



Mémoire d'habilitation à diriger des recherches: The Russian Dolls

Nolwenn M. Dheilly

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Université de Montpellier
Ecole Doctorale GAIA
2020

Mémoire d'Habilitation à Diriger des Recherche



DHEILLY Nolwenn M

**Université de Montpellier
Ecole Doctorale GAIA
2020**

Mémoire d'Habilitation à Diriger des Recherche

DHEILLY Nolwenn M

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1. Curriculum vitae

1.1. Education

- 2010 **PhD in Biological Sciences** – Macquarie University, Sydney, Australia (April 9, 2010)
- 2005 **Master (M.Sc) in Biology and Biotechnology**, specialization in Proteomics – Université des Sciences et technologies de Lille, France
- 2004 **Maitrise (M.Sc) in Population Biology and Marine Ecosystems**, specialization in Biodiversity and Biochemical Analysis – Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, France
- 2003 **Licence (B.Sc) in Biology of Aquatic Organisms**, specialization in Toxicology - Université de Bretagne Occidentale, France
- 2001 **Diplome Universitaire et Technologique (DUT) in Biological and Biochemical analyses** - Université de Bretagne Occidentale, France

1.2. Professional experience

PROJECT LEADER

Since July 2019

Unité de Génétique Virale et Biosécurité, Laboratoire de Ploufragan-Plouzané-Niort de l' Agence Nationale de Sécurité Sanitaire (ANSES) de l'alimentation, de l'environnement et du travail, France

Awards

- 2020-2021 Dispositif SAD "Stratégie d'Attractivité Durable" of Région Bretagne : *Microbiome and Macrobiome of swine: identification of biomarkers of health and disease* (Beneficiary, Principal investigator : Yannick Blanchard) **76000 €**

TENURE TRACK ASSISTANT PROFESSOR

August 2015-January 2020

School of Marine and Atmospheric Sciences at Stony Brook University, NY, USA.

The Dheilly Lab: Host-Parasite-Microbes interactions.

Awards

- 2017-2018 Minghua Zhang Early Career Faculty Innovation Fund : *Real-time investigation of fish host adaptation to infection by a helminth parasite* (Principal Investigator) **USD\$ 37,000**
- 2018-2021 Marsden Fund Research Grant: *Microbes at the helm: are microbiomes shaping parasite phenotypes?* (Associate Investigator, Principal investigator : Prof. Robert Poulin) **NZD\$ 890,000**
- 2017 CHILDCARE Award by the Society for Molecular Biology and Evolution to facilitate attendance and presentation **USD\$ 1000**

- 2017-2018 AWARD from the EPPLEY Foundation For Research: *Microbiome-Parasite Interactions: The tapeworm-Stickleback Fish Parasite-Host System* (Principal Investigator) **USD\$ 7,832**
- 2016-2017 Minghua Zhang Early Career Faculty Innovation Fund: *A model system to study the role of parasite microbiome role in infection* (Principal Investigator) **USD\$ 36,660**
- 2016-2017 AWARD from the EPPLEY Foundation For Research: *Microbiome-Parasite Interactions: The tapeworm-Stickleback Fish Parasite-Host System* (Principal Investigator) **USD\$ 24,893**
- 2015 Starting fund for lab renovation, equipment and salary **USD\$ 330,000**

Teaching

Member of the Marine Sciences graduate program and of the Ecology and Evolution graduate program, Stony Brook University, USA

- *Fall 2015* Master and PhD students in Marine Sciences - development of a new course entitled: Functional and Integrative Marine Genomics (30 h)
- *Fall 2016 and 2017* Master and PhD students in Marine Sciences, Biomedical Engineering, Ecology and Evolution, Molecular and Cellular Biology - development of a new course entitled: Next Generation sequencing Application in Functional Genomics (45 h)
- *Spring 2017 and 2018* Master and PhD students in Marine Sciences: Revision and teaching of a course entitled: Practical skills for scientists and Responsible conduct of Research (45 h)

Synergistic activities

- Co-chair of Stony Brook University Research Committee
 - ✓ Elected member of Stony Brook University Research Committee (2016-2018)
 - ✓ Recipient of the 2018 Service Award from the Research Committee of the University Senate
 - ✓ Elected co-chair of Stony Brook University Research Committee (since 2018)
- Completed the Academic Advising Certificate Program (provided by the Division of Undergraduate Education, Stony Brook University), Spring 2017
- Attended Grant writing workshops
 - ✓ Write Winning Grant Proposals seminar, hosted by the Office of Proposal Development and presented by Grant Writers' Seminars and Workshops, LLC
 - ✓ Write NIH Research proposals, hosted by the Office of Proposal Development and presented by Beth Schuchter
- Participate in the development of Genomics education at SBU
 - ✓ Founding member of SBU Genomics and Member of the Steering Committee
 - ✓ Maintenance of SBU Genomics Website, Twitter and Scoop it!
 - ✓ Organize Genomics-related events for undergraduates, graduates and faculty members: Data Carpentry Genomics Workshop, C-STEP meet Genomics Researchers
- Participate in Departmental activities:
 - ✓ Volunteer Science Judge at the Bay scallop bowl
 - ✓ Presentation during the prospective students weekend events
 - ✓ Volunteer at the new graduate students breakout sessions
 - ✓ Open lab for visiting prospective undergraduate students

POSTDOCTORAL FELLOW

March 2011 - August 2013

**UMR MIVEGEC «Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle»,
UMR 5244 CNRS «Ecologie et Evolution des Interactions», Université de Perpignan Via Domitia**

Research projects

- Integrative study of a parasitic manipulation: the case of the ladybeetle *Coleomegilla maculata* that protects the cocoon of the parasitic wasp *Dinocampus coccinellae*
- Characterizations of the effects of acid stress and thermal stress on the immune system of the coral *Pocillopora damicornis* and on its susceptibility to *Vibrio coralliilyticus*
- Investigations on the specificity of *Biomphalaria glabrata* immune system: identification of the molecular determinants of specificity and role of epigenetic regulations

Teaching

- 2012: Lectures, Bachelor courses, at the Institut Universitaire et Technologique of Perpignan
 - Biological and Biochemical analyses, first year, ABB 1: Digestion and absorption (11 hours)
 - Biological and Biochemical analyses, first year, ABB 1: Reproduction (11 hours)
- 2011-2012: Lectures, Graduate course, at the Université de Perpignan Via Domitia
 - Master 2 of Integrative Biology: Shotgun proteomics (10 hours)

POSTDOCTORAL FELLOW

March 2010 - February 2011

UMR 100 IFREMER «Physiologie et Ecophysiologie des Mollusques Marins». Université de Caen – Basse Normandie, Institut de Biologie Fondamentale et Appliquée / IFREMER Centre de Brest, Plouzané.

Research project

- Development of a microarray for *Crassostrea gigas* and comparative analysis of gametogenesis in diploid and triploid oysters: towards a better understanding of the mechanisms behind infertility of triploid oysters.

PhD CANDIDATE

May 2006 – November 2009

Marine and Freshwater Biology Laboratory, Macquarie University, Sydney, Australia

Thesis title: Proteomic analysis of sea urchin immune responses and characterization of highly variable immune response proteins (313 p)

Thesis Supervisors: Prof. David A. Raftos and Dr. Sham V. Nair

Awards

- 2009 TRAVEL Grant by the 11th International Congress of the International Society for Developmental and Comparative Immunology to facilitate attendance and presentation
- 2008 TRAVEL Grant by the 33rd Lorne conference on Protein Structure and Function to facilitate attendance and presentation
- 2007 Macquarie University Postgraduate Research Fund (PGRF grant): Proteomic analysis of defensive proteins from sea urchins. **AUD\$ 3,904**

- 2006-2009 International Macquarie University Research Excellence Scholarship (iMQRES), 3.5 years of salary.

Research assistant

- August 2008 - Department of Biological Sciences, Macquarie University (Sydney, Australia). *Proteomic analyses of the Sydney rock Oyster Saccostrea glomerata response to heavy metals.*
- January - April 2006 - Department of Biological Sciences, Macquarie University (Sydney, Australia). *Proteomic analyses of highly variable defensive proteins in sea urchins.*

Teaching assistant

- 2007-2008: Practicals in High School: Science Partnership Program (Sydney, Australia)
 - ✓ The partnership is a model of University and School collaboration designed to provide students with opportunities to engage and excel in science, technology, engineering and mathematics (STEM) subjects (40 hours)
- 2006-2008: Practicals, Bachelor courses, Macquarie University, Sydney, Australia
 - ✓ First year, Biol 115: The Thread of Life (215 hours over three semesters)
 - ✓ Second year, Biol 246: Human Physiology (38 hours)

1.3. Scientific expert activities

Virus Taxonomy

- ICTV study group member – Jingchuvirales 2020

Member of Professional Societies

- ✓ the European Society for Evolutionary Biology (ESEB)
- ✓ the Society for Molecular Biology and Evolution (SMBE)
- ✓ the American Society for Microbiology (ASM)
- ✓ the Genomic Standard Consortium (GSC)
- ✓ the Canadian society of Zoology (CSZ)
- ✓ Société Française de Parasitologie (SFP)
- ✓ Société Française de Microbiologie (SFM)

Organization of National and International seminars and workshops

- European Multicolloquium of Parasitologists (EMOP) – Belgrade 2020.
Symposium title « Microbes of parasites and role in diseases »
co-organized by **Dheilly NM** and Cinzia Cantacessi.
- Joint Congress on Evolutionary Biology – Montpellier 2018.
Symposium «S18 : Evolution of hosts and parasites with their microbiomes : a problem of unfaithful relationships »
co-organized by **Dheilly NM**, Douglas A, Martinez-Martinez J, Schulenburg H
- The first Parasite Microbiome Project Workshop - Clearwater, USA. 10-15 Janvier 2019.
Funded by the Gordon and Betty Moore Foundation.
Organized by **Dheilly NM** and Martinez-Martinez J, and Jon Kaye.

Invited speaker at a conference

- Evolutionary Hologenomics Mini-Conference , Copenhagen, Denmark 2020 *The conference has been reported to Spring 2021 due to the SARS-CoV-2 Pandemic*
- European Multicolloquium of Parasitologists (EMOP) – Belgrade 2020. *The conference has been reported to Spring 2021 due to the SARS-CoV-2 Pandemic*
- Collaboration workshop on the Ecology and Evolution of Infectious Diseases – Montpellier 2020. *The conference has been reported to Spring 2021 due to the SARS-CoV-2 Pandemic*
- Fédération de recherche en Infectiologie, FÉRI scientific days, Tours, France 2019.
- Annual Meeting of the Canadian Society of Zoologists, Parasitism, Immunity and Environment (PIE) section, Windsor, Canada 2019
- Annual Meeting of the American Society of Parasitology, President's symposium, Cancun, Mexico 2018
- Genomics Standard Consortium, San Diego 2018
- Genomic approaches for aquatic species, La Rochelle, France 2011

Invited departmental seminars

- Institute of Parasitology, University of Berne, Berne, Switzerland, 2020
- Unit Viral Genetics and Biosecurity, ANSES, Ploufragan, France 2019
- MIVEGEC, UMR CNRS/IRD/UM1/UM2 5290, Montpellier, France, 2019
- Department of Biological Sciences and Pathology, Vanderbilt University, Tennessee, USA 2018
- Bigelow Laboratory for Ocean Sciences, Maine, USA 2017
- Department of Ecology and Evolution, Stony Brook University, New York, USA 2016
- Department of Molecular and Cell Biology, University of Connecticut, Connecticut, USA 2016
- Department of Biology, Brooklyn college, New York, USA 2015
- Department of Biology, University of New Mexico, New Mexico, USA 2015
- Institut de Recherche sur la Biologie de l'Insecte (IRBI), Université de Tours, France 2013
- Institut de Biologie Moléculaire et cellulaire (IBMC), Université de Strasbourg, France 2013
- Laboratoire d'Ecologie et Evolution des Interaction (2EI), UMR CNRS 5244, Université de Perpignan, France 2010

Reviewer for peer review journals

see <https://publons.com/author/1331885/nolwenn-dheilly#profile>:

- Basic Research Journal of Agricultural Science and Review
- Biological control
- Biology letters
- BMC Genomics
- Chinese Journal of Oceanology and Limnology
- Comparative Biochemistry and Physiology
- Conservation Physiology
- Current opinion in insect science
- Ecological Entomology
- Environmental microbiology
- European Journal of Entomology
- Evolutionary Applications

- FEMS Microbiology Ecology
- Frontiers In Physiology
- Human Immunology
- International Journal of Molecular Sciences
- The ISME Journal
- Journal of Experimental Zoology part A
- Journal of Proteomics
- Journal of the World Aquaculture Society
- Parasitology
- PLoS Neglected Tropical Diseases
- PLoS Pathogen
- PLoS One
- Science Advance
- Scientific reports
- Virus Journal

Associate Editor in Microbial Symbiosis

- Frontiers in Microbiology
- Frontiers in Marine Sciences

Ad-hoc and Panel reviewer for grant funding agencies

- For the National Science Foundation (USA):
 - ✓ Minority Postdoctoral Research Fellowships (ad hoc reviewer)
 - ✓ Integrative Organismal Systems (ad hoc and panel reviewer)
 - ✓ Division of Environmental Biology (ad hoc reviewer)
- For the Israel Science Foundation (ad hoc reviewer)
- For the German Academic Exchange Service (DAAD) (ad hoc reviewer)

1.4. Public and media outreach

Children book:

- The Secret of the Scuba Diving Spider... and More! (Animal Secrets Revealed!) by Ana Maria Rodriguez: ISBN: 978-0766086296: In each chapter, the Author presents a scientist and a curious animal under study. One chapter was prepared with my help and describes the discovery of *D. coccinellae* associated I flavivirus.

On the radio:

- France Inter “la tête au carre” by Mathieu Vidard – February 13, 2015
<http://www.franceinter.fr/emission-la-tete-au-carre-le-club-coccinelles-e-penser-daniel-pennac-et-bilinguisme>: Interview

On Television:

- ici Radio Canada – Découverte – November 8, 2015 <http://ici.radio-canada.ca/tele/decouverte/2015-2016/episodes/360905/vie-mort> : Interview

In the Press:

- Science et Vie Junior numero 320: «Spellbound by the wasp». The double page was prepared with my help and describes the role of *D. coccinellae* associated Iflavirus in behavioral manipulation
- Science et Vie Junior numero 320: «Nightmare in Alaska». The double page was prepared with my help and describes the ability of *S. solidus* in altering the behavior of Threespine sticklebacks
- Science Magazine «Wasp [virus turns ladybugs into zombie babysitters](#)» by Nicholas Weiler
- National Geographic News «Wasp zombifies ladybugs using a virus as a bio-weapon – a first» by James Owen
- Discover Blogs: «Biological warfare: parasitic wasp uses a virus to control its host» by Christie Wilcox
- TheScientist : «Cooperative control» by Sandhya Sekar
- Discovery : «Parasites uses Bio weapon to create zombie bodyguard» by Jennifer Viegas
- The Huffington Post : «Le virus d'une guêpe transforme en zombie une coccinelle» By Marine Le Breton
- Le Monde : «La coccinelle otage d'une guêpe et d'un virus» by Herve Morin
- National Geographic Phenomena: « Parasitic wasps infected with mind-controlling viruses» by Carl Zimmer
- PhysOrg «Study shows example of a parasite using a bioweapon to control host» by Bob Yirka
- IFLS «Parasitoid wasp gives ladybirds a virus that turn them into a zombie bodyguard» by Justine Alford
- and many more in various languages...

1.5. Publications

- Peer-reviewed articles: 33 (19 as first or last author; 14 co-authored with students*, 5 co-authored with students under my supervision*)
- Book chapters: 1
- Citations (Google scholar): 1414
- h index (number h of publications with at least h citations): 20
- i10 index (publications with at least 10 citations): 28
- Relative Citation ratio (iCite, NIH): 1.90 (>75th percentile)

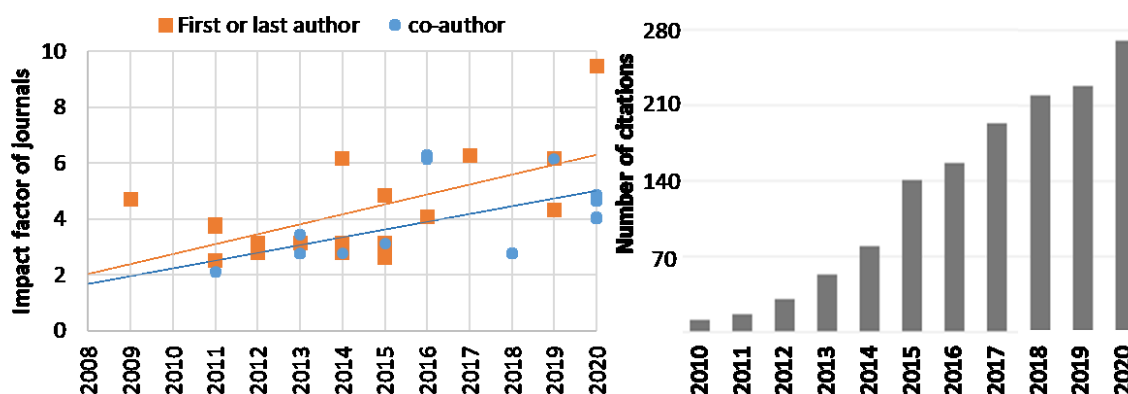


Figure 1. Increase in impact factor and number of citations as I develop an independent research program (data collected on November 10, 2020).

Complete list of peer-reviewed articles, from the most recent to the oldest. The impact factor (IF) of the journal is provided. **Bold:** myself, students and postdocs under my supervision. ***Students**

33. **Speer K***, **Dheilly NM**, Perkins S. 2020 Microbiomes are integral to arthropod parasite conservation. *Biological conservation* 250: 108695 IF 4.66
 32. **Jorge F**, **Dheilly NM**, Poulin R. 2020 Persistence of a core microbiome through the ontogeny of a multi-host parasite. *Frontiers in Microbiology* 11:954 IF 4.08
 31. **Huang I***, **Dheilly NM**, Sirotkin H, McElroy A. 2020 Comparative transcriptomics implicate mitochondrial and neurodevelopmental impairments in larval zebrafish (*Danio rerio*) exposed to two selective serotonin reuptake inhibitors (SSRIs). *Ecotoxicology and Environmental Safety* 203: 110934 IF 4.87
 30. **Hahn MA***, Rosario K, Lucas P, **Dheilly NM**. 2020 Characterization of viruses in a tapeworm: Phylogenetic position, vertical transmission and transmission to the parasitized host. *The ISME Journal* 14 : 1755–1767 IF 9.49
 29. Thomas F, Giraudeau M, **Dheilly NM**, Gouzerh F, Boutry J, Beckmann C, Biro PA, Hamede R, Abadie J, Labrut S, Bieuville M, Misse D, Bramwell G, Shultz A, Le Loc'h G, Roche B, Renaud F, Russell T, Ujvari B. **2020** Rare and unique adaptation to cancer in domesticated species: an untapped resource? *Evolutionary applications* 00, 1– 10 IF 4.03
 28. **Dheilly NM**. Martinez Martinez J, Rosario K, Brindley PJ, Fichorova RN, Kaye JZ, Kohl KD, Knoll LJ, Lukes J, Perkins SL, Poulin R, Schriml L, Thompson LR **2019** Parasite microbiome project: Grand challenges. *Plos Pathogens* 15(10): e1008028 IF 6.16
- ** Highlighted on PlosChannels**
27. **Dheilly NM**, Ewald PW, Brindley PJ, Fichorova RN, Thomas F. **2019** Parasite-microbe-host interactions and cancer risk. *Plos Pathogens* 15(8), e1007912 IF 6.16
 26. **Hahn MA***, **Dheilly NM**. **2019** Genome characterization, prevalence and transmission mode of a novel picornavirus associated with the Threespine Stickleback fish (*Gasterosteus aculeatus*). *Journal of Virology* 93(9), e02277-18 IF 4.32
 25. **Porter A***, **Pinaud S***, Chaparro C, Galinier R, **Dheilly NM**, Portela J, Charriere GM, Allienne J-F, Duval D, Gourbal B. **2019** Sympatric versus allopatric evolutionary contexts shape differential immune response in *Biomphalaria*/*Schistosoma* interaction. *Plos Pathogens* 15(3), e1007647 IF 6.16
 24. **Fuirst M***, Veit RR, **Hahn M***, **Dheilly NM**, Thorne L. **2018** Effects of urbanization on the foraging ecology and microbiota of the generalist seabird *Larus argentatus*. *Plos One* 13(12), e0209200 IF 2.78
 23. **Dheilly NM**, Bolnick D, Bordenstein S, Brindley PJ, Figueres C, Holmes EC, Martinez Martinez J, Phillips AJ, Poulin R, Rosario K **2017** Parasite Microbiome Project: Systematic investigation of microbiome dynamics within and across parasite-host interactions *mSystems* 2(4) IF 6.28

22. Hahn MA*, Dheilly NM 2016 Experimental models to study the role of microbes in host-parasite interactions. *Frontiers in microbiology* 7(1300) IF 4.08

21. Theis KR, **Dheilly NM**, Klassen JL, Brucker RM, Baines JF, Bosch TCG, Cryan JF, Gilbert SF, Goodnight CJ, Lloyd EA, Sapp J, Vandenkoornhuyse P, Zilber-Rosenberg I, Rosenberg E, Bordenstein SR. **2016** Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems* 1 (2), e00028-16 IF 6.28

** Faculty of 1000 recommendation

20. **Pinaud S*, Portela J***, Duval D, **Nowacki F***, Olive M-A, Allienne J-F, Galinier R, **Dheilly NM**, Kieffer-Jaquinod S, Mitta G, Théron A, and Gourbal B. **2016** A shift from cellular to humoral responses contributes to innate immune memory in the vector snail *Biomphalaria glabrata*. *Plos Pathogens*. 12(1): e1005361 IF 6.16

19. Dheilly NM, Poulin R, Thomas F. **2015** Biological warfare: Microorganisms as drivers of host-parasite interactions. *Infections Genetic and Evolution* 34: 251-259 IF 2.61

18. **Cardinaud M***, **Dheilly NM**, Huchette S, Moraga D, Paillard C. **2015** The early stages of the immune response of the European abalone *Haliotis tuberculata* to a *Vibrio harveyi* infection. *Journal of Developmental and Comparative Immunology* 51(2):287-297 IF 3.13

17. Dheilly NM, **Maure F***, Ravallec M, Galinier R, Doyon J, Duval D, Leger L, Volkoff A-N, Misse D, Nidelet S, Demolomb V, Brodeur J, Gourbal B, Thomas F, Mitta G. **2015** Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behavior manipulation. *Proceedings of the Royal Society B Biological Sciences*. 282(1803) IF 4.85

** Highlighted in international press, Radio (France Inter and Radio Canada), TWiV #443, popular science articles (e.g. National Geographic) and blogs, and in children science articles and books (Science et Vie junior N°320, and "The Secret of the Scubadiving Spider... and More!" By Ana Maria Rodriguez)

16. Dheilly NM, Duval D, Mouahid G, Emans R, Allienne J-F, Genthon C, Dubois E, Du Pasquier L, Adema C, Grunau C, Mitta G, and Gourbal B. **2015** A family of variable immunoglobulin and lectin-domain containing molecules in the snail *Biomphalaria glabrata*. *Journal of Developmental and Comparative Immunology*. 48(1):234-243 IF 3.13

15. Dheilly NM, **Jouaux A***, Boudry P, Favrel P, Lelong C. **2014** Comparative transcriptomic profiling of gametogenesis of diploid and triploid Pacific oyster *Crassostrea gigas*: towards an understanding of partial sterility associated with triploidy. *Plos One* 9(11): e112094 IF 2.78

14. **Vidal-Dupiol J***, **Dheilly NM**, **Rondon R***, Grunau C, Cosseau C, Smith K, Freitag M, Adjeroud M, Mitta G. **2014** Thermal Stress Triggers Broad *Pocillopora damicornis* Transcriptomic Remodeling, while *Vibrio coralliilyticus* Infection Induces a More Targeted Immuno-Suppression Response. *Plos One* 9(9): e107672 IF 2.78

13. Dheilly NM 2014 Holobiont-holobiont interactions: redefining host-parasite interactions. *Plos Pathogens* 10(7): e1004093 IF 6.16

12. **Dheilly NM**, Adema C, Raftos DA, Gourbal B, Grunau C, Du Pasquier L. **2014** No more non-model species: the promise of Next Generation Sequencing for comparative immunology. *Journal of Developmental and Comparative Immunology*. 45(1): 56-66 IF 3.13
11. **Fneich S***, **Dheilly NM**, Adema C, Rognon A, Reichelt M, Bulla J, Grunau C, Cosseau C. **2013**. 5-methyl-cytosine and 5-hydroxy-methyl-cytosine in the genome of *Biomphalaria glabrata*, a snail intermediate host of *Schistosoma mansoni*. *Parasites and Vectors*. 6:167 IF 3.43
10. **Vidal-Dupiol J***, Zoccola D, Tambutté E, Grunau C, Cosseau C, Smith KM, Freitag M, **Dheilly NM**, Allemand D, Tambutté S. **2013**. Genes related to ion-transport and energy production are upregulated in response to CO₂-driven pH decrease in corals: new insights from transcriptome analysis. *PLoS One*. 8(3):e58652 IF 2.78
9. **Dheilly NM***, Haynes PA, Smith LC, Raftos DA, Nair SV. **2013**. Shotgun proteomic analysis of the coelomic fluid of the purple sea urchin, *Strongylocentrotus purpuratus*. *Journal of Developmental and Comparative Immunology*. 40:35-50 IF 3.13
8. **Dheilly NM**, Lelong C, Kellner K, Dubos M-P, Riviere G, Huvet A, Boudry P, Favrel P. **2012**. Gametogenesis in an alternative hermaphrodite mollusk, the Pacific oyster *Crassostrea gigas*: a microarray-based analysis identifies stage and sex specific genes. *Plos One* 7(5): e36353 IF 2.78
7. **Dheilly NM***, Haynes PA, Raftos DA, Nair SV. **2012** Time course proteomic profiling of cellular responses to immunological challenge in sea urchins (*Heliocidaris erythrogramma*). *Journal of Developmental and Comparative Immunology* 37(2): 243-256 IF 3.13
6. Smith L, **Ghosh J***, **Buckley KM***, Clow LA, **Dheilly NM***, Haug T, Henson JH, Li C, Lun CM, Majeske AJ, Matranga V, Nair SV, Rast JP, Raftos DA, Roth M*, Sacchi S, Schrankel CC*, Stensvåg K*. **2011**. Echinoderm Immunity. *Advances in Experimental Medicines and Biology*. 708: 260-301. IF 2.13
5. **Dheilly NM***, Birch D, Nair SV, Raftos DA. **2011**. Ultrastructural localization of a highly variable immune response protein (185/333) within coelomocytes of sea urchins. *Journal of Immunology and Cell biology*. 89: 861-869. IF 3.79
4. **Dheilly NM**, Lelong C, Huvet A, Favrel P. **2011**. Development and application of a 31,952-feature microarray: Identification of reference genes and tissue-enriched expression patterns. *BMC Genomics*. 12: 468. IF 3.73
3. **Dheilly NM***, Haynes P, **Bove U***, Nair SV, Raftos DA. **2011**. Comparative proteomic analysis of a sea urchin (*H. erythrogramma*) antibacterial response revealed the Involvement of Apextrin and Calreticulin. *Journal of Invertebrate Pathology*. 106(2): 223-229. IF 2.51
2. **Dheilly NM***, Nair SV, Smith LC and Raftos DA. **2009**. Highly variable immune-response proteins (185/333) from the sea urchin, *Strongylocentrotus purpuratus*: Proteomics analysis identifies diversity within and between individuals. *Journal of Immunology*. 182(4): 2203-12. IF 4.72
1. Bowden L, **Dheilly NM***, Raftos DA and Nair SV. **2007**. New Immune Systems: Pathogen-specific host defence, life history strategies and hypervariable immune-response genes of invertebrates. *Invertebrate Survival Journal*. 4: 127-36. IF 0.78

Book chapter

Smith, LC, Arriza V, Barela Hudgell MA, Barone G, Bodnar AG, Buckley KM, Cunsolo V, **Dheilly NM**, Franchi N, Fugmann SD, Furukawa R, Garcia-Arraras J, Henson JH, Hibino T, Irons ZH, Li C, Lun CM, Majeske AJ, Oren M, Pagliara P, Pinsino A, Raftos DA, Rast JP, Samasa B, Schillaci D, Schrankel CS, Stabili L, Stensväg K, Sutton E. **2018**. Complexity of the Immune System in Echinoderms. In "Comparative Immunology", EL Cooper, ed. Springer Publisher.

1.6. Conferences

Oral presentations (6 with students under my supervision*)

20. Hahn M*, Dheilly NM (2019) *Schistocephalus solidus* and Threespine stickleback holobiont coevolution. Holobiont 2 Conference- Montreal, QC

****Received best student oral presentation award**

19. Hahn M*, Dheilly NM (2018) *Schistocephalus solidus* interaction with microbes. 9th international conference on stickleback behavior and evolution, Kyoto, Japan

18. Hahn M*, Dheilly NM (2017) A potential role for microbes in the infectious process of the cestode parasite *Schistocephalus solidus*. NYC Parasitology 2. New-York city, USA

17. Dheilly NM, Hahn M* (2017). Evolution of hosts, parasites and their microbiomes. NYC Parasitology 2, New-York city, USA

16. Hahn M*, Dheilly NM (2017) A potential role for microbes in the infectious process of the cestode parasite *Schistocephalus solidus*. European Society of Evolutionary Biology. Groningen, The Netherlands

15. Dheilly NM, Hahn M* (2017) Evolution of hosts, parasites and their microbiomes. European Society of Evolutionary Biology. Groningen, The Netherlands

14. Dheilly NM, Maure F*, Brodeur J, Mitta G, Gourbal B, Thomas F (2014) Rôle du changement d'hôte et du changement de tissu cible dans l'évolution virale. REID : Réseau Ecologie des Interactions Durable, Montpellier, France

13. Dheilly NM, Maure F*, Ravallec M, Doyon J, Galinier R, Duval D, Nidelet S, Demolombe V, Volkoff N, Brodeur J, Mitta G, Gourbal B, Thomas F (2014) Qui est le marionnettiste? REID: Réseau Ecologie des Interactions Durable, Montpellier, France

12. Vidal-Dupiol J*, Dheilly NM, Rondon R, Grunau C, Cosseau C, Smith K, Freitag M, Adjeroud M, Mitta G. (2014) Deeper inside into the coral diseases puzzle: evidences of high temperature induced immune-deficiency while bacterial virulence leads to immunosuppression. REID: Réseau Ecologie des Interactions Durable, Montpellier, France

11. Dheilly NM, Maure F*, Brodeur J, Doyon J, Galinier R, Mitta G, Gourbal B, Thomas F. (2013) La coccinelle "garde du corps": La manipulation du transcriptome de la coccinelle *C. maculata* par la guêpe parasitoïde *D. coccinellae*. REID : Réseau Ecologie des Interactions Durable, Talence, France

10. **Dheilly NM**, Duval D, Emans R, Grunau C, Mitta G, Gourbal B. (2012) A complex immune system revealed by generation of the *B. glabrata* transcriptome *de novo* from RNA-seq data. Immuninv: Immunité des invertébrés, Perpignan, France
9. **Vidal-Dupiol J***, Grunau C, Cosseau C, Destoumieux-Garzon D, **Dheilly NM**, Duval D, Smith K, Freitag M, Sautière P-E, Ladrière O, Tambutté S, Allemand D, Mitta G. (2012) The innate immune response of *Pocillopora damicornis* confronted to *Vibrio coralliilyticus*. Immuninv: Immunité des invertébrés, Perpignan, France
8. **Dheilly NM**, Lelong C, Kellner K, Dubos M-P, Riviere G, Huvet A, Boudry P, Favrel P. (2012) Gametogenesis in an alternative hermaphrodite mollusk, the Pacific oyster *Crassostrea gigas* :Physiomar 12, Santiago de Compostela, Spain
7. **Vidal-Dupiol J***, Ladrière O, **Dheilly NM**, Destoumieux-Garzon D, Tambutté S, Grunau C, Cosseau C, Freitag M, Adjeroud M, Mitta G. (2012) The immune response of *Pocillopora damicornis* confronted to *Vibrio coralliilyticus*: 12th International Coral Reef Symposium, Cairns, Australia
6. **Vidal-Dupiol J***, Zoccola D, Tambutté E, Grunau C, Coasseau C, Freitag M, Adjeroud M, **Dheilly NM**, Allemand J, Mitta G, Tambutté S. (2012) Global coral transcriptomic responses to ocean acidification: 12th International Coral Reef Symposium, Cairns, Australia
5. Kellner K, Jouaux A, Heude-Berthelin C, Lelong C, **Dheilly NM**, Sourdain P, Mathieu M. (2010). Gametogenic stages in triploid oysters *Crassostrea gigas*: irregular locking of gonial proliferation and subsequent reproductive effort: Physiomar10, Québec city, Canada
4. **Dheilly NM***, Raftos DA, Haynes P, Nair SV. (2009). Comparative proteomic profiling of the wound and LPS immune response in sea urchins: 11th International Congress of the International Society of Developmental and Comparative Immunology, Prague, Czech Republic
3. **Dheilly NM***, Nair SV, Smith LC, Birch D, Raftos DA. (2009). Generation of diversity and immunity in sea urchins: 11th International Congress of the International Society of Developmental and Comparative Immunology, Prague, Czech Republic
2. Nair SV, **Roth M***, **Dheilly NM***, Raftos DA. (2009). Diversity and defense : characterisation of a highly variable gene family in the sea urchin *Heliocidaris erythrogramma*: 11th International Congress of the International Society of Developmental and Comparative Immunology, Prague, Czech Republic
1. Raftos DA, Adlard RD, Aladaileh S, Butt DT, **Dheilly NM***, Nell JA, O'Connor WA, Simonian M, Nair SV (2008). New paradigms for disease resistance in aquaculture. ASP & ARC/NHMRC Research Network for Parasitology Annual Conference, Glenelg, Australia

Poster presentations (5 with students under my supervision*)

15. **Peña-Lobel M***, Nye J, **Hahn M***, **Schwemmer TG***, Baumann H, **Dheilly NM** (2020) Changes in gene expression of ion regulatory proteins in developing Atlantic Silversides (*Menidia menidia*) subjected to elevated temperature and acidity. Ocean Sciences Meeting, San Diego, USA

14. **Hahn M***, **Dheilly NM** (2019) Discovery of viruses in parasitic Platyhelminths opens new perspectives for therapeutic strategies. Holobiont 2 Conference- Montreal, QC
13. **Huang I***, **Dheilly NM**, Sirotkin H, McElroy A (2018) Using zebrafish (*Danio rerio*) to develop biomarkers for pharmaceutical pollutants. International Zebrafish Society, Madison, USA
12. **Dheilly NM**, **Hahn M*** (2017) Evolution of hosts, parasites and their microbiomes. Society for Molecular Biology and Evolution. Austin, USA
11. **Dheilly NM**. (2017). Parasite Microbiomes: The missing link in parasite ecology and associated pathologies. BioGenomics2017 Washington DC, USA
10. **Tucker C***, **Hahn M***, Nye J, Baumann H, **Dheilly NM** (2017) How will climate change affect the health of marine fish? Undergraduate Research and Creative Activities (URECA) Stony Brook University, Stony brook, USA
9. **Dheilly NM**, **Maure F***, Brodeur J, Mitta G, Gourbal B, Thomas F (2014) The holobiont parasite: A parasite-associated virus replication correlates with host behavioral manipulation. Jacques Monod Conference: Infectious disease as drivers of evolution : The challenge ahead. Roscoff, France
8. **Dheilly NM**, **Portela J***, Galinier R, Grunau C, Emans R, Duval D, Gourbal B, Mitta G. (2012) Caractérisation du transcriptome de *Biomphalaria glabrata* par NGS : un pré-requis pour mieux comprendre les mécanismes moléculaires régissant son interaction avec le schistosome. Immuninv: Immunité des invertébrés, Perpignan, France
7. **Dheilly NM**, **Maure F***, Gourbal B, Emans R, Deleury E, Brodeur J, Thomas F, Mitta G. (2012) La coccinelle « garde du corps » : la manipulation du transcriptome de la coccinelle *Coleomegilla maculata* par la guêpe parasitoïde *Dinocampus coccinellae* Immuninv: Immunité des invertébrés, Perpignan, France
6. **Fneich S***, **Lepesant J***, Cosseau C, Reichelt M, **Dheilly NM**, Dejean B, Grunau C. (2012). DNA methylation in *Biomphalaria glabrata* the intermediate host of the human parasite *Schistosoma mansoni*: role of epigenetics in the coevolution host / parasite interactions. 13th International symposium on Schistosomiasis, Brésil
5. **Fneich S***, **Lepesant J***, Cosseau C, Reichelt M, **Dheilly NM**, Dejean B, Grunau C. (2012). DNA methylation in *Biomphalaria glabrata* the intermediate host of the human parasite *Schistosoma mansoni*: role of epigenetics in the coevolution host / parasite interactions. Society for Molecular Biology and Evolution, Dublin, Ireland
4. Bigot L, Dubos MP, **Dheilly NM**, Favrel P, Kellner K, Lelong C, Martinez AS, Riviere G, Santerre C, Sourdain P, Degremont L, Flahauw E, Hatt P-J, Heurtebise S, Lapegue S, Azzouin N, Galibert F, Boudry P, Corporeau C, Fabioux C, Guevelou E, Huvet A, Sussarellu R. (2012). Etude transcriptomique, génétique et fonctionnelle de la gamétogénèse chez l'huître *Crassostrea gigas*. Colloque AGENAE-GENANIMAL, La Rochelle, France
3. **Jouaux A***, Heude-Berthelin C, Lelong C, **Dheilly NM**, Sourdain P, Mathieu M, Kellner K. (2010). Gametogenesis and storage strategies in triploid and diploid Pacific oysters *Crassostrea gigas*. Physiomar10, Québec city, Canada

2. **Dheilly NM***, Nair SV, Raftos DA. (2008). 185/333 immune response proteins : predicted protein structure and origins of variability. 13th Lorne Proteomics Symposium, Lorne, Australia
1. **Dheilly NM***, Nair SV, Raftos DA. (2008). 185/333 immune response proteins : predicted protein structure and origins of variability. 33rd Lorne Conference on Protein Structure and Function, Lorne, Australia

1.7. Student supervision on research projects

1.7.1. Students enrolled in a PhD program

- **Megan A Hahn PhD in Marine Sciences (29/06/2020)**

2015-2020 School of Marine and Atmospheric Sciences, Stony Brook University, USA.

Thesis title: The role of microbes in the interaction between the cestode *Schistocephalus solidus* and its Threespine stickleback host

Thesis Supervisor: Nolwenn M Dheilly

Jury: Prof Robert Cerrato, Prof Christopher Gobler, Prof Daniel Bolnick, Prof Seth Bordenstein, Dr Karyna Rosario

Megan joined my lab as a MS student in August of 2015, only a few weeks after I started working at Stony Brook University. When she started, I could ensure a 1,5 years of Teaching assistantship from my start-up fund. During that first year, and despite a heavy and challenging teaching and course load required by the department, Megan and I initiated a literature review aiming at identifying model systems to study the role of microbes in host-parasite interaction. During this literature review, we identified the organismal system she would study for her thesis: the interaction between Threespine sticklebacks and the cestode *Schistocephalus solidus*. We published her analysis in *Frontiers in Microbiology* in August 2016. She also obtained excellent grades in classes and the department strongly supported her request to transfer to our PhD program in order to pursue working in my lab.

I obtained a first research grant that allowed me to cover her Research Assistant salary for longer, and to fund our first field sampling expedition in Alaska to characterize the viral and bacterial microbiome of *S. solidus*. I then obtained a couple of innovation prizes that provided the necessary funding to build fish rooms and maintain the parasite life-cycle locally. It allowed us to conduct experimental infections, and in vitro breeding of parasites to determine viruses transmission mode. The first two research chapters of Megan's PhD thesis focus on characterizing the fish and parasite virome. We published these findings in the *Journal of Virology* in 2019 and in *The ISME Journal* in 2020.

From this initial field sampling, we showed that *S. solidus* plerocercoids harbor a complex microbiome, and that *S. solidus* infection is associated with significant changes in the composition of the microbiome of Threespine stickleback hosts. In spring 2017, she spent a semester at Max Planck Institute (MPI) for Evolutionary Biology in Plön, Germany to conduct a follow-up collaborative study with Dr. Martin Kalbe. At MPI, she experimentally infected threespine

sticklebacks with *S. solidus* and collected phenotypic information and tissue samples in order to 1. Characterize the role of the environment (host microbiome or host origin) and genetics (parasite origin) on the parasite microbiome, 2. Characterize the role of host and parasite origin on the host microbiome and 3. Characterize the effect of *S. solidus* on the host microbiome over the first few hours of infection. Three manuscripts are in final phase of preparation to present these results.

Megan also contributed to collaborative studies being undertaken in my laboratory. As such, she is co-author on a published manuscript lead by Matthew Furst for her help characterizing the bacterial microbiome of a seabird published in *Plos One*, and for help characterizing the bacterial microbiome of Atlantic silversides developing at different pH and Temperatures representative of current and future stressful conditions (manuscript in preparation).

When all her thesis work is published, Megan will be author on eight manuscripts, a terrific achievement. Megan has developed extensive fundamental knowledge in parasitology, microbiology, ecology and evolution with broad applications. Over the course of the past four years, she has acquired a diversity of laboratory and computational skills, including experience in designing complex experiments with appropriate controls, and running Linear Mixed Models and other statistical analyses. There is a crucial need for such experiments as we increasingly recognize the complexity of organismal systems and multi-level interactions between host, microbes, parasites and the immune system.

Megan is also a skilled communicator. During her teaching practicum in my class on Next Generation Sequencing, she has acquired great skills at communicating complex concepts, and sometime Jargon-heavy bioinformatics methods in simple terms that would be understandable to non-expert audience. In May of 2019, the STRIDE program selected her for a fellowship. STRIDE is a competitive NSF funded initiative that provides graduate students with interdisciplinary skills to assist, create, and eventually lead in the translation of complex data-enabled research to a broad variety of audiences. Her communication skills also led her to receive an award for best student presentation at the last International Holobiont Conference.

Megan successfully defended her thesis in June 2020 and since July 2020, she is a postdoctoral Scholar at NYC health center.

Publications:

Hahn MA, Dheilly NM 2016 Experimental models to study the role of microbes in host-parasite interactions. *Frontiers in microbiology* 7(1300)

Furst M, Veit RR, **Hahn MA**, Dheilly NM, Thorne L. 2018 Effects of urbanization on the foraging ecology and microbiota of the generalist seabird *Largus argentatus*. *Plos One* 13(12), e0209200

Hahn MA, Dheilly NM. 2019 Genome characterization, prevalence and transmission mode of a novel picornavirus associated with the Threespine Stickleback fish (*Gasterosteus aculeatus*). *Journal of Virology* 93(9), e02277-18

Hahn MA, Rosario K, Lucas P, Dheilly NM. 2020 Characterization of viruses in a tapeworm: Phylogenetic position, vertical transmission and transmission to the parasitized host. *The ISME Journal*.

Conferences:

- Peña-Lobel M, Nye J, **Hahn M**, Schwemmer TG*, Baumann H, Dheilly NM (2020) Changes in gene expression of ion regulatory proteins in developing Atlantic Silversides (*Menidia menidia*) subjected to elevated temperature and acidity. Ocean Sciences Meeting, San Diego, USA
- Hahn M**, Dheilly NM (2019) *Schistocephalus solidus* and Threespine stickleback holobiont coevolution. Holobiont 2 Conference- Montreal, QC **Received best student oral presentation award
- Hahn M**, Dheilly NM (2019) Discovery of viruses in parasitic Platyhelminths opens new perspectives for therapeutic strategies. Holobiont 2 Conference- Montreal, QC
- Hahn M**, Dheilly NM (2018) *Schistocephalus solidus* interaction with microbes. 9th international conference on stickleback behavior and evolution, Kyoto, Japan
- Tucker C, **Hahn M**, Nye J, Baumann H, Dheilly NM (2017) How will climate change affect the health of marine fish? Undergraduate Research and Creative Activities (URECA) Stony Brook University, Stony brook, USA
- Hahn M**, Dheilly NM (2017) A potential role for microbes in the infectious process of the cestode parasite *Schistocephalus solidus*. NYC Parasitology 2. New-York city, USA
- Dheilly NM, **Hahn M** (2017). Evolution of hosts, parasites and their microbiomes. NYC Parasitology 2, New-York city, USA
- Hahn M**, Dheilly NM (2017) A potential role for microbes in the infectious process of the cestode parasite *Schistocephalus solidus*. European Society of Evolutionary Biology. Groningen, The Netherlands
- Dheilly NM, **Hahn M** (2017) Evolution of hosts, parasites and their microbiomes. European Society of Evolutionary Biology. Groningen, The Netherlands
- Dheilly NM**, Hahn M (2017) Evolution of hosts, parasites and their microbiomes. Society for Molecular Biology and Evolution. Austin, USA

Invited seminar:

Postdoctoral training program interview at Regeneron Pharmaceuticals, Inc (February 2020)

- **Irvin Huang PhD in Marine Sciences (22/11/2019)**

2014-2019 School of Marine and Atmospheric Sciences, Stony Brook University, USA.

Thesis title: Sublethal effects of neuroactive pharmaceuticals in developing zebrafish (*Danio rerio*), an aquatic vertebrate model organism

Thesis Supervisor: Prof Anne McElroy

Jury: Prof Bruce Brownawell, Prof Howard Sirotkin, Dr Cheryl Murphy, Dr Nolwenn M Dheilly

Irvin started his PhD in the laboratory of Anne Mc Elroy before I started working at Stony Brook University, but I rapidly got involved in his PhD, allowing him to use RNA sequencing and qPCR approaches to identify differentially expressed genes and test their efficiency as biomarkers. Irvin's dissertation research investigated the developmental and behavioral effects of pharmaceuticals associated with wastewater effluent. He used an adverse outcome pathway framework to develop tools for assessing potential impacts of emerging contaminants.

I have been involved in two chapters of Irvin's thesis. In the first chapter, published in *Ecotoxicology and Environmental Safety*, we used a ultra-low input RNA sequencing approach to identify neuronal biological pathways impacted by zebrafish exposure to selective serotonin reuptake inhibitors (SSRIs) (fluoxetine and paroxetine).). I supervised all the technical aspects associated with using RNA sequencing technology, as well as all the corresponding data analyses (bioinformatics processing and statistical analyses). Weighted gene co-expression network analysis allowed us to show that toxic effects of SSRI exposure are likely caused by mitochondrial dysfunction and subsequent neurodevelopmental effects.

In a follow-up study, we compared the performance of using changes in the spontaneous swimming behavior during a visual motor response (VMR) behavioral screen and larval brain gene expression of candidate biomarker genes markers indicative of pharmaceutical exposure. Again, I provided the technical supervision necessary for using the RT-qPCR approach, and the expertise necessary to ensure accurate interpretation of the data. Because results were unexpected, I contributed to discussions to determine the follow-up experiments. By comparing the biological response of individuals from different parental pairs and from different zebrafish strains, we showed that using non-lethal methods, such as behavioral screening, and using repetitive measures is more sensitive than qPCR approaches that require fish euthanasia and provide a unique endpoint. The manuscript is currently in preparation for publication. As a member of Irvin's thesis committee, I reviewed his entire thesis and contributed to preparing a final report and guidance before earning his PhD.

Co-authored publications:

Huang I, Dheilly NM, Sirotkin H, McElroy A. Comparative transcriptomics implicate mitochondrial and neurodevelopmental impairments in larval zebrafish (*Danio rerio*) exposed to two selective serotonin reuptake inhibitors (SSRIs). *Ecotoxicology and Environmental Safety*

Co-authored conferences:

Huang I, Dheilly NM, Sirotkin H, McElroy A (2018) Using zebrafish (*Danio rerio*) to develop biomarkers for pharmaceutical pollutants. International Zebrafish Society, Madison, USA

Irvin successfully defended his thesis on November 26, 2019 and I was a member of his thesis committee. Since then, he won a John A. Knauss Marine Policy Fellowship from NOAA and Sea Grant and began a new journey in Washington DC as an Ocean and Coastal Policy Analyst at National Ocean Services, Policy and Constituent Affairs Division.

- **Lisa M Crawford PhD candidate in Marine Sciences**

Since 2016 School of Marine and Atmospheric Sciences, Stony Brook University, USA.

Thesis supervisor: Prof Anne McElroy

Provisional thesis title : Exposure & Molecular Responses to Bioaccumulative Contaminants in Northwest Atlantic Sharks

Committee members: Prof Michael Frisk, Prof Nicholas Fisher, Dr James Gelsleichter, Dr Nolwenn Dheilly

Lisa started her PhD in the laboratory of Anne Mc Elroy in fall 2016 and attended that first year both of my classes in application of NGS to functional genomics, and in science communication. She told me I was the best teacher she ever had and wanted me to be involved in her PhD. She asked me to help her write a grant for the NSF Graduate Research Fellowship program and to review her personal statement. Lisa demonstrated early a scientific maturity superior to many of her classmates and I was happy to provide her guidance. Unfortunately, she did not receive the funding, but her PhD supervisor Anne McElroy was convinced and the project she wrote then is at the heart of her thesis project. I also wrote support letters for Lisa applications to various Fellowships. In 2019, she was the recipient of a Jerry R Schubel Fellowship, and as such was recognized as an ambassador for SoMAS, for her role at informing public policy. Her PhD aims at developing non-invasive methods to monitor contaminant levels in wild-caught sharks.

As a member of her thesis committee, I maintain frequent discussions with Lisa and discuss all aspects of her thesis, but now that I have moved out of Stony Brook University, I do not provide as much personal day-to-day supervision and my role is mostly to provide guidance and expert advice regarding the transcriptomic work that she is conducting. Over the past few years, Lisa has been continually sampling sharks with multiple groups from DEC, OCEARCH and the Fisheries Research Foundation. She was invited as collaborating scientist and principal investigator with OCEARCH to conduct hands-on research with large marine predators, and participated in expeditions Nova Scotia 2018 & NASFA 2019. She now has muscle samples for chemical analysis and preserved muscle RNA samples from nearly 100 individual sharks from seven species of North Atlantic sharks including the shortfin mako (*Isurus oxyrinchus*), common thresher (*Alopias vulpinus*), white (*Carcharodon carcharias*), tiger (*Galeocerdo cuvier*), sandbar (*Carcharhinus plumbeus*), sand tiger (*Carcharias taurus*), and dusky (*Carcharhinus obscurus*). Non-invasively sampled muscle tissues were analyzed for total mercury (THg, ppm dw) content, showing a positive correlation between body length and THg in all species except sandbars, suggesting that these species accumulate mercury as they grow. Future work will examine relationships between health markers (white blood cell counts, granulocyte : lymphocyte ratios, protein electrophoresis, and acute phase protein levels stress) in white and tiger sharks with the other variables being measured.

Additionally, RNA sequencing is being conducted on white, tiger, and shortfin mako muscle samples to investigate relationships between gene expression and contaminant concentrations. My main role now is to ensure that Lisa selects the best samples for RNA sequencing to reduce the confounding factors and ensure she has enough replicates to find pathways differentially expressed depending on contaminant exposure. For tiger and shortfin mako, the transcriptomes were assembled *de novo*. For white shark, sequenced reads were aligned against the reference genome. A preliminary comparative analysis of gene expression combined with functional profiling was conducted to determine the most appropriate analysis pipeline. It reveals cellular pathways differentially expressed depending on contaminant exposure. Lisa is planning on advancing to candidacy by the end of 2020.

1.7.2. Students enrolled in a Master program

- **Matthew Furst Master in Marine Sciences**

2016-2018 Stony Brook University, Stony Brook, USA under the supervision of Lesley H. Thorne.

Matthew's master thesis focused on the foraging behavior of the generalist feeding herring gull along a gradient of urbanization. His preliminary data rapidly showed that gulls foraging on landfills and other urban environment differed with the degree of urbanization. I rapidly got involved in his project and we decided to investigate together the composition of the microbiome of herring gulls by collecting swabs from the cloacae. While Lesley Thorne initially conceptualized the project and supervised fieldwork, I supervised all the laboratory work. Matthew had no lab experience and needed to learn how to use a pipette and how to behave in a molecular biology lab, as well as how to conduct bioinformatics analyses of amplicon-based sequencing data using QIIME. It was a steep learning curve but under my supervision, and by working in my lab next to other students with advanced skills in molecular biology, he eventually got some good habits and processed his samples. I organized the laboratory experiments and sequencing and supervised the microbial data analyses. Matt successfully achieved his objectives and observed high microbial diversity at all colonies, though microbial diversity was highest at the least urban colony where gulls used a wider variety of foraging habitats. Matthew defended his thesis and I was a member of his committee. His master thesis was soon after published in *Plos one*.

Co-authored publications:

Furst M, Veit RR, Hahn MA, Dheilly NM, Thorne L. 2018 Effects of urbanization on the foraging ecology and microbiota of the generalist seabird *Largus argentatus*. *Plos One* 13(12), e0209200

In august 2018, Matthew started a PhD in Integrative biology in Norris Lab, University of Guelph, Guelph, ON where he is studying the breeding success and dispersal movements of Canada jays. He recently told me that his experience in studying the bird bacterial microbiome during his master allowed him to get involved in a side project to investigate if the saliva of Canada jays has antibacterial properties that can be exploited for food preservation.

- **Glawdys Almayrac Master in integrative biology**

2013 Master 2 of Integrative Biology: Molecules, populations and sustainable development, Laboratory of Ecology and Evolution of Interactions, Université de Perpignan Via Domitia, France

Glawdys conducted her Master's research project during my postdoc. I used RNA sequencing to assemble the transcriptomes of the ladybeetle *Coleomegilla maculata* and its associated parasitoid wasp *Dinocampus coccinellae*, and to extract lists of genes differentially expressed at different time points over the course of parasitoid larval development within the ladybeetle abdomen. Her project aimed at extracting functional information from these lists of differentially expressed genes. I taught her how to do so and supervised all her analyses. Glawdys successfully conducted a very detailed analysis showing the impact of the parasitoid on ladybeetle immune response genes, digestion, and neuronal pathways and wrote a high quality thesis. Unfortunately, this part of our research results have not been published, mostly because of the low number of technical replicates that were used for RNA sequencing, a decision that was made before Glawdys started working on the project.

- **Jennifer Jankowiak Master in Marine Sciences**

2015-2017 Stony Brook University, Stony Brook, USA under the supervision of Chirstopher Gobler.

I was not technically involved in Jennifer's thesis, but I was a member of her thesis defense committee. I provided expert review of Jennifer's thesis on the effects of Nitrogen, phosphorus and temperature on Cyanobacterial bloom intensification, diversity and toxicity in Lake Eerie. My comments helped her improve her data analysis before publication (Published in PLoS One in 2019). In a very short time she analysed a large quantity of data that merited my sincere congratulations.

1.7.3. Undergraduate and high school student supervision

Summer 2018: Maya Pena-Lobel, high school student in Suffolk County. Now undergraduate student at Harvard. *Impact of pH and temperature on the expression of genes involved in ion regulation in the silverside fish Menidia menidia during the first few days of development.*

Spring 2018: Nita Wong, freshman at Stony Brook University. Now enrolled in medical program. *Development of RT-PCR for the detection of viruses associated with S. solidus*

Spring 2018: Stefanie Sforza, freshman at Stony Brook University. Now graduate student at Ohio State university. *Experimental infections of copepods with the cestode S. solidus*

Spring, summer and fall 2017, spring 2018: Clara Tucker, freshman at Stony Brook University. Now master student in the department of Ecology and Evolution. *Impact of pH and temperature on the microbiome of the fish menidia menidia over the first few days of development.*

Spring 2017: Wilderness Obermann, senior at Stony Brook University, graduated with Honors for her research project in my lab. Now undergraduate in computer sciences at Columbia University. *Field study of the impact of pH and temperature on stress response genes of Oysters.*

Summer 2015: Karin Schweitzer, senior at Stony Brook University. Graduate with a Master degree in marine Sciences at Stony brook University. *Impact of infection by S. solidus on the density and buoyancy of the fish host G. aculeatus.*

Spring 2006: Alex Meguid, senior at Macquarie University, Sydney, Australia. *Introduction to comparative proteomics*

Conferences:

Peña-Lobel M, Nye J, Hahn M, Schwemmer TG, Baumann H, Dheilly NM (2020) Changes in gene expression of ion regulatory proteins in developing Atlantic Silversides (*Menidia menidia*) subjected to elevated temperature and acidity. Ocean Sciences Meeting, San Diego, USA

Tucker C, Hahn M, Nye J, Baumann H, Dheilly NM (2017) How will climate change affect the health of marine fish? Undergraduate Research and Creative Activities (URECA) Stony Brook University, Stony brook, USA

2. Overview of doctoral and postdoctoral research activities (2006-2013)

2.1. PhD training in comparative immunology and highly variable immune response molecules (2006-2010)

I completed my PhD at Macquarie University, in Sydney, Australia under the supervision of Prof David A. Raftos and Sham V. Nair. In 3.5 years, I contributed to a total of seven peer-reviewed articles, and was first author on five of these. My thesis is untitled “proteomic analysis of sea urchin immune responses and characterization of highly variable immune response molecules”.

2.1.1 Context

For a long time, the prevailing theory was that invertebrates had a very simple immune system, efficient for host protection but non-specific, and without adaptive capabilities, in contrast with chordates that can mount an adaptive immune response¹⁻⁴. But, invertebrates would also benefit from a specific immune system, and from having the ability to resist re-infection. Indeed, starting in the late 1990's, classical immunization experiments demonstrated the efficiency of priming, suggesting the existence of immune processes providing both specificity and memory⁵⁻⁷. Any receptor showing high degrees of intra-individual variability could in principle, be a candidate recognition molecule in a pathogen-specific innate immune system. In addition, post-genomic recombination could increase diversity and opportunity for specific recognition of pathogen, while selective expression after immunological challenge and clonal expansion would allow immune memory.

Sea urchins are deuterostome invertebrates, members of the echinoderm phylum, a sister group to chordate, and as such they can be studied to better understand the evolutionary transition that lead to an adaptive immune system in vertebrates. When I started my PhD, the first genome of the purple sea urchin (*Strongylocentrotus purpuratus*) had just been sequenced⁸. The genome annotation revealed an unprecedented expansion of many immune gene families, including 253 Toll-like receptors, 208 NACHT domain and leucine-rich repeat (NLR) proteins, 218 Scavenger-receptor cysteine rich containing proteins (with a total of 1095 SRCR domains), 46 ficolins, and a coordinated expansion of signaling adaptors^{1,9}. In addition, the genome encloses 15-50 genes encoding for TrF (previously 185/333 proteins), uniquely found in sea urchins, and overexpressed in response to challenge¹⁰⁻¹². TrF genes are small, composed of one or two exons, representing a family of linked genes that occupy long stretches of repeats along the genome¹²⁻¹⁴. A combination of gene conversion, DNA recombination and post-transcriptional diversification appears to contribute to Trf transcripts diversity¹⁵.

2.1.2. Proteomic analysis of sea urchin immune responses

The sea urchin genome sequence provided a static image, and homologies to other species was used to infer the role of proteins in immune responses. The newly sequenced genome made it possible to use proteomics approaches to provide a dynamic image of sea urchin immune response mechanisms. Initially, I implemented proteomic approaches to provide the first proteome maps of the coelomic fluid of two sea urchin species, *Heliocidaris erythrogramma* and *S. purpuratus*^{16,17}. Of significance, the complement components C3, the complement receptor 2 CR2 (also known as CD21), and the major yolk protein (MYP) were among the most abundant proteins. The interaction between C3d and CR2 is key to complement immune system activation and a link between innate and adaptive

immunity, but the role of the MYP remains unknown to date. My results revealed a diversity of SRCR and Trf (185/333), with different variants expressed in different individuals ^{16,17}.

I then used comparative proteomics to identify proteins involved in anti-pathogen response. I initially used 2DE gel electrophoresis to identify proteins with increase abundance in response to bacteria, and revealed the role of apexrin and calreticulin ¹⁸. In order to increase sensitivity and specificity of detection, I obtained funding (Macquarie University Postgraduate Research Fund) to develop a shotgun proteomics approach based on separating proteins on 1D SDS-PAGE, in-gel trypsin digestion and LC-MS ¹⁶. This study revealed a rapid expression of proteins involved in the response to injury (MYP and large lipid transfer molecules, von Willebrand factor, cyclophilin B and selectins) suggesting cell migration to the site of injury and adhesion to collagen. In response to LPS challenge, changes in protein abundance occur 48h after injection, and consist in an increase in vesicular transport proteins, C3, dual oxidase maturation factor, dual oxidase, α 2macroglobulin, and three SRCRs¹⁷. Some proteins were identified only after LPS injection, such as Fibrocystin L, involved in regulating phagocytosis, and aminopeptidase N (also called CD13) involved in peptide degradation, antigen trimming and antigen processing. Further studies are now needed to assess the effect of different immune challenges on the sea urchin immune response, and in particular on the expression of highly variable families such as TLR, NLR, SRCRs, lectins and Trf proteins.

2.1.3. Characterization of highly variable immune response molecules

The second half of my PhD describes my research efforts into characterizing the diversity of Trf, a family of highly variable molecules, with no homology to other known proteins, and of unknown function. Interest in Trf sparked after a study using subtractive hybridization to identify genes overexpressed in *S. purpuratus* coelomocytes upon LPS challenge revealed their inherent variability¹¹. To allow alignment of transcripts, large gaps had to be inserted, revealing blocks of shared sequence, called "elements". A follow-up study investigating the diversity of Trf transcripts showed that individual sea urchin expressed a different set of transcripts, and that this profile changed upon challenge with LPS and β -1,3-glucan¹⁹.

I used a proteomic approach to build upon these findings²⁰. I initially confirmed that Trf proteins are overexpressed upon challenge and then investigated Trf proteins diversity within and among individual sea urchins. All individuals expressed a unique set of Trf proteins, with up to 264 unique protein spots identifiable on 2DE gels. Trf proteins had a much higher molecular weight and more acidic pH than expected. There was numerous variants with similar molecular masses but different isoelectric points (pI), regularly spaced every 0.1 to 0.2 pH units. Given that there are numerous conserved sites for both N-linked and O-linked glycosylation, we suggested that Trf are glycosylated, and form complexes covalently linked with carbohydrates, or form oligomers resistant to reducing treatment used. Indeed, the expression of recombinant Trf in vitro results in bands on 1DE with MW that correspond to homodimers and higher order oligomers ²¹. Using the shotgun proteomics approach that I developed with Macquarie University Postgraduate Research Fund, I identified peptides of 185/333 proteins by mass spectrometry and showed that multiple Trf proteins were present with a given MW, further supporting the hypothesis that they form multimers. Finally, I observed changes in the set of Trf proteins expressed after challenge with LPS or peptidoglycan, with changes in expression of shared proteins, and appearance / disappearance of Trf variants suggesting that sea urchins may be able to alter the expression of different Trf to tailor specific response against different pathogens.

Previous studies showed that Trf proteins are expressed on the surface of a subpopulation of coelomocytes, called small amoebocytes, and within perinuclear vesicles of small amoebocytes and polygonal amoebocytes²¹. The authors speculated that Trf are secreted from amoebocytes and subsequently associate with the cell surface, forming aggregates that lead to the encapsulation of pathogens. I followed-up on this study by using gold immunolabelling and transmission electron microscopy to reveal the subcellular localization of Trf proteins within coelomocytes in the coelomic fluid and in the gut of sea urchins²². The Trf was associated predominantly on the cell surface and perinuclear vesicles of small amoebocytes and filopodial amoebocytes, and within large spherules of colorless spherule cells²². Trf proteins were associated directly with the membrane of a range of different vesicles, originating from the trans face of the golgi apparatus, later fusing with plasma membrane. This association with membrane is unexpected because Trf lack transmembrane domain, but it may be possible if Trf associates with negatively charged lipids as suggested above. Upon challenge, spherule cells were affected and appeared to release the content of the spherules, including Trf proteins within the coelomic fluid. I also observed Trf-positive cells within the gut epithelium of *H. erythrogramma*, and observed Trf-positive vesicles filled with granular content and structures resembling bacteria²². These results suggest that Trf proteins can be either humoral or membrane bound, and have different biological functions in different cell types. Follow-up studies confirmed the association of Trf with coelomocytes in the pharynx, oesophagus, intestine and axial organ of *S. purpuratus*²³.

Since then, further studies have been conducted as an attempt to understand the function of Trf. Interestingly, the recombinant SpTrf protein is a disordered protein that fold into a α helical upon binding to pathogen-associated molecular patterns or cell membrane-associated motifs, opsonizes bacteria and increases phagocytosis²⁴⁻²⁷. Comparison of Trf transcripts and genes in sperm cells and coelomocyte cells showed that both spherule cells and small amoebocytes undergo somatic diversification by gene deletion, duplication, and single nucleotide polymorphism^{28,29}. However, it remains to be determined if the Trf gene diversification results from exposure to pathogens and allows immunological memory.

2.2. Postdoctoral formation in comparative transcriptomics applied to host-parasite interactions (2010-2013)

I returned to France for my postdocs and completed my formation in functional genomics. I used various approaches of comparative transcriptomics to study diploid and triploid oyster gametogenesis^{30,31}, the response of corals to a temperature-dependent *Vibrio*, to heat stress that mimic global warming³², and to ocean acidification³³ that I will not discuss further in the present document. However, I will provide more details below regarding two studies that greatly influenced the research program I developed later as an Assistant Professor by initiating my transition from comparative immunology to parasitology and microbiology. I contributed to the initiation of a large project aiming at characterizing the role of highly variable molecules in the specificity and memory of a snail immune response to a trematode parasite that show the role of local adaptation to the parasite in driving the diversification of the snail immune system. I was also involved in a project characterizing the mechanisms allowing a parasitoid wasp to manipulate the behavior of its coccinellid host that lead me to the discovery of a new virus species.

2.2.1. The role of highly variable immune response molecules in immune specificity and memory

By the end of my PhD, I started wondering about the level of specificity of invertebrate immune responses and the potential for immune memory. I wished to join the laboratory of Ecology and Evolution of Interactions (2EI, now IHPE, Interactions Hôtes-Pathogènes-environnements) that was studying the interaction between *Biomphalaria glabrata* and *Schistosoma mansoni* and were interested in developing a new project on the diversity, and role of a family of highly variable immune response molecules, called Fibrinogen-related proteins, FREPs^{34,35}. In the freshwater snail *B. glabrata*, evidence showed that FREPs play a role in snail resistance to infection by Schistosomes and diversify somatically by point mutation and alternative splicing events³⁵⁻³⁹. Researchers at IHPE had been working on the epidemiology of *S. mansoni* for over 30 years and maintained over 15 strains of the mollusks and parasites that could be used to test the specificity of the interaction, and even role of FREPs in local adaptation of hosts to parasites. Together with Dr Benjamin Gourbal, we designed an experiment to test the role of host genotype, priming (infection, and re-exposure to the parasite), and parasite genotype on the response of *B. glabrata* to *S. mansoni* infection. Dr Gourbal, his PhD student Julien Portela and myself prepared the experimental design, conducted all the experimental infections, extracted RNA and used RNAseq to sequence the transcriptome of control and infected individuals. I designed the bioinformatics analysis pipeline with Dr Christoph Gruneau and assembled the first high-quality transcriptome of *B. glabrata*. It led to the discovery of many new subfamilies of FREPs. Most interestingly, while analyzing the data, I identified new molecules that contained Immunoglobulin domains similar to the ones in FREPs, but a different lectin domain (Galectin or C-type lectin instead of Fibrinogen). I named these CREPs, for C-type lectin related proteins and GREP for galectin-related proteins (Figure 2). Phylogenetic analyses of the shared Immunoglobulin domains revealed that all these molecules are closely related and constitute together a new family of Variable immunoglobulin and Lectin domain-containing proteins, that I called VIgGs⁴⁰. A follow-up study then demonstrated that the highly diversified FREPs variants 2, 3 and 12⁴⁰, GREP and CREPs were among the most abundant plasma proteins capable of binding to *S. mansoni* larva⁴¹. Most interestingly, GREP and CREP2 present consistent strain-specific substitutions and differential expression in resistant and susceptible snails suggesting that they could play a role in host-parasite compatibility⁴¹.

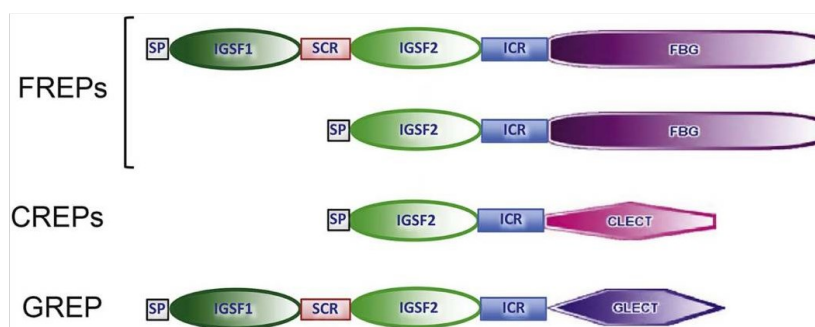


Figure 2. Similar domain structure of members of the VIgG family. SP: signal peptide, IgSF1 Immunoglobulin superfamily domain 1, IgSF2: Immunoglobulin superfamily domain 2, SCR: small connecting region, ICR: interceding region, FBG: fibrinogen domain, CLECT: C-type lectin domain, GLECT: Galectin domain.

The remaining sequencing data were used as part of the thesis of three different PhD students. I was not involved in their supervision. However, I contributed to the discovery of methylation-based epigenetic mechanisms in *B. glabrata*⁴², participated in characterizing the role of VIgGs in immune memory in *B. glabrata*⁴³, and revealed that locally adapted parasites trigger a rapid immunosuppression in the snail that is associated with increased parasite growth⁴⁴.

Follow-up studies of VlgL structure and diversity demonstrated their presence in various species, and suggested that these molecules constitute a complement-like complex specific of Apogastropoda⁴⁵. Characterizing the cellular and humoral mechanisms involved in snails immune response mechanisms to infection by trematodes will allow the development of new strategies preventing infection. For instance, we have identified humoral mechanisms, involving VlgLs, that allows snails to gain resistance against parasites that they have previously encountered⁴³, which suggest that a « vaccine » could be created to protect snails against infection by the trematode. We also identified a mechanism allowing parasites to infect local snails that could be targeted for the development of novel biocontrol strategies.

2.2.2. A virus, in a parasitoid wasp, in a ladybeetle

During my postdoc in IHPE, I also worked on an ANR directed by Dr Frederic Thomas (MIVEGEC, Montpellier) aiming at characterizing the underlying molecular mechanisms that allow the parasitoid wasp *Dinocampus coccinellae* to manipulate the behavior of its coccinellid host. Many species of parasitoid wasps induce the Bodyguard behavior, where the host remains on top of the pupating parasitoid to protect it from predators^{46,47}. The most intriguing about Bodyguard manipulations is probably that the parasitoid pupae and the host are not in contact anymore at the time of the manipulation (Figure 3). My goal was to determine how the parasitoid can distantly control the behavior of its host.

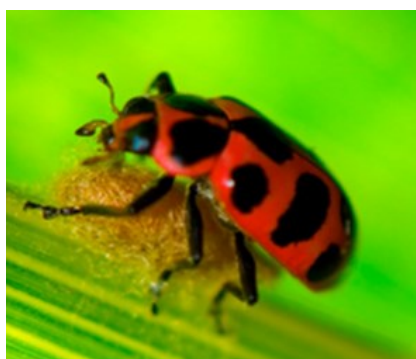


Figure 3. A Bodyguard ladybeetle (*Coleomegilla maculata*) attending a parasitoid cocoon (*Dinocampus coccinellae*).

I discovered a new positive strand RNA virus of the family Iflaviridae associated with *Dinocampus coccinellae*: The virus was abundant in parasitoid wasp larvae and in parasitized ladybeetles, but it was absent in healthy non-infected ladybeetles. I hypothesized that the virus was responsible for the behavioral manipulation in lieu of the parasitoid wasp. To test this, I designed strand-specific qPCRs that allowed me to show that the *Dinocampus coccinellae* paralysis virus (DcPV), is stored in abundance, but do not replicate actively, in the oviduct of healthy adult wasps. However, the virus replicates in developing larvae and is transmitted to the ladybeetle host during larval development within the host abdomen. Using electron microscopy, I visualized the virus in the ladybeetle nervous tissue demonstrating its neurotropism, a characteristic that could induce severe neuropathy responsible for the bodyguard behavior (Figure 4). Indeed, all the symptoms related to this behavior (starvation, paralysis, tremors) could be attributed to DcPV replication in the nervous tissue. Finally, given that some ladybeetles recovered from the behavioral manipulation, these were likely able to fight off the virus, so why did they get infected by the virus in the first place? I hypothesized that the parasitoid injects an immunosuppressive venom at the time of oviposition that makes the ladybeetle susceptible to the virus. I used qPCR to compare the expression of antiviral immune response genes

over the course of parasite development. As expected, I observed a rapid immunosuppression, most probably induced by the parasitoid venom proteins⁴⁸. At the time of the bodyguard manipulation, the antiviral immune response is returning to normal level (Figure 4), indicating that the ladybeetle paralysis might actually result from the antiviral immune response associated with an accumulation of phagosomes in the nervous tissue.

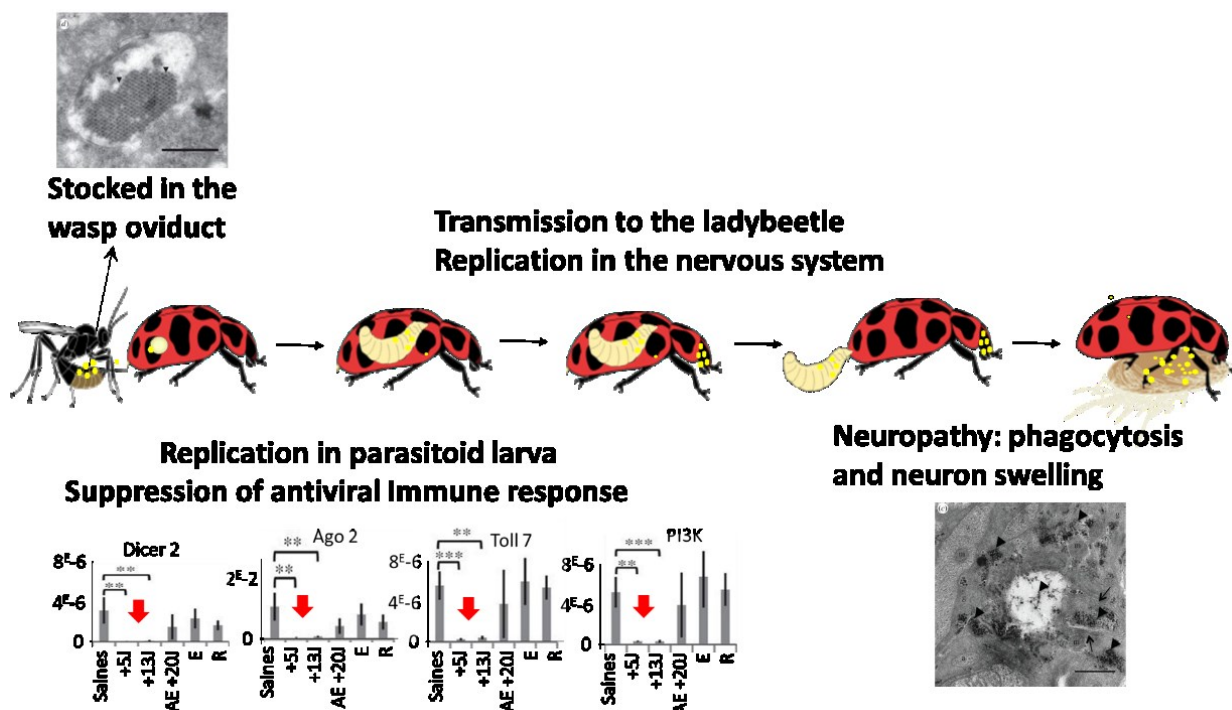


Figure 4: Concerted role of the *Dinocampus coccinellae* paralysis virus (DcPV) and *Dinocampus coccinellae* in initiating the Bodyguard behavior

This finding is a departure from the status quo by proposing that the parasitoid wasp and virus work together to manipulate the ladybeetle behavior. The discovery of the DcPV has extensively been discussed in the media and I have personally been interviewed on multiple occasions for blogs, journal articles, radio and TV documentaries. I have also assisted a journalist with the redaction of an article in Science et Vie Junior, and an author in writing a children book within which a chapter focuses on this discovery.

The discovery of the concerted role of the parasitoid immunosuppressive effect, the neurotropic parasite-associated virus, and the host immune response to the viral infection in inducing the behavioral manipulation led me to reconsider the role of microbes in driving host-parasite interactions. It initiated a departure from the field of comparative immunology and brought me to initiate a novel research program that integrates the role of microbes in the ecology and evolution of host-parasite interactions.

3. The Dheilly lab: host-parasite-microbes interactions (2015-2020)

In spring 2014, I received an offer to join the School of Marine and Atmospheric Sciences of Stony Brook University, in NY, USA, as a Tenure Track Assistant Professor. I moved to the USA with my family in the fall of 2015. This position has presented me with many opportunities to demonstrate my leadership abilities, at the university, departmental and laboratory level. I was given an empty space and start-up funds to renovate the laboratory, buy small equipment, pay my summer salary and the first year of my student's stipend. In addition to teaching, I was expected to facilitate access to genomics to my colleagues, and to develop an independent research program.

3.1. The birth of the Parasite Microbiome Project

3.1.1. Building a conceptual framework

My discovery of the role of DcPV in behavioral manipulation significantly influenced the way I understood host-parasite interactions and led me to conduct literature reviews of our current body of knowledge on parasite-microbe interactions. I quickly realized that 1. we have very limited knowledge of the microbiome of parasites and 2. when the role of microbes has been investigated, they play key role in the host-parasite interaction. Clearly, any host-parasite interaction could be considered a holobiont (host)- holobiont (parasite) interaction⁴⁹. Thus, I decided to focus the following part of my career on filling this important gap in knowledge by studying host-parasite-microbe interactions.

In a follow-up article, in collaboration with Dr. Robert Poulin and Dr. Frederic Thomas, I considered how microbes could drive host-parasite co-evolution⁵⁰. Indeed, host-associated microbes and parasite-associated microbes can directly or indirectly contribute to the outcome of host-parasite interactions (Figure 5). I describe the disruption strategy, where a parasite alters its host microbiome to its advantage, and the biological weapon strategy, where a parasite-associated microbe is transmitted to the host and alters the parasite pathogenicity. Because of the independent evolutionary paths taken by host, parasites and microbial species, we also consider that conflicts of interests may arise between microbes and their parasitic hosts, resulting in more or less well adapted holobionts. Finally, we discuss how metacommunities of microbes vary at different spatial scales and can contribute to variation in compatibility between host and parasite. In this review, we proposed ecological and evolutionary research topics that emerge from the consideration of the role of host- and parasite-associated microbes in host-parasite interactions and offer an initial list of research questions that need to be answered.

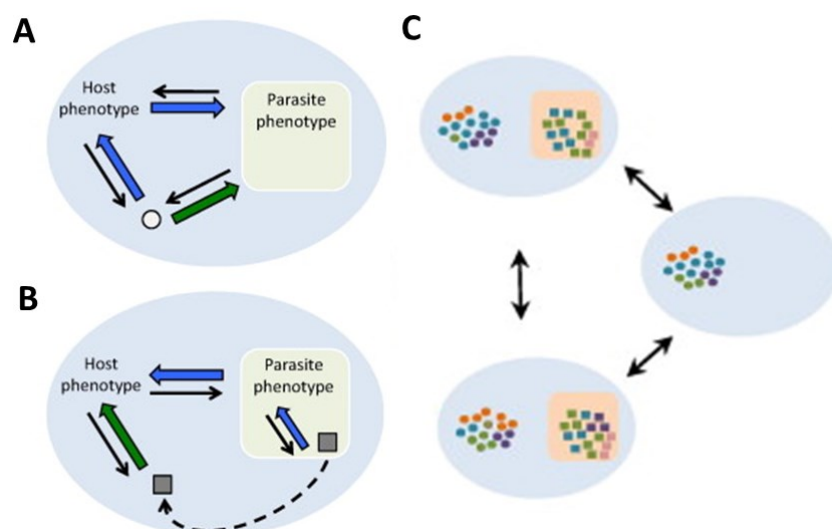


Figure 5: Role of host- and parasite-associated microorganisms in host–parasite interactions. The three-partner interaction consists in three direct effects between (i) the host and the parasite, (ii) the host and the microorganism and (iii) the parasite and the microorganism. Host-associated microorganisms (A) and parasite-associated microorganisms (B) can directly (green) or indirectly (blue) participate in the host–parasite interaction. (C) Within a population, host individuals serve as local communities of host and parasite –associated microbial communities that are connected by dispersal or transmission from host to host (healthy and parasitized hosts are shown)

I was then invited to contribute to reflections on the hologenome theory of evolution that considers that the combination of all the genomes of the holobiont is a unit of natural selection. I, and others, argue that this new concept is necessary to embrace the pluralistic attributes of any plants and animals, whose phenotype results from multilevel selection on the host genome, the microbe genomes, and the hologenome. The significance of my contribution justified my second authorship on the high impact manuscript «Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes.»⁵¹ that clarifies misconceptions about the theory. In particular, we emphasized that the holobiont definition encompasses all microbes associated with the host at a given time. It includes microbes that are beneficial and detrimental to the host, with microbes that have coevolved with the host (known as symbionts), and microbes that have not coevolved with the host and might be transient. It makes the distinction between holobiont, a structural definition for an entity composed of microbes and a host, and processes such as coevolution, evolution, selection and adaptation that are processes that will result in either a stochastic or deterministic assembly of microbiome (although it is often a mix of both).

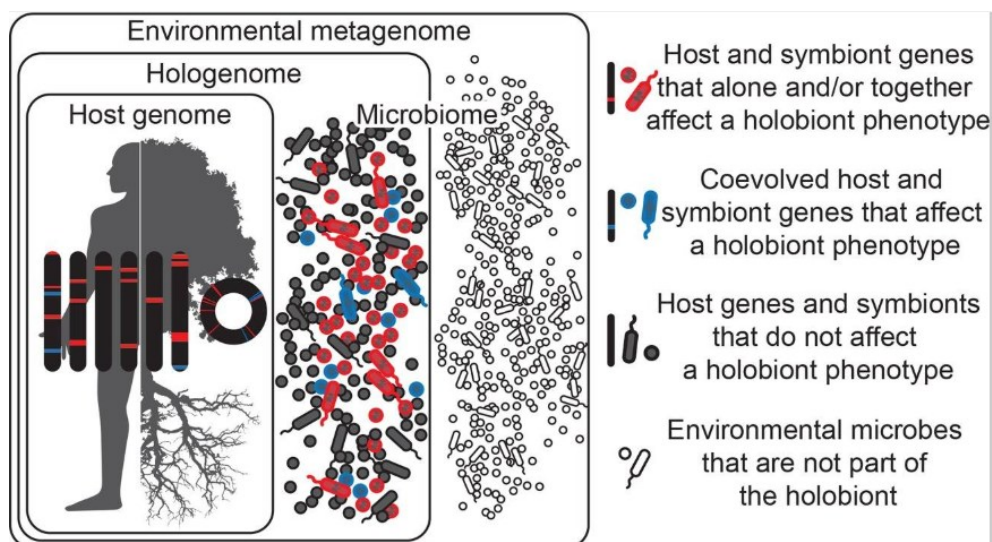


Figure 6: Hologenomes encompass the genomes of the host and all of its microbes at any given time point. Holobionts are entities comprised of the host and all of its symbiotic microbes, including those which affect the holobiont's phenotype and have coevolved with the host (blue), those which affect the holobiont's phenotype but have not coevolved with the host (red), and those which do not affect the holobiont's phenotype at all (gray). Therefore, holobiont phenotypes can change in time and space as microbes come into and out of the holobiont. Microbes in the environment are not part of the holobiont (white).

3.1.2. Building a consortium

While I started investigating parasite-microbe interactions in my laboratory, I realized that to advance the field of parasitology forward quickly, we needed to investigate parasite-microbes interactions in a diverse range of parasite species. To do so, I launched the **Parasite Microbiome Project (PMP)** aiming at connecting researchers with diverse expertise in immunology, parasitology, microbiology and evolutionary biology to facilitate characterizing the microbiome of parasites across phyla and the effect of parasites on their host microbiome.

Following the publication of an initial manuscript where I justify the need for the PMP⁵², I obtained **funding from the Gordon and Betty Moore Foundation** to organize the First Parasite Microbiome project Workshop and bring together experts from complementary fields, and members from the Genomic Standard Consortium, the Unified Microbiome Initiative, and the Earth Microbiome Project. The workshop, co-organized by myself, Joaquin Martinez Martinez and Jon Kaye, took place on January 9-14, 2019 Clearwater, Florida, USA and hosted 13 researchers from across continents and disciplines of expertise. As an initial step in establishing the PMP consortium towards driving scientific discourse, the participants wrote together a manuscript invited in Plos Pathogen to define the vision, structure, and objectives of the PMP. We identify knowledge gaps and grand challenges in the field of host-parasite-microbe interactions⁵³. Additional manuscripts are being written to outline the benefit of the PMP to diverse research fields. I have led the redaction of a Pearl Plos Pathogen that reveals the role of microbes in cancer associated with parasites⁵⁴ and contributed to an analysis of the importance of considering the microbiome in parasite conservation⁵⁵. Together with the Genomic Standard Consortium, we are currently preparing a MlxS symbiont-associated package: a MlxS extension defining the minimum information standard for sequence data from mutualistic and parasitic symbiotic organisms, and their hosts.

Together with both proponents and supporters of the hologenome theory of evolution, I organized a symposium at the last Joint Congress in Evolutionary Biology (Montpellier 2018, co-organized with Douglas A, Martinez-Martinez J, and Schulenburg H). The symposium entitled “Evolution of hosts and parasites with their microbiomes: a problem of unfaithful relationships” brought together evolutionary biologists focusing on coevolution, microbial adaptation, animal and plant immune defense, with parasitologists, researchers working on infectious diseases, and also ecologists. We discussed the evolutionary and ecological processes that result in the formation of a mutually beneficial host-microbiome association, the functionally relevant characteristics of such interaction, and the influence of microbes on host-parasite co-evolutionary dynamics.

At the next European Multicolloquium of Parasitology (EMOP2020, moved to 2021) that will take place in Belgrade, I am co-organizing a session entitled “the microbiome of parasites and their role in diseases” with Dr Cinzia Cantacessi, while other colleagues (Harnett W and Sfronic-Milosavljevic L) are proposing a session on “helminth immunomodulation and interactions with the microbiome”. Clearly, the parasitology community is now embracing this new research field and major progress is being made.

Other upcoming events (that have been delayed due to the on-going sanitary crisis) that will foster discussions and collaborations include my invitation by Tom Gilbert to a small, by invitation only, Evolutionary Hologenomics meeting in Copenhagen, organized as part of the creation of a research center in Evolutionary Hologenomics. I have also been pre-selected to attend a Collaboration workshop on the Ecology and Evolution of Infectious Diseases that will be held in Montpellier and aims at creating opportunities for collaborations.

Since I have returned to France, I have also initiated discussions to set up a COST Action in Europe to catalyze the scientific discourse towards an integrated understanding of the viral and bacterial microbiota of protozoan and helminth parasites and their impact on human and animal health and disease. To achieve this goal, the action will establish a structured network of researchers with complementary expertise in Parasitology, Virology, Bacteriology and Genetics and facilitate interactions and collaborations among research areas that historically rarely interact with each other and with biomedical industries. The action will propel this new research field forward by connecting the newest research with applied use by enabling collaboration among experts involved in basic and strategic research, together with R&D companies. The Cost action will also provide opportunities for minorities, female and young researchers, and enable a new generation of top-level interdisciplinary investigators, and constitute an incubator for innovation on the role, impacts and utilization of parasite interactions with microbes on human and animal health.

3.2. *Schistocephalus solidus* interaction with microbes

3.2.1. An ideal model system

To initiate studies on host-parasite-microbe interactions, I had to identify a model system for my lab. *Schistocephalus solidus* is a cestode parasite specific to the threespine stickleback *Gasterosteus aculeatus* that I have been using as model system since I joined Stony Brook University in 2015. The threespine stickleback species complex has proven to be powerful for ecological studies and evolutionary biology⁵⁶. By association, *S. solidus*, is broadly used for eco-evo studies of host-parasite interactions^{57,58}. *S. solidus* definitive host is any piscivorous bird, within which the parasite

reproduces sexually. Eggs are released with feces and hatch into coracidia infective for cyclopoid copepods, the first intermediate host. They develop into procercoids and become infective for the second intermediate host, the threespine stickleback. The life cycle is complete when an infected stickleback with a mature plerocercoid is ingested by a bird. We have determined that *S. solidus* is an ideal model system for ecological and evolutionary studies of host-parasite-microbes interactions⁵⁹ because:

- The life cycle of *S. solidus* can be reproduced in laboratory conditions⁵⁷. A method to breed *S. solidus* in vitro in an artificial bird gut was developed in 1946⁶⁰. After breeding, parasite eggs can be stored in the fridge, in darkness for a few years. Eggs can be hatched in vitro and used to experimentally infect copepods, that are themselves used to experimentally infect threespine sticklebacks (Figure 7).

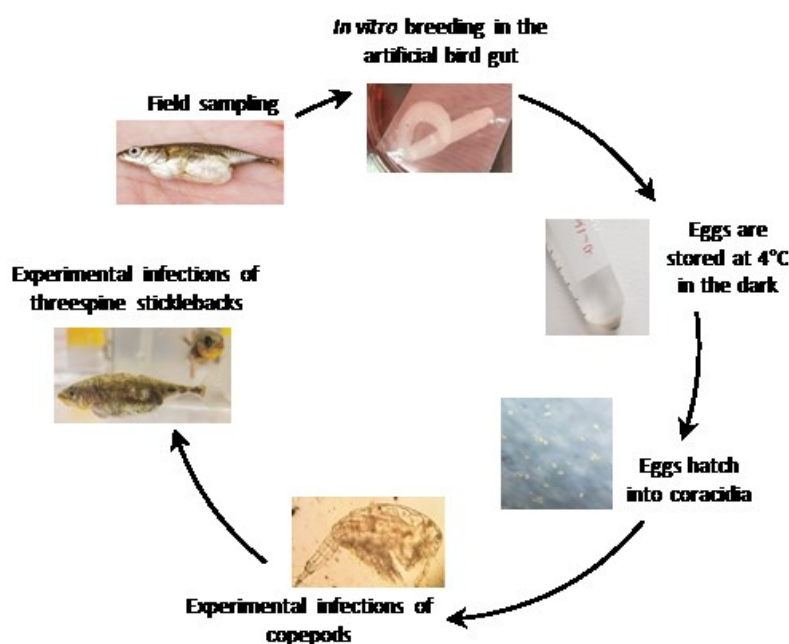


Figure 7: The life-cycle of *Schistocephalus solidus* is reproduced in laboratory conditions

- Tissue samples of *S. solidus* can be collected before breeding without altering its reproductive abilities⁶¹. Parasites can self-fertilize if stimulated alone, or mate in pair when presented to a size-matched partner^{62,63}.
- *S. solidus* is ubiquitous and prevalent in freshwater environment in Europe, Canada and Alaska^{61,64} with pronounced genetic structuring among lakes and regions⁶⁵⁻⁶⁷.
- The closely related species *Schistocephalus pungitii*⁶⁶ is specific to Ninespine sticklebacks. *S. solidus* and *S. pungitii* can hybridize suggesting that there is an unknown mechanism of postzygotic isolation^{68,69}.
- Cross-infection experiments have shown that sticklebacks adapt to *S. solidus* by acquiring a global resistance mechanisms while *S. solidus* locally adapts to its fish host⁶¹.
- The genomes of threespine sticklebacks and *S. solidus* have been sequenced and assembled⁷⁰⁻⁷².

Thus, this system could be used to test various hypotheses regarding the role of microbes in host-parasite interactions, as discussed in a review co-written with my PhD student, Megan Hahn⁵⁹. However, the presence of microbes associated with *S. solidus* was then unknown, and the impact of *S. solidus* on its host microbiome had not yet been characterized.

I obtained **funding from the Eppley Foundation for Research** and **two Innovation Funds provided by the Laurie Landeau Foundation** that allowed me to support graduate and undergraduate students salaries, and to purchase equipment for *in vitro* culture of *S. solidus*, to culture fish in the laboratory, and to conduct experimental infections. We built two fish rooms equipped with temperature control, water quality monitors (pH and conductivity), water chiller, and a fully automated water change and dosing system.

3.2.2. *S. solidus* interaction with viruses

We initially used virus purification coupled with RNA sequencing to characterize the virome of four individual *S. solidus* and corresponding sticklebacks. We discovered one new virus in Threespine sticklebacks called the Threespine stickleback picornavirus (TSPV) and showed that it is vertically transmitted in fish⁷³. This is the first demonstration of vertical transmission of a picornavirus in vertebrates. The TSPV is not transmitted to *S. solidus*. We also assembled complete genome sequences of six new virus species associated with *S. solidus* (Figure 8)⁷⁴. Using *in vitro* culturing and experimental infections, we showed that five of the six viruses of *S. solidus* are vertically transmitted, sometime with very high efficiency, but we did not find evidence of horizontal transmission in co-infected fish⁷⁴. We also showed that the Schistocephalus solidus rhabdovirus (SsRV) has a basal position to rhabdoviruses of vertebrates and is transmitted to parasitized hosts, suggesting that parasites could facilitate host shifts of viruses to vertebrate host. This very first characterization of the virome of a parasitic flatworms, conducted on a limited number of individuals from a small geographic region reveals that these organisms may host a very diverse array of viruses.

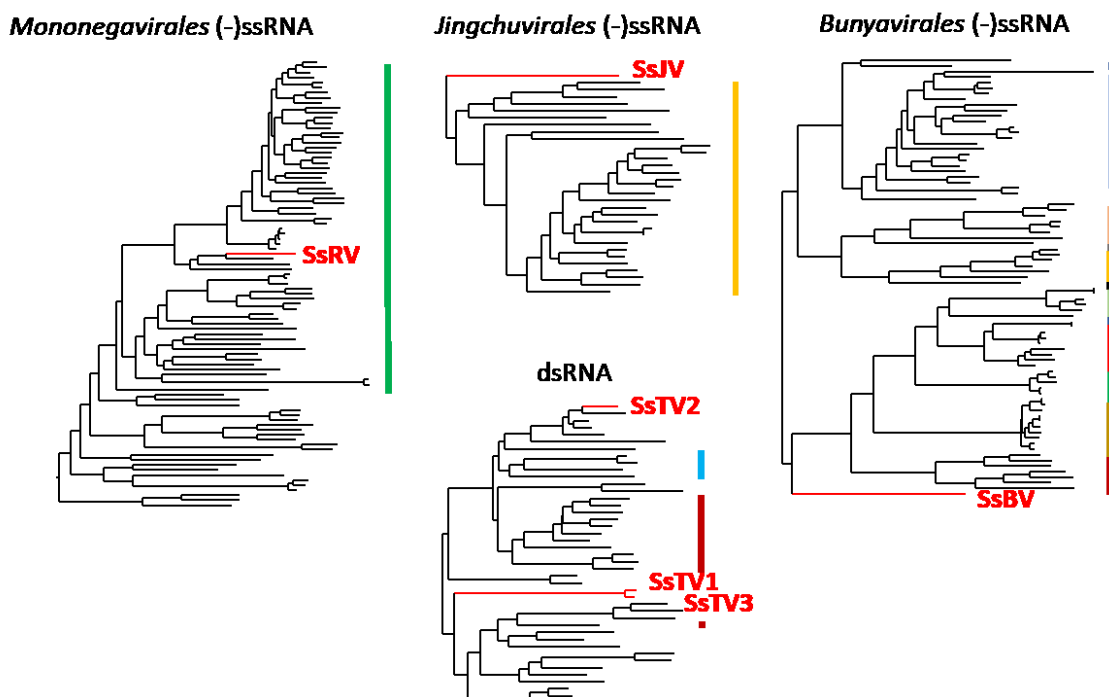


Figure 8: *S. solidus* hosts a complex virome. The trees provide the phylogenetic positions of *S. solidus* viruses from Alaska (in red) within the broader diversity of known RNA viruses. The name of the major clades is shown at the top of the trees. The colored bars on the right of the trees indicate recognized families.

3.2.3. *S. solidus* bacterial microbiome

Using field sampled *S. solidus* from Alaska, we revealed the presence of a complex bacterial microbiome in *S. solidus*. I established a new collaboration with Prof. Martin Kalbe at Max Planck Institute of Evolutionary Biology, in Plön (Germany) to conduct experimental infections with hosts and parasites from very distinct origins to determine the processes regulating parasite microbial assembly. We used fish and parasites from two locations in Alaska and from Europe (Figure 9).

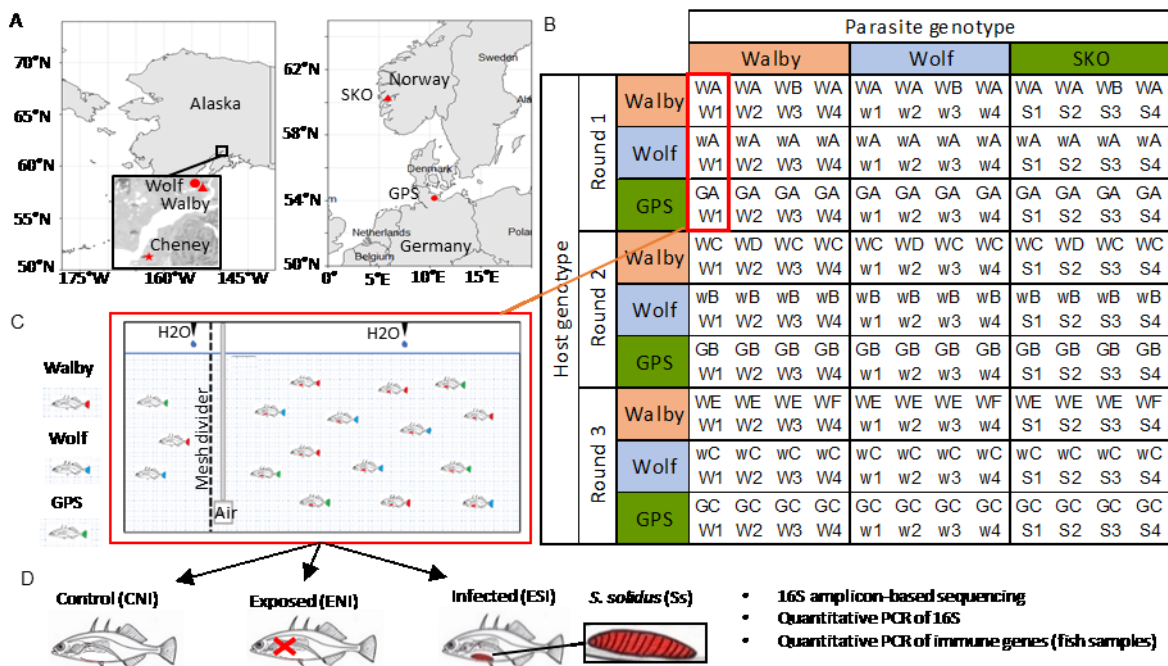


Figure 9: Summary of experimental design. (A) The map displays all sampling locations. The field study was conducted on sticklebacks and *S. solidus* from one location in Alaska (Cheney). The three genotypes of stickleback hosts (Walby (W), Wolf (w) and GPS(G)) and three genotypes of *S. solidus* (Walby (W), Wolf (w) and SKO (S)) used for experimental infection were collected from lakes in Alaska and Europe. (B) Design matrix showing the organization of rounds and tanks based on fish genotype (denoted by letters) and parasite genotype (denoted by numbers). A total of 36 tanks were used. One example is squared in red. (C) Each of the tanks contained three sham control fish (left) from Wolf (Red Tail), Walby (Green Tail), and GPS (Blue Tail) separated by a mesh divider from 5 Wolf fish, 5 GPS fish, and 4 Walby fish that had been exposed to parasites (right). (D) Upon dissection, the success of infection was assessed, and exposed individuals were classified as exposed non-infected (ENI) or exposed successfully infected (ESI). All successfully infected fish were processed, and corresponding ENI and control non-infected fish from the same tanks and fish origin were processed as controls.

Our results show that *S. solidus* microbiome is not a random assemblage of microbes present in the parasite environment and suggest that parasite genotype-based selective forces play a role in shaping the parasite microbiome composition and diversity. Our results indicate that the *S. solidus* microbiome composition is markedly different from its host microbiome. Differences are increasingly evident as we compared the microbiome of fish and parasites at the family, and then the ASV level. For instance, the most prevalent and most abundant family in both host and parasite is Bacillaceae. But, phylogenetic evidence shows distinct clustering of ASVs associated with the two partners, indicating that the bacteria found in the parasite are distinct from those present in the fish host (Figure 10). The presence in *S. solidus* of ASVs that were rare in Threespine sticklebacks suggest that some of

these bacteria could be vertically transmitted. In support of this, we found that *S. solidus* microbiome composition varies depending on parasite origin. Finally, we revealed a positive correlation between some of the highly prevalent bacterial families and immune gene expression in infected fish. Most specifically, the relative abundance of the family of *Chloroflexi* in *S. solidus* was correlated with higher expression of *stat6*, *foxp3*, *tnfr1*, *cd97*, and *marco* demonstrating the impact of parasite-associated bacteria on host immune response to infection (Figure 11)

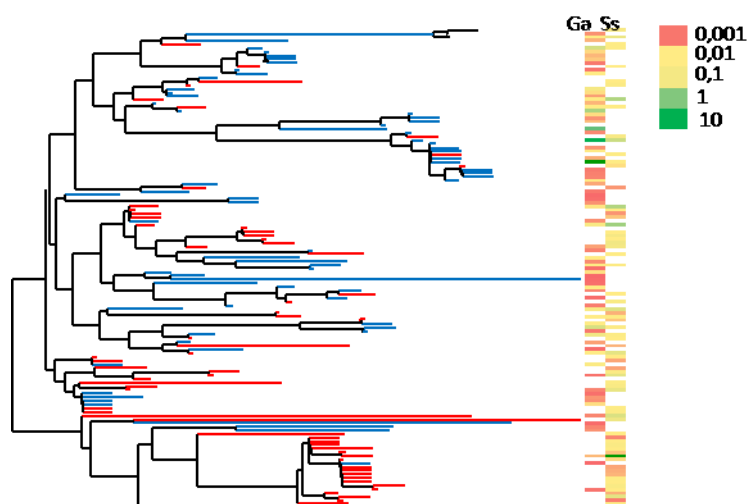


Figure 10: Distinct clustering of ASVs more prevalent in *S. solidus* (red) and ASVs more prevalent in the fish host (blue). The heat map provides information on ASV relative abundance in the parasite (Ss) and the fish host (Ga) and shows congruence with prevalence information.

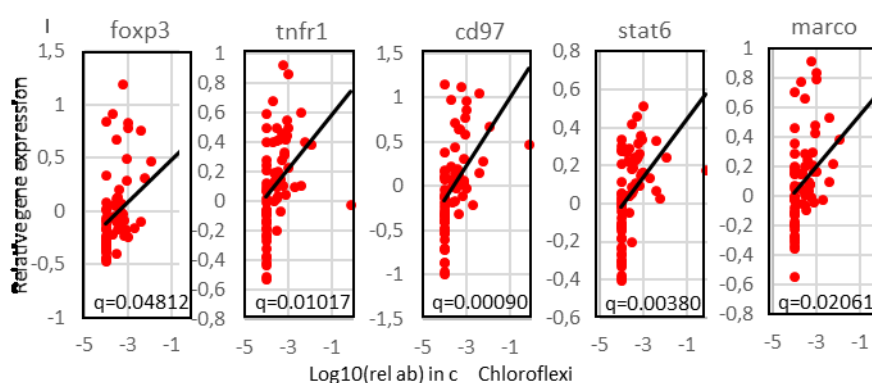


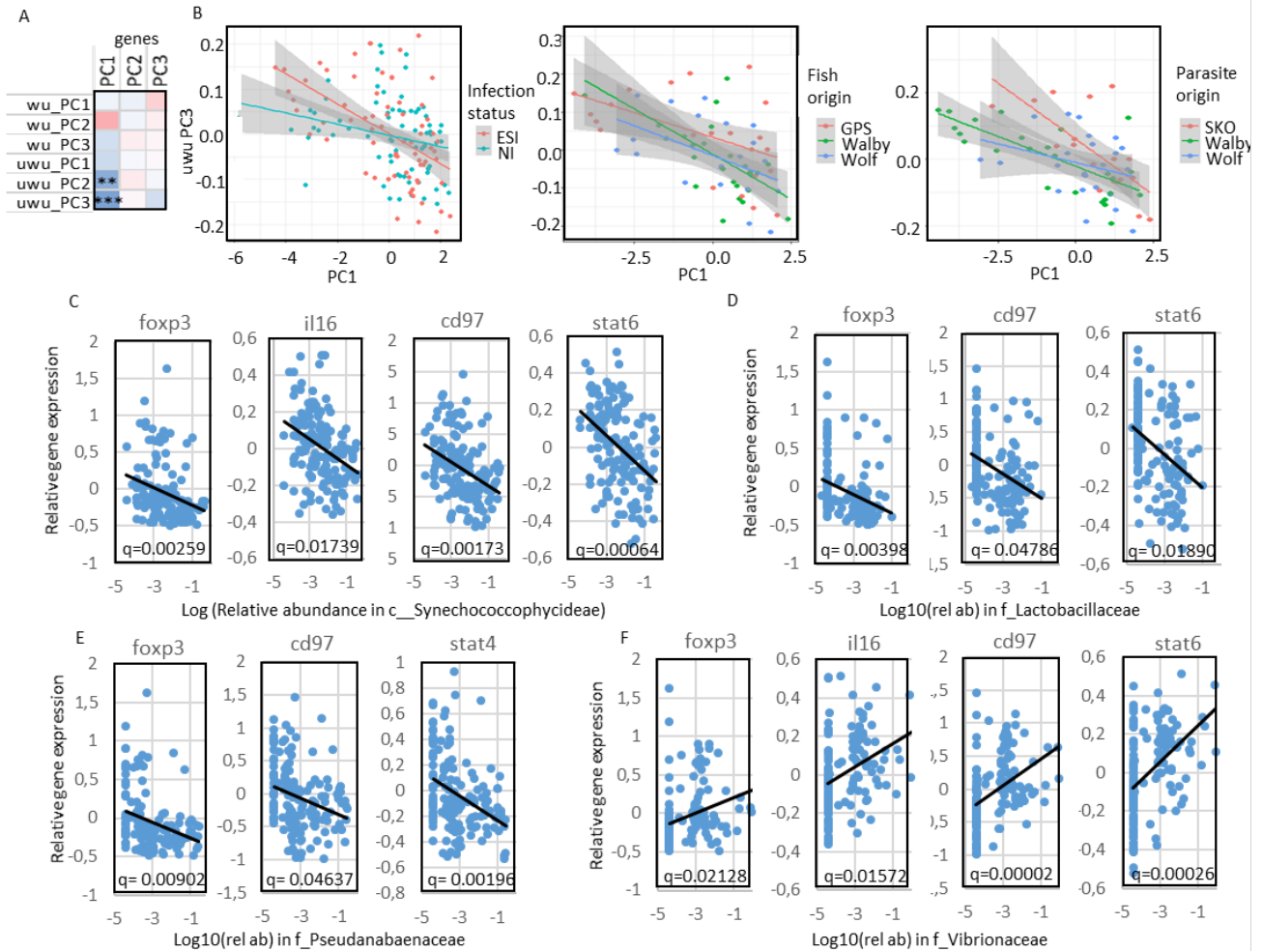
Figure 11: Immune response gene expression in infected fish correlates with the relative abundance in an unknown family of *Chloroflexi* associated with *S. solidus*. The significant Spearman correlation (with Bonferroni correction), was found between the relative abundance of *Chloroflexi* in the parasite and relative abundance in *foxp3*, *tnfr1*, *cd97*, *stat6*, and *marco* (N=69).

3.2.4. *S. solidus* interaction with the host microbiota

We initially characterized the bacterial microbiome of the stomach and intestine of field sampled parasitized sticklebacks and found that infection by the cestode parasite correlates with a disruption of the bacterial microbiome of sticklebacks. The association between helminth infections and changes in the composition and diversity of the host gut microbiota has been investigated in many systems⁷⁵⁻⁷⁹. Results differ greatly, with some studies showing an increase in microbial diversity associated with infection⁸⁰⁻⁸², others showing a decrease in microbial diversity^{75,83-85}, and some showing no changes^{77,86,87}. The differences that have been reported likely result from profound differences in the nature of parasite interaction with microbes (direct or indirect) and in host and parasite eco-evolutionary history. Our experimental design allowed us to test for the role of host origin, and parasite origin on various epidemiological traits, including host microbiome composition.

Our results indicate that the microbiome composition of fish exposed to *S. solidus* depends on both host genotype and parasite genotype. In control non-infected hosts, we observed that the microbiome vary with host-genotype and confirm previous studies in sticklebacks and various other organisms showing that the host-based selective processes shape the organism microbiota⁸⁸⁻⁹⁰, probably due to constitutive differences in immune gene expression pathways between populations⁹¹⁻⁹⁴. Surprisingly, we found that exposure to *S. solidus* increased differences based on host genotypes. In contrast, we found limited differences between infected sticklebacks of different genotype. But, genetic variations in parasites influences the ways the microbiota changes in response to infection. This parasite-genotype effect was observed in all exposed Threespine sticklebacks, but it was stronger in successfully infected fish than in non-infected fish.

Finally, we showed that the correlation between host immune gene expression profile and host microbiome composition varied with infection status, host origin and parasite origin (Figure 12). In particular the immune genes *foxp3*, *il16*, *cd97*, *stat4* and *stat6*, and key bacterial families, such as *Synechocophycideae*, *Lactobacillaceae*, *Pseudanabaenaceae* and *Vibrionaceae* showed significant correlation. Previous studies have shown that *S. solidus* is locally adapted to immunological characteristics of its sympatric host^{61,94}, and that Threespine sticklebacks can mount parasite genotype specific innate and adaptive immune responses to trematodes and cestodes^{92,93,95,96}. Thus, differences in microbial communities observed in successfully infected individuals compared to exposed individuals could result from different immune responses the fish mount during *S. solidus* infection⁹⁷. Through this, the parasite likely alters the interaction between the host and its microbiome. The more limited parasite genotype effect on the microbiome of exposed but non-infected fish supports the hypothesis of an indirect effect via differential modulation of the immune response by different parasite genotypes.



3.3. The microbiome of other parasitic Platyhelminthes

3.3.1: The bacterial microbiome of Trematodes and impact on their life-cycles

With Professor Robert Poulin, we obtained a substantial **grant from the Marsden Foundation** to test the potential for microbes to manipulate their parasitic hosts life cycle to increase their own fitness. This project, untitled « Microbes at the helm : *are microbiomes shaping parasite phenotypes?* » started in 2019 and is expected to be completed in 2021. Using two flatworms, we are testing for ontogenetic, inter-individual and geographic variation in parasite microbiomes and experimentally quantify their impact on parasite development and, ultimately, on host and parasite phenotype.

The persistence of microbial communities throughout the life of a parasite is particularly challenging for helminths with complex life cycles. These parasites undergo major morphological changes during their life, and parasitize host species that are immunologically, physiologically, and phylogenetically very different. We started by characterizing the microbiome of the trematodes *Coitocaecum parvum* and *Philophthalmus sp.* across all of their life stages: sporocysts or redia, metacercariae and adults, as well as free-living cercariae⁹⁸. Our results demonstrate that the parasite possesses a phylogenetically diverse microbiome, distinct from that of its hosts or the external environment. This result clearly indicate that the microbiome of these worms is not a random subsampling of microbes from the parasite direct environment, but that it is under constraint by parasite selective forces.

The *C. parvum* microbiome comprises bacterial taxa specific to each life stage, as well as a small core that persists across the parasite's whole life. The existence of an ontogenetically and vertically transmitted core microbiome is supported by our finding that the main source of microbial taxa at any life stage is the previous life stage⁹⁸. On-going studies include the comparison of the microbiome of different *C. parvum* individual parasites with a normal or a precocious life-cycle. The latest mature precociously in the amphipod host and produce eggs via selfing, thus skipping sexual reproduction in the definitive host entirely.

In addition, we are comparing the microbiome of different castes of *Philophthalmus sp.* and testing experimentally (following antibacterial treatments) the role of microbes in determining the clonal parasite development into reproductive morphs or into mobile soldier morphs. Preliminary results indicate that an alteration of the parasite microbiome composition can alter the development of redia into different morphs. This body of work will allow us to assess the role of bacteria in shaping parasite phenotypes and life-cycles.

3.3.2: The bacterial microbiome of the tapeworm *Eubothrium sp.*

In collaboration with Dr Morten Limborg, we characterized the surface and endomicrobiome of the tapeworm *Eubothrium sp.*, a parasite of salmon that constitute a growing threat to salmon aquaculture due to its negative impact on fish growth. Consistent with other studies, we determined that presence of tapeworm altered the salmon gut microbiota, and observed a correlation between tapeworm burden and an increase in pathobionts and a decrease in the dominating commensal *Mycoplasma*. We also established that despite lacking a gut, *Eubothrium sp.* hosts a distinct endomicrobiome, while its surface tegument microbiome was intermediate between the salmon gut and endomicrobiome, suggesting that selective forces play a role in regulating the parasite microbial communities. The tapeworm microbiome was dominated with distinct and novel *Mycoplasma* phylotypes that differed from those abundant in the salmon gut. Currently, the genome of this new *Mycoplasma* bacteria is being assembled from shotgun metagenomics of eight endomicrobiome

samples. The genome of the *Eubothrium*-associated *Mycoplasma* bacteria will be compared with the recently assembled genomes of *Mycoplasma* phylotypes associated with the Atlantic salmon host. Further studies will be designed to determine the role of the *Mycoplasma* symbiont in the cestode, as well as its possible interactions with the salmon gut microbiome and the immune system.

3.3.3: The virome of parasitic Platyhelminthes

Immediately after the first PMP Workshop, I started investigating the presence of unidentified viruses within freely available *de novo* assembled transcriptomic data of parasites available on NCBI. Indeed, most comparative transcriptomics studies consider transcripts mapping to bacteria and viruses as contaminants, and these are removed before further analyses are undertaken. I rapidly discovered many novel viral sequences. While some parasite taxa are currently poorly represented within databases, the transcriptome of many different species of free-living and parasitic Platyhelminthes have been sequenced. In collaboration with Dr Karyna Rosario and Dr Yannick Blanchard, we downloaded the raw transcriptomic data of a total of 149 species of free-living and parasitic Platyhelminthes that we re-analyzed to extract full-length reliable genome sequences. This body of work has allowed me to discover over a hundred new species of viruses, and to assemble full-length genome sequences for most of them. These viruses constitute the preliminary data for the research projects that I propose below.

4. Research projects and perspectives for the future

4.1. Context and objectives

In January 2020, I have officially quit my position at Stony brook University and I have returned to France. Together with Yannick Blanchard, we have obtained funding from Région Bretagne to cover my salary to join the “Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du travail” (ANSES), in his team of Viral Genetics and Biosecurity. This temporary position provides an ideal environment to continue my research endeavor and provide opportunities to apply this novel field of research to animal health and food security. While I can’t predict the future in terms of where I will be pursuing my research career, and what funding I will receive, I hope I can pursue working on parasite-virus interactions and I have identified key gaps in knowledge and research questions that apply to various parasite species and will constitute the framework of my future research endeavors. For each section, I propose model systems that would be appropriate to test the stated hypotheses.

Viruses infect all cellular life, including free-living organisms but also parasitic organisms. Indeed, evidence show that viruses are widespread components of the microbiota of parasitic arthropods⁹⁹⁻¹⁰², nematode¹⁰³⁻¹⁰⁷, and protozoans¹⁰⁸⁻¹¹⁰ and that they contribute to their parasitic host virulence and ecology. **It is now necessary to consider how parasites contribute to virus evolution, and how viruses of parasites contribute to the ecology and evolution of parasites and their hosts.** Viruses can be beneficial to the parasitic host, but they can also be costly, thus mediating divergent selection between parasite populations¹¹¹. By imposing additional local selection pressures, viruses could also contribute to parasite speciation, competition among parasites, parasite reproductive behavior, and host range. When transmitted to the parasitized host, parasite viruses can divert the host immune response in ways that may aid the parasite survival, or trigger inflammatory responses that participate in parasite pathogenicity^{54,112,113}. Equally, because of parasites adaptation to viruses, and because virus diversity is nested within the parasitic host, parasite biodiversity, ecology and population structure will affect virus populations. Parasites could serve as reservoirs and vectors of viruses, facilitating host shifts among host species from distantly related taxa. Parasite geographical diversity, resulting from local adaptation to hosts and on-going ecological speciation could drive virus diversification and variable prevalence in host populations¹¹⁴. There is thus potential for detrimental and beneficial impact of viruses, and reciprocal adaptation from the host or the parasite. This bidirectional interdependence is at the heart of my research projects whose **general objective** is to reveal the role of nested systems - viruses within parasites, within hosts - in the ecology and evolution of each of the partners in the interaction.

4.2 Role of parasites in driving the evolution of viruses

Parasites should be privileged hosts for viruses given that parasitism is the most successful way of life if we consider solely the diversity of parasite species, and the number of times it has evolved independently. Yet, our current knowledge of viral diversity is strongly biased towards viruses of free-living organisms. Herein, I propose to fill this major gap in knowledge. Parasites represent very diverse groups of organisms that share one major common feature: a very specialized feeding behavior and intimate association with the host. Other characteristics of parasites are likely to greatly influence the evolution of their viruses. 1. They are often hermaphrodite, or use parthenogenesis or

asexual reproduction. 2. They usually have a shorter generation time than their host. 3. Parasite populations tend to be highly fragmented. All these characteristics could drive virus diversification by preventing host switching.

But, parasites also display major ecological and life-history differences that could differentially impact parasite-virus evolution and co-evolution. Parasite Intrapopulation size (the size of the population within a given host individual) vary greatly among parasite species, and would either favor, or constraints horizontal transmission of viruses and genetic drift. Parasite infra-population diversity influenced by the reproduction mode, including the rate of self-fertilization, the presence of asexual amplification in some trematodes, and the number of maternal founders would also impact the potential for virus transmission. A high parasite genetic structure is likely to be associated with a greater virus diversity. Some parasites present very high genetic structure, often associated with complex life-cycle, self-fertilization, immobile or asocial hosts, highly aggregated distribution, short generation time, high host specificity, and vertical transmission¹¹⁵. Whereas other parasites have low genetic structure associated with highly motile hosts, long-lived life-cycle, large effective population size, long generation time, low host specificity, high mobility¹¹⁵. Parasite mobility would influence virus gene flow, with ectoparasites allowing greater dispersal than endoparasites, or than parasites infecting hosts with low mobility.

I propose to discover and characterize viruses in a broad range of parasites. The newly assembled viral genomes can then be analyzed, and compared, to test a range of hypotheses regarding the role of parasites in driving the diversity and evolution of viruses. RNA viruses are of special interest since they dominate the diversity of viruses infecting eukaryotes, including numerous human, plant and animal pathogens of health and economic significance. To characterize RNA viruses, I propose to use two complementary approaches. First, by mining through parasite transcriptomic data, I have shown that it is possible to identify viruses, and even to assemble full-length new viral genomes. Secondly, by working with researchers that conduct epidemiological studies on a diverse array of parasites, fresh samples of parasites and of the corresponding hosts can be obtained to determine the presence and diversity of viruses that infect those parasites.

4.2.1. The phylogenetic position of parasite viruses relative to host-associated viruses and to closely-related free-living organisms

Hypothesis: Host shifts occur frequently from and to hosts and parasites

Hypothesis: The virome changed upon transition from free-living to a parasitic life style.

Across taxa, from nematode, to plants, insects, protozoans, crustaceans, or Platyhelminthes, organisms have independently transitioned to a parasitic life style. These transitions have been associated with more or less dramatic changes in the organism's behavior, phenotype, and genotype. The transition to parasitism would impose new selection pressures on viruses and would likely reduce the rate of horizontal transmission, but it would also provide new opportunities for host shifts. Indeed, the emergence of new viral strains, which in turn may lead to virus host shifts and new diseases, is dependent on host species interactions and opportunities for cross-species transmission. Organisms with a parasitic life style have a close intimate relationship with their hosts that would provide such opportunities for virus transmission. Transcending phylogenetic boundaries, and resulting from convergent evolution, six general parasitic strategies have been described: parasitoid, parasitic castrator, directly transmitted parasite, trophically transmitted parasite, vector-transmitted parasite and micropredator^{116,117}. From an evolutionary perspective, it can be expected that micropredators that can infect multiple host individuals, sometime of different species, over the

course of their life would often serve as vectors for viruses. This is true for many ectoparasites such as ticks, mites, lice, bed bugs, and fleas that are common vectors of diseases and source of virus emergence and re-emergence^{118,119}. Along the same train of thought, trophically transmitted parasites that infect different host species over the course of their life cycles, such as parasitic Platyhelminthes could provide opportunities for viruses to complete major host shifts across distantly related taxa. This would be true as well for vector-transmitted parasites that infect at least two different species over the course of their life-cycle. In contrast, parasitoids, parasitic castrators and directly transmitted parasites that infect one host, from one species over the course of their life are not expected to be involved in major host shifts.

By characterizing the phylogenetic position of viruses associated to parasites relative to viruses associated with closely-related free-living relatives, we will be able to determine whether an ancestral virus infected their common ancestor. Viruses acquired after the transition to parasitism would only be found in parasites, and be absent from distant relatives. By characterizing the phylogenetic position of viruses associated to parasites relative to viruses associated with their host, we will be able to determine whether host shifts have happened between viruses and parasites. If a given parasite has acquired viruses from its host, the host-associated viruses will have an ancestral position to the parasite-associated viruses. Conversely, when host-associated viruses have ancestors in a parasite, the diversity of viruses from the host will be nested within the diversity of viruses discovered within the parasite.

Preliminary results:

I started with parasitic flatworms (Phylum Platyhelminthes, group Neodermata), specialized in parasitizing vertebrates. Platyhelminthes also known as flatworms, constitute a diverse phylum estimated to contain up to 100,000 species with diverse body-plans, life-style and ecological roles^{120,121}. It includes species of biomedical significance such as *Schmidtea mediterranea* and *Macrostomum lignano* used for stem cell research, and to study aging, tissue regeneration and homeostasis¹²²⁻¹²⁴. The clade Neodermata, representing more than half of Platyhelminthes biodiversity, is composed of obligate vertebrate parasites. The clade groups endoparasitic trematodes (Digenea and Aspidogastrea) and tapeworms (Cestoda), and ectoparasitic monogeneans (Polyopisthocotylea and Monopisthocotylea)¹²⁵. While monogeneans typically have a single vertebrate host, trematodes and tapeworms are trophically transmitted and present complex life-cycles that involve a first invertebrate intermediate host (molluscs and crustaceans typically, but also cnidarians or polychaetes), and sometime a second intermediate host (mollusks, crustaceans, plants, fish, amphibians)¹²⁵⁻¹²⁸. By infecting humans, cattle and other domesticated animals, neodermatans represent a significant economical and health burden. We mined transcriptomic data and found over a hundred new virus species in ancestral free-living platyhelminthes and neodermatan parasites. By comparing the virome of free-living and parasitic Platyhelminthes, we revealed that the virome of flatworms changed dramatically during the transition to a parasitic lifestyle. We discovered a greater diversity of positive-strand RNA viruses and double-stranded RNA viruses of the families *Picornavirales* and *Ghabrivirales* in Rabditophora than in Neodermata. In contrast, Neodermatan parasites harbored a greater diversity of negative-strand RNA viruses. When viruses of a given clade were found in both Rabditophora free-living species and Neodermatan parasites, they clustered separately on phylogenetic trees, further suggesting that the transition to a parasitic life-style that occurred over 500Ma ago in a protovertebrate drove virus evolution¹²⁹.

While I have not yet started characterizing the virome of the parasite's hosts, comparing the phylogenetic position of parasite-associated viruses relative to the known diversity of viruses

provides some indication that host shifts from and to parasites and their host has occurred. The diversity of viruses of other invertebrates and vertebrates was often nested within the diversity of viruses of parasitic flatworms, implying their ancestral nature (Figure 13). In fact, all the known rhabdovirus diversity is nested within the diversity of viruses of Neodermata implying the ancestral nature of flatworm-associated viruses. Our phylogenetic analysis clearly shows that rhabdoviruses of flatworms have a basal position to *Lyssavirus* (rabies virus) and more generally to the dimarhabdovirus supergroup. The most parsimonious explanation is that a rhabdovirus associated with a Neodermatan parasite was transmitted to the parasitized host and acquired later the ability to maintain its life cycle without the parasite, becoming the ancestral *Lyssavirus*.



Figure 13: Phylogenetic positions of parasitic flatworm viruses (in red) within the broader diversity of RNA viruses (in black) based on full length RdRP. The name of the major clade (phylogeny) is shown at the top of the tree.

In addition, a preliminary analysis of Nematode transcriptomic data revealed the presence of new viruses in *Trichinella pseudospiralis*, *Trichinella patagoniensis*, *Ascaris suum*, *Ascaridia galli*, *Dirofilaria immitis*. Viruses of Nematodes appear to cluster together closely. A thorough analysis of their phylogenetic position relative to other known diversity of viruses will provide further insight on nematode-virus evolutionary history and potential for spillover. But, as stated above, mining transcriptomic data does not allow a thorough characterization of the viral diversity in a given parasite species, and the number of species for which transcriptomic data are available is limited. Therefore, I am in contact with diverse research teams in Anses with who I am collaborating to collect samples of parasites of significance and characterize their virome. Through this, I will increase the diversity of viruses of nematodes and initiate a study on the viruses associated with pathogenic protozoans.

4.2.2. The phylogenetic position of parasite viruses relative to viruses in other parasites

Hypothesis 1: Viruses co-diversify with their parasitic hosts

Hypothesis 2: Host shifts occur frequently between parasites co-infecting the same host

Viruses ecology and evolutionary history is tightly linked with the ecology and evolution of their hosts. As a result, viruses from different host taxa tend to cluster within separate clades. But viruses,

and most particularly RNA viruses, also often display complex history of both co-diversification with their host, and host switching¹³⁰⁻¹³³. Hosts form isolated islands of parasites, and in absence of co-infection, horizontal transmission of viruses will be impossible. Thus, if parasite infrapopulation is small, viruses will be under selection pressures to transmit vertically. We recently showed experimentally that most, if not all, viruses of the cestode *S. solidus* are vertically transmitted⁷⁴. As a result, a pattern of co-diversification of viruses with their parasitic hosts is expected to be common. But, co-infection by multiple individuals, or by multiple parasites is a common feature that can offer opportunities for host shifts. Parasite aggregation, where a few hosts harbor most of the parasite population whereas most hosts remain uninfected is commonly observed. A single virus, the *Schistocephalus solidus* rhabdovirus (SsRV) was excreted by *S. solidus* proceroids and plerocercoids and could in theory infect other parasites co-infecting the same hosts⁷⁴. Both theoretical models and experimental evidence indicate that host shifts decreases with phylogenetic distance between the donor and recipient hosts¹³⁴. Thus, viruses are more likely to infect new parasitic hosts of closely related taxa than distantly related species, which could also result in apparent co-diversification of hosts and parasites.

By characterizing the phylogenetic position of viruses associated to parasites relative to viruses associated with closely related parasites, and unrelated parasites, we will be able to determine whether the identified viruses tend to co-diversify with their parasitic host or whether they are able to shift host often. Codiversification between viruses and parasites will be characterized by a virus speciation events that correlate with parasite speciation events. Ideally, large-scale cophylogenetic analyses will be conducted by including hundreds of pairs of viruses and corresponding parasitic hosts.

Preliminary results:

Among the diversity of novel viruses discovered in Platyhelminthes, most were found within Neodermatan parasites. Viruses of neodermata often clustered together based on their parasitic host phylogenetic relationship: viruses of cestodes and monogenean clustered closely together and distantly from viruses of trematodes. Within the family of Bunya-like viruses, viruses of trematodes further clustered together depending on the parasite's family: Viruses associated with the *Schistosomatidae* clustered together, separately from viruses associated with the liver flukes of the family *Opisthorchiidae* that clustered together and separately from viruses associated with the *Psilostomidae* (Figure 14).

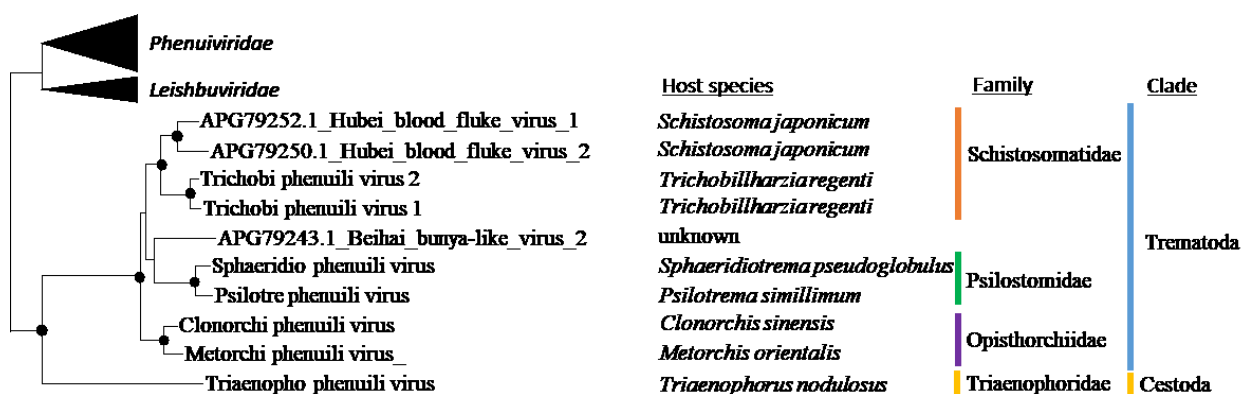


Figure 14. Bunya-like viruses of trematodes and cestodes cluster together depending on their host phylogenetic relationship. The figure provides the Phylogenetic trees of the RNA-directed RNA polymerase (RdRPs) of relative to the most closely related known families of viruses. The tree was

inferred in PhyML using the LG substitution model. Branch dots indicate that result of Shimodaira-Hasegawa branch test was > 0.98 . The host species, family and clade is provided next to the branches. The Beihai bunya-like virus 2 was discovered in a mix of molluscs, among which one was likely parasitized by a trematode.

In contrast, rhabdoviruses of trematodes and cestodes did not codiversify strictly with their parasitic host (Figure 15). Indeed, our analysis did not give rise to two distinct branches of viruses of cestodes and trematodes, but indicate a dynamic history of virus host-shift between cestodes and trematodes, and of subsequent diversification. Up to four different rhabdovirus species were found within a given parasitic host. This result is in accordance with our previous finding that the SsRV of *S. solidus* is excreted by the parasite, which would facilitate host switch⁷⁴. Given that cestodes and trematodes use different type of intermediate hosts, it is likely that these host shifts occurred mostly within the gut of the definitive vertebrate host.



Figure 15. Phylogenetic position of rhabdoviruses of trematodes and cestodes relative to the known diversity of viruses within the family rhabdoviridae. The figure provides the Phylogenetic trees of the RNA-directed RNA polymerase (RdRPs). The tree was inferred in PhyML using the LG substitution model. Branch dots indicate that result of Shimodaira-Hasegawa branch test was > 0.98 . The host species, family and clade is provided next to the branches. The fujan dimarhabdovirus, the Beihai dimarhabdovirus 1, the wenling dimarhabdovirus 8, the fox fecal rhabdovirus and the wenling dimarhabdovirus 10 were discovered in feces, the gut or gill of vertebrates and were assumed to be

associated with their food. This study suggest that they are associated with Neodermatan parasites infecting these organisms at the time of sampling.

4.2.3. Characterizing intra-specific viral diversity

Hypothesis: The diversity of viruses within a species is dependent on the parasite genetic diversity and structure

Due to their short generation time, viruses can rapidly diverge when physically isolated in different host populations, increasing viral diversity. Given the diversity of parasites, and the high genetic structure of many parasite species, resulting often in cryptic species, parasites could host a very diverse virome at the species level. With their short generation time and high mutation rate, RNA viruses are capable of rapid evolution through local adaptation or genetic drift, which would result in greater genetic structuring and diversity than their parasitic hosts. In addition to divergent selection and genetic drift, gene recombination and re-assortment also contribute to virus diversification and to new emergence^{133,135,136}. Hybridization of distinct parasite strains, or of closely related parasite species could provide opportunities for viruses to shift hosts and for re-assortments.

Thus, as a key step forward to understand how parasites might drive virus evolution and diversification, I propose to characterize the virome of target parasite species that present different life-history traits to test the hypothesis that greater parasite genetic structure is associated with greater virus diversity. I will then test if there is an association between parasite genetic divergence and virome composition at different spatial scale. Phylogenetic analyses will be employed to determine the position of newly discovered viruses relative to each other and to compare virus phylogenies with phylogenies of the parasitic hosts. Therefore, I will test for diversifying selection, purifying selection and gene recombination. Ecological isolation would result in signals of positive selection on proteins that directly interact with the host.

Proposed model systems:

- As described in previous chapters, I have recently discovered various viruses associated with the cestode parasite *S. solidus*. Of specific interest, *S. solidus* is ubiquitous and prevalent in freshwater environment in Europe, Canada and Alaska^{61,64} with pronounced genetic structuring among lakes and regions⁶⁵⁻⁶⁷. Both intermediate hosts are aquatic freshwater organisms with mobility limited to the lake, but the highly motile definitive bird hosts could provide opportunities for gene flow. As such, it is an ideal system to investigate the impact of parasite genetic structure on virus diversity. To do so, the virome of *S. solidus* individuals will be characterized across their geographic range (Figure 16). In addition, the closely related parasite *Schistocephalus pungitii*⁶⁶ is specific to Ninespine sticklebacks. Threespine sticklebacks and ninespine stickleback can be found in the same lake, in sympatry, as well as their corresponding parasites *S. solidus* and *S. pungitti*. Both species can co-infect the same definitive host and hybridization results in viable individuals^{68,69}. Co-infection of definitive hosts and hybridization provide opportunities for viruses of *S. solidus* and *S. pungitii* to shift host and to recombine. To test this, the virome of a dozen of sympatric parasite species will be characterized and compared.



Figure 16: Candidate sampling locations of *S. solidus* in North America and Europe. Field sampling in North America will be facilitated by Dr Milligan Myhre in Alaska, Dr Bolnick in Vancouver and Dr Aubin-Horth in Quebec and in Europe by Dr Kristjansson in Iceland, Dr Peichel in Switzerland, Dr Per Jakobsen in Norway, Dr Byström in Sweden, and Dr Webster in United Kingdom

- *Trichinella* is a food-born disease present throughout the world. In humans, infection occurs following ingestion of insufficiently cooked meat. *Trichinella britovi*, *T. nativa*, *T. nelsoni*, *T. murrelli*, *T. pseudospiralis*, *T. papuae*, T6, T8, and T9 are predominantly found in wildlife and are rarely found in humans, whereas *T. spiralis* that infects domestic pigs and horses is the main source of human infections. Mixed infections, and some level of hybridization occur between species and could contribute to virus evolution^{137,138}. Overall *Trichinella* species harbor low level of genetic variations, probably due to the high host motility, large population size, long generation time, diversity of hosts and high availability, and that parasite load can be very high, with hundreds of individuals per gram in wildlife. But still, geographically isolated populations present low level of genetic admixture that could allow some level of virus differentiation^{139,140}. I have recently discovered rhabdoviruses in *T. pseudospiralis*, *T. nativa*, and *T. patagoniensis*, and toti-like viruses in *T. pseudospiralis*, T8, and *T. Murelli*. In collaboration with Gregory Karadjan, UMR Biologie Moléculaire et Immunologie Parasitaire à Maison-Alford, Anses, I propose to further characterize the virome of all 12 different species of *Trichinella*, and 30 different strains already present in the laboratory collection, and to conduct a study of *Trichinella* viruses in wildlife across Europe to provide a more contemporary picture of the parasite virome and of its evolution.
- *O. viverrini* and *C. sinensis* are food-born diseases endemic to Northern Thailand, Laos, Vietnam and china, in particular in the region of Khon Kaen where incidence of Cholangiocarcinoma associated with the liver fluke is the highest in the world. The trematode encysts in cyprinoid fish and infection occurs when people ingest raw or undercooked fish with metacercariae. A high genetic diversity and strong genetic structuring of *O. viverrini*, with distinct groups associated with different major wetlands in Thailand and lao PDR has previously been reported¹⁴¹. Similarly, different region-specific lineages of *C. sinensis* have been described^{142,143}. This high genetic structuring is predicted to be associated with a greater diversity of viruses. Both species are found in sympatry in the north-east region of Thailand and in Vietnam. Thus, co-infection could provide opportunities for virus host shifts. I have discovered two different rhabdoviruses, a bi-fragmented bunya-like virus and three virga-like viruses in *C. sinensis*. In a closely related species of Opsithorchiidae that preferentially infect ducks, *Metorchis orientalis*, I also found closely related species of Rhabdoviruses, a closely-related Bunya-like virus and a new Jingchuvirus, that indicate that various viruses are associated with this family of parasites. In collaboration with Dr Laha Thewarach (Department of Parasitology, Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine at Khon Kaen University, Thailand) and Paul Brindley (Department of Microbiology, Immunology and Tropical Medicine at George

Washington University), I seek to characterize the virome of these flukes across their geographic range.

4.3. Role of viruses in parasite speciation.

The holobiont/hologenome revolution is rapidly changing the field of ecology and evolution as researchers realize that much of an organism's phenotype can be attributed to its microbiome^{51,144-146}. It is also increasingly recognized that viruses are not all pathogenic and that some of them establish mutualistic symbiosis with their hosts¹⁴⁷⁻¹⁴⁹. Viruses of parasites can either be beneficial for the parasite by increasing infectivity and transmission to the next host, or they can be hyperparasitic and reduce parasite fitness⁵⁰. **The nature of parasite-virus interactions will have a critical effect on the virulence and evolution of parasites.**

Studies of the general patterns and processes of parasite speciation have mainly focused on their life-history traits, and the role of their interaction with the host. Parasite speciation can occur through congruent co-speciation with the host, thus reducing opportunities for mating. Parasite speciation can also result from host switching and the specialization to different host species that lead to reproductive isolation. It has been recently suggested that microbes could also contribute to speciation, through hybrid lethality¹⁵⁰ or behavioral isolation¹⁵¹. The discovery of viruses within parasites leads me to question the role of virus-mediated divergent selection. Indeed viruses may reduce the fitness of immigrants and hybrids. Three prerequisite must be made for virus-mediated divergent selection¹⁵²: (i) infection should differ within or between host populations. Currently, there are indication that this is the case, at least in *S. solidus*⁷⁴, and studies proposed above will provide additional data to further test this point. (ii) These differences should remain constant through time to allow or divergent selection. Clearly, field sampling proposed above (4.2) will need to be conducted over multiple years to test the stability of viral community associated with parasite populations under study (iii) Infection imposes significant fitness consequences. These effects result from infection by a single pathogenic virus, or result from the joint effects of co-infection by different viruses. Thus, as a prerequisite before testing experimentally the role of viruses in parasite speciation, I will start by assessing the impact of viruses on parasite fitness and test for local adaptation.

4.3.1. Characterizing the impact of viruses on parasite fitness

Hypothesis: Parasites are locally adapted to some viruses

Hypothesis: Some viruses are locally adapted to the parasite

As a result of local adaptation and co-evolution, the impact of a given virus on a parasite can vary depending on the strain of viruses and strain of parasites used. Cross-infection experiments, wherein parasites from different geographic locations are experimentally infected with local (sympatric) and foreign (allopatric) viruses will be used to identify patterns of evolution as previously done for host-parasite interaction^{44,92,94,153,154}. Virus local adaptation would be revealed when the virus has higher replication in local than foreign parasite. Similarly, parasite local adaptation to viruses would be associated with higher parasite fitness when infected by local viruses than viruses isolated from another population.

By combining cross-infection experiments with proteomics, gene expression analyses and next generation sequencing technologies, I will move beyond phenomenology to dissect the underlying molecular mechanisms of parasite resistance and virus virulence that result from the geographic

mosaic of coevolution^{44,92,153-155}. The antiviral immune system of most parasites has not yet been studied, simply because no virus had been described until now. But there are indication that most have a functional antiviral immune system. For instance, the efficiency of RNA interference¹⁵⁶⁻¹⁶⁵, the presence of miRNA^{166,167} and the identification in their genomes of core genes of the miRNA pathway¹⁶⁸⁻¹⁷⁰ demonstrate the presence of a functional antiviral immune system in parasitic flatworms, thus allowing us to compare the specificity of parasite immune response to local and foreign viruses. By comparing the transcriptomic response of parasites from different locations to different strains or species of viruses, I will identify genes involved in parasite resistance to viral infection.

Proposed model system:

In *S. solidus* I have previously demonstrated that I can generate virus(-) and virus(+) families of parasite⁷⁴. I will test the impact of local and foreign strain of target virus species on parasite fitness. I will use viruses isolated from *S. solidus* parasites from different lakes in Europe, Canada and USA. I will generate families of *S. solidus* infected with local and foreign viruses before measuring their fitness. The impact of the viruses on parasite fitness can be assessed by comparing the number of eggs, hatching rate, infection success, proceroid volume, while controlling for the effect of viral load and parasite genotype. I expect significant variation in virus effects on parasite fitness among viral species and parasite populations. When considering strictly vertically transmitted viruses, I expect that parasites will be locally adapted to their viruses, and reciprocally that viruses will be locally adapted to parasites. As a result, viruses will be more successful and replicate better in sympatric hosts and parasites will have a better fitness when infected by sympatric parasites. Considering the SsRV that is excreted by the parasite and may be horizontally transmitted. I expect that *S. solidus* will be locally adapted to SsRV, and thus better resist infection by local SsRV virus strains, but that SsRV will be able to replicate successfully in foreign parasites, and cause more damages to foreign hosts.

4.3.2. Testing for virus mediated parasite speciation

Hypothesis: The viability of immigrants and hybrids exposed to local viruses decreases along a continuum of speciation

Viruses biodiversity is driven by stochastic processes and evolutionary adaptation to specific hosts. Because they have shorter generation time, and parasite-associated virus infrapopulations are nested within a parasite, within a host, virus speciation is likely to occur more rapidly within populations of parasites that are geographically isolated from one another. It would eventually lead to the emergence of novel viruses locally coevolving with their hosts. I hypothesize that parasite reproductive isolation could result from virus-mediated reduction in the fitness of immigrants and/or hybrids.

Immigrants result from host mobility between parasite populations. For example, *S. solidus* definitive hosts are fish-eating birds that can easily travel long distance and shed parasite eggs in different lakes. Variations in the ability of immigrants to resist infection by local viruses, and maintain a high fitness might constraint gene flow. Actually, theoretical considerations predict that some viruses could facilitate parasite speciation, while others could constrain speciation, depending on the outcome of parasite-virus coevolution¹⁵². When the virus adapt to its local parasite population, immigrant parasites could provide new genetic variants that allow it to escape infection. In contrast, when parasite populations are locally adapted to the sympatric virus, or viral community, a foreign parasite would be maladapted and gene flow will be limited.

The impact of viruses on hybrids should also be investigated. These individuals result from the breeding of parasites from different populations or species that co-infect the same definitive hosts. Intra- and inter-specific hybridization has been observed in various parasite species including trematodes^{115,171-178}, cestodes¹⁷⁹, monogeneans^{180,181}, and nematodes¹⁸². These hybrids sometime show increased fitness compared to their parents, a phenomenon known as hybrid vigor. For instance, hybrids often retain the ability to infect the hosts of both parental lines, which extends significantly the range of suitable hosts of parasites^{68,69,115,177,183}. Other important aspects of parasite life history traits are often impacted by hybridization. For example, hybrids of Schistosomes have diurnal shedding pattern and host finding behavior that are intermediate to the parental strains^{184,185}. When two distinct parasite populations or species eventually interact, and inter-breed, forming a parasite hybrid zones, viruses could impose selection pressures that prevent hybridization, or on the contrary, hybrids may gain resistance to viruses infecting both parental strains.

The impact of local (sympatric) and foreign (allopatric) viruses on immigrant and hybrids fitness will be measured in various contexts, along a continuum of speciation in order to assess how, and when along the speciation process (from more or less distant populations, to closely related species), do viruses contribute.

Proposed model system:

As discussed above, *S. solidus* has a very broad geographical distribution and genetically distinct populations can be found in closely located lakes (despite a very mobile bird definitive host), and different haplotypes are found on different continents, resulting in a very diverse intra-specific genetic diversity. *S. solidus* is specific to threespine sticklebacks whereas *S. pungitii* is specific to Ninespine sticklebacks⁶⁶. Both species have a broad range of avian definitive hosts and can be found in sympatry within the same lakes. Therefore, the two species very likely encounter each other quite frequently in definitive hosts. *S. solidus* and *S. pungitii* can hybridize in laboratory conditions indicative of the absence of prezygotic isolation^{68,69}. In fact, mate choice experiments showed a preference for a different species over conspecifics. Hybrids are able to increase their host range compared to parental strains: they are able to infect both threespine sticklebacks and ninespine sticklebacks.

Thus, we could first conduct experimental infection experiments with viruses from *S. solidus* from Europe, Canada and the USA, and from the closely related species *S. pungitii* infecting parasitic hosts from closely located population, from populations on different continents, and from a different species to test whether viruses could be selection agents preventing gene flow. In addition, with this same organismal system, we can generate hybrid parasite families and test their fitness in response to infection by viruses from either parental lineages.

4.4. A nested system – The impact of viruses on parasite ecology

I hypothesize that viruses could have an important ecological and evolutionary role in parasite community ecology. Indeed, viruses could have very diverse impact on their parasitic hosts life history traits by directly manipulating them to improve their own fitness and transmission, or by inducing adaptive responses from their parasitic hosts. Here, I propose to investigate the role of viruses in competition among parasites, in parasite-associated behavioral manipulation, and in parasite reproduction.

4.4.1. Viruses role in competition among parasites

Hypothesis: Viruses can be used as biological weapons during within-host parasite competition

Hypothesis: Viruses can facilitate co-infections

Co-infections with multiple strains, or species of parasites are common in the wild, and within-host competition among parasites contributes to determining how parasites evolve. Within-host competition can affect infection success, parasite fitness, and successful transmission to new hosts. When within-host competition is high, parasites have evolved various arrays of mechanisms to improve their chances over, or despite, competitors. Three mechanisms have been described so far^{186,187}. (i) In exploitation competition, related strains, or closely related species, of parasite compete for access to resources. The parasite that is better equipped to exploit those resources will outgrow others. (ii) In immune-mediated apparent competition, both parasites compete to evade a detrimental host immune response. A non-specific immune response could target both parasites regardless of who triggered it. But, in some cases, parasites can trigger an immune response to which they are immune but that prevent infection by competitors¹⁸⁸. (ii) Interference competition refers to situations where the parasite releases compounds that are toxic to its competitors. I argue that through variations of these mechanisms, viruses associated with parasites can either contribute to within-host competition, or decrease within-host competition.

First, a virus-mediated interference competition might occur. When within-host competition is high, the parasite that excretes a viruses that has a negative fitness impact on other parasite species, or other strains of the same species, will have an advantage. But, such mechanism would probably only be beneficial in the presence of a competitor. In absence of competitors, the cost of viral infection is expected to reduce parasite fitness. Through this, viruses can contribute to within-host competition.

In contrast, following a virus-driven facilitation hypothesis, viruses that contribute to host exploitation (by facilitating degradation of host tissues), or deter the host immune system (towards an antiviral immune response) could reduce parasite competitive ability. Indeed, parasites could achieve greater infection success, or greater host exploitation when associated with a virus. In some cases, infection success may even be dependent on the production of viruses. But, if those mechanisms are non-specific and beneficial to parasites that do not carry the virus (most likely different individuals of the same species), then within-host competition will favor cheaters that do not carry the virus, and avoid the fitness cost associated with viral infection. Such mechanism would both maintain the virus within the parasite population, but prevent the rise to 100% prevalence by benefiting virus (-) cheaters. As such, within-host competition could actually be a driver of parasite holobiont diversity.

To assess the role of viruses in competition among parasites, I will assess the fitness of parasites in presence and absence of viruses, and in presence or absence of competitors. In addition, the direct fitness impact of a parasite-associated virus on the parasite competitors will be tested.

Proposed model systems

Some trematodes appear to be better equipped than others to face competition in snails. After a single miracidium infects the snail, it can produce through clonal expansion a large number of daughter parthenitae. These are called sporocysts when they lack a pharynx and gut, or rediae when they have a pharynx and a gut. The later are able to physically compete with competitors, which explains that they tend to be dominant over species with sporocysts¹⁸⁹. Among those trematodes, some present a surprising phenotypic plasticity that further increase their competitive ability. At least

15 different species of trematodes, belonging to the families *Himasthlidae*, *Philophtalmidae* and *Heterophyidae* form colonies with distinct reproductive and non-reproductive 'soldier' castes¹⁹⁰⁻¹⁹⁷. The presence of much smaller, potentially immature, non-reproductive rediae had long been observed^{198,199}. More recently, studies have shown that small rediae, that maintain a large pharynx constitute a caste of soldiers that actively defend reproductive rediae against other parasites co-infecting the same host¹⁹⁰⁻¹⁹⁷. Soldiers tend to populate the snail mantle (entry site of competitors), whereas reproducers safely isolate in other snail tissue compartment. First, virus mediated interference competition could have contributed to the evolution of distinct castes. Indeed, selection pressures against the production of large quantities of viruses in soldiers might be reduced, compared to reproductive rediae that need to save energy for reproduction. I hypothesize that viruses could then be 'domesticated' by the parasite but retain infectivity against con-specific or immature parasites, thus contributing to competition. I propose to characterize the virome of trematodes from these families through RNA sequencing, to measure viruses replication in different castes, and to experimentally test virus transmission, and impact on the fitness of competing parasites.

In the fish eye flukes *Diplostomum*, the variable outcome of co-infection by recently diverged sympatric species could be explained by a virus-driven facilitation. The parasite life cycle involves a two intermediate hosts, a snail and a fish, and fish eating birds serve as definitive hosts. Following mixed infection with multiple strains of *D. pseudospathaceum*, the probability of parasite establishment in the next host is higher, but transmission success of each genotype is lower relative to a single genotype infection²⁰⁰. Furthermore, some strains of *D. spathaceum* and *D. gasterostei* perform better in mixed exposures, while other combinations decrease infection success²⁰¹. Therefore, I propose to characterize viruses within these parasite species, and conduct experimental infections in presence of one or two strains/species of parasites, with or without virus, to assess the role of virus presence in parasite establishment.

4.4.2. Viruses impact on parasite reproduction

Hypothesis: Viruses manipulate parasite reproduction to increase their own transmission

Hypothesis: Parasite respond to viral infection by changing their reproductive behavior

Coevolution between host and parasite is recognized as a major driver in the evolution of sex, host reproduction and reproductive behavior²⁰². Indeed parasites can manipulate their host reproduction and behavior to increase their own transmission. For instance, the mite *Unionicola ypsilophora* increases male midges mating chance, which increases its transmission to female midges that will then transport the mite back to water²⁰³. The virus LbFV induces superparasitism in the parasitoid wasp *Leptopilina boulardii*, where the wasp lays eggs in already infected hosts, which increases horizontal transmission of the virus²⁰⁴. And the insect iridovirus IIV-6/CrIV increases the cricket *Gryllus texensis* mating behavior, effectively increasing its transmission through sexual contact²⁰⁵. In response to parasite prevalence and associated fitness cost, hosts develop strategies to evade infection and tend to select for healthy mates, a 'parasite avoidance behaviour'. Typically, females tend to avoid males infected with parasites, probably to increase chances of mating with individuals resistant to parasites, and to avoid transmission of diseases²⁰⁶⁻²⁰⁸. As a result, males display appendage or behaviors indicative of good health^{209,210}. Parasitism also appears to be a selective force in the evolution of mating system. It has been suggested that sexual reproduction, that increases the genetic diversity of offspring might have evolved in response to the need to resist parasites²¹¹. Interestingly, polygamy, or polyandry can further increase the genetic diversity of the

offspring and increases chances that some of these survive²¹². In the nematode *Caenorhabditis elegans*, that can either self-fertilize or outcross, outcrossing is preferred in presence of pathogens and provides adaptive fitness benefits²¹³.

The reproductive behaviors of parasites varies greatly and no generalities could be made. Yet, it could be expected that viruses of parasites could affect mate choice, the rate of self-fertilization in hermaphrodite species, or even the faithfulness of monogamous parasitic hosts. Platyhelminthes are again great model systems because while many species are able to reproduce by self-fertilization and cross-fertilization, others have strict sexual reproduction. Thus, I will use mate-choice experiments in presence or absence of viruses, and test the rate of self-replication and crossing with virus-free and infected parasites.

Proposed model systems

The cestode *S. solidus* is a simultaneous hermaphrodite within which both out-crossing and self-fertilization occur at the same time²¹⁴. The parasite appears to favor out-crossing that increases hatching rate, infection success and competitive ability^{215,216}. The hermaphrodite's dilemma, encountered when two hermaphrodites meet for reproduction is often solved with reciprocity and both partners contribute to providing eggs and sperms and mostly outcross²¹⁴. But cheaters have also been observed: these fertilize all eggs of their partner and in addition self-fertilize their own eggs²¹⁴. Small, selfed individuals are better selfers and tend to produce a greater proportion of selfed progeny even when given the opportunity to breed with a partner⁶². In addition, outcrossed worms prefer outcrossed mating partners over a selfed one²¹⁷. Mate choice by *S. solidus* individuals present other peculiarities: *S. solidus* chooses to mate within individuals within the same size range, or with a slightly larger worm if given the option. They also prefer breeding with siblings than with unrelated partner²¹⁸. I have previously discovered a range of vertically transmitted viruses within *S. solidus* that may play a role in their parasitic host reproductive behavior and could have contributed to some of the observations that I relate above. First, it will be necessary to assess whether viruses are transmitted by eggs or sperm, and whether outcrossed infected individuals better resist the viral infection than selfed individuals. Then, if a virus is transmitted with sperm, the virus may manipulate the parasite and force it to cheat by refusing reciprocity so that 100% of the parasite progeny is infected. It may also be expected that *S. solidus* individuals will chose to self-fertilize over breeding with another virus-infected worm. Alternatively, especially for viruses transmitted with eggs, the parasite may have the option to cheat by selfing all its eggs, but reproduce sexually with the infected mate. The mixed mating strategy of *S. solidus* may be beneficial in its race with viruses by limiting the rate of local adaptation of viruses, as previously demonstrated with *C. elegans* and a bacterial parasite²¹³.

Schistosomes, that are responsible for one of the most prevalent parasitic disease called Schistosomiasis, are unique among trematodes because they have separate sexes. Mating of male and female schistosome in definitive host is obligatory for the maintenance of this trematode life cycle. Indeed, females of *S. mansoni* grow and mature only after prolonged physical residence in the gynecophoral canal of males. Mate choice and mate change has previously been reported in *S. mansoni*^{219,220}. When given the choice, schistosomes prefer pairing with individuals of the same species²²¹. But within a given species, divorce rate increases when females are given the opportunity to mate with a more dissimilar partner²²⁰. In addition, aggressive competition for access to females has been shown at the interspecific level²²¹. This system could be used to test whether parasite-associated viruses increase mate change, or the rate of polygyny (multiple females for one male). As described above, it could be expected that parasites will chose to mate with healthy mates rather

than virus-infected individuals. If sexually transmitted, the virus itself could manipulate males or females in order to favor mate change and polygyny, in order to increase its own transmission.

4.4.3. Viruses role in parasite-associated behavioral manipulation

Hypothesis: Viruses contribute to behavioral manipulation

One of the most curious outcome of the selection pressures that are imposed on parasites to ensure their own transmission, is the ability of some parasites to dramatically alter their host behavior²²². These behavioral manipulations can be classified into three different groups called the suicidal behavior, the bodyguard behavior, and reduced sociality/spontaneity²²³. Being able to modulate the host behavior in very subtle ways that increase the parasite fitness, and with precise timing, is tricky and the underlying mechanisms have long been studied to reveal this mystery. Yet, our knowledge of the proximate mechanisms remains limited²²³. Research indicate that the underlying mechanisms fall within three main categories. First, it can be immunological where the parasite modulates the host immune system that impact behavior²²⁴. Secondly, it can be neuropharmacological, where parasites secrete neuromodulators that impact the host Central Nervous System²²⁴. Finally, through the discovery of the *Dinocampus coccinellae* Paralysis Virus that contribute to its parasitoid host behavioural manipulation of parasitized ladybeetles⁴⁸, I revealed a third mechanism called symbiont-mediated. Because of its novelty, this last hypothesis has not yet been investigated in other systems. The discovery of a broad diversity of viruses within cestodes and trematodes make these organisms ideal candidates to test whether other parasites have similarly evolved towards using one of their associated virus as a biological weapon to alter their host behavior.

To test this hypothesis, I will quantify parasite-associated virus replication and abundance just before, during and just after the behavioral manipulation. I will also assess virus presence in the CNS of the manipulated host. Finally, the virus role in behavioral manipulation will be experimentally tested by infecting the host with virus alone, or with a virus-free parasite.

Proposed model systems

The lancet liver fluke (*Dicrocoelium dendriticum*) manipulates the behavior of its second intermediate host, ants, forcing them to climb to the tip of a blade of grass, in order to increase its transmission to the definitive herbivorous animal host²²⁵. Within each ant, a single parasite migrate to the brain, and is assumed to control the behavior of the parasitized ant. The underlying mechanisms remain unknown and could be investigated with novel sequencing technologies, thus allowing the discovery of putative associated viruses related to other viruses of trematodes. Next, the role of these viruses in contributing to the behavioral manipulation would be investigated using a combination of experimental infection with parasites and viruses, alone, and together.

The trematode *Microphallus papillorobustus* encyst in the brain of the crustacean *Gammarus insensibilis* and alters its response to various stimuli that increases predation by birds, the definitive host of the parasite²²⁶. The timing of the behavioral manipulation of gammarids is very precise: the behavior is altered a few weeks after infection, once metacercariae are mature and infective to the definitive host. Infection was found to induce chronic inflammation in the brain of gammarids, which can explain the neuropathological and behavioral symptoms that characterize the manipulation²²⁷. I have discovered two different species of viruses associated with *Microphallus sp.*, including a rhabdovirus. The role that *Microphallus*-associated viruses play in neuro-inflammation and behavioural manipulation merits further investigation.

4.5 Tripartite evolution - The impact of viruses on parasite pathogenicity

Evidence show that viruses contribute to their parasitic host virulence. Key examples include Polydnviruses of Braconid wasps that control host immunity and development^{100,228,229} the *Trichomonavirus* that modulates the pathogenicity of the human protozoan parasite *Trichomonas vaginalis*²³⁰⁻²³², and the *Leishmanivirus* that is predictive of the severity of Leishmaniasis and is associated with treatment failure^{233,234}. **It is now necessary to consider how viruses contribute to parasite pathogenicity.** The pathogenicity of a parasite depends and vary with parasite virulence, and host response to the parasite infection. Therefore, to accurately assess the effect of parasite-associated viruses on parasite pathogenicity, we need to test the virulence of viruses interacting directly with the host, the effect of viruses on the production of parasite virulence factors, and how these interfere with host response.

4.5.1. Parasite-associated viruses impact on host immune response

Hypothesis: Viruses excreted by parasites deter the host response to infection

Most parasite-associated viruses will likely have limited impact on the host, but viruses transmitted to parasitized host (such as rhabdoviruses of flatworms) are expected to be associated with changes in host immune response to infection and fitness. Parasite-associated viruses excreted and transmitted to the parasitized host are of particular interest because the host is then co-infected by virus and parasite. Co-infection can exacerbate virulence as observed with the *Trichomonavirus*, and *Leishmanivirus*^{112,113}. Both viruses induce Type I interferon (IFN) and elevated proinflammatory response that contribute to the severity of the diseases^{112,113}. The IFN system, conserved in all vertebrates, is the first line of defence against virus infection²³⁵⁻²³⁷. A similar proinflammatory responses to parasite-associated viruses can be expected in other systems. In fact, previous studies have shown induction of IFN during helminth infection that might have been caused by an undetected flatworm virus²³⁸. Therefore, upon testing the ability of viruses of parasites to replicate in host tissue, I will follow the host immune response and inflammatory response to infection by the virus alone, and parasites infected or not by virus using a combination of transcriptomic approaches. Finally, the parasite-virus association brings up the question of whether the host co-evolve with the parasite only, the virus only, or the holobiont parasite (parasite + virus). To test for host-virus co-evolution, I propose to employ cross-infection experiments, wherein hosts from different geographic locations are experimentally infected with viruses isolated from local (sympatric) and foreign (allopatric) parasites.

Proposed model system:

The *Schistocephalus solidus* rhabdovirus constitutes an ideal model to get started on this question. Indeed, we have already demonstrated that SsRV is excreted by *S. solidus* and is transmitted to the threespine stickleback fish host. First, we will compare the stickleback immune response to infection by SsRV(+) and SsRV(-) parasites. Experimental infections will be conducted as well to specifically determine what aspects of the fish immune response is due to the virus alone and to test the virus ability to replicate in sticklebacks. Next, I will assess the ability of SsRV to replicate in leucocytes isolated from stickleback head kidney²³⁹. The fish cell cultures will then be used to conduct a cross-infection experiment, where leucocytes from freshwater sticklebacks from different lakes are exposed to viruses isolated from parasites from the same locations. The resistance of the cells to viral infection will allow me to assess the fish local adaptation to *S. solidus* associated virus. In addition, I will test the resistance of marine threespine sticklebacks to the parasite-associated viruses, because these fish represent naïve ancestors that have not been exposed and have not adapted to *S. solidus*.

The effect of virus infection on host cells will also be compared by RNA sequencing. By comparing virus replication in cells of fish from different origin, we will test the virus ability to locally adapt to the fish host of the parasite it infects.

4.5.2. Parasite-associated viruses impact on parasite virulence

Hypothesis: Parasite-associated viruses alter the production of Excretory/secretory products to their own benefit

Viral infection could have profound effects on parasitic flatworms that will be partially characterized through the comparative transcriptomic analysis of virus(-) and virus(+) parasites proposed earlier. Many viruses exploit, disrupt or otherwise modulate host membrane trafficking²⁴⁰. These virus-induced changes in parasite biology could alter the production of excretory/secretory (E/S) products, including virulence factors. In particular, parasite-associated viruses that are transmitted to the parasitized host and replicate in host cells, will be under selection pressures to increase the production of E/S products that facilitate their transmission and reproduction, including growth factors and anti-apoptotic molecules produced by some parasites. Therefore, the production of E/S products by parasites in presence and absence of viruses will be compared through comparative proteomics. I will also experimentally assess the effect of target viruses on parasite pathogenicity in host, by measuring the infection success, parasite index, and indices representative of host health or behavior.

Proposed model systems

Three parasitic helminths have been identified as group I carcinogens by the International Agency for research on cancer (IARC): *Schistosoma haematobium*, *O. viverrini* and *C. sinensis*²⁴¹. But, are these parasites essential causes of cancer? Intracellular microbes such as viruses, and some bacteria or protozoans could directly benefit from metabolic reprogramming and would be under selection pressures to disrupt the cell-cycle if that can increase their fitness. In contrast, for extracellular parasites, the evolutionary path that would lead them to break the cellular barriers to cancer is unclear. Co-infection with intracellular viruses could explain why and how these parasites breach host barriers to cancer, and parasite-microbe association would explain association between parasite infection and increased risk of cancer occurrence⁵⁴. I propose to focus on *O. viverrini* and *C. sinensis*, and to assess the role of viruses of parasites in oncogenesis. For instance, I will assess the impact of viruses on the production of E/S proteins that contribute to the parasite oncogenicity, such as the growth factor granulin and the anti-apoptotic Thioredoxine produced by *O. viverrini*²⁴²⁻²⁴⁴. Upon determining which viruses are transmitted to parasitized hosts, I will also assess the role of identified viruses in compromising the cellular barriers to cancer: I will for instance compare in vitro the migration and proliferation of cholangiocytes in the presence of target viruses alone, or in combination with E/S products.

4.6 Concluding remarks

Infectious diseases, including helminths and protozoan parasites are a threat to food security and human health²⁴⁵⁻²⁴⁷. Parasite infections are associated with a range of diseases and symptoms that remain mostly unexplained²⁴⁸⁻²⁵⁰. The recent discovery of viruses of parasites could explain why some people develop debilitating diseases while others do not. The payoff for identifying the real culprits for parasite-associated diseases is evident as it would allow prediction of complications associated with infections, and treatments. The pay-off is even more compelling with respect to new leads for

clinical intervention and prevention: if a virus plays a causal role in diseases associated with infection by a parasite, vaccines could reduce the health burden associated with parasite infection²⁵¹. Parasites carrying viruses may constitute an underexplored source of virus diversification and viral emergence. Finally, given that viruses are themselves agents of emerging and re-emerging diseases, there is a need to better understand viral diversity, evolution, and source of cross-species transmission²⁵². Therefore, determining the nature of parasite interaction with viruses has implications for epidemiology, control and diagnostics. The fundamental knowledge I will gain is needed to jumpstart this new field of research.

This project advances the state-of-the-art by applying the holobiont concept to host-parasite interactions. Students working with me will be exposed to a diversity of novel concepts and contribute to developing theoretical knowledge. Crossing disciplinary boundaries, my research project is inspired by approaches and fundamental knowledge from the fields of immunology, parasitology, virology, genomics and evolutionary biology. By focusing on parasites that constitute a global threat for food security and human health, and play a significant role in natural ecosystems, this project will provide opportunities for students with various fundamental and applied interests in epidemiology, control, diagnostics and development of novel therapeutic strategies.

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Annexes

1. Résumé en Français du mémoire
2. Tirés à part de cinq publications choisies