



Investigation of a novel assay for the proteomic screening of the secretory and excretory products of individual salmon lice *Lepeophtheirus salmonis*

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ABSTRACT

Secretions are central to the ability of the salmon louse *Lepeophtheirus salmonis* to parasitize salmonid hosts by promoting immunomodulation and feeding. Previous characterizations of secretory and excretory products (SEPs) have relied on pooled individuals, preventing assessment of inter-individual variation and responses to host-derived cues. A novel SEP collection method was developed to obtain samples from individual adult female *L. salmonis*, enabling evaluation of secretory profiles following exposure to conditioned seawater from a susceptible host, Atlantic salmon (*Salmo salar*), or a resistant host, coho salmon (*Oncorhynchus kisutch*). SEP concentrations ranged from 328 to 1597 µg mL⁻¹, with no effects of host species or conditioning treatment on protein yield. Across 16 individuals, a mean of 101.4 proteins were detected per replicate, including 61.2 secretory proteins, indicating substantial inter-individual variation. Conditioning did not alter protein richness, yet conditioned treatments showed clear qualitative differences in composition. *S. salar*-conditioned lice uniquely secreted 40 proteins, including proteases, protease inhibitors, C-type lectins, gamma crystallins, and labial gland factors, whereas controls yielded a single unique protein. *O. kisutch* conditioning was further associated with additional proteases, protease inhibitors, epidermal growth factor-like proteins, and other putative virulence factors. Detection of chitin deacetylase-7 and an LY6/uPAR domain protein across multiple conditions highlights previously uncharacterised candidates relevant to louse–host interactions. In total, these findings establish individual-level SEP collection as a robust and sensitive approach for resolving secretomic diversity in *L. salmonis* and detecting host-associated modulation of parasite secretory activity obscured by pooled analyses.

1. Introduction

The salmon louse (*Lepeophtheirus salmonis* (Krøyer, 1837)) is an ectoparasitic Caligid with a distribution across the Northern Hemisphere that primarily feeds upon the mucus, skin, and blood of its hosts (Pike & Wadsworth, 1999). It represents a significant economic burden to the global salmonid aquaculture industry, causing approximately €790 million in cumulative annual damages to the Atlantic salmon (*Salmo*

salar (Linnaeus, 1758)) farming industry alone (Boxaspen et al., 2022).

Similarly to other ectoparasites, *L. salmonis* utilizes immunomodulatory secretory proteins in order to facilitate feeding and to assist survival on the host. Coupled with the open wounds that *L. salmonis* causes by feeding, this immunomodulation may make the host more susceptible to potentially lethal secondary infections (Braden et al., 2020). Early work identified low molecular weight proteases and prostaglandin E2 synthase in *L. salmonis* SEPs, implicating these secretions in vasodilation

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and immunomodulation (Fast et al., 2004; Fast et al., 2003). Subsequent *in vitro* studies demonstrated suppression of pro-inflammatory gene expression and chemotaxis in salmonid leukocytes, while the investigation of recombinant labial gland proteins have further revealed roles in anti-coagulation and immune modulation (Lewis et al., 2014; Piesz et al., 2020; Midtbø et al., 2024a). Beyond *L. salmonis*, comparatively few fish ectoparasites have been examined at the level of secreted/excreted proteins using proteomics, although SEPs from the branchiuran fish louse *Argulus foliaceus* have been recently characterized via LC-ESI-MS/MS, identifying a small but functionally informative protein profile including proteases (e.g., trypsin), metalloproteases (including astacin), peroxidases, and serine protease inhibitors (including serpins), with SignalP predicting secretion-associated signal peptides in a subset of proteins (AmbuAli et al., 2020).

Proteomic investigations of *L. salmonis* SEPs have elucidated the presence of further putative immunomodulatory proteins. In a proteomic investigation of adult and pre-adult *L. salmonis* SEPs, Hamilton et al. (2018) identified proteins, including peroxidases, which may play a role in protecting *L. salmonis* from host reactive oxygen species, and serine-type endopeptidases, which may play diverse roles in immunomodulation by cleaving host proteins. Most recently, Dindial et al. (2025) used liquid chromatography tandem mass spectrometry (LC MS/MS) to characterize the protein composition of SEPs acquired from *L. salmonis* copepodids, revealing the presence of diverse potential virulence factors, including proteases, peroxidases, and protease inhibitors with homologies to those used by other ectoparasites to inhibit host proteases implicated in such diverse functions as coagulation or leukocyte proliferation.

However, all of these studies have relied upon acquiring *L. salmonis* SEPs by extracting supernatant from high densities of pooled salmon lice, with or without the presence of a secretory inducer (e.g., dopamine) (Dindial et al., 2025). While Fast et al. (2004) implemented a protocol in which individual adult *L. salmonis* were set upon a droplet of dopamine solution for 25 min prior to SEP collection, this protocol was focused narrowly on the characterization of secretory prostaglandin E2 and was further conducted in conjunction with typical pooled extractions. Critically, no data comparing the two protocols were reported.

While pooled protocols are useful for acquiring workable concentrations of SEPs proteins (e.g., with reported concentrations of ~31–66 µg mL⁻¹ prior to further concentration) and providing broad overviews of secretory protein profiles across a sample of multiple *L. salmonis*, they are inherently unable to provide an overview of individual-level secretomic variation (Fast et al., 2007; Lewis et al., 2014). Consequently, the degree to which individual variation exists within the *L. salmonis* secretome remains opaque. Accordingly, the extent to which different host-associated and wider environmental conditions affect the modulation of the *L. salmonis* secretome at the individual level remains unknown. Additionally, both previously reported pooled and individual SEP extraction protocols have required either the complete immersion of entire *L. salmonis* or the near-complete coverage of the ventral surface, potentially increasing the burden of faecal contamination.

Furthermore, while the primary host of *L. salmonis* is *S. salar*, it is well-documented to also infect a diverse array of salmonids, including taxa in the genera *Oncorhynchus* and *Salvelinus* (Fast et al., 2003). However, different species within these taxonomic grouping exhibit differing levels of resistance to *L. salmonis* infection. In particular, the coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)) is known to exhibit a robust immune response to *L. salmonis* infection, including increased inflammatory cell proliferation at the site of infection relative to *S. salar* and the rejection of lice via a hyperplastic response driven partly by chitin sensing (Sveen et al., 2025; Braden et al., 2023). Fast et al. (2003) further reported different zymogram profiles in SEPs from *L. salmonis* treated with mucus from *S. salar* and *O. kisutch*, suggestive of differential secretory responses to the presence of either potential host. However, the identities of these differentially secreted proteases remain

unknown, as is the full scope of the differences in secretomic profile between *L. salmonis* exposed to susceptible host species like *S. salar* and resistant species like *O. kisutch*, which may provide further leads relating to differential response of salmonids to *L. salmonis*. Differential louse activity has previously been noted to different salmonid hosts at the transcriptomic level, highlighting the fact that expression of cysteine proteases such as cathepsin L, and serine proteases such as trypsin, are significantly influenced by the host species (Braden et al. 2017 Jan).

Here, a novel method to extract SEPs from individual adult female *L. salmonis* has been developed to (i) characterise individual-level secretomic variation and (ii) evaluate how exposure to conditioned seawater from a susceptible host (*S. salar*) and a relatively resistant host (*O. kisutch*) affects the composition of the SEP secretome. Using LC-MS/MS and *in silico* annotation of secreted proteins, we compare richness, functional composition, and the presence of known and putative virulence factors across treatments.

2. Methods

2.1. *L. salmonis* and salmonid conditioned seawater collection

Adult female *L. salmonis* were collected from the skin of adult sea-farmed rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) at Kames Fish Farming Ltd., Scotland using forceps and were transferred in seawater to the Institute of Aquaculture in Stirling, Scotland, where they were incubated in aerated seawater at 10 °C for 24 h prior to treatment.

O. kisutch conditioned seawater was collected from the United Kingdom Centre for Environment, Fisheries, and Aquaculture Science facilities in Weymouth, England. 50 fish with an average mass of 243.6 g were allowed to incubate in 1000 L 10 °C seawater for ~7 mos prior to conditioned water collection. *S. salar* conditioned seawater was collected from the University of Stirling's Marine Environmental Research Laboratory in Machrihanish, Scotland. 70 fish of ~250 g each were allowed to incubate in 700 L of 10 °C seawater for 48 h prior to conditioned water collection. Samples of ambient, unconditioned seawater were also harvested from each collection site to serve as negative controls. All conditioned seawater samples and their corresponding controls were vacuum filtered at 0.45 µm prior to usage to mitigate contamination with extraneous debris and microorganisms that may have been present.

As a consequence of differing conditioning regimes between host species in duration, tank volume/stocking context, and facility site, contrasts between host species treatments are framed as exploratory, as differences in dissolved metabolites, microbial communities, organic load, or fish physiological state could contribute independently of host species identity.

2.2. SEP collection and extraction

Following the 24-h incubation period, individuals were rinsed in 0.45 µm filtered seawater at 10 °C for 20 min to decrease extraneous contamination. Individual lice (n = 4 per condition) were then carefully transferred with forceps into single, dry wells in sterile 12-well tissue culture plates (Sarstedt, Nümbrecht, Germany), with one louse per well. Individuals positioned on their backs in the wells, leaving their ventral surface exposed. At this point, 15 µL of 10 °C *S. salar* conditioned seawater, *O. kisutch* conditioned seawater, or one of the two control seawater media was carefully pipetted onto the ventral cephalothorax, directly over the mouth to form a droplet constrained within the bounds of the marginal membrane and the third swimming leg. No additional media were added to any other part of the body. The presence of these media allowed the continuance of percutaneous gas exchange and further served as the solvent for the SEPs that would later be collected. Care was taken to avoid pipetting media over the anus to prevent faecal material contaminating the media. Following treatment with media, lice were incubated at 10 °C for 3 h.

After three hours, the well plates containing the lice were removed from incubation and ~12 µL of the SEPs-containing media was carefully pipetted off the ventral cephalothorax of each louse and transferred to 1.5 mL tubes (Eppendorf, Hamburg, Germany) on ice. Lice were then returned to 10 °C aerated filtered seawater for 24 h, after which they were assessed for survival (i.e., motility).

To remove excess salts (e.g., sodium chloride) and any debris that could interfere with downstream liquid chromatography tandem mass spectrometry (LC MS/MS) as well as to denature any proteases that could contribute to sample degradation, each SEP sample was subjected to trichloroacetic acid (TCA) precipitation. 100% TCA solution was added to each sample to yield a 20% TCA solution and left to incubate on ice for one hour, after which the samples were centrifuged at 15,000 x g and 4 °C for 10 min to pellet precipitated proteins. The supernatant was then removed, and protein pellets were washed three times with -20 °C acetone, with centrifugation under the same conditions after each wash. The acetone was then removed from each protein sample, after which each sample was reconstituted in 10 µL of ultrapure water.

2.3. Protein quantification and SDS PAGE

The protein concentration of each sample was determined using the protein A280 function on a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). A280 was selected due to the low sample volumes and because TCA precipitation followed by acetone washing was used to reduce salt carryover prior to quantification. Blank correction was performed using identically processed negative control seawater (i.e., control media subjected to the same precipitation and wash steps). Protein concentration data (µg mL⁻¹) were analyzed using a two-way ANOVA with salmonid species (*S. salar* vs. *O. kisutch*) and condition (control vs conditioned seawater) as fixed factors. Normality and homogeneity of variance were verified by Shapiro-Wilk and Levene's tests, respectively.

Samples were standardized to a total protein concentration of 250 µg mL⁻¹ using ultrapure water to ensure sufficient volumes were available for downstream analysis.

To evaluate protein quality, samples were subjected to sodium dodecyl sulfate gel electrophoresis (SDS PAGE). SEP protein samples were diluted 1:3 with 4x Laemmli buffer (Thermo Fisher Scientific, Waltham, USA), heated at 100 °C for five minutes, and centrifuged at 4 °C and 13,000 x g to prepare them for gel electrophoresis. 10 µL of each buffered sample was then loaded into 15-well SurePAGE 10 × 8 bis-tris gels (GenScript, Piscataway, USA) in 1 × 3-(N-morpholino)propane-sulfonic acid sodium dodecyl sulfate (MOPS SDS) running buffer along with 1 µL of dual color protein standard (Bio-Rad, Hercules, USA) and negative controls comprising the two salmon conditioned water samples and their two corresponding unconditioned controls. Gels were then allowed to run at 200 V for 40 min, were rinsed in ultrapure water, and were left to fix in a solution of 40% ethanol and 10% acetic acid in ultrapure water overnight. Gels were then silver stained using a Silver-Quest (Thermo Fisher Scientific, Waltham, USA) kit according to the manufacturer's instructions and immediately imaged.

All SEP protein samples were then immediately frozen at -80 °C and subsequently transferred to Moredun Research Institute in Penicuik, Scotland for LC MS/MS analysis.

2.4. LC MS/MS and in silico analysis

Samples were digested using an S-Trap micro column (Protifi) kit following the manufacturer's instructions. Briefly, 1–2 µg of sample was mixed with the supplied 2X SDS lysis buffer, proteins were reduced with 5 mM Tris (2-carboxyethyl) phosphine (TCEP) and alkylated with 20 mM methyl methanethiosulfonate (MMTS). Proteins were then acidified with 2.5% phosphoric acid and bound to the S-Trap column in 6 volumes of S-Trap binding/washing buffer (100 mM TEAB in 90% methanol). The column was centrifuged (4000 x g for 30 s) and then

washed a further 4 times in wash buffer. Proteins were digested using sequencing grade trypsin (in 50 mM TEAB) in a 1:10 ratio to trapped protein for 16 h at 37 °C. Tryptic peptides were eluted in three steps: firstly, 40 µL of 50 mM triethylammonium bicarbonate (TEAB) buffer, followed by 40 µL 0.2% formic acid (FA), and finally 40 µL 50% acetonitrile (ACN) in water and 0.2% FA. Elutions were pooled and dried in a vacuum drier. Dried peptides were reconstituted in 20 µL of 0.1% FA prior to analysis by mass spectrometry.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis was performed using a Vanquish Nano-LC system (Thermo Fisher Scientific) coupled to a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific). The LC system was equipped with an Acclaim Pepmap nano-trap column (C18 PepMap100, 300 µm × 5 mm, 5 µm, 100 Å, Thermo Fisher Scientific) and an Acclaim Pepmap RSLC analytical column (C18, 100 Å, 75 µm × 50 cm EasySpray, Thermo Fisher Scientific). The tryptic peptides were loaded onto the trap column in a volume of 20 µL at an isocratic flow of 60 µL/min, peptides were then separated on the Easy Spray analytical column. The eluents used were 0.1% v/v FA (solvent A) in H₂O and 80% v/v acetonitrile in 0.1% v/v FA (solvent B). A 110-minute gradient was used at 350 nL min⁻¹ from (i) 0–95 min, 1–35% B; (ii) 95–115 min, 99% B.

Data were acquired in positive mode using a data dependent approach, MS1 scans were acquired at 280,000 resolution over a mass range of *m/z* 380–1500 with an AGC of 3e6 and a maximum IT of 50 ms. In each cycle, the 15 most intense ions with charge states of ≥ 2 and intensity thresholds of ≥ 2.0e4 were selected for MS/MS and subjected to high-energy collision dissociation (HCD) at a normalized collision energy of 30%. The isolation window was set at 1.2 *m/z*, at a resolution of 17,500, an AGC target of 1e5 and a maximum IT time of 50 ms. The dynamic exclusion time was set to 45 s.

The MS raw data was processed using the Proteome Discoverer platform (version 2.4, Thermo Fisher Scientific) and Sequest HT algorithm. The MS data was searched against a combined database comprising the current *L. salmonis* v1.4 annotation, the *S. salar* v3.1 annotation, and the UniProt Knowledgebase (UniProtKB/Swiss-Prot, reviewed entries; release 2025_04). A maximum of two missed tryptic cleavages were allowed. The oxidation of methionine and protein N-terminal acetylation were set as variable modifications, as was the methylthio addition to cysteines. MS and MS/MS ion tolerances were set at 10 ppm and 0.02 Da, respectively. A maximum false discovery rate (FDR) of 1% at both the peptide and the protein levels was set and only proteins identified with ≥ 2 peptides were accepted.

Following the filtration of proteomics data, amino acid sequence data were analyzed using SignalP and InterPro to identify secretory proteins bearing signal peptides and to further annotate proteins with their corresponding gene ontology (GO) terms for biological process (BP), molecular function (MF), and cellular component (CC), respectively (Blum et al., 2025; Nielsen, 2025). 'Secretory proteins' were operationally defined as proteins containing predicted N-terminal signal peptides as identified using SignalP (default eukaryotic parameters). Proteins predicted to contain transmembrane domains were not additionally excluded from this classification. Accordingly, the secretory subset reported here reflects proteins bearing canonical signal peptides and may include proteins that are membrane-associated or retained within secretory pathways. This operational definition captures classically signal peptide-directed secretion but does not distinguish between soluble secreted proteins and membrane-integrated proteins, nor does it capture proteins released via non-classical secretion pathways or extracellular vesicles. Given that the SEP extraction protocol samples extracellular milieu following incubation, proteins predicted to bear signal peptides are interpreted as compatible with secretion into the sampled environment, though intracellular or membrane-associated contributions cannot be excluded.

The number of distinct protein identifications per sample post-filtration (henceforth referred to as protein richness) was quantified for both the full proteome and the secreted proteome across all

experimental conditions and replicates. Richness values were computed at the replicate level ($n = 4$ per condition) and aggregated to the condition level. To evaluate whether conditioning affected protein richness within hosts, richness values were compared between coho salmon control vs. coho salmon conditioned and Atlantic salmon control vs. Atlantic salmon conditioned lice using Welch’s two-sample t -tests. For each comparison, four biological replicates per group were used. Statistical assumptions (independence, approximate normality) were considered to have been satisfied given the nature of replicate-level proteomic richness values. Comparisons were conducted separately for whole-proteome richness and SP-only richness. Significance was evaluated at $\alpha = 0.05$.

Sequence similarity searches of the amino acid sequences of 22 known labial gland proteins were performed using BLASTp (NCBI BLAST+ v2.17.0) with an e -value threshold of $1e^{-5}$ (Øvergård et al., 2022; Midtbø et al., 2024a). Matches were required to meet $\geq 90\%$ sequence identity over $\geq 80\%$ of the query length. Because closely related paralogs may yield high identity values, results are interpreted as highly similar homologs rather than definitive ortholog assignments.

3. Results

3.1. *L. salmonis* survival and SEP protein concentrations

All evaluated lice survived the experimental treatments and remained alive and healthy following the elapse of 24 h post-treatment.

Across all 16 samples, the concentrations of SEP proteins ranged from $328 \mu\text{g mL}^{-1}$ to $1597 \mu\text{g mL}^{-1}$, with a mean ($\pm$ SD) of $716 \pm 300 \mu\text{g mL}^{-1}$ (Table 1).

There was no significant effect of host salmonid species or their corresponding control seawater source ($F_{1,12} = 0.46$, $p = 0.51$), conditioning ($F_{1,12} = 1.83$, $p = 0.20$), or their interaction ($F_{1,12} = 0.65$, $p = 0.44$) on the protein concentrations of SEP samples (two-way ANOVA, $n = 4$ per group). Mean ($\pm$ SD) values were $706 \pm 128 \mu\text{g mL}^{-1}$ for the *S. salar* control seawater treatment, $624 \pm 129 \mu\text{g mL}^{-1}$ for the *S. salar* conditioned seawater treatment, $930 \pm 469 \mu\text{g mL}^{-1}$ for the *O. kisutch* control seawater treatment, and $605 \pm 330 \mu\text{g mL}^{-1}$ for the *O. kisutch* conditioned seawater treatment. In addition, direct comparison of the two control seawater sources revealed no significant difference in protein concentration between *S. salar* and *O. kisutch* control treatments (Welch’s t -test, $t = 0.98$, $p = 0.39$).3.2. SEP protein profiles

Analysis of SDS PAGE banding patterns revealed subtle, yet discernible differences in protein profiles between all SEPs samples, albeit with considerable variation within treatment groups (Fig. 2).

Table 1
Protein concentrations in $\mu\text{g mL}^{-1}$ as measured by the protein A280 function on a Nanodrop 2000 spectrophotometer. Blank correction was performed using identically processed negative control seawater.

Sample ID	Protein Concentration ($\mu\text{g mL}^{-1}$)
Atlantic salmon control 1	833
Atlantic salmon control 2	543
Atlantic salmon control 3	778
Atlantic salmon control 4	671
Atlantic salmon conditioned seawater 1	706
Atlantic salmon conditioned seawater 2	667
Atlantic salmon conditioned seawater 3	691
Atlantic salmon conditioned seawater 4	432
Coho salmon control 1	507
Coho salmon control 2	1597
Coho salmon control 3	746
Coho salmon control 4	870
Coho salmon conditioned seawater 1	721
Coho salmon conditioned seawater 2	351
Coho salmon conditioned seawater 3	328
Coho salmon conditioned seawater 4	1019

Samples from lice treated with *S. salar* conditioned seawater uniquely showed distinct banding at both ~ 80 kDa and ~ 17 kDa, whereas those from lice treated with *O. kisutch* conditioned seawater (apart from replicate C3) uniquely showed distinct banding at ~ 70 kDa.

All SEP samples showed banding associated with the presence of low molecular weight proteins (i.e., ≤ 10 kDa) and proteins with a mass of ~ 27 kDa, ~ 30 kDa, ~ 125 kDa, and ~ 150 kDa. Two faint bands associated with a mass of ~ 50 kDa and ~ 60 kDa were noted in the negative control seawater samples from the collection sites of the conditioned seawater samples from both salmonid species, whereas no banding was detected in any lane associated with either of the salmonid conditioned seawater samples.

3.2. Overall SEP protein content

In total, 254 unique proteins were identified across all treatments and replicates. At the treatment condition level, a range of 113–219 proteins were detected, (mean 158.3 ± 44.3 S.D.). Analysis of the presence of signal peptides via SignalP revealed that across all conditions and replicates, a total of 148 proteins were secretory in nature (hereafter referred to as ‘secretory proteins’), with a range of 67–129 proteins per condition (mean 101.8 ± 25.7 SD).

Comparable degrees of variability were observed at the replicate level. Across all 16 replicates from all tested conditions, a range of 49–175 proteins (mean 101.4 ± 38.9 SD) were detected, of which 24–98 proteins (mean 61.2 ± 23.3 SD) were secretory in nature. Coho control replicates yielded 136 ± 41.6 proteins, coho conditioned yielded 115.8 ± 23.0 , Atlantic conditioned yielded 93.5 ± 27.3 , and Atlantic control yielded 60.3 ± 12.1 proteins. Corresponding counts for secretory proteins were 64.0 ± 30.7 , 71.8 ± 10.6 , 69.0 ± 23.0 , and 40.0 ± 13.0 , respectively.

Protein richness varied across replicates, and conditioning effects were evaluated separately within host species. For the coho conditioned seawater group and the corresponding control, conditioning did not significantly alter whole-proteome richness (coho control mean = 87.3; coho conditioned mean = 115.8; Welch’s $t = 1.07$, $p = 0.338$). Similarly, no significant difference was observed for secretory protein only protein richness (coho control mean = 64.0; coho conditioned mean = 71.8; $t = 0.47$, $p = 0.666$).

In contrast, the Atlantic salmon conditioned seawater group and the corresponding control showed higher richness in conditioned individuals relative to controls for both whole-proteome (Atlantic salmon control mean = 60.3; Atlantic salmon conditioned mean = 93.5) and secretory protein only datasets (Atlantic salmon control mean = 40.0; Atlantic salmon conditioned mean = 69.0). However, these differences did not reach statistical significance (whole proteome: $t = 2.29$, $p = 0.082$; secretory proteins only: $t = 2.08$, $p = 0.094$).

3.3. Profiles of secretory proteins shared between all conditions

A total of 14 distinct secretory proteins were detected in all 16 replicates. Most of these proteins were proteases (i.e., 91.67% of the 12 proteins with valid GO terms for biological process), including serine-type endopeptidases and astacin-like domain-bearing metalloendopeptidases and metalloproteinases. These proteases had NCBI annotations corresponding to low choriolytic enzyme (4 total), trypsin-1 (3 total), or bradyrin-like, bradyrin, chymotrypsinogen A, and hatching enzyme 1.2 (1 total of each). The remaining 3 proteins were annotated as vitellogenin-6 (associated with lipid transfer), matrix metalloproteinase-9 (associated with a fibronectin type-II domain), and gamma-crystallin 1.

Additionally, 64 secretory proteins were detected across all four treatment conditions, for which 49, 74, and 10 valid GO terms for biological function, molecular process, and cellular component, respectively, were available (Fig. 3). This shared secretory protein profile was dominated by proteases, with 30% of all proteins with assigned

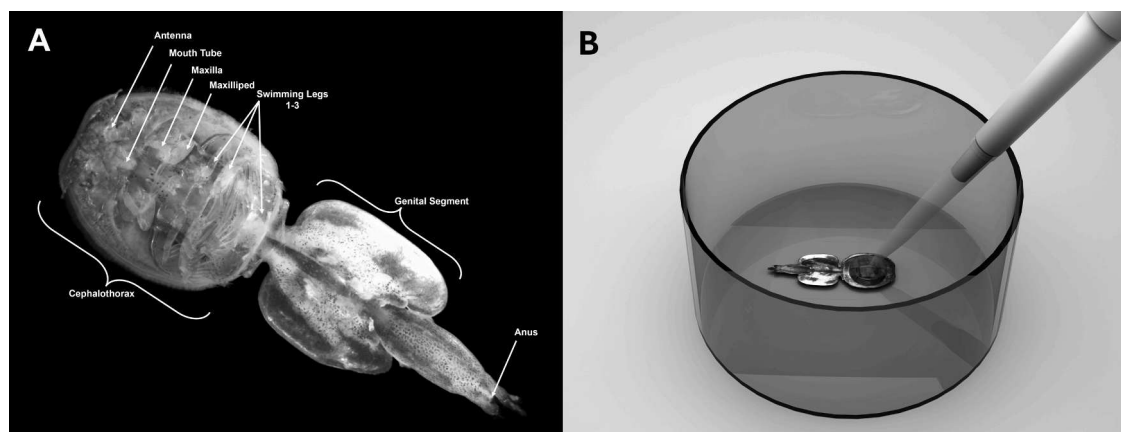


Fig. 1. A Focus-stacked image of ventral aspect of mature adult female salmon louse showing key anatomical features. B Diagram of experimental set-up, showing adult female placed on dorsal surface in culture dish, with ventral surface of cephalothorax holding collection medium. Focus-stacking of stereomicrograph images performed using Helicon Focus 8.3.9.

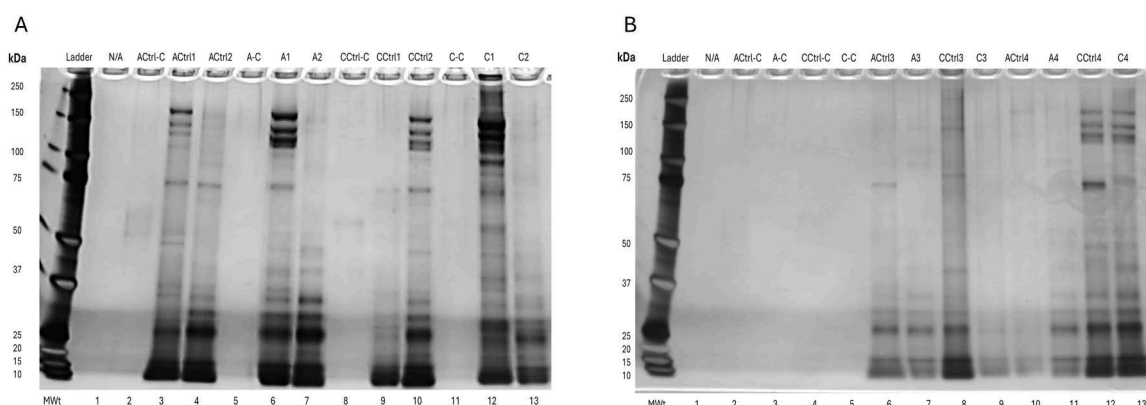


Fig. 2. Protein profiles of individual adult female *L. salmonis* SEPs as visualized through silver stained SDS PAGE. Abbreviations: kDa: kilodalton; MWt: molecular weight; ACtrl-C: unconditioned seawater negative control from the Atlantic salmon conditioned seawater collection site; A-C: Atlantic salmon conditioned seawater negative control; CCtrl-C: unconditioned seawater negative control from the coho salmon conditioned seawater collection site; C-C: coho salmon conditioned seawater negative control; ACtrl#: SEP proteins from lice incubated in unconditioned seawater from the Atlantic salmon conditioned seawater site; A#: SEP proteins from lice incubated in Atlantic salmon conditioned seawater; CCtrl#: SEP proteins from lice incubated in unconditioned seawater from the coho salmon conditioned seawater site; C#: SEPs proteins from lice incubated in coho salmon conditioned seawater. '#' represents the replicate number corresponding to each sample.

functions implicated in proteolysis. This included 17 serine-type endopeptidases, 7 metalloendopeptidases, 7 metalloproteases, 3 cysteine-type peptidases, 2 metalcarboxypeptidases, 1 serine-type exopeptidase, and 1 dipeptidyl-peptidase. In addition to the proteases found commonly across all replicates, these included proteins annotated as chymotrypsin-like elastase family member 2 A, putative serine protease K12H4.7, carboxypeptidase B, cathepsin L-like proteinase and peptidase, chymotrypsin-like protease CTRL-1, chymotrypsin BI-like, and chymotrypsin-BI. A further 9 proteins were found to be associated with zinc ion binding, 3 with heparin binding or O-glycosyl hydrolase activity, 2 with protein binding, and 1 with cell adhesion molecule binding, alpha-1,4-glucosidase activity, catalytic activity, phosphatase activity, alkaline phosphatase activity, peroxidase activity (i.e., phospholipid hydroperoxide glutathione peroxidase), lysozyme activity, sterol binding, enzyme activator activity, carbohydrate metabolism (i.e., chitin deacetylase-7), beta-N-acetylglucosaminidase activity, or beta-N-acetylhexosaminidase activity.

Of these 64 secretory proteins, 17 held NCBI annotations of 'uncharacterized protein.' However, 11 of these proteins had valid InterPro annotations that may contribute to the elucidation of their function. For example, two of these proteins (NCBI accessions XP_071747775.1 and XP_040572125.1) were found to be associated with Kelch-type beta propeller domains and may accordingly be

implicated in protein binding, while another three proteins (XP_040574440.2, XP_040569647.1, XP_040577721.1) were found to be associated with lipid binding and transport, with the latter further associated with ganglioside catabolism. Furthermore, XP_040566367.1 was associated with cellular adhesion. Two further uncharacterized proteins (XP_040579408.1 and XP_040571310.1) may be implicated in the *L. salmonis* immune system, with the former possessing a C-type lectin fold, and the latter associated with lysozyme activity and the presence of a lysozyme domain and C-terminal pesticin domain. Finally, XP_040572657.1 is annotated as gamma-crystallin-like, XP_071746852.1 is associated with heparin binding and possesses a fibronectin type II domain, while XP_040579422.1 is annotated as a sleep homeostasis regulator.

3.4. Comparison of SEP protein profiles from *S. salar* conditioned water treated *L. salmonis*

In total, 66 proteins were found to be shared between the secretory protein profiles of *L. salmonis* SEPs in both the *S. salar* conditioned water and *S. salar* control treatments. 50 of these proteins had valid GO terms for biological process, of which, 62% were associated with proteolysis. This included 17 serine-type endopeptidases, 8 metalloendopeptidases, 8 metalloproteases, 3 cysteine-type peptidases, 2

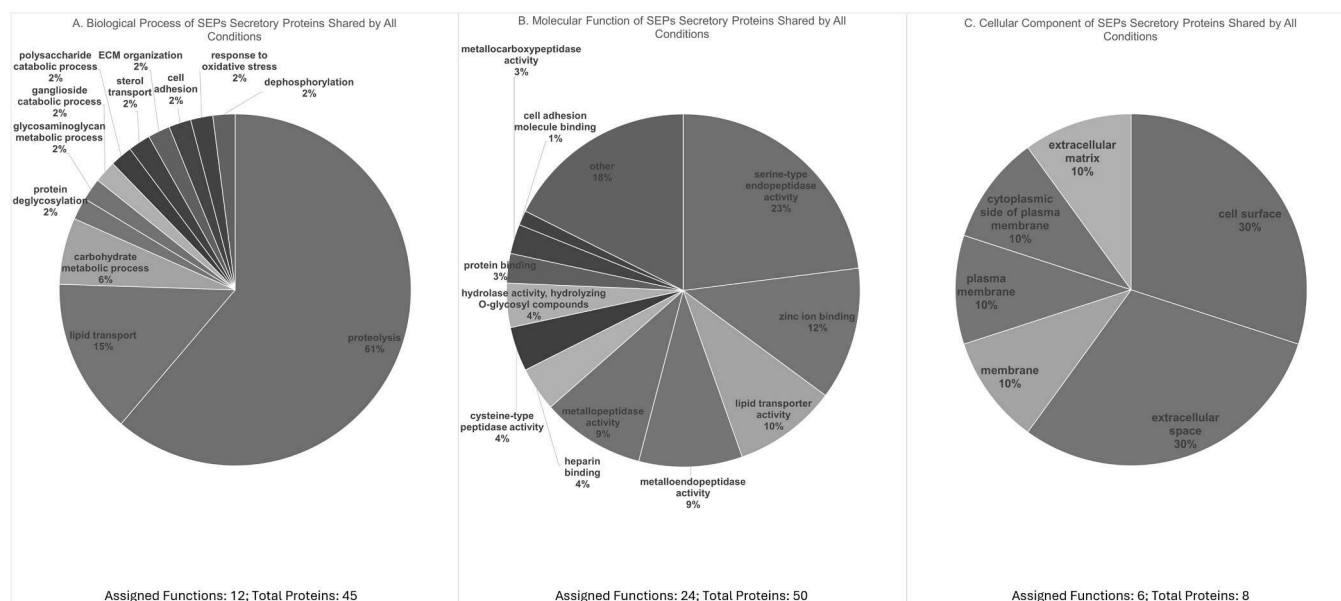


Fig. 3. Pie charts showing proportion of proteins assigned to selected Gene Ontology (GO) terms for the set of secretory proteins shared between all four of the investigated treatment conditions. For GO term domains (biological process, molecular function, or cellular component) for which over 12 terms were available, the top 12 terms are included in the chart while the remaining terms are included in an 'other' category.

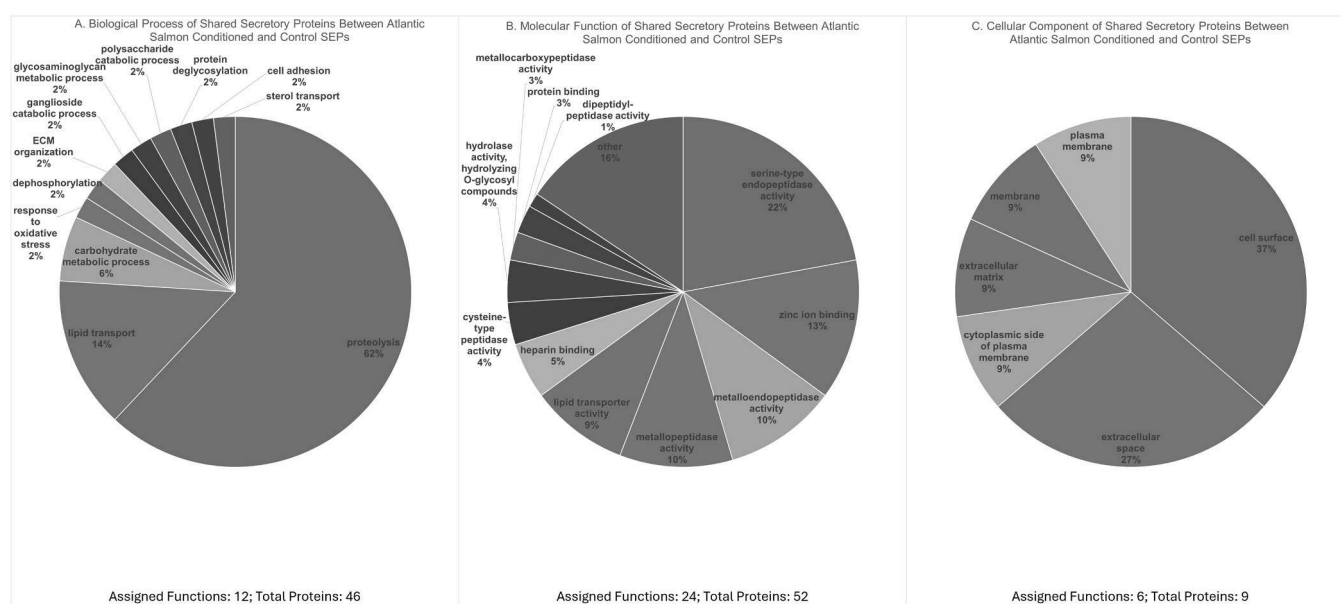


Fig. 4. Pie charts showing proportion of proteins assigned to selected Gene Ontology (GO) terms for the set of secretory proteins identified both in SEPs from the *S. salar* conditioned seawater and control treatments. For GO term domains (biological process, molecular function, or cellular component) for which over 12 terms were available, the top 12 terms are included in the chart while the remaining terms are included in an 'other' category.

metalcarboxypeptidases, 1 dipeptidyl-peptidase, and 1 serine-type exopeptidase. A further 10 were associated with zinc ion binding, 7 with lipid binding, 4 with heparin binding, 3 with hydrolase activity, 2 with protein binding, and of 1 of each associated with alkaline phosphatase activity, alpha-1,4-glucosidase activity, beta-N-acetylglucosaminidase activity, beta-N-acetylhexosaminidase activity, carbohydrate binding, catalysis, cell adhesion, enzyme activation, lysozyme activity, peroxidase activity, phosphatase activity, and sterol binding.

In contrast, 40 secretory proteins were detected uniquely in the protein profiles of *S. salar* conditioned seawater treated *L. salmonis* SEPs, whereas just 1 secretory protein was detected uniquely in the control.

These 'unique' proteins reflect detection in this dataset and should be interpreted alongside the non-significant richness comparisons reported in Section 3.3 ($n = 4$ per group). While this single unique protein found among the control SEPs samples is uncharacterized and lacks any NCBI or InterPro annotations, the secretory protein profile of the SEPs from the conditioned water treated *L. salmonis* presents a diverse array of proteins. Eighteen of these were proteases (variously annotated as cathepsin D, seminal metalloprotease 1, carboxypeptidase I, legumain, trypsin, trypsin-I, aspartic protease 4, xaa-Pro aminopeptidase 1, carboxypeptidase B, hatching enzyme 1.2, chymotrypsin BI, zinc metalloproteinase nas-7, and phenoloxidase-activating factor 2), and also present were 3 protease inhibitors (thrombin inhibitor hemalin, an

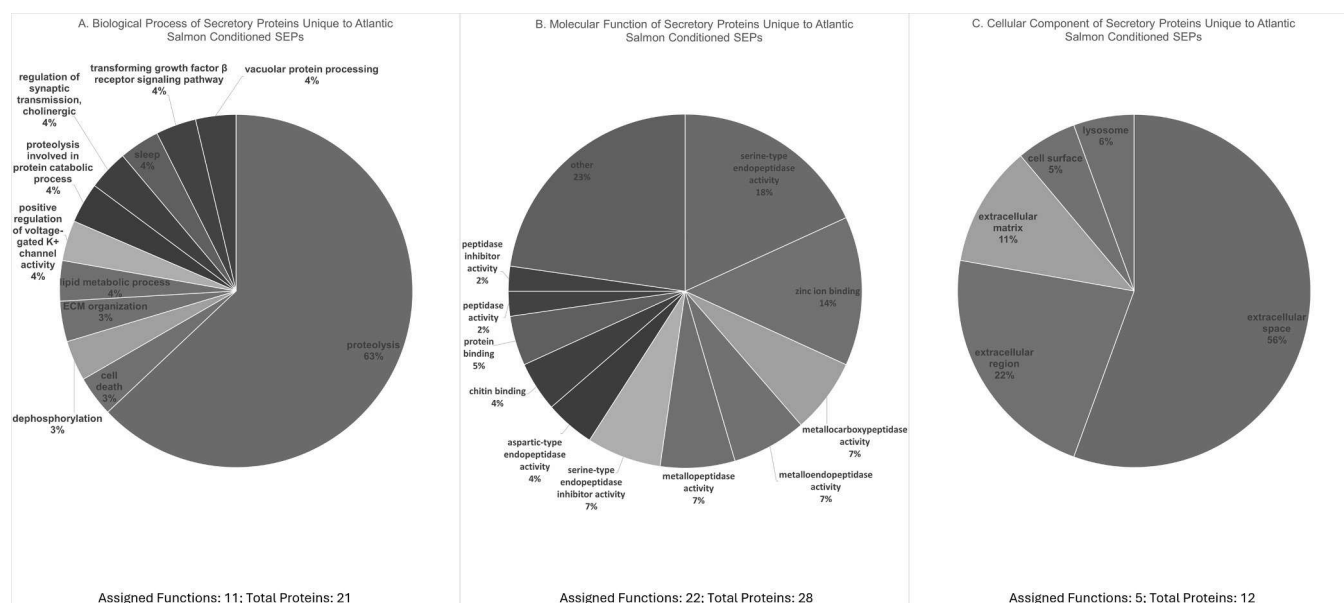


Fig. 5. Pie charts showing proportion of proteins assigned to selected Gene Ontology (GO) terms for the set of secretory proteins identified in SEPs from the *S. salar* conditioned seawater treatment, but not in those from the control. For GO term domains (biological process, molecular function, or cellular component) for which over 12 terms were available, the top 12 terms are included in the chart while the remaining terms are included in an 'other' category.

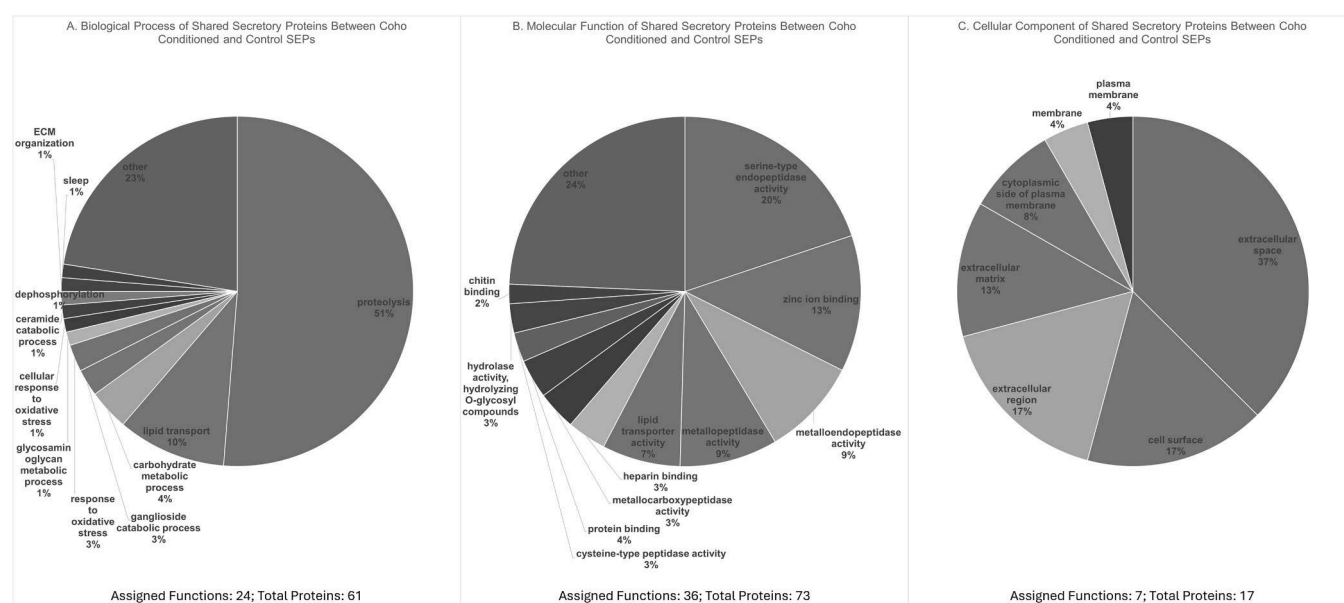


Fig. 6. Pie charts showing proportion of proteins assigned to selected Gene Ontology (GO) terms for the set of secretory proteins identified both in SEPs from the *O. kisutch* conditioned seawater and control treatments. For GO term domains (biological process, molecular function, or cellular component) for which over 12 terms were available, the top 12 terms are included in the chart while the remaining terms are included in an 'other' category.

uncharacterized cystatin-domain bearing protein, and a papilin-1-like protein), 3 thrombospondins (SCO-spondin isoform X1 and A disintegrin and metalloproteinase with thrombospondin motifs adt-1), 3 *L. salmonis* immune system proteins (murinoglobulin-1, papilin and C-type lectin Bfl-1 isoform X1), 3 gamma crystallin-like proteins, 2 proteins implicated in chitin and protein binding (hemocytin-like), as well as 1 protein each implicated in heparin binding, RNA endonuclease activity, alkaline phosphatase activity, glycerophosphodiester phosphodiesterase activity, and hydrolase activity. Additionally, 1 protein was annotated as UPAR/Ly6 domain-containing protein crok and contained domains associated with the sleep homeostasis regulator and the snake toxin-like superfamilies of domains. An additional single

uncharacterized protein (NCBI accession XP_040568779.1) was detected but lacked any further annotation.

3.5. Comparison of SEP protein profiles from *O. kisutch* conditioned water treated *L. salmonis*

A total of 92 secretory proteins were found to be shared between the secretory protein profiles of adult female *L. salmonis* treated with *O. kisutch* conditioned seawater and the corresponding control seawater. While this shared secretory protein profile carried a range of proteases (including 22 serine-type endopeptidases, 10 metalloendopeptidases, 10 metallopeptidases, 4 metalcarboxypeptidases, 3 cysteine-type

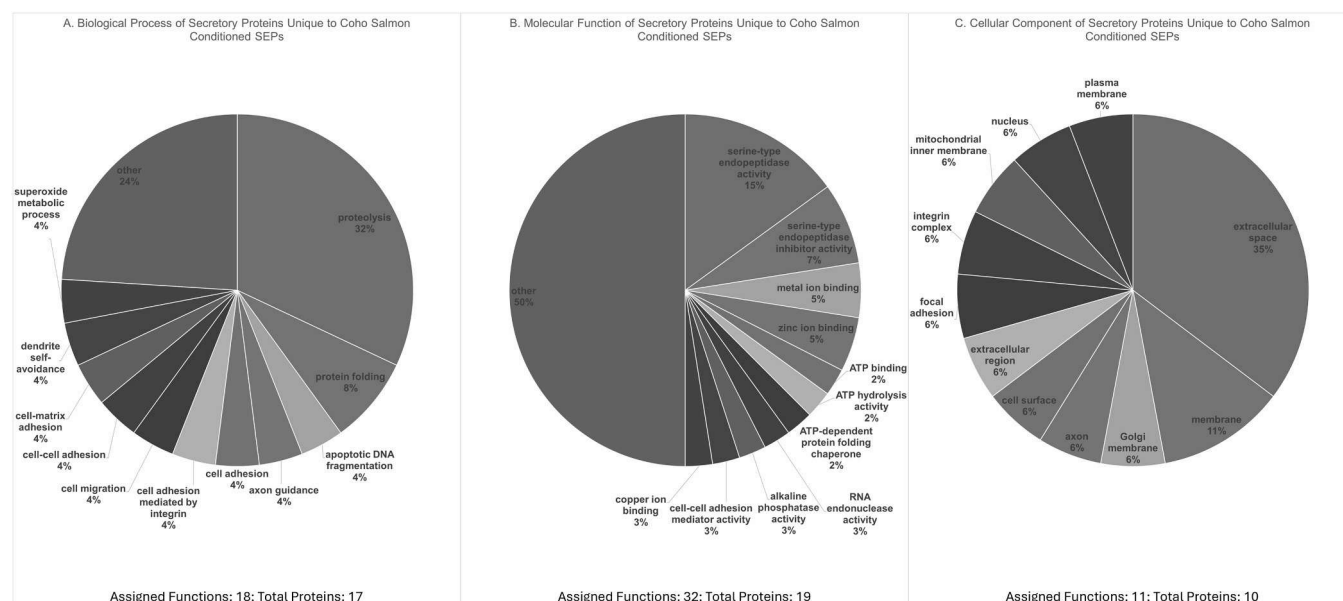


Fig. 7. Pie charts showing proportion of proteins assigned to selected Gene Ontology (GO) terms for the set of secretory proteins identified in SEPs from the *O. kisutch* conditioned seawater treatment, but not in those from the control. For GO term domains (biological process, molecular function, or cellular component) for which over 12 terms were available, the top 12 terms are included in the chart while the remaining terms are included in an 'other' category.

peptidases, 1 cysteine-type endopeptidase, 1 dipeptidyl-peptidase, 1 serine-type exopeptidase, 1 metalloaminopeptidase, and 1 peptidase), it was nevertheless rich in proteins with diverse molecular functions. This included 14 proteins associated with zinc ion binding, 8 with lipid transporter activity (predominantly vitellogenins), 4 with heparin binding or protein binding, 3 with hydrolase activity, 2 with chitin binding, enzyme activation, serine-type endopeptidase inhibitor activity, or peroxidase activity, and 1 with ATP binding, ATP hydrolysis, N-acylsphingosine amidohydrolase activity, alkaline phosphatase activity, alpha-1,4-glucosidase activity, beta-N-acetylglucosaminidase activity, beta-N-acetylhexosaminidase activity, carbohydrate binding, catalysis, cell adhesion molecule binding, endopeptidase inhibitor activity, glycerophosphodiester phosphodiesterase activity, heme binding, lysozyme activity, phosphatase activity, phosphoric diester hydrolase activity, or sterol binding.

Additionally, 37 secretory proteins were detected uniquely in the SEPs of individuals treated with the *O. kisutch* conditioned seawater, while 14 secretory proteins were detected among the corresponding control SEPs samples. The proteins unique to the control-treated *L. salmonis* SEPs included 4 different proteases (annotated with such NCBI descriptions as trypsin, trypsin-1, aspartic protease 4, and cathepsin D), 2 hydrolases, a C-type-lectin-like protein, a gamma-crystallin-like protein, a serpin-domain bearing protease inhibitor, a putative ascorbate peroxidase, a FAS-1-domain bearing protein, a fibronectin type II-domain bearing protein, and a saposin-like protein.

In contrast, the secretory protein profile of the SEPs of *L. salmonis* treated with *O. kisutch* conditioned seawater was associated with a greater degree of diversity of protein function. This included 8 proteases (serine protease 48 isoforms X1 and X2, a CLIP domain-containing serine protease HP8, hatching enzyme 1.2, venom serine protease Bi-VSP, carboxypeptidase B, and chymotrypsinogen A), 4 protease inhibitors (inter-alpha-trypsin inhibitor heavy chain H3-like and uncharacterized proteins bearing cystatin, serpin, and Kunitz domains), 2 fibronectin domain containing proteins (type II and type III), 2 gamma-crystallin-like proteins, 2 proteins with epithelial growth factor-like domains, 1 heat shock protein 90 domain containing protein, 1 neurotransmitter-gated ion channel, 1 thioredoxin, 1 endonuclease, 1 protein containing a cellular repressor of E1A-stimulated genes domain, 1 alkaline phosphatase, 1 protein containing a sleep homeostasis regulator and

snake toxin-like superfamily domain, and 1 calycin.

3.6. Detection of labial gland proteins

Two distinct labial gland protein sequences were detected in the secretory protein profiles observed in this experiment. The proteins XP_040574063.2 and XP_040574064.2 (both bearing the NCBI annotation of 'uncharacterized protein') had 94.36% sequence identity and 94.87% sequence identity, respectively, with the amino acid sequence for LsLGP3. These two proteins were detected in all four of the treatment conditions.

XP_040571157.1 (NCBI annotation chymotrypsinogen A), detected solely in the *O. kisutch* conditioned seawater treatment group, was further found to bear a 97% sequence identity with the amino acid sequence for LsLGP1.

4. Discussion

Analysis of secretory and excretory products (SEPs) provides a functionally relevant snapshot of the wide array of proteins produced by *L. salmonis* at the host-parasite interface, capturing the diversity of parasite-derived biomolecules in their native, secreted form. In this context, the novel technique explored in this study to extract SEPs from individual adult female *L. salmonis* yields robust qualitative proteomic data while capturing the diversity of individual secretory dynamics obscured in studies implementing pooled extraction approaches while mitigating fecal contamination. The concentrations of protein in SEP samples were comparable to those observed in studies using previously characterised pooled extraction techniques, with the range of 328–1597 $\mu\text{g mL}^{-1}$ reported here aligning closely with values of 100–1900 $\mu\text{g mL}^{-1}$ and 125–1189 $\mu\text{g mL}^{-1}$ reported by Hamilton et al. (2018) and Dindial et al. (2025), respectively. Notably, the number of distinct *L. salmonis* proteins identified in this study (254) was considerably greater than those reported for adult lice by Hamilton et al. (2018) and Dindial et al. (2025) (187 and 117, respectively), with the former also including proteins detected in the preadult stage).

While the range of proteins detected across the 16 replicates in this study (49–175 proteins per replicate, mean 101.4 ± 38.9 SD) was suggestive of a wide range of variability in the investigated individual SEP

samples, such large degrees of variability agree with the variation in secretome observed for secretomic and sialomic studies involving other individual ectoparasites. This is underscored by early work in ixodid ticks, which demonstrated pronounced molecular individuality in salivary gland protein profiles, with marked polymorphism between individual ticks even under similar conditions and is further exacerbated by differences in feeding state (Wang et al., 2001; Kim et al., 2016). Furthermore, transcriptomic analysis of individuals has confirmed that salivary gland expression profiles can vary greatly between ticks of the same species and life stage, a pattern often obscured in pooled-sample studies (Perner et al., 2018).

The secretory protein profiles observed in this study revealed an array of putative virulence factors. First, both the labial gland proteins LsLGP3 and LsLGP1 were identified, the former across all tested conditions and the latter solely in the group treated with coho salmon conditioned seawater, respectively (Midtbø et al., 2024a). LsLGP3 is known to bind to an as yet undetermined surface receptor on salmonid leukocytes to bring about downstream apoptosis, whereas LsLGP1 is a serine protease thought to dampen the host immune response, as its knock-down is associated with the heightened presence of cytokine transcripts at the *L. salmonis* attachment site (Midtbø et al., 2024a; Midtbø et al., 2024b). Their consistent detection among individual adult females in this study reinforces earlier observations that these proteins represent core components of the virulence repertoire of *L. salmonis*.

Beyond these well-studied labial gland factors, the individual-level SEP samples investigated in this study included a broad array of proteases and protease inhibitors. While these functional classes of proteins have been previously observed in *L. salmonis* SEPs, the present study provides direct evidence of their inter-individual variability (Hamilton et al., 2018; Dindial et al., 2025). Secreted proteases are widely recognized as key virulence factors across ectoparasites like ticks and mites, in which they contribute to tissue penetration, nutrient acquisition, and immunomodulation. For example, the Asian blue tick *Rhipicephalus microplus* cysteine protease BmCL1 inhibits clotting via induction of fibrinogen hydrolysis, while the scabies mite *Sarcoptes scabiei* serine protease Sar s 3 cleaves the human skin protein flaggrin to promote digestion (Beckham et al., 2009; Xavier et al., 2019; Fernando and Fischer, 2020). Furthermore, with respect to immunomodulation, the cat flea *Ctenocephalides felis* serine protease CfSP28 is known to cleave host immunoglobulin G, while the *S. scabiei* serine protease paralogue SMIPP-Ss is known to modulate host immunity via binding to mannose binding lectins, properdin, and C1q (Silver et al., 2002; Reynolds et al., 2014; Fernando & Fischer, 2020). The diverse array of trypsin, metalloproteases, and cysteine proteases (*inter alia*) detected here is consistent with a potential strategy employed by *L. salmonis* to negotiate host barriers and inflammatory responses. Furthermore, this study identified multiple protease inhibitors, including serpins and Kunitz-type inhibitors, whose presence suggests an additional layer of regulatory control. These molecules may protect parasite tissues from autodigestion while simultaneously modulating host protease cascades associated with coagulation, leukocyte proliferation, and cytokine production (Chmelař et al., 2017; Jmel et al., 2023; Dindial et al., 2025). For instance, the *Ixodes ricinus* serpin Iris binds macrophages to suppress tumour necrosis factor secretion, inhibits T-cell proliferation, and interferes with haemostasis in vertebrate hosts, whereas the *Rhipicephalus microplus* Kunitz-type inhibitor boophilin binds thrombin to directly impede normal hemostatic processes in mammalian hosts (Prevot et al., 2006; Corral-Rodríguez et al. 2009; Prevot et al., 2007). Furthermore, while the limited fish ectoparasite SEP proteomes make cross-taxon comparison challenging; the only branchiuran SEP proteomic study to date (on *Argulus foliaceus*) similarly reported secreted/excreted proteases, metalloproteases (including astacins), and protease inhibitors (including serpins), alongside a subset bearing predicted signal peptides (AmbuAli et al., 2020). This convergence across divergent crustacean ectoparasites supports the plausibility that proteolysis- and immune-modulation-adjacent protein families form recurring components of

ectoparasite secretory repertoires, while still requiring functional validation in *L. salmonis*.

In addition to proteases and protease inhibitors, several oxidative stress-associated enzymes, including peroxidases, were identified in this study, as they were by Hamilton et al. (2018) and Dindial et al. (2025). Although their functions in *L. salmonis* remain incompletely understood, peroxidases in other hematophagous ectoparasites play central roles in detoxifying reactive oxygen species generated by inflammation associated with tissue damage, thus mitigating host-induced oxidative stress in the parasite. (Pawłowska et al. 2023). For example, peroxiredoxin has been observed to be localized around the pharynx of the sheep mite *Psoroptes ovis*, and the deer tick *Ixodes scapularis* is known to secrete both peroxiredoxins and superoxide dismutases (McNair et al., 2009; Das et al. 2001). Accordingly, the upregulation of host glutathione peroxidase 1b and metallothionein has been observed at the attachment site of copepodids on coho salmon, which may serve to protect host tissues from reactive oxygen species generated in response to infection, underscoring the potential importance of secreted factors to mitigate oxidative stress in *L. salmonis* (Sveen et al., 2025).

The detection of multiple fibronectin II domain metalloproteases (such as matrix metalloproteinase-9, identified under all conditions in this study) highlights the multifunctionality of secreted proteases in *L. salmonis*. While such proteins have been established to be secreted by *L. salmonis* tegumental glands and have been suggested to play a role in mitigating tegumental biofouling, spatial transcriptomic study of the copepodid attachment site in coho salmon has shown increased expression of coho metalloproteinase inhibitor 2-like proteins, which may suggest that *L. salmonis* metalloproteinases may further play a role in the response of coho salmon to infection (Øvergård et al., 2016; Harasimczuk et al., 2018; Sveen et al., 2025).

These data also contain insights into potential *L. salmonis* virulence factors that remain wholly uncharacterized. For example, one secretory protein identified in this study was XP_040579469.1 (NCBI annotation UPAR/Ly6 domain-containing crok), detected in all conditions excepting the *S. salar* control. This protein was annotated by InterPro as ‘sleep homeostasis regulator, snake toxin-like superfamily,’ and ‘UPAR/Ly6 domain-containing protein Quiver-like.’ This LY6/uPAR-like protein is predicted based on structure to bear a characteristic three-finger fold supported by multiple disulfide bonds, structurally paralleling mammalian LY6 family neuromodulators and venom-derived α -neurotoxins that modulate nicotinic acetylcholine receptors, immune cell activation, and epithelial barrier signalling (Vasilyeva et al., 2017). While such proteins are largely uncharacterized in ectoparasites, their diverse roles in neuro- and immunomodulation in other systems make them intriguing candidates for future functional work on *L. salmonis* virulence.

The data collected in this study also revealed the presence of a chitin deacetylase-7 (CDA7; NCBI accession XP_040580790.1) across all treatment conditions. Chitin deacetylases convert chitin to chitosan, a less immunogenic polymer, which may limit host pattern-recognition receptor binding and the activation of chitinase-mediated inflammatory pathways (Liu et al., 2019). It has further been established that *S. salar* susceptible to *L. salmonis* infection exhibit downregulated acidic mammalian chitinase, while *O. kisutch* exhibit a robust, macrophage-driven response to *L. salmonis* chitin (Braden et al., 2023; Usmasuthan, et al., 2020). This CDA7 may function to mask exposed cuticular chitin or to deacetylate shed chitin fragments before they engage host lectins and epithelial pattern recognition receptors, thereby potentially contributing to a broader strategy of chitin-sensing avoidance in this species.

While no statistically significant difference in protein richness was found between the SEPs from lice in salmonid conditioned seawater treatment conditions and their corresponding controls, the secretory protein profiles of the conditioned seawater treatment SEPs nevertheless contained more proteins with a greater diversity of functions relative to the controls. This was most pronounced in the *S. salar* conditioned

seawater treatment condition, which exhibited 40 uniquely detected secretory proteins in comparison with one in its control. This included a wide array of putative and potential virulence factors, including Kunitz-type and cystatin protease inhibitors. Correspondingly, the *O. kisutch* conditioned water treated condition exhibited ~2.6X more secretory proteins than its control counterpart, and was also accordingly richer in proteases (e.g., LsLGSP1, chymotrypsin-like peptidases, and astacin-like metalloproteases) and protease inhibitors (e.g., Kunitz and serpin domain-containing proteins). However, it must be noted that the variation in the collection conditions of the salmonid conditioned seawater samples in this study means that it is not possible to directly make comparisons between the differential effects of the treatments associated with the two salmonid species investigated in this work. Future work employing comparatively controlled conditions is necessary to more thoroughly establish these comparisons. Furthermore, it must be noted that the detection of certain proteins in this work (such as vitellogenins) could reflect genuine release into the sampled secretory milieu; however, they may also arise from hemolymph leakage or handling-associated mechanical stress during droplet incubation/collection. These proteomic data alone do not discriminate among these possibilities.

While all individuals examined in the present study were adult females, secretory protein profiles in *L. salmonis* may be influenced by sex and reproductive state. Transcriptomic analyses have revealed pronounced sex-biased gene expression, including the differential expression and expansion of Kunitz-domain-containing protease inhibitors in males, alongside evidence for sex-specific evolutionary trajectories in genes implicated in host–parasite interactions (Poley et al., 2016; Michaud, 2025). These findings raise the possibility that male lice deploy distinct secretory strategies, potentially reflecting differences in feeding behavior, mating biology, or immunomodulatory requirements. Among adult female lice, transitions between pre- and post-copulatory states or shifts associated with egg production could plausibly drive changes in secretory composition as energetic resources are reallocated toward reproduction. Further application of the SEP extraction protocol developed here provides the methodological framework for investigating the sex- and reproduction-associated dynamics in the *L. salmonis* secretome.

Despite substantial inter-individual variability, 64 secretory proteins were consistently detected across all conditions, including rich array of proteases and proteins like CDA7. The replicate-level detection frequencies of these proteins are summarised in Table S1. Given the variance in SEP protein profiles from individual lice observed in this work and in other studies with other ectoparasites, the consistency in this array of core secretory proteins may thus warrant further investigation and characterization to better elucidate the dynamics of host–parasite interactions in *L. salmonis*, while the methodology may provide the basis for future higher throughput analysis of treatment impacts on the *L. salmonis* secretome.

5. Conclusions

This study presents a novel SEP collection approach that allows isolation of uncontaminated SEPs from individuals and thereby permits estimates of variability in SEP production within a sampled population. This study has highlighted an array of putative virulence factors and elucidating new proteins potentially implicated in virulence while capturing the large degree of variation in the secretome of individual lice across salmonid conditioned water treatments. This individual-level variation in SEP secretory protein profile is an important consideration for the further research of targeted therapies and vaccines against SEPs proteins. However, such work in therapeutic development requires functional characterization of the described SEP proteins, using techniques such as *in situ* hybridization, gene knockouts, and transcriptomics in both host and parasite to better establish the roles of these proteins.

CRedit authorship contribution statement

Kevin McLean: Software, Data curation. **Ava Drake:** Resources. **Michael McGowan:** Resources. **James E. Bron:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Sean J. Monaghan:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Diego Robledo:** Project administration, Funding acquisition. **Andre Van:** Resources. **William Roy:** Supervision. **Dorota Androsuk:** Software, Data curation. **Alexander Dindial:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mark D. Fast:** Writing – review & editing, Project administration. **Claire Joiner:** Resources, Project administration. **Richard Paley:** Resources, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2026.110752.

Data availability

Due to the author's ongoing PhD, raw proteomic data will be made available upon thesis publication or upon reasonable request to the corresponding author.

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