

University of Prince Edward Island

Faculty of Veterinary Medicine
Summary of Dissertation

Submitted in Partial Fulfilment
of the Requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

Dylan Michaud

Department of Pathology & Microbiology

Supervisory Committee

Dr. Fred Kibenge, Chair
Dr. Mark Fast, Supervisor
Dr. Spencer Greenwood
Dr. David Speare
Dr. Mark Braceland

Examination Committee

Dr. Chelsea Martin, Chair
Dr. Spencer Greenwood
Dr. Mark Fast, Supervisor
Dr. David Speare
Dr. James Bron, External Examiner

**Investigations of Development, Virulence, and Evolution of the
Salmon Louse (*Lepeophtheirus salmonis*)**

Sea lice are important ectoparasitic copepods of wild and farmed salmonids. Mirroring trends observed in terrestrial agriculture and livestock industries, the intensification of aquaculture practices has led to the emergence of resistance to multiple chemotherapeutics. Moreover, these conditions may exacerbate the selection pressures favoring traits related to virulence, development, transmission, or reproduction in parasites. The development of novel management strategies has been hindered by knowledge gaps in functional and genomic characterizations of key pathways, such as virulence, development, and sexual dimorphism, for sea lice compared to similar pests in other industries. In the present thesis, we address these knowledge gaps through targeted qPCR, RNA interference (RNAi), and RNA sequencing coupled with evolutionary analyses. Transcriptomic analyses of pre-adult and adult stages of *L. salmonis* revealed both a conservation of sex-biased expression patterns across stage or sex-specific differences for several candidate reproductive transcripts. Previous suggestions of elevated evolutionary rates for male-biased transcripts were confirmed using sophisticated empirical evidence. Male-biased transcripts were confirmed to have greater relaxation of evolutionary constraint, while female-biased transcripts had the most optimal codon usage. The nuanced aspects of mating-responsive gene expression were also highlighted by the lack differential expression for female lice which received a spermatophore when compared to those who did not. The development of *L. salmonis* was demonstrated to be most significantly impacted at three major junctures within the chitin synthesis pathway (CSP). RNAi mediated expression knockdown for CHS1, CDA5956 and GFAT resulted in the complete abrogation of infectivity, while other CSP transcript knockdowns did not, highlighting dynamic nature of the CSP and underscoring the need for

functional studies to inform novel management strategies. The synergistic effects of several chemotherapeutics following initial treatment of lufenuron, the latest chemotherapeutic to be introduced in aquaculture, was also assessed using bioassays. The most notable reduction for both adult female and male *C. rogercresseyi* occurred for lice treated with lufenuron and 5ppb azamethiphos. Furthermore, 44k microarrays were constructed and used to evaluate differential gene expression for lice treated with 100 or 1000 ppb lufenuron across 3-, 5- and 7-dph. Overall, 178 DEGs were associated with lufenuron treatment, the majority of which were downregulated, while 3687 DEGs were associated with copepodid age. Functions of three putative virulence factors assumed to be involved at the host-parasite interface were assessed using RNAi. Trypsin 1c were revealed to significantly reduce the hematophagous behavior and survival of adult female *L. salmonis*. Links between intensive farming and elevated pathogen virulence have been shown in the agricultural and livestock industries and recently similar trends have been observed for intensive aquaculture farming. An assessment for the evolution of virulence for lice from the Bay of Fundy were assessed by comparing whole transcriptome data via RNAseq for farm- and wild-collected lice. Farm lice were associated with increased lesion severity on host Atlantic salmon where 871 transcripts were differentially expressed between the two sources, several of which were had ontologies associated with virulence. The works described in this dissertation provide comprehensive transcriptomic profiling of virulence, development, and sexual dimorphism of sea lice. It also provides the first empirical assessment of evolutionary trends and of elevated virulence for Canadian farm-source *L. salmonis*.

Publications

Braden L, Michaud D, Igboeli O, Dondrup M, Hamre L, Dalvin S, Purcell S, Kongshaug H, Eichner C, Nilsen F and Fast M. 2020. Identification of critical enzymes in the salmon louse pathway as revealed by RNA interference-mediated abrogation of infectivity. *Int. J. Parasitol.* 50(10-11): 873-889. <https://doi.org/10.1016/j.ijpara.2020.06.007>

Michaud DR, Poley JD, and Fast MD. 2019. Sex-biased gene expression and evolution of candidate reproductive transcripts in adult stages of salmon lice (*Lepeophtheirus salmonis*). *FACETS* 4: 254–274. <https://doi.org/10.1139/facets-2018-0016>

Braden, L.M., Michaud, D., Groman, D. et al. 2023. Rejection of *Lepeophtheirus salmonis* driven in part by chitin sensing is not impacted by seawater acclimitization in Coho salmon (*Oncorhynchus kisutch*). *Sci Rep* 13, 9685. <https://doi.org/10.1038/s41598-023-36632-0>

Groves L, Whyte SK, Purcell SL, Michaud DR, Cai WC, Garber AF, Fast MD. 2023. Temperature impacts Atlantic salmon's (*Salmo salar*) immunological response to infectious salmon anemia virus (ISAv). *Fish and Shellfish Immunology Reports* 4, 100099. <https://doi.org/10.1016/j.fsirep.2023.100099>

Awards

Bioscience Presentation - UPEI Graduate Studies and Research Conference, May 2018 Awarded second overall bioscience presentation at the UPEI Graduate Studies and Research Conference held at the University of Prince Edward Island.

Science Atlantic Graduate Research Award, March 2017 Awarded second overall graduate student presentation at the Science Atlantic Aquaculture & Fisheries and Biology Conference held at St. Xavier University.

UPEI Diamond Award, April 2017 Awarded the University of Prince Edward Island's Diamond Award for Health & Wellness Leadership for the 2016-2017 academic year. This award recognizes a student's personal development and achievements outside the classroom, which positivity impacts campus life and external community.

CERC Early Career Development Grant, June 2016 Among the few awarded the CERC Early Career Development Grant which funded attendance at the "Exploratory Analysis of Biological Data using R" workshop hosted by Canadian Bioinformatics Workshops in Toronto, Ontario.

Biographical Data

Born: Timmins, Ontario. Undergraduate: BSc: Honors Specialization in Biology, University of Western Ontario. 2015.