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Secretory/Excretory Protein (SEP) profiling of Lepeophtheirus salmonis (Gulen strain) copepodites induced by Dopamine: A comparative analysis between natural and artificial sea water

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Abstract

Background

Lepeophtheirus salmonis (*L. salmonis*) is a crustacean ectoparasite that significantly impacts the salmon aquaculture industry. The copepodite stage is critical for successful host infestation, as failure to attach to the host results in the parasite's death. Once attached, copepodites must evade the host immune system to establish infestation and grow. Secretory/Excretory Proteins (SEPs) released by copepodites are hypothesized to play a role in immune evasion, supported by the mild inflammatory response observed in certain salmon species, particularly Atlantic salmon.

Methods

SEPs were collected from 1000 copepodites (n = 3) incubated in filtered artificial seawater (ASW) and filtered natural seawater (NSW), both with and without dopamine stimulation. After incubation, copepodites were removed, and SEP samples were buffer-exchanged, trypsin-digested, and analyzed using LC-MS/MS for protein identification and quantified. Identified proteins were classified into various functional categories.

Results

A higher number of protein classes were identified in SEPs from the artificial seawater group with dopamine stimulation (ADC) compared to the natural seawater group (NDC). Specifically, 1039 proteins were identified in the ADC group, while 263 proteins were identified in the artificial seawater group without dopamine stimulation (AC). Identified proteins included proteases (metalloproteases, serine proteases, cysteine proteases, aspartic proteases), protease inhibitors, peroxidases, oxidoreductases, vitellogenins, calcium-binding proteins, ferritin, NK-lysin, metabolite interconversion enzymes, cytoskeletal proteins, and heat-shock proteins.

Conclusions

This study highlights the diversity of SEPs released by *L. salmonis* copepodites. However, further characterization of these proteins is necessary to identify their specific roles in host immune evasion and infestation success and might inform strategies to mitigate its impact on aquaculture.

Background

Lepeophtheirus salmonis, commonly known as salmon lice, is a crustacean ectoparasite belonging to the family Caligidae which infests salmonid fish mostly in the northern hemisphere [1]. Salmon lice pose a significant threat to the Norwegian aquaculture industry, contributing to substantial economic losses

and environmental concerns. These impacts arise from fish mortality, secondary infection, reduced growth due to decreased feed intake and increased operational costs associated with treatment and management measures. In addition to farmed salmon, *L. salmonis* also affects wild Atlantic salmon (*Salmo salar*) further exacerbating its ecological and economic consequences [2]. The life cycle of *L. salmonis* consists of eight stages: two free living nauplius stage, one infective copepodite stage, two attached chalimus stage, two motile pre-adult stage and the final adult stage, with each stage separated by moulting [3]. Among these, the copepodite stage is particularly critical, as it represents the infectious phase during which the parasite must successfully locate and attach to its salmonid host to survive. Failure to attach results in the death of the parasite, making this stage a key determinant of infestation success. Once attached, the copepodite must overcome the host's immune defenses to establish a successful infestation and continue its development [4].

Among salmonid fish, Atlantic salmon are more susceptible to *L. salmonis* compared to other species such as coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*) [5]. Experimental infestations have demonstrated significantly lower copepodite attachment rates in chum (*O. keta*), pink (*O. gorbuscha*), and coho salmon compared to Atlantic salmon [6]. Notably, Atlantic salmon showed epithelial tissue damage at the site of copepodite attachment, accompanied by an initial infiltration of polymorphonuclear cells into dermal blood vessels. However, this inflammatory response reduced after 36 h post-infection, suggesting that copepodites actively modulate the host immune response to facilitate their survival [6].

The interaction between *L. salmonis* and its host is a complex process involving the secretion of bioactive molecules known as secretory/excretory proteins (SEPs). These bioactive molecules are hypothesized to play a pivotal role in modulating the host immune response, facilitating attachment, and ensuring the parasite's survival [7–9]. SEPs are either actively secreted from specialized glands or excreted as waste products onto the host environment, they interfere with the host defenses, aid in feeding and counter immune response through immunomodulation [10]. In blood-feeding parasites, SEPs include enzymes and other proteins that facilitate blood acquisition and digestion, while also excreting excess water, ions and potentially harmful host proteins [11]. The role of parasite SEPs in modulating host immune system has been extensively studied in ticks and helminths. These studies have identified diverse proteins, including proteases, protease inhibitors, and immunomodulatory molecules, which are critical for host-parasite interactions [11–16]. However, the specific roles of *L. salmonis* SEPs in host immune modulation remain poorly understood and require further characterization.

In *L. salmonis*, exocrine glands, such as type 3 tegumental and labial glands have ducts that open onto the host attachment surface. Secretions from these glands are hypothesized to play a role in immunomodulation [4, 17]. Previous studies have identified enzymes such as serine protease, apyrase, astacins, and other proteins from the labial glands of adult *L. salmonis*. Knockdown experiments targeting some of these proteins have demonstrated their importance in successful infestation [9, 18, 19]. Additionally, genes encoding proteins such as cathepsin B, cathepsin L, heme peroxidase,

fibronectin II domain, heat-shock proteins (HSPs) and ferritins have been identified in the *L. salmonis* genome, which could be important in host-parasite interactions [20–25].

The proteomic analyses of SEPs from pre-adult and adult stages of *L. salmonis* using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have identified key molecules such as prostaglandin E2 (PGE2), serine endopeptidase, cysteine endopeptidase, metalloendopeptidase, vitellogenins, calreticulin, antioxidant enzymes, and metabolic enzymes, all of which may contribute to immune evasion and host tissue degradation [8, 26]. Furthermore, exposure of peripheral blood leucocytes to SEPs from the chalimus stage of *L. salmonis* has been shown to reduce chemotactic response, indicating their immunomodulatory potential [27]. Unlike Atlantic salmon, coho salmon exhibit rejection of copepodites within the first 48 h of infestation due to robust inflammatory response and epithelial hyperplasia, highlighting the importance of early immune responses in controlling lice infestations [6].

The success of salmon lice infestation depends heavily on the copepodite stage, making research on the composition of copepodite SEP essential. Identifying proteins in the SEPs of the copepodite stage could provide insights into the very early mechanisms of immunomodulation employed by this infective stage to ensure successful parasitism. A recent study has identified SEP proteins in copepodites of the IoA 00 strain from Scotland using isophorone as inducer. However, deeper understanding of the infestation mechanism of different copepodites strains is necessary to develop effective control strategies. Additionally, the choice of seawater (artificial or natural) for efficient in vitro SEP profiling remains an important consideration for future studies.

In this study, we aimed to identify and compare the protein composition of SEPs released by copepodites of the Gulen strain of *L. salmonis* incubated in artificial and natural seawater utilizing LC-MS/MS based proteomics. Dopamine, a neurotransmitter commonly used to stimulate SEP release, was employed to enhance SEP yield by activating specific receptors in the parasites [8, 28]. This study provides valuable insights into the molecular composition of copepodite SEPs and their potential roles in host-parasite interactions and immunomodulation, contributing to the broader understanding of *L. salmonis* parasitism and its control.

Material and Methods

Copepodites

Copepodites of *L. salmonis* (Gulen strain) were obtained from the Aquaculture Research Station land facility at Kårvika, Tromsø. The egg strings were incubated in seawater with temperature 12.4 ± 0.1 ^{o}C and the copepodites were collected on the 12th day post-incubation. The collected copepodites were transported to the laboratory in sterile cell culture flasks and kept 7 ^{o}C for 1–2 h until processing.

Experimental seawater

Incubation of copepodites for SEP collection was performed in both 0.22 µm membrane filtered natural seawater (NSW) and artificial seawater (ASW). The NSW was collected from the same water source as egg string incubation from the Aquaculture Research Station land facility at Kårvika. The NSW had a salinity of 35 ppt and an osmolarity of 1012 mOsm. The ASW was prepared by dissolving specific salts as shown in Supplementary Table S1. The salinity and osmolarity of ASW were adjusted to match those of NSW.

SEP collection

Prior to incubation, copepodites were washed three times with fresh and filtered water (NSW or ASW). Washed copepodites (n = 1000) were transferred to 10 mL of filtered NSW or ASW per replicate. Dopamine stimulation was performed by adding 0.5 mM dopamine (Sigma Aldrich, St. Louis, USA) to NSW or ASW and were incubated in the dark for 2 h at 10 ^{o}C on a shaker at 60 rpm. Parallel unstimulated controls (ASW or NSW without dopamine) were also included. Additionally, NSW or ASW containing only dopamine (without copepodites) was used as a control (Fig. 1). The experimental sample groups were as follows: ASW dopamine stimulated copepodites (ADC), ASW unstimulated copepodites (AC), ASW dopamine control (AD), NSW dopamine stimulated copepodites (NDC), NSW unstimulated copepodites (NC), NSW dopamine control (ND). Each experimental condition was prepared in triplicate. Detailed experimental setup is shown in Fig. 1.

SEP buffer exchange and concentration

Following incubation, copepodites were removed from the samples and the SEP containing seawater including controls, was transferred to 3 kDa molecular weight cut-off (MWCO) protein concentrator columns (Pierce,Thermo Fisher Scientific, Rockford, USA). Buffer exchange was performed three times with 50 mM ammmonium hydrogen carbonate (Sigma Aldrich, St. Louis, USA) by centrifugation at 3500 x g for 1.5 h at 4 ^{o}C, for each exchange. After the final buffer exchange, samples were concentrated to a final volume of 200–400 µL by centrifugation for an additional 1.5 h.

Sample preparation for proteomics

Sample preparation and the Mass spectrometry-based proteomic analyses were performed at UiT Proteomics and Metabolomics Core Facility (PRiME). The total volume of concentrated SEP samples was evaporated in a vacuum concentrator (Centrivap Concentrator, Labconco, Kansas city, USA) and reconstituted in 20 µL of 2% Sodium dodecyl sulfate (SDS) in 50 mM Triethylammonium bicarbonate (TEAB) (Thermo Fisher Scientific, Rockford, USA). Reduction of disulfide bonds was achieved by adding 1,4-dithiothreitol (Sigma Aldrich, St. Louis, USA) to a final concentration of 20 mM, followed by boiling at 95°C for 10 minutes. The disulfide bridges were then alkylated by adding iodoacetamide at a final concentration of 40 mM and incubated for 30 min at room temperature in the dark (Amresco, Ohio, USA).

Protein trapping and digestion were performed using the S-Trap micro protocol (Protifi, New York, USA) with minor modifications. After the initial spin-through, samples were reloaded onto the column to ensure efficient protein trapping, and the number of wash steps increased to five. Proteins were digested overnight at 37 ^{o}C with 2.5 µg trypsin (Promega, Madison, USA) in 40 µL of 50 mM TEAB per sample. Post-digestion, peptides were eluted from the S-Trap column, evaporated to dryness, and reconstituted in 100 µL of 0.5% Trifluoracetic acid (TFA) (Thermo Fisher Scientific, Rockford, USA). Peptides were further concentrated and purified using Omix C18 pipette tips (Agilent, Santa Clara, USA) according to manufacturer's protocol. Purified peptide samples were dried in a vacuum concentrator and reconstituted in 15 µL of 0.1% formic acid. Peptide concentrations were measured on a spectrophotometer (Nanodrop ONE, Thermo Fisher Scientific, Rockford, USA) at 205 nm.

LC-MS/MS

For peptide separation, 0.25 µg peptides per sample were fractionated using 2-100% acetonitrile gradient in 0.1% formic acid at a flow rate of 300 nL/min over 93 min on an EASY-nLC1200 system (Thermo Fisher Scientific, Bremen, Germany) equipped with an EASY-Spray RP column (50 cm x 75 µm ID, C18, 2 µm particles, 100 Å pore size, Thermo Fisher Scientific, Bremen, Germany). The separated peptides were analyzed using an Orbitrap Exploris 480 (Thermo Fisher Scientific, Bremen, Germany) mass spectrometer. Ionization was performed with a spray voltage of 1500 volts and the temperature of the ion transfer tube was set at 275ºC. Peptides were analyzed in positive mode with peptide scan range of 350–1200 m/z, followed by 40 data-dependent high collision dissociation (HCD) MS/MS scans (1 µscan), using collision energy of 28% and an isolation window of 1.6 m/z. Dynamic exclusion was applied for 45 s after the second fragmentation event and unassigned charged ions were excluded from analysis.

Data processing and analysis

Raw data files were processed in Proteome Discoverer 3.1.1.93 (Thermo Fisher Scientific, Massachusetts, USA) as described by [29]. The protein identification was performed by searching MS/MS data against *Lepeophtheirus salmonis* Uniprot Proteomes FASTA database (UP000675881, containing 17,041 protein entries as of 28/08/2024) using SEQUEST-HT algorithm (Proteome Discoverer 3.1.1.93). Search parameters used included: enzyme digestion- trypsin, maximum missed cleavage = 2, minimum peptide length = 6, precursor mass tolerance and fragment mass tolerance = 10 ppm and 0.06 Da respectively. The variable modifications allowed were oxidation of methionine, acetylation of protein N-terminus and fixed modification was carbamidomethylation of cysteine.

The protein abundance ratio between the sample groups was determined using the label-free quantification (LFQ) workflow in Proteome Discoverer, employing the Minora Feature Detector node and the analysis of variance (ANOVA) method (individual proteins). The false discovery rate (FDR) was obtained using a decoy database and only proteins with high confidence and master proteins, identified

by more than one peptide were included in further analysis. Statistical evaluation was performed using the Percolator node, with the FDR threshold set below 1%. Proteins identified in AD group (control) were eliminated from ADC and AC groups. Similarly, proteins found in ND group (control) were removed from NDC and NC groups. Only proteins identified with high confidence in at least two out of three replicates were selected. The filtering steps are shown in Fig. 3 and S2

The final list of non-redundant protein IDs was then classified into functional protein classes using the PANTHER 19.0 annotation tool (http://www.pantherdb.org). The presence of signal peptides was determined using SignalP 6.0 [30].

Result

Protein identification

The LC–MS/MS analysis identified a total of 1815 non-redundant protein groups. The combined proteome data set (ASW and NSW) was generated by merging 45248 identified spectra (Peptide Spectrum Matches, PSMs) from 10118 different peptide groups.

To the best of our knowledge, this dataset constitutes the largest collection of proteins and peptides of *L. salmonis* SEP from ASW and NSW identified to date.

Raw data files from all experimental groups (ASW and NSW) were analyzed using Proteome Discoverer 3.1.1.93, applying default filters for Master proteins and protein FDR high confidence. In the ASW dataset, 1479 proteins were identified in dopamine stimulated group (ADC), followed by 545 proteins in the unstimulated group (AC), of which 454 proteins were common to both the groups, as shown in Fig. 2a. Whereas, in the NSW dataset, 997 proteins were identified in the dopamine stimulated group (NDC), while 659 proteins were identified in unstimulated (NC) group, sharing 501 proteins in common (Fig. 2b). To evaluate the suitability of ASW and NSW for downstream analysis, we compared the control groups (AD and ND). The result showed lower number of total proteins IDs in ASW control group compared to NSW control group, indicating reduced background noise in ASW control group (Fig. 2c).

Downstream filtering and protein quantification

The downstream filtering steps were applied uniformly for both ASW and NSW dataset. Post- filtering, the ASW dataset revealed 1039 proteins in the dopamine stimulated (ADC) group, 263 proteins in the unstimulated (AC) group (Fig. 3). The protein abundance ratio between ADC and AC groups showed that 74 proteins were at least 1.5-fold more abundant in the ADC group (log2 fold change of 1.5 or higher with $P > 0.05$). Similarly, the protein abundance ratio between NDC and NC groups showed 108 proteins were at least 1.5-fold more abundant in the NDC group ($P > 0.05$). When comparing protein abundance ratio between ADC and NDC groups, 24 proteins were at least 1.5-fold more abundant ($P > 0.05$) in the ADC group. Further, the results for NSW post-filtering showed 638 proteins in the NDC group and 364 in

unstimulated (NC) group (Supplementary Figure S2). The details of the protein classes found in NSW are provided in Supplementary Figure S3.

Functional annotation of proteins

Functional annotation of proteins in the ADC/NDC and AC/NC groups was performed using the PANTHER classification system. The analysis identified several important protein classes, including proteases (metalloproteases, serine proteases, cysteine proteases, aspartic proteases), protease inhibitors, peroxidases, vitellogenin related proteins and ferritin as shown in Fig. 4a and S3. Among the important protein classes identified, metalloproteases represent the largest proportion of proteases identified, followed by serine proteases and other proteases. These protein classes are known to play important roles in host-parasite interactions, as reported in previous studies on other parasite species. Among total proteins IDs in ADC group, 249 protein IDs (24%) were categorized as 'uncategorized', while the majority (61%) of proteins other than important ones listed in Fig. 4a, are classified as 'other protein classes' (Fig. 4b) in this study. Details of the proteins in the 'other protein classes' category are provided in Supplementary figure S1. Similar protein classes were identified in NDC and NC groups, although the total number of protein IDs differed compared to the ADC and AC (Figs. 4, S1, and S3). Notably, not all protein IDs within the identified classes contained signal peptides. The percentage of proteins with signal peptide varied from 0–100% as indicated next to the bars in Figs. 4, S1 and S3.

Discussion

This study provides a comprehensive proteomic analysis of secretory/excretory proteins (SEPs) released by *L. salmonis* copepodites from the Gulen strain, with a comparative evaluation of artificial seawater (ASW) and natural seawater (NSW) as incubation media. Using LC-MS/MS, we identified 1815 non-redundant protein groups, constituting the largest dataset of *L. salmonis* SEPs reported to date. Our findings demonstrate the important role of dopamine stimulation in enhancing SEP release and highlight the suitability of ASW for in vitro SEP profiling due to its lower background protein noise compared to NSW.

Suitability of artificial seawater for SEP profiling

The comparison of control groups (AD and ND) revealed a significantly lower number of protein identifications in ASW controls compared to NSW controls. This indicates that ASW provides a cleaner background, minimizing contamination and non-specific protein noise, thereby improving the reliability of downstream analyses. Such controlled environments are particularly important for studying SEPs, as seen in other parasitic species like *Fasciola hepatica, Trichinella spiralis*, and *L. salmonis*, where proteomic analyses have been used to identify immunomodulatory proteins critical for host-parasite interactions [8, 10, 31]. The reduced background in ASW enhances the detection of parasite-derived proteins, making it a more suitable medium for SEP collection and characterization.

Impact of dopamine stimulation on SEP release

Dopamine stimulation at the concentration used, significantly increased the number of proteins identified in both ASW and NSW groups, with the ADC group yielding 1039 proteins compared to 263 proteins in the unstimulated AC group. Similarly, the NDC group yielded 997 proteins compared to 659 proteins in the NC group. These results aligning with previous studies, demonstrated the role of dopamine in stimulating secretory activity by activating specific receptors in parasitic organisms like blacklegged tick *Ixodes scapularis* and adult salmon lice *L. salmonis* [26–28, 32], thus showing dopamine-induced SEP release may mimic the physiological conditions encountered by copepodites during host attachment.

Comparison between ASW and NSW

The higher number of protein identifications in the ADC group compared to the NDC group (1039 *vs*. 638 proteins) suggests that ASW may provide a more conducive environment for SEP release under dopamine stimulation. This finding is consistent with the observed lower background noise in ASW controls, which likely enhances the detection of parasite-derived proteins. Additionally, the identification of 24 differentially ($P < 0.05$) more abundant proteins (at least 1.5-fold change in abundance) in the ADC group compared to the NDC group, further supports the suitability of ASW for in vitro SEP profiling. These findings underscore the importance of selecting an appropriate incubation medium for SEP studies, as it can significantly influence the quality and quantity of the proteomic data obtained.

Functional insights into identified protein classes and implications for host-parasite interactions

Functional annotation of the identified proteins in copepodite SEPs revealed several key classes, including metalloproteases, serine proteases, cysteine proteases, aspartic proteases, protease inhibitors, and peroxidases. This is consistent with previous studies on *L. salmonis* secretions, which have identified a wide range of secretory proteins across different life stages, including pre-adults, adults, and recently in copepodites. These proteins are thought to play critical roles in immunomodulation, tissue degradation and survival on the host [8, 9, 26, 32, 33]. For instance, proteomic analyses of adult *L. salmonis* SEPs have identified serine endopeptidases, cysteine endopeptidases, metalloproteases, and prostaglandin E2 (PGE2), which are believed to contribute to immune suppression and tissue invasion [8, 26]. Recent study on copepodite SEPs of IoA 00 strain have also highlighted the presence of proteases and other bioactive molecules that may facilitate host attachment and immune evasion [33].

These protein classes are known to play critical roles in host-parasite interactions in other parasitic species, such as ticks and helminths, where SEPs have been shown to modulate host immune responses, degrade tissue, inhibit coagulation and inflammation, enabling prolonged feeding, immune evasion and promote parasite survival, [11–16]. For example, metalloproteases and serine proteases have been implicated in tissue invasion and degradation in various parasites, such as *Ancylostoma caninum* and *Trichuris muris*, facilitating parasite attachment and feeding at the early infection process [34, 35]. Similarly, cysteine and aspartic proteases found in *Trichinella spiralis* SEP degrades host

immunoglobulins contributing to immunomodulation [36, 37]. Protease inhibitors, on the other hand, may counteract host proteases, thereby protecting the parasite from host immune responses [13, 38].

The identification of other proteins such as vitellogenin-related proteins, ferritin and calcium binding protein in the SEPs is particularly noteworthy. Vitellogenins, which have been also identified in adult *L. salmonis* SEPs, are known to possess antimicrobial and antioxidant properties in other organisms, such as honeybees (*Apis cerana*), and may contribute to the parasite's defense against host immune responses [39]. Ferritin, an iron-binding protein, plays a crucial role in iron metabolism and oxidative stress regulation, which are essential for parasite survival and reproduction, as reported in *L. salmonis* and other parasites like *Fasciola hepatica* [8, 25, 40, 41]. Similarly, calcium binding proteins are important immunomodulatory molecules that aid in immune evasion and parasite survival in host, as reported in *L. salmonis* and other parasites [8, 33, 42, 43]. The identification of HSPs in copepodite SEPs in this study further supports their role in stress responses, facilitating the parasite’s adaptation to the host environment. HSPs have been systematically identified in *L. salmonis* and are known to be upregulated during environmental stress, suggesting their importance in parasite survival [24]. These findings underscore the multifunctional nature of SEPs in *L. salmonis* copepodites and provide valuable insights into the molecular mechanisms underlying parasitism and their potential roles in host-parasite interactions and immune modulations.

Interestingly, not all identified proteins within key functional classes contained signal peptides, with the percentage of signal peptide-containing proteins varying from 0% to 100%. This observation raises questions about the mechanisms of protein secretion in *L. salmonis* and suggests the potential involvement of non-classical secretion pathways, as reported in other parasitic species such as *Fasciola hepatica and Clonorchis sinensis* [30, 41, 44]. Further studies are needed to elucidate the alternative secretion mechanisms and to determine the functional relevance of these proteins in host-parasite interactions.

Limitations and future directions

While this study provides valuable insights into the protein composition of *L. salmonis* copepodite SEPs, certain limitations must be acknowledged. For instance, it is unclear whether all the proteins identified in this study are released onto the host surface, as the SEP samples were not collected directly from the site of attachment. Additionally, some proteins involved in blood meal acquisition and processing were identified, even though the copepodite stage does not feed on blood. These findings may reflect damage to the copepodites during the SEP collection process. Future research should focus on functional characterization of the identified proteins, particularly their roles in immune modulation and host attachment, through gene knockdown or overexpression experiments. Furthermore, the observed differences in protein profiles between ASW and NSW highlight the need for further exploration of how environmental factors influence SEP composition and function.

Finally, the identification of strain-specific differences in SEP composition, as demonstrated in this study and others [33], underscores the importance of studying multiple *L. salmonis* strains to gain a

comprehensive understanding of their parasitic strategies. Such insights could inform the development of effective control strategies to mitigate the impact of *L. salmonis* on aquaculture.

Conclusion

This study represents a significant advancement in our understanding of *L. salmonis* copepodite SEPs, providing the largest dataset of proteins identified from this life stage to date. The findings highlight the utility of ASW for in vitro SEP profiling and underscore the importance of dopamine stimulation in enhancing SEP release. The identification of key protein classes with potential roles in host-parasite interactions offers valuable insights into the molecular mechanisms underlying *L. salmonis* parasitism. Future research should focus on functional characterization of these proteins and their roles in immune modulation, as well as the development of effective control strategies to combat *L. salmonis* infestations in aquaculture.

Abbreviations

ANOVA
Analysis of variance
ASW
Artificial seawater
FDR
False Discovery rate
HSPs
Heat-shock proteins
LC-MS
Liquid chromatography-mass spectrometry
L. salmonis
Lepeophtheirus salmonis
LFQ
Label-free quantification
MWCO
Molecular weight cut-off
NSW
Natural seawater
PGE2
Prostaglandin E2
SDS
Sodium dodecyl sulfate
SEP
Secretory/Excretory Protein
TEAB

Triethylammonium bicarbonate
TFA
Trifluoracetic acid

Declarations

Ethics approval and consent to participate

Not applicable. Formal approval of the experimental protocol by the Norwegian Animal Research Authority (NARA) is not required since it falls under the purpose of recognized animal husbandry. These practices are exempt from the European convention on the protection of animals used for scientific purposes (EU Directive (2010/63/EU, cf. article 5d), which Norway has subscribed and implemented.

Consent for publication

Not applicable

Availability of data and materials

The data which support the conclusions are included within the article and its additional files. Other proteomic dataset will be made available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

VNT and JKS conceived and designed the experiments. VNT performed the experiment. TAG and J-AB carried out proteomics sample preparation and LC-MS/MS run. VNT, J-AB, MC, MP, and JKS analyzed and visualized the data. VNT and JKS wrote the original manuscript draft. VNT, J-AB, TAG, MC, MP and JKS revised the manuscript. JKS Supervision, funding acquisition and Project administration. All authors read and approved the final version of the manuscript.

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Figures

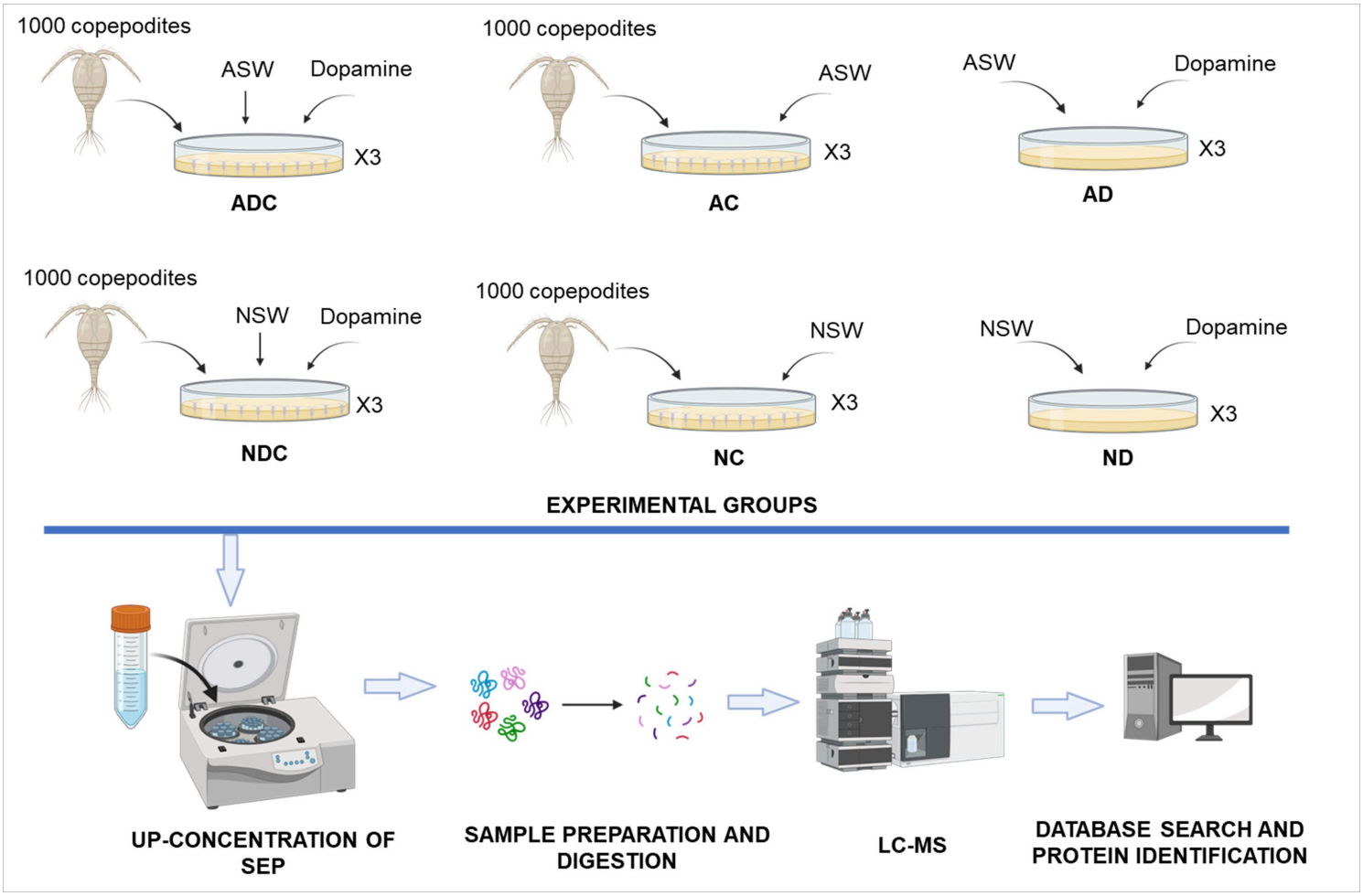


Figure 1

Experimental setup showing different groups and downstream sample preparation steps for SEP profiling. Graphical illustrations were created using the BioRender web tool (BioRender.com)

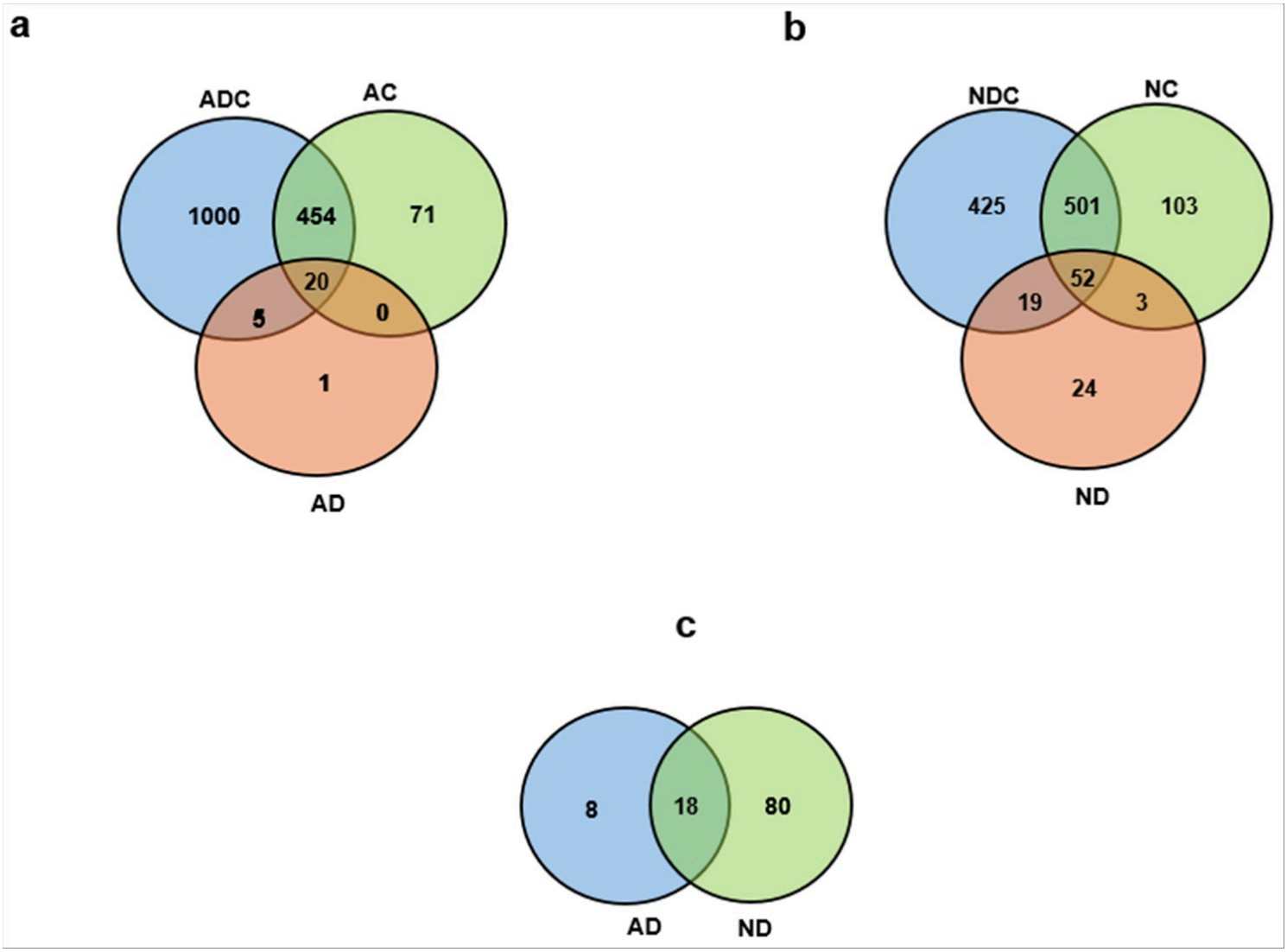


Figure 2

Venn diagram showing protein identified in (a) different ASW groups, (b) different NSW groups and (c) ASW and NSW controls

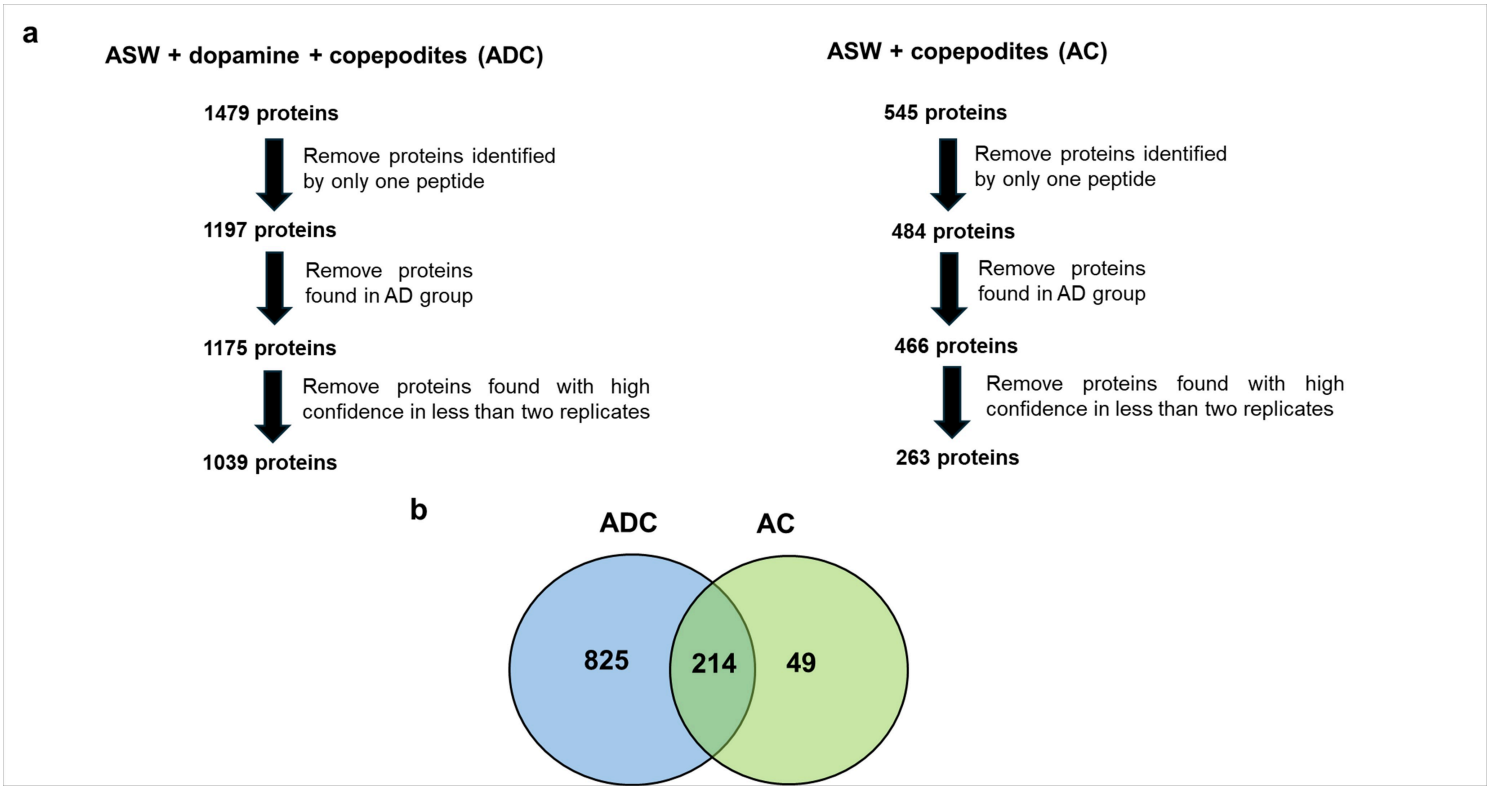


Figure 3

Final number of protein IDs obtained for ASW groups, (a) using filtering steps in ADC and AC (b) represented in Venn diagram

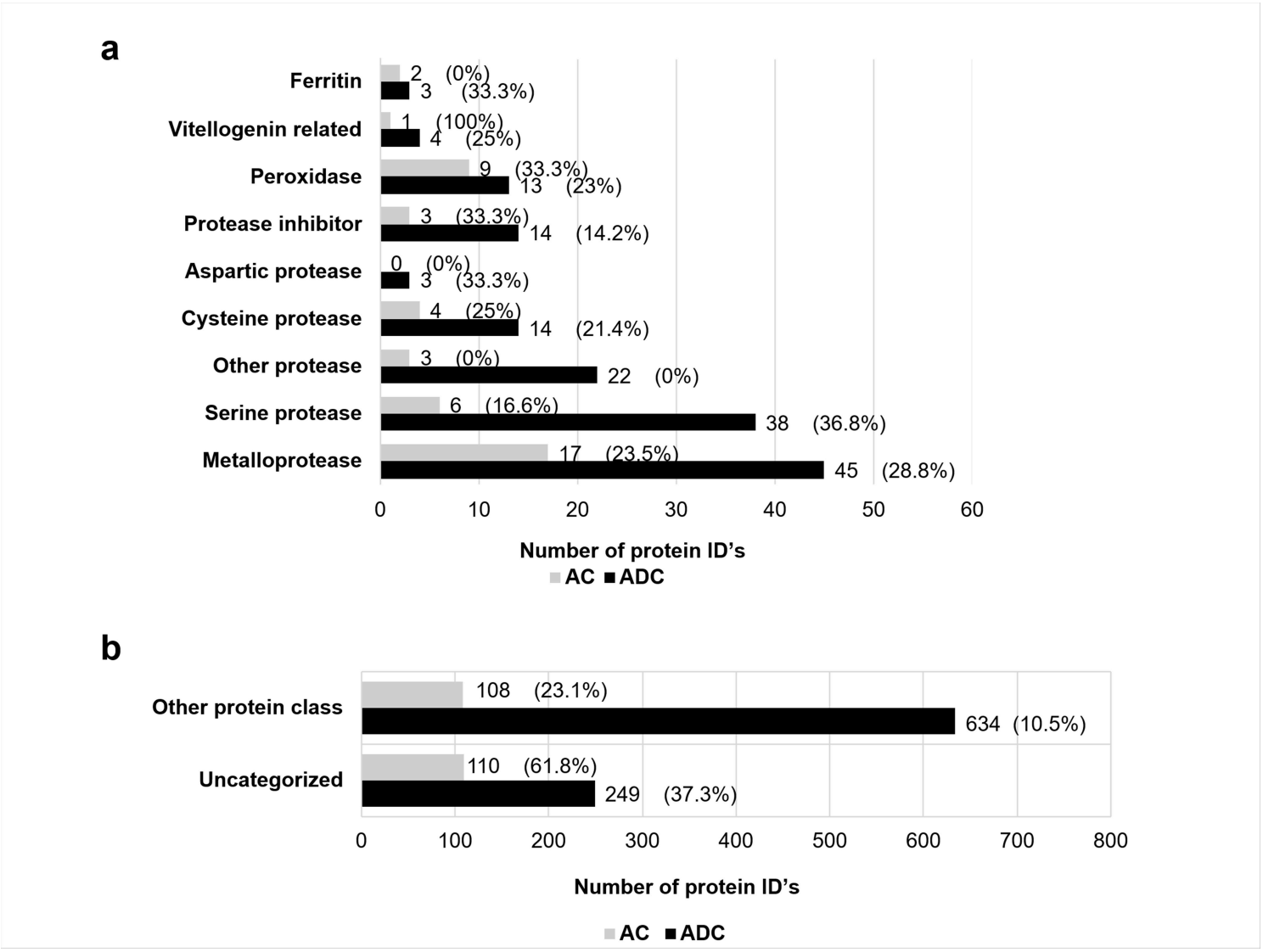


Figure 4

Bar graph (a) shows important protein classes identified in ADC and AC groups, (b) distribution of uncategorized and 'other protein classes' in the ADC and AC groups. The percentage (%) of proteins with signal peptides is shown next to the bars.

Supplementary Files

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