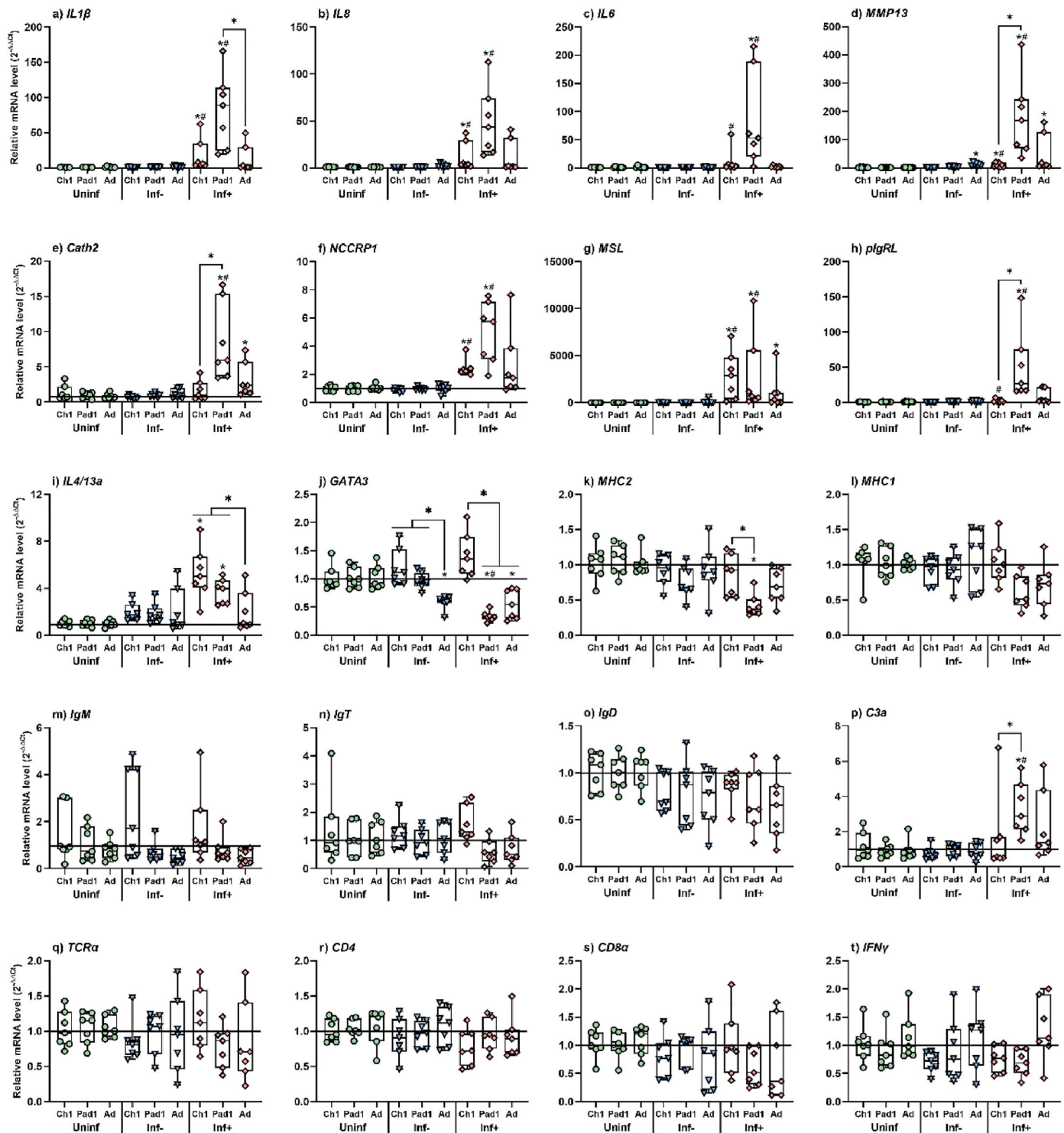
Fig. 6. qPCR analysis of selected immune genes in skin infested with different salmon louse life stages. Relative transcript level of 20 selected immune genes in skin of Atlantic salmon (*Salmo salar*) infested with salmon lice (*Lepeophtheirus salmonis*). Samples were collected at 7, 23 and 43 days post infestation, corresponding to the chalimus 1 (Ch1), preadult 1 (Pad1) and adult (Ad) stages, respectively. The expression level in infested fish, both in unaffected (Inf-, blue dots) and affected (Inf+, red dots) sites, was calculated as $2^{-\Delta\Delta Ct}$ (N = 7), related to uninfested control fish (Uninf, green dots). Boxplot that shows median ± interquartile range, with values for each fish plotted above. Statistical significance from untreated control fish is denoted with an * and between unaffected and infested sites with #. * also denotes significant difference between the different time points in each sample type.

at least the expression of *IL8* was sustained underneath the larger louse stages analysed, similar to observations in rainbow trout [15]. The influx of immune cells to the site of copepodid attachment has been reported to be minor in Atlantic salmon and rainbow trout, though, increasing as the louse develops [12,14,15]. Influx of both neutrophils and lymphocytes

underneath copepodids were reported by Johnson and Albright (1992), and immune cell specific genes found to be elevated in the present study include the *non-specific cytotoxic cell receptor P1 (NCCRP1), granulocyte colony-stimulating factor receptor (G-CFS-R), macrophage mannose-receptor 1, C5a anaphylatoxin chemotactic receptor 1-like* and various T-cell
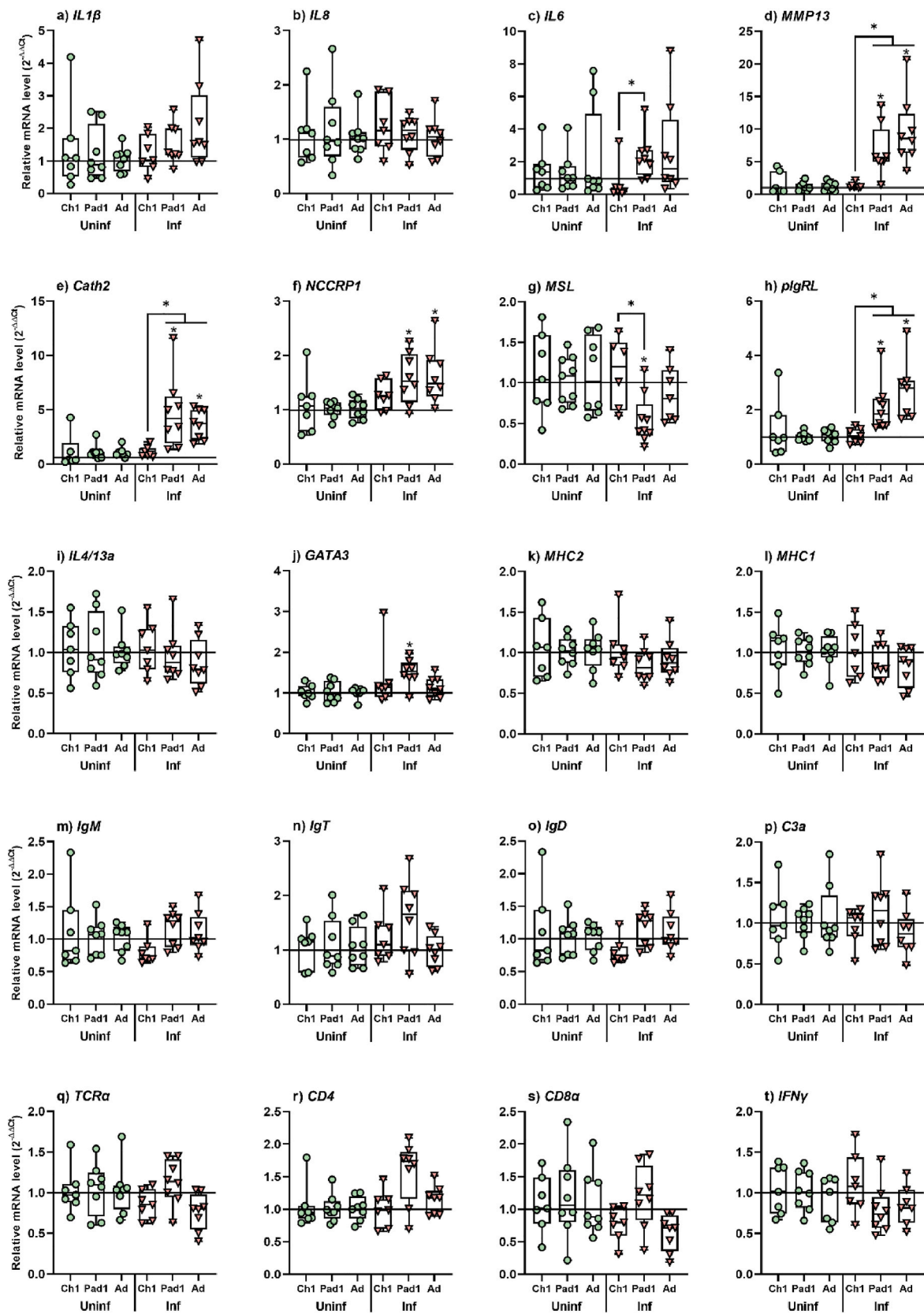
Fig. 7. qPCR analysis of selected immune genes in head kidney infested with different salmon louse life stages. Relative transcript level of 20 selected immune genes in head kidney of Atlantic salmon (*Salmo salar*) infested with salmon lice (*Lepeophtheirus salmonis*). Samples were collected at 7, 23 and 43 days post infestation, corresponding to the chalimus 1 (Ch1), preadult 1 (Pad1) and adult (Ad) stages, respectively. The expression level in infested fish (Inf, red dots) was calculated as $2^{-\Delta\Delta Ct}$ (N = 7), related to uninfested control fish (Uninf, greed dots). Boxplot that shows median ± interquartile range, with values for each fish also plotted. Statistical significance from untreated control fish and between the different time points are denoted with an *.

markers as *CD3, CD8, lymphocyte-specific protein tyrosine kinase* (*Lck*) and *zeta-chain-associated protein kinase 70* (*ZAP-70*). This indicates some influx of neutrophils, macrophages, non-specific cytotoxic cells and T-cells to the site of copepodid attachment. The elevation of transcripts encoding the CD8-chains in addition to *IL12β, Tc-cell originated protein kinase* and *granzyme K-like* indicate that mainly T cytotoxic (T_C) cells are recruited to the site of infestation. However, T-cell related genes did not show any significant regulation underneath older salmon louse stages in line with previous publications [25,31], suggesting that T-cells are not activated even though T_C-cells seems to be initially recruited. In general, T_C-cells are related to intracellular viral infections, and *interferons* (*IFN*) and *IFN regulated* genes also exhibited higher expression in response to salmon louse copepodids. Interestingly, salmon lice, including the laboratory strain used in the present study, are commonly infected with several viruses that presumably are non-pathogenic to the louse [55–57]. Moreover, an involvement of two *L. salmonis* rhabdoviruses (LsRV) in the host-parasite interaction have been reported [17]. Thus, louse virus could be secreted onto the host skin and might up-regulate viral induced genes in the fish. The low magnitude of regulation indicated, similar to previous results [17,55], that louse virus does not replicate in salmon cells. Nevertheless, this emphasises that we should bear in mind that louse infestations also bring in additional microorganisms, not only viruses but also bacteria and other parasites [58,59], that might contribute to the immune responses at the site of infestation.

As previously shown for a limited number of immune genes [15,17, 19,23,30,31], the present study confirms that the immune response towards salmon louse is mainly localized to the site of louse attachment. *Interleukin 4/13A* was, however, upregulated also in unaffected sites on infested fish when compared to uninfested control fish, as previously reported for both Atlantic salmon and rainbow trout [15,23,32,60]. Piscine IL4/13A is one of two cytokines evolutionary related to both IL4 and IL13 in higher vertebrates [61,62]. As IL4/13A has been found to promote T_H2-type immune responses by increasing B-cell proliferation and antibody production in zebrafish [63], and promote IgM secretion in rainbow trout [62], it has been suggested that teleost's have a T helper (T_H) 2-skewed skin environment due to a constitutive skin expression of *IL4/13A* and the transcription factor *GATA3* [64]. However, serum titers of specific anti-salmon louse antibodies have been found to be low after a natural infestation with salmon louse [65], indicating that the salmon louse induction of IL4/13A are not resulting in a protective antibody response. This was also evident in the transcript data, as no elevation of either *IgM, IgD* or *IgT* was detected at any timepoints analysed, in contrast to what was reported by Tadiso et al. [24]. Interestingly, an overall decrease of *GATA3* was observed at the louse attachment sites in rainbow trout, especially after the louse had moulted into the preadult stage [15], and this was also seen in the present study. As other leukocyte populations such as NKT cells, eosinophils, basophils and mast cells may produce IL4 and IL13 in mammals [66,67], the present results indicate that the louse induced elevation of *IL4/13A* comes from alternative sources besides T_H2-cells. Moreover, the level of *IL4/13A* declined underneath the louse as it matured towards the adult stage, mirroring the decrease of *GATA3*. Though, *GATA3* was also downregulated in unaffected skin on fish infested with preadult 1 and adult lice, contrary to what was seen in rainbow trout [15]. In higher vertebrates, GATA3 is also an important transcription factor in skin keratinocytes, necessary to establish an intact epidermal barrier during mouse embryonic development [68], known to inhibit proliferation and induce differentiation of human keratinocytes and is decreased in skin diseases like psoriasis where keratinocyte hyperproliferation is a hallmark [69, 70]. In salmon, GATA3 expression has been detected in gill epithelial cells [71], indicating a similar involvement of piscine GATA3 in regulating epithelial processes. Thus, the downregulation of salmon *GATA3* in both affected and unaffected sites represents an interesting aspect for further exploration.

GATA3 was, however, not the only transcript found to be decreased at the louse attachment site. Lice induced down modulation of gene expression is of special interest as the lice is found to supress immune responses [20,23], and identifying such genes could provide important targets for immune based treatment strategies. There were, however, more genes displaying an increased rather than a decreased transcript level, with a stronger regulation seen for genes displaying elevated expression levels. Moreover, the GO term analysis revealed a lesser significance in enrichment of down modulated genes than those with a higher expression, indicating that there is not a comprehensive down regulation of certain processes at this early stage of louse infestation. Also, looking specifically at the processes significantly enriched amongst the down modulated genes, it seems the down modulation is caused by the feeding activity of the lice resulting in fewer epithelial cells in the samples from the attachment site. All layers of fish epidermal epithelial cells are alive, closely attached to each other and are constantly renewed [45]. Therefore, a slight reduction in transcripts involved in cell proliferation, mobility, adhesion and anatomical structure morphogenesis is expected if there are less epidermal cells present. Thus, identifying genes that are truly dampened by louse secretions might be difficult at the stages where the lice mainly feed on the salmon epidermis. Also, the present study indicates that the suppression of immune responses at the early stage of infestation primarily dampens activated immune responses rather than causing a decline in transcript levels below that of uninfested fish. A more thorough analysis of the response underneath more mature blood-feeding louse stages is therefore warranted, as these stages tend to graze less on the epidermis [14,15].

Salmon louse attach to the skin surface of the host fish, but the skin of a fish is, however, not a homogenous surface, and, depending on the location, it varies in thickness, composition and how it respond to salmon louse [28,46]. Interestingly, several transcript levels were found to be higher in scale samples as compared to whole skin and especially fin samples. The epidermal to dermal factor would be highest in scales and decreasing in fin and whole skin (scales > fin > skin). This because the scale samples would include mainly epidermal cells in addition to some connective tissue that is likely to follow during sampling as the scales lies within the stratum spongiosum, while the fin samples would include a thin layer of stratum compactum and the whole skin would include both spongious and compact dermal tissue. NCCRP1 is expressed on the surface of non-specific cytotoxic cells (NCCs), but also other types of innate immune cells in fish [72,73]. Thus, the elevation of *NCCRP1* transcripts indicate an influx of innate immune cells in general. The higher increase of *NCCRP1* in the scale samples further augments that the influx of salmon immune cells is more prominent in the part of dermis just underneath the lice, as seen in rainbow trout [15]. If so, the proportion of immune cell might be higher in the scale samples if they follow in the connective tissue surrounding the scales during sampling, and as the scale samples are more concentrated around the site of attachment. Consequently, immune cell transcripts will have a smaller dilution factor in the scale samples as compared to whole skin, and in line with this, the level of inflammatory transcripts was also found to be higher in scale samples as compared to skin. A larger variation of the transcript levels was, however, seen, indicating that there are differences between how individual fish respond to lice. This has also been observed in other experiments [15,17,19,23,25,31], and selective salmon breeding for enhanced anti-louse immune response has been attempted [27,74,75], as to see if it is possible to exploit such differences. However, in these studies louse numbers and the general skin response have been used as parameters for selection, whereas examining local immune responses towards the salmon louse could increase the possibility of selecting the family that mounts the most appropriate response. As it is not optimal to take skin biopsies on living fish, sampling of scales as a nonlethal sampling method can have the potential to enhance breeding trials. The transcript level measured in skin and scales on a given fish were, however, not correlated, thus it appears that the response towards lice has a large intra-fish variation. This indicates that the variation is caused by other factors, e.g. that the inclusion of immune cells varies in the scale samples, or the severity of erosions varies as

copepodids are quite mobile and might have infested a given site at various times. Thus, scale sampling as a nonlethal sampling method should be evaluated in the chalimus stage before it can be implemented in breeding trials. On the opposite, implementing fin sampling should be avoided, as the immune gene transcript level here seems to be unaffected by the louse infestation. Especially lice in the chalimus stage are more commonly located on fins on both susceptible and resistant salmonid species [5,9,12,18]. Considering the present results, it is tempting to believe that the preference to fin could be immune dependent. Johnson and Albright [12] also reported a general lack of tissue responses in infested fin, even though the lice had exposed the dermis and underlaying fin rays to the environment. A more recent study showed a general downmodulation of immune gene transcripts underneath chalimi when attached to salmon fins [76], supporting this, indicating that the immune modulation mounted by the lice might be strengthened as the louse grows and attach the frontal filament that restricts it to a smaller region of the skin. Nevertheless, further studies are needed to see if the apparent lack of immune responses in fin causes a louse attachment preference towards fin after infestation. Alternatively, the immune response in skin is sufficient to cause some clearance of lice decreasing the body to fin ratio of louse during the copepodid stage.

The mobile preadult and adult salmon lice also display a distinct spatial distribution on the salmons body surface, where adult females are commonly found in groups behind the dorsal, adipose and anal fins, while males are often seen on the head and opercula area [77,78]. This implies that most louse changes location after the development to the preadult stage, infesting new skin sites. Preadult lice has developed a suction cup shaped cephalothorax for attachment allowing it to move freely over the body surface of the fish, it starts feeding more frequently on host blood, and additional gland types believed to be involved in the host parasite interaction appears [11,18,22]. Hence, the local immune response could be altered accordingly. Also right after the louse inserts its frontal filament, the immune response could be expected to be somewhat elevated, though, previous reports have shown similar levels of *IL1β* and *IL8* underneath copepodid and chalimus lice stages [15,17, 31]. Also in the present study, immune gene transcripts were not elevated to very high levels underneath newly moulted chalimus 1 (Fig. 6). The increase in the pro-inflammatory genes was, however, considerable higher underneath newly moulted preadult 1 louse, indicating that the preadult lice induce a stronger immune response right after it has changed its skin location. Preadult lice are known to be more virulent for the salmon and can induce mortality in smaller fish, coinciding with a stage of development where the lice initiate blood feeding [3,15,18]. Accordingly, lice at the preadult stage appear to activate a systemic immune response, as an elevation of head kidney *MMP13*, *CATH2*, *NCCRP1* and *pIgRL* transcripts were detected in fish infested with preadult 1 and adult stages only. The transcript level in head kidney was, however, only marginally elevated as previously reported [25]. Also, the increase of skin immune gene transcripts underneath the young preadult 1 louse appeared to be rather transient, as the transcript levels were restored to that observed in response to chalimi when the lice became adult. Moreover, relatively stable cytokine transcript levels were recorded in rainbow trout when the response was analysed slightly later when preadults were in the middle of the moult cycle [15]. Hence, the current study does not support the view that the higher virulence of preadult lice is immune dependent.

Overall, this comprehensive study of the Atlantic salmon transcript response against salmon louse presents new knowledge of the salmon's inadequate immune response against the louse. As the mode of attachment is reversible in the copepodid stage, and clearance of lice in resistant (pacific) salmonid species can be achieved in this stage (Jones 2011), the first initial establishment phase of the copepodids is likely where immune-based anti-louse counter measures can be most effective. Thus, the thorough transcriptomic analysis of the copepodid attachment site presented here is of high importance. Moreover, the results indicate

that the shift to the preadult phase induces a higher immune response and can also represents a challenging time point worth further evaluation. Finally, this study confirms that most responses elicited by the salmon, particularly towards the juvenile salmon louse stages, are localized to under the louse attachment site. All the presented data should, however, be further validated by a more thorough investigation of immune cell responses and protein expression analysis.

CRediT authorship contribution statement

Aina-Cathrine Øvergård: Conceptualization, Writing – original draft, Writing – review & editing, Investigation. **Christiane Eichner:** Conceptualization, Investigation, Writing – review & editing. **Noelia Nuñez-Ortiz:** Conceptualization, Investigation, Writing – review & editing. **Heidi Kongshaug:** Investigation, Writing – review & editing. **Andreas Borchel:** Investigation, Writing – review & editing. **Sussie Dalvin:** Conceptualization, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2023.108835.

References

[1] S.E. Barker, I.R. Bricknell, J. Covello, S.L. Purcell, M.D. Fast, W. Wolters, Sea lice, *Lepeophtheirus salmonis* (Krøyer 1837), infected Atlantic salmon (*Salmo salar* L.) are more susceptible to infectious salmon anemia virus, PLoS One 14 (2019), e0209178, https://doi.org/10.1371/journal.pone.0209178.

[2] B. Finstad, P.A. Bjørn, A. Grimnes, N.A. Hvidsten, Laboratory and field investigations of salmon lice [*Lepeophtheirus salmonis* (Krøyer)] infestation on Atlantic salmon (*Salmo salar* L.) post-smolts, Aquacult. Res. 31 (2000) 795–803, https://doi.org/10.1046/j.1365-2109.2000.00511.x.

[3] A. Grimnes, P.J. Jakobsen, The physiological effects of salmon lice infection on post-smolt of Atlantic salmon, J. Fish. Biol. 48 (1996) 1179–1194, https://doi.org/10.1006/jfbi.1996.0119.

[4] L. Tort, Stress and immune modulation in fish, Dev. Comp. Immunol. 35 (2011) 1366–1375, https://doi.org/10.1016/j.dci.2011.07.002.

[5] P.A. Bjørn, B. Finstad, The development of salmon lice (*Lepeophtheirus salmonis*) on artificially infected post smolts of sea trout (*Salmo trutta*), Can. J. Zool. Rev. Can. Zool. 76 (1998) 970–977, https://doi.org/10.1139/cjz-76-5-970.

[6] J.M. Bowers, A. Mustafa, D.J. Speare, G.A. Conboy, M. Brimacombe, D.E. Sims, J. F. Burka, The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*, J. Fish. Dis. 23 (2000) 165–172, https://doi.org/10.1046/j.1365-2761.2000.00225.x.

[7] S.M. Aaen, K.O. Helgesen, M.J. Bakke, K. Kaur, T.E. Horsberg, Drug resistance in sea lice: a threat to salmonid aquaculture, Trends Parasitol. 31 (2015) 72–81, https://doi.org/10.1016/j.pt.2014.12.006.

[8] K. Overton, T. Dempster, F. Oppedal, T.S. Kristiansen, K. Gismervik, L.H. Stien, Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review, Rev. Aquacult. 11 (2019) 1398–1417, https://doi.org/10.1111/raq.12299.

[9] J.E. Bron, C. Sommerville, M. Jones, G.H. Rae, The settlement and attachment of early stages of the salmon louse, *Lepeophtheirus salmonis* (Copepoda, Caligidae) on the salmon host, *Salmo salar*, J. Zool. 224 (1991) 201–212.

[10] M.W. Jones, C. Sommerville, J. Bron, The histopathology associated with the juvenile stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L, J. Fish. Dis. 13 (1990) 303–310.

[11] S.C. Johnson, L.J. Albright, The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda, Caligidae), Can. J. Zool. 69 (1991) 929–950, https://doi.org/10.1139/Z91-138.

[12] S.C. Johnson, L.J. Albright, Comparative susceptibility and histopathology of the response of naive Atlantic, Chinook and Coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda, Caligidae), Dis. Aquat. Org. 14 (1992) 179–193, https://doi.org/10.3354/dao014179.

[13] Z. Kabata, Copepoda (Crustacea) parasitic on fishes - problems and perspectives, Adv. Parasitol. 19 (1982) 1–71, https://doi.org/10.1016/S0065-308x(08)60265-1.

[14] H. Jonsdottir, J.E. Bron, R. Wootten, J.F. Turnbull, The histopathology associated with the preadult and adult stages of *Lepeophtheirus salmonis* on the Atlantic Salmon, *Salmo salar* L, J. Fish. Dis. 15 (1992) 521–527, https://doi.org/10.1111/j.1365-2761.1992.tb00684.x.

[15] S. Dalvin, L.V.G. Jorgensen, P.W. Kania, S. Grotmol, K. Buchmann, A.C. Øvergård, Rainbow trout *Oncorhynchus mykiss* skin responses to salmon louse *Lepeophtheirus salmonis*: from copepodid to adult stage, Fish Shellfish Immunol. 103 (2020) 200–210, https://doi.org/10.1016/j.fsi.2020.05.014.

[16] R. Wootten, J.W. Smith, E.A. Needham, Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment, P Roy Soc Edinb B 81 (1982) 185–197, https://doi.org/10.1017/S0269727000003389.

[17] A.C. Øvergård, L.A. Hamre, S. Grotmol, F. Nilsen, Salmon louse rhabdoviruses: impact on louse development and transcription of selected Atlantic salmon immune genes, Dev. Comp. Immunol. 86 (2018) 86–95, https://doi.org/10.1016/j.dci.2018.04.023.

[18] E.I. Heggland, M. Dondrup, F. Nilsen, C. Eichner, Host gill attachment enables blood-feeding by the salmon louse (*Lepeophtheirus salmonis*) chalimus larvae and alters parasite development and transcriptome, Parasites Vectors 13 (2020).

[19] L.M. Braden, B.F. Koop, S.R. Jones, Signatures of resistance to *Lepeophtheirus salmonis* include a TH2-type response at the louse-salmon interface, Dev. Comp. Immunol. 48 (2015) 178–191, https://doi.org/10.1016/j.dci.2014.09.015.

[20] A. Mustafa, C. MacWilliams, N. Fernandez, K. Matchett, G.A. Conboy, J.F. Burka, Effects of sea lice (*Lepeophtheirus salmonis* Kroyer, 1837) infestation on macrophage functions in Atlantic salmon (*Salmo salar* L.), Fish Shellfish Immunol. 10 (2000) 47–59, https://doi.org/10.1006/fsim.1999.0229.

[21] M.D. Fast, N.W. Ross, A. Mustafa, D.E. Sims, S.C. Johnson, G.A. Conboy, D.J. Speare, G. Johnson, J.F. Burka, Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*, Dis. Aquat. Org. 52 (2002) 57–68, https://doi.org/10.3354/dao052057.

[22] A.C. Øvergård, L.A. Hamre, E. Harasimczuk, S. Dalvin, F. Nilsen, S. Grotmol, Exocrine glands of *Lepeophtheirus salmonis* (Copepoda: Caligidae): distribution, developmental appearance, and site of secretion, J. Morphol. 277 (2016) 1616–1630, https://doi.org/10.1002/jmor.20611.

[23] A.C. Øvergård, H.M.D. Midtbø, L.A. Hamre, M. Dondrup, G.E.K. Bjerga, O. Larsen, J.K. Chettri, K. Buchmann, F. Nilsen, S. Grotmol, Small, charged proteins in salmon louse (*Lepeophtheirus salmonis*) secretions modulate Atlantic salmon (*Salmo salar*) immune responses and coagulation, Sci. Rep. 12 (2022) 7995, https://doi.org/10.1038/s41598-022-11773-w.

[24] T.M. Tadiso, A. Krasnov, S. Skugor, S. Afanasyev, I. Hordvik, F. Nilsen, Gene expression analyses of immune responses in Atlantic salmon during early stages of infection by salmon louse (*Lepeophtheirus salmonis*) revealed bi-phasic responses coinciding with the copepod-chalimus transition, BMC Genom. 12 (2011), https://doi.org/10.1186/1471-2164-12-141. Artn 141.

[25] S. Skugor, K.A. Glover, F. Nilsen, A. Krasnov, Local and systemic gene expression responses of Atlantic salmon (*Salmo salar* L.) to infection with the salmon louse (*Lepeophtheirus salmonis*), BMC Genom. 9 (2008) 498, https://doi.org/10.1186/1471-2164-9-498.

[26] M.D. Fast, D.M. Muise, R.E. Easy, N.W. Ross, S.C. Johnson, The effects of *Lepeophtheirus salmonis* infections on the stress response and immunological status of Atlantic salmon (*Salmo salar*), Fish Shellfish Immunol. 21 (2006) 228–241, https://doi.org/10.1016/j.fsi.2005.11.010.

[27] H. Holm, N. Santi, S. Kjoglum, N. Perisic, S. Skugor, O. Evensen, Difference in skin immune responses to infection with salmon louse (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar* L.) of families selected for resistance and susceptibility, Fish Shellfish Immunol. 42 (2015) 384–394, https://doi.org/10.1016/j.fsi.2014.10.038.

[28] H.J. Holm, S. Skugor, A.K. Bjelland, S. Radunovic, S. Wadsworth, E.O. Koppang, O. Evensen, Contrasting expression of immune genes in scaled and scaleless skin of Atlantic salmon infected with young stages of *Lepeophtheirus salmonis*, Dev. Comp. Immunol. 67 (2017) 153–165, https://doi.org/10.1016/j.dci.2016.10.008.

[29] A. Krasnov, S. Skugor, M. Todorcevic, K.A. Glover, F. Nilsen, Gene expression in Atlantic salmon skin in response to infection with the parasitic copepod *Lepeophtheirus salmonis*, cortisol implant, and their combination, BMC Genom. 13 (130) (2012), https://doi.org/10.1186/1471-2164-13-130.

[30] L.M. Braden, D.E. Barker, B.F. Koop, S.R. Jones, Comparative defense-associated responses in salmon skin elicited by the ectoparasite *Lepeophtheirus salmonis*, Comp. Biochem. Physiol., Part D: Genomics Proteomics 7 (2012) 100–109, https://doi.org/10.1016/j.cbd.2011.12.002.

[31] S. Dalvin, C. Eichner, M. Dondrup, A.C. Øvergård, Roles of three putative salmon louse (*Lepeophtheirus salmonis*) prostaglandin E_2 synthases in physiology and host-parasite interactions, Parasites Vectors 14 (2021) 206, https://doi.org/10.1186/s13071-021-04690-w.

[32] M.S. Ugelvik, S. Maehle, S. Dalvin, Temperature affects settlement success of ectoparasitic salmon lice (*Lepeophtheirus salmonis*) and impacts the immune and

[33] R. Skern-Mauritzen, O. Torrissen, K.A. Glover, Pacific and Atlantic *Lepeophtheirus salmonis* (Krøyer, 1838) are allopatric subspecies: *Lepeophtheirus salmonis salmonis* and *L. salmonis oncorhynchi* subspecies novo, BMC Genet. 15 (2014), https://doi.org/10.1186/1471-2156-15-32. Artn 32.

[34] L.A. Hamre, K.A. Glover, F. Nilsen, Establishment and characterisation of salmon louse (*Lepeophtheirus salmonis* (Krøyer 1837)) laboratory strains, Parasitol. Int. 58 (2009) 451–460, https://doi.org/10.1016/j.parint.2009.08.009.

[35] L.A. Hamre, F. Nilsen, Individual fish tank arrays in studies of *Lepeophtheirus salmonis* and lice loss variability, Dis. Aquat. Org. 97 (2011) 47–56, https://doi.org/10.3354/dao02397.

[36] A.C. Øvergård, A.H. Nerland, S. Patel, Evaluation of potential reference genes for real time RT-PCR studies in Atlantic halibut (*Hippoglossus Hippoglossus* L.); during development, in tissues of healthy and NNV-injected fish, and in anterior kidney leucocytes, BMC Mol. Biol. 11 (2010) 36, https://doi.org/10.1186/1471-2199-11-36.

[37] E. Afgan, D. Baker, M. van den Beek, D. Blankenberg, D. Bouvier, M. Cech, J. Chilton, D. Clements, N. Coraor, C. Eberhard, B. Gruning, A. Guerler, J. Hillman-Jackson, G. Von Kuster, E. Rasche, N. Soranzo, N. Turaga, J. Taylor, A. Nekrutenko, J. Goecks, The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update, Nucleic Acids Res. 44 (2016) W3–W10, https://doi.org/10.1093/nar/gkw343.

[38] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (2015) 357–360, https://doi.org/10.1038/nmeth.3317.

[39] Y. Liao, G.K. Smyth, W. Shi, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics 30 (2014) 923–930, https://doi.org/10.1093/bioinformatics/btt656.

[40] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 550, https://doi.org/10.1186/s13059-014-0550-8.

[41] C. Xie, X. Mao, J. Huang, Y. Ding, J. Wu, S. Dong, L. Kong, G. Gao, C.Y. Li, L. Wei, KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases, Nucleic Acids Res. 39 (2011) W316–W322, https://doi.org/10.1093/nar/gkr483.

[42] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001), https://doi.org/10.1093/nar/29.9.e45. ARTN e45.

[43] Y. Benjamini, A.M. Krieger, D. Yekutieli, Adaptive linear step-up procedures that control the false discovery rate, Biometrika 93 (2006) 491–507, https://doi.org/10.1093/biomet/93.3.491.

[44] F. Supek, M. Bosnjak, N. Skunca, T. Smuc, REVIGO summarizes and visualizes long lists of gene ontology terms, PLoS One 6 (2011), e21800, https://doi.org/10.1371/journal.pone.0021800.

[45] L. Sveen, C. Karlsen, E. Ytteborg, Mechanical induced wounds in fish - a review on models and healing mechanisms, Rev. Aquacult. 12 (2020) 2446–2465, https://doi.org/10.1111/raq.12443.

[46] L.R. Sveen, G. Timmerhaus, A. Krasnov, H. Takle, S. Handeland, E. Ytteborg, Wound healing in post-smolt Atlantic salmon (*Salmo salar* L.), Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-39080-x. ARTN 3565.

[47] M. Agard, S. Asakrah, L.A. Morici, PGE$_2$ suppression of innate immunity during mucosal bacterial infection, Front. Cell. Infect. Microbiol. 3 (2013), https://doi.org/10.3389/fcimb.2013.00045. ARTN 45.

[48] A.A. Gamil, T.C. Guo, M. Konig, O. Evensen, Distribution of EP4 receptor in different Atlantic salmon (*Salmo salar* L.) tissues, Dev. Comp. Immunol. 48 (2015) 143–150, https://doi.org/10.1016/j.dci.2014.09.013.

[49] M.D. Fast, S.C. Johnson, T.D. Eddy, D. Pinto, N.W. Ross, *Lepeophtheirus salmonis* secretory/excretory products and their effects on Atlantic salmon immune gene regulation, Parasite Immunol. 29 (2007) 179–189, https://doi.org/10.1111/j.1365-3024.2007.00932.x.

[50] M.D. Fast, N.W. Ross, C.A. Craft, S.J. Locke, S.L. MacKinnon, S.C. Johnson, *Lepeophtheirus salmonis*: characterization of prostaglandin E_2 in secretory products of the salmon louse by RP-HPLC and mass spectrometry, Exp. Parasitol. 107 (2004) 5–13, https://doi.org/10.1016/j.exppara.2004.04.001.

[51] C.J. Oliveira, A. Sa-Nunes, I.M. Francischetti, V. Carregaro, E. Anatriello, J.S. Silva, I.K. Santos, J.M. Ribeiro, B.R. Ferreira, Deconstructing tick saliva: non-protein molecules with potent immunomodulatory properties, J. Biol. Chem. 286 (2011) 10960–10969, https://doi.org/10.1074/jbc.M110.205047.

[52] N.M. Poole, G. Mamidanna, R.A. Smith, L.B. Coons, J.A. Cole, Prostaglandin E_2 in tick saliva regulates macrophage cell migration and cytokine profile, Parasites Vectors 6 (2013) 261, https://doi.org/10.1186/1756-3305-6-261.

[53] C. Eichner, A.C. Øvergård, F. Nilsen, S. Dalvin, Molecular characterization and knock-down of salmon louse (*Lepeophtheirus salmonis*) prostaglandin E synthase, Exp. Parasitol. 159 (2015) 79–93, https://doi.org/10.1016/j.exppara.2015.09.001.

[54] E.A. Dennis, P.C. Norris, Eicosanoid storm in infection and inflammation (vol 15, pg 511, 2015), Nat. Rev. Immunol. 15 (2015), https://doi.org/10.1038/nri3928.

[55] A.L. Økland, A. Nylund, A.C. Øvergård, S. Blindheim, K. Watanabe, S. Grotmol, C.E. Arnesen, H. Plarre, Genomic characterization and phylogenetic position of two new species in Rhabdoviridae infecting the parasitic copepod, salmon louse (*Lepeophtheirus salmonis*), PLoS One 9 (2014), e112517, https://doi.org/10.1371/journal.pone.0112517.

[56] A.L. Økland, A. Nylund, A.C. Øvergård, R.H. Skoge, H. Kongshaug, Genomic characterization, phylogenetic position and *in situ* localization of a novel putative

mononegavirus in *Lepeophtheirus salmonis*, Arch. Virol. 164 (2019) 675–689, https://doi.org/10.1007/s00705-018-04119-3.

[57] A.C. Øvergård, L.A. Hamre, H. Kongshaug, F. Nilsen, RNAi-mediated treatment of two vertically transmitted rhabdovirus infecting the salmon louse (*Lepeophtheirus salmonis*), Sci. Rep. 7 (2017), 14030, https://doi.org/10.1038/s41598-017-14282-3.

[58] E. Harasimczuk, A.C. Øvergård, S. Grotmol, F. Nilsen, S. Dalvin, Characterization of three salmon louse (*Lepeophtheirus salmonis*) genes with fibronectin II domains expressed by tegumental type 1 glands, Mol. Biochem. Parasitol. (2017), https://doi.org/10.1016/j.molbiopara.2017.12.002.

[59] S. Nylund, A. Nylund, K. Watanabe, C.E. Arnesen, E. Karlsbakk, *Paranucleospora theridion* n. gen., n. sp (Microsporidia, Enterocytozoonidae) with a life cycle in the salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic salmon (*Salmo salar*), J. Eukaryot. Microbiol. 57 (2010) 95–114, https://doi.org/10.1111/j.1550-7408.2009.00451.x.

[60] M.S. Ugelvik, S. Dalvin, The effect of different intensities of the ectoparasitic salmon lice (*Lepeophtheirus salmonis*) on Atlantic salmon (*Salmo salar*), J. Fish. Dis. 45 (2022) 1133–1147, https://doi.org/10.1111/jfd.13649.

[61] M. Ohtani, N. Hayashi, K. Hashimoto, T. Nakanishi, J.M. Dijkstra, Comprehensive clarification of two paralogous interleukin 4/13 loci in teleost fish, Immunogenetics 60 (2008) 383–397, https://doi.org/10.1007/s00251-008-0299-x.

[62] T. Wang, P. Johansson, B. Abos, A. Holt, C. Tafalla, Y. Jiang, A. Wang, Q. Xu, Z. Qi, W. Huang, M.M. Costa, P. Diaz-Rosales, J.W. Holland, C.J. Secombes, First in-depth analysis of the novel Th2-type cytokines in salmonid fish reveals distinct patterns of expression and modulation but overlapping bioactivities, Oncotarget 7 (2016) 10917–10946, https://doi.org/10.18632/oncotarget.7295.

[63] L.Y. Zhu, P.P. Pan, W. Fang, J.Z. Shao, L.X. Xiang, Essential role of IL-4 and IL-4Ralpha interaction in adaptive immunity of zebrafish: insight into the origin of Th2-like regulatory mechanism in ancient vertebrates, J. Immunol. 188 (2012) 5571–5584, https://doi.org/10.4049/jimmunol.1102259.

[64] F. Takizawa, E.O. Koppang, M. Ohtani, T. Nakanishi, K. Hashimoto, U. Fischer, J. M. Dijkstra, Constitutive high expression of interleukin-4/13A and GATA-3 in gill and skin of salmonid fishes suggests that these tissues form Th2-skewed immune environments, Mol. Immunol. 48 (2011) 1360–1368, https://doi.org/10.1016/j.molimm.2011.02.014.

[65] T.H. Grayson, P.G. Jenkins, A.B. Wrathmell, J.E. Harris, Serum responses to the salmon louse, *Lepeophtheirus salmonis* (Kroyer, 1838), in naturally infected salmonids and immunised rainbow trout, *Oncorhynchus mykiss* (Walbaum), and rabbits, Fish Shellfish Immunol. 1 (1991) 141–155, https://doi.org/10.1016/S1050-4648(06)80014-8.

[66] D. Piehler, W. Stenzel, A. Grahnert, J. Held, L. Richter, G. Kohler, T. Richter, M. Eschke, G. Alber, U. Muller, Eosinophils contribute to IL-4 production and shape the T-helper cytokine profile and inflammatory response in pulmonary Cryptococcosis, Am. J. Pathol. 179 (2011) 733–744, https://doi.org/10.1016/j.ajpath.2011.04.025.

[67] T. Yoshimoto, The hunt for the source of primary Interleukin-4: how we discovered that natural killer T cells and basophils determine T helper type 2 cell

[68] differentiation *in vivo*, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.00716.

[68] C.D. Strong, P.W. Wertz, C.W. Wang, F. Yang, P.S. Meltzer, T. Andl, S.E. Millar, I. C. Ho, S.Y. Pai, J.A. Segre, Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3, J. Cell Biol. 175 (2006) 661–670, https://doi.org/10.1083/jcb.200605057.

[69] I. Masse, L. Barbollat-Boutrand, M. El Kharbili, O. Berthier-Vergnes, D. Aubert, J. Lamartine, GATA3 inhibits proliferation and induces expression of both early and late differentiation markers in keratinocytes of the human epidermis, Arch. Dermatol. Res. 306 (2014) 201–208, https://doi.org/10.1007/s00403-013-1435-5.

[70] E. Racz, D. Kurek, M. Kant, E.M. Baerveldt, E. Florencia, S. Mourits, D. de Ridder, J. D. Laman, L. van der Fits, E.P. Prens, GATA3 expression is decreased in psoriasis and during epidermal regeneration; induction by narrow-band UVB and IL-4, PLoS One 6 (2011), e19806, https://doi.org/10.1371/journal.pone.0019806.

[71] H. Chi, X.H. Meng, R.A. Dalmo, GATA-3 in Atlantic salmon (*Salmo salar*): tissue distribution and its regulation of IL-4/13a promoter, Front. Cell. Infect. Microbiol. 12 (2022), 1063600, https://doi.org/10.3389/fcimb.2022.1063600.

[72] Y. Ishimoto, R. Savan, M. Endo, M. Sakai, Non-specific cytotoxic cell receptor (NCCRP)-1 type gene in tilapia (*Oreochromis niloticus*): its cloning and analysis, Fish Shellfish Immunol. 16 (2004) 163–172, https://doi.org/10.1016/S1050-4648(03)00059-7.

[73] A. Cuesta, M.A. Esteban, J. Meseguer, Molecular characterization of the nonspecific cytotoxic cell receptor (NCCRP-1) demonstrates gilthead seabream NCC heterogeneity, Dev. Comp. Immunol. 29 (2005) 637–650, https://doi.org/10.1016/j.dci.2004.11.003.

[74] H.Y. Tsai, A. Hamilton, A.E. Tinch, D.R. Guy, J.E. Bron, J.B. Taggart, K. Gharbi, M. Stear, O. Matika, R. Pong-Wong, S.C. Bishop, R.D. Houston, Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations, Genet. Sel. Evol. 48 (2016) 47, https://doi.org/10.1186/s12711-016-0226-9.

[75] K. Gharbi, L. Matthews, J. Bron, R. Roberts, A. Tinch, M. Stear, The control of sea lice in Atlantic salmon by selective breeding, J. R. Soc. Interface 12 (2015), 0574, https://doi.org/10.1098/rsif.2015.0574.

[76] N. Umasuthan, X. Xue, A. Caballero-Solares, S. Kumar, J.D. Westcott, Z. Chen, M. D. Fast, S. Skugor, B.F. Nowak, R.G. Taylor, M.L. Rise, Transcriptomic profiling in fins of Atlantic salmon parasitized with sea lice: evidence for an early imbalance between chalimus-induced immunomodulation and the host's defense response, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21072417.

[77] C.D. Todd, A.M. Walker, J.E. Hoyle, S.J. Northcott, A.F. Walker, M.G. Ritchie, Infestations of wild adult Atlantic salmon (*Salmo salar* L.) by the ectoparasitic copepod sea louse *Lepeophtheirus salmonis* Krøyer: prevalence, intensity and the spatial distribution of males and females on the host fish, Hydrobiologia 429 (2000) 181–196, https://doi.org/10.1023/A:1004031318505.

[78] A. Jaworski, J.C. Holm, Distribution and structure of the population of sea lice, *Lepeophtheirus salmonis* Krøyer, on Atlantic salmon, *Salmo salar* L., under typical rearing conditions, Aquacult. Fish. Manag. 23 (1992) 577–589, https://doi.org/10.1111/j.1365-2109.1992.tb00802.x.