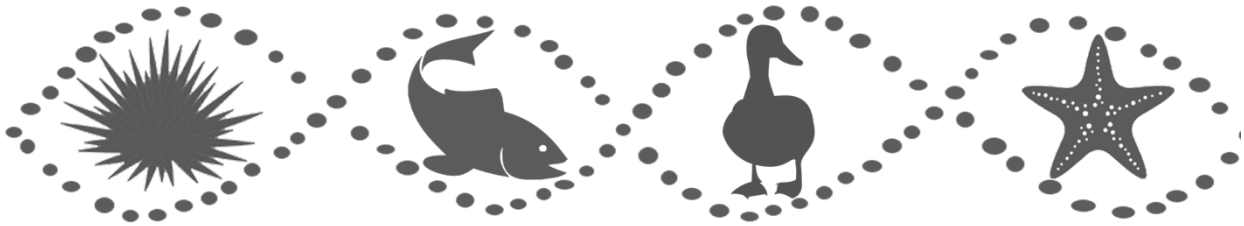
Programme

Virtual

NACI WORKSHOP



June 8th – 9th 2020

Access Details:

	Date	Time	Zoom link	Meeting password	Moderator
Session 1:	08-Jun	8 am PST	https://unm.zoom.us/j/97042823715	167225	Kate Buckley (59)
Session 2:	08-Jun	9 am PST	https://unm.zoom.us/j/99584963764	109259	Brian Dixon (63)
Session 3:	08-Jun	12 pm PST	https://unm.zoom.us/j/92686657777	838291	Irene Salinas (71)
NSF IOS info session	08-Jun	1pm PST	https://unm.zoom.us/j/92731923895	53630	Joanna Sisler
Happy hour social mixer	08-Jun	1.30 pm PST	https://unm.zoom.us/j/91185860414	839293	Speakers/Attendants
Session 4:	09-Jun	8 am PST	https://unm.zoom.us/j/98024048212	612506	Jacques Robert (77)
Session 5:	09-Jun	9 am PST	https://unm.zoom.us/j/91727748491	121163	Brian Dixon (76)
Session 6:	09-Jun	12 pm PST	https://unm.zoom.us/j/91401840184	316040	Helen Dooley (74)
Happy hour social mixer	09-Jun	1.30pm PST	https://unm.zoom.us/j/91516224139	226974	Speakers/Attendants

Time Zone Converter:

	Date	PST	MST	CST	EST	AST	Nfld ST
Session 1:	08-Jun	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	12:30 PM
Session 2:	08-Jun	9:00 AM	10:00 AM	11:00 AM	12:00 PM	1:00 PM	1:30 PM
Session 3:	08-Jun	12:00 PM	1:00 PM	2:00 PM	3:00 PM	4:00 PM	4:30 PM
NSF Session"	08-Jun	1:00 PM	2:00 PM	3:00 PM	4:00 PM	5:00 PM	5:30 PM
Happy Hour:	08-Jun	1:30 PM	2:30 PM	3:30 PM	4:30 PM	5:30 PM	6:00 PM
Session 4:	09-Jun	8:00 AM	9:00 AM	10:00 AM	11:00 AM	12:00 PM	12:30 PM
Session 5:	09-Jun	9:00 AM	10:00 AM	11:00 AM	12:00 PM	1:00 PM	1:30 PM
Session 6:	09-Jun	12:00 PM	1:00 PM	2:00 PM	3:00 PM	4:00 PM	4:30 PM
Happy Hour:	09-Jun	1:30 PM	2:30 PM	3:30 PM	4:30 PM	5:30 PM	6:00 PM

Sessions:

1: Innate invertebrate immunity	Alkie	Tamiru	Establishment and characterization of shrimp and crayfish primary and continuous cells
	Monod	Emma	PACAP-38 as an Immunostimulant in aquatic invertebrates
	Shaw	Chloe	Progression and recovery of bald sea urchin disease in *Strongylocentrotus purpuratus*
	Poynter	Sarah	Broad-spectrum shrimp antivirals using dsRNA-nanoparticle therapies
2: Innate immunity vertebrates I	Bakke	Fiona	Plasma proteome immune responses of the nurse shark, *Ginglymostoma cirratum*, using a high quality de novo transcriptome.
	Semple	Shawna	The exterior coating of telemetry tags influences the chronic inflammation observed in rainbow trout following surgical implantation.
	Campbell	Lee	Transcriptomic analysis of the duck TRIM repertoire finds tissue specific patterning of 51 TRIM or TRIM-like genes
	Evseev	Danyel	Species-specific regulation of RIG-I by influenza A viruses
3: Innate immunity vertebrates II	Heimroth	Ryan	SKIN INNATE IMMUNITY IS ESSENTIAL FOR LUNGFISH TERRESTRIALIZATION
	Hauser	Kelsey	Discovery of granulocyte-lineage cells in the skin of the amphibian *Xenopus laevis*
	Kraus	Aurrora	Olfactory sensory neurons regulate mucosal antiviral immune responses
	Yaparla	Amulya	CXCL12 mediates the homing/retention of *Xenopus laevis* macrophage-granulocyte precursors to the bone marrow

4: Immune regulation	Soliman	Amro	Role of Fever in Tissue Repair
	Wang	Jiahui	Alternative splicing contributes to diversification of leukocyte immune-type receptors (CaLITRs) of the goldfish (*Carassius auratus L.)*
	McGuire	Connor	Thyroid Disrupting Chemicals Perturb Thymocyte Development in *Xenopus laevis* tadpoles
	Frenette	Aaron	Antigen presentation genes in Gadoid species (haddock: *Melanogrammus aeglefinus* and Atlantic cod: *Gadus morhua*) raise questions about cross-presentation pathways and glycosylated beta-2-microglobulin.
5: Innate lymphocytes and MHC	Das	Pankoj	Discovery of rainbow trout nasal innate lymphocytes
	Khan	Adil	Characterization of iT cells interacting with the MHC class I-like XNC4 during mycobacteria infection
	Paiola	Matthieu	Characterization of MHC class I-like XNC4 function in host resistance against *Mycobacterium marinum*
	Wcisel	Dustin	The Holostean Major Histocompatibility Complex
6: B cells and antibodies	Matz	Hanover	Primordial B Cell Selection Sites in the Nurse Shark Spleen
	Salazar	Linda	Characterization of immunoglobulins and B lymphocytes in the olfactory epithelium and nasal mucus of the Nurse shark (*Ginglymostoma cirratum*)
	Waly	Doaa	Antibody affinity modification in fish
	Jenik	Kristof	ENHANCING INOSINE MONOPHOSPHATE DELIVERY USING NOVEL PHYTOGLYCOGEN-BASED NANOPARTICLES IN BOVINE AND RAINBOW TROUT MODELS
	Aradana	Muthupanian	Antigen trapping by cells in the putative germinal centre of fish

Abstracts

(in order of presentation)

Establishment and characterization of shrimp and crayfish primary and continuous cells

Alkie, T.N.[1]; Poynter, S.J.[1]; Monod, E.[2]; DeWitte-Orr, S.J.[1]

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Abstract: As a tool, *in vitro* cell culture facilitates the study of host-pathogen interactions and efficacy of therapeutic and prophylactic compounds under controlled conditions. In shrimp, attempts have been made to establish continuous cell lines, however, there have been no successful continuous cultures from shrimp. The aim of this study was to establish and characterize shrimp cells derived from salt and freshwater shrimp species, and crayfish at different developmental stages. These species include white leg shrimp (*Litopenaeus vannamei*), giant freshwater prawn (*Macrobrachium rosenbergii),* dock shrimp (*Pandalus danae*), black sakura shrimp (*Neocaridina davidi*) and rusty crayfish (*Orconectes rusticus*). Different culture conditions were tested based on Leibovitz L-15 medium in chitin-coated or tissue-culture treated flasks or dishes. Cell cultures were attempted from gill, hepatopancreas, testis and intestine of mature shrimp and from whole shrimp, head, thorax, or abdominal regions of the larval stages using explants, impression smears, cell suspensions or a combination of these methods. From the larval stages, cells were viable for 2 weeks and passaged thrice when obtained from the whole or parts of shrimp larvae. Hepatopancreas from *Pandalus danae* provided immature adipocyte-like cells that survived for over 4 weeks. Our preliminary results showed that primary cells can be easily generated from larval stages of shrimp. Interestingly, the extent of bacterial contamination varied greatly, being lower grade in larvae than from cells originated from mature shrimp. One of the puzzling challenges for establishing shrimp cell lines was obtaining sterile tissues. All tissues cultured, had extensive bacterial and/or fungal contaminants that even proliferated in the presence of a cocktail of different classes of antibiotics and antimycotics. Indeed, tissues that in other aquatic organisms would be sterile, in shrimp housed substantial numbers of microorganisms. This may be an example of pathogen tolerance in shrimp whereby shrimp survive and grow in the presence of bacteria throughout their tissues. Though a hinderance to future successful production of shrimp cell lines, the concept of innate immune tolerance is worthy of further study.

PACAP-38 as an Immunostimulant in aquatic invertebrates

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Abstract: A rise in microbial resistance to antibiotics is motivating the identification of alternative antimicrobial compounds. A promising alternative is emerging in highly conserved, cationic antimicrobial peptides (AMPs). The present study focuses on the application of a highly conserved, multifunctional neuropeptide with antimicrobial properties – pituitary adenylate cyclase activating polypeptide (PACAP) – as an immunomodulator and antiviral agent in an invertebrate model. PACAP has been shown to disrupt bacterial membranes, regulate pro and anti-inflammatory cytokine production through cAMP signalling cascades, and interfere directly with viral protein transcription. In this study, PACAP's function as an antiviral immunomodulator was investigated using primary hemocytes collected from *Macrobrachium rosenbergii*, *Pandalus danae*, and *Orconectes rusticus.* Immune markers used to measure its efficacy include reactive oxygen species generation, phenoloxidase production and melanization, and phagocytic activity. Initial findings support PACAP's function as an immunomodulator as it decreased melanisation and maintained cell viability at high concentrations in hemocytes collected from untreated groups. These results contrasted with what was observed in dsRNA (high molecular weight polyinosinic:polycytidylic acid) injected control animals, which enhanced melanisation in collected hemocytes. This work provides a fundamental understanding of PACAP's immuno-modulatory role in shrimp species that will help develop immune treatments that can reduce the use of antibiotics worldwide.

Progression and recovery of bald sea urchin disease in *Strongylocentrotus purpuratus*

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Department of Biological Sciences, George Washington University, Washington, DC, U.S.A.

Abstract: Bald sea urchin disease (BSUD) affects many species of sea urchins and is characterized by black and/or green lesions on the surface of the animal and loss of primary spines, tube feet, and pedicellaria. Physiological stresses from changes in water temperature or salinity impact the sea urchin immune system, which makes them susceptible to disease. Previously, reports of BSUD from infected species in natural settings have shown that various microbes are associated with the disease without determining whether symptoms are caused by a single or multiple pathogens. After a stress-inducing shipment, sea urchins developed BSUD after about three months. We evaluated this group of sea urchins because they were isolated in a single aquarium and could be observed for the duration of the disease. Initial symptoms included disorientation of the spines and a "sleeping" behavior in which their spines drooped. The disease progressed over the course of two months to the loss of some or all of the primary spines and other surface structures. Recovery was apparent when sea urchins started to regrow their spines that progressed over the course of three months. Full recovery was evident when the sea urchins appeared healthy with fully functional tube feet, and few or none showing "sleeping" behavior. We have extracted DNA from both diseased, healthy, and recovered sea urchins plus water from the aquaria housing the healthy and diseased animals. The isolated genomic DNA supports 16S gene amplification, which will be sequenced in the future to identify the bacteria associated with the disease. Because this study was carried out in a closed system compared to diseased animals sampled from marine habitats, sequencing data is expected to identify one or a few pathogens rather than the confusing set of microbes that have been documented previously from diseased animals in the ocean.

Broad-spectrum shrimp antivirals using dsRNA-nanoparticle therapies

Poynter, S.J.[1]*, Alkie, T.N.[1]*, Monad, E.C.[2], DeWitte-Orr, S.J.[1]
**Authors contributed equally to this work*

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Abstract: Shrimp aquaculture across the world is threatened by pathogenic infection. Shrimp are infected by a large number of aquatic viruses, viruses that lead to rapid spread throughout shrimp farms, mass die-offs, and vast economic impacts. Double-stranded (ds)RNA is an immune modulator in shrimp that induces both non-specific, broad-spectrum antiviral responses and virus-specific RNA interference-mediated responses. The potential for dsRNA's effects to be optimized is currently being explored in a variety of shrimp and prawn species, using a natural, phytoglycogen nano-carrier to provide increased stability and enhanced immune responses. Brine shrimp (*Artemia franciscana*) were fed dsRNA-nanoparticle conjugates and was observed in the intestine; this resulted in an increase in immune gene expression. In haemocytes isolated from dock shrimp (*Pandalus danae)* and native Ontario crayfish uptake of the dsRNA-nanoparticle was observed, and this uptake lead to morphological changes indicating activation of the cells, as well as increased phenoloxidase activity. Further analysis will explore other markers of immune activation and the potential for the dsRNA-based therapy to be applied to shrimp as a prophylactic or antiviral treatment against a wide range of aquatic viruses.

Plasma proteome immune responses of the nurse shark, *Ginglymostoma cirratum*, using a high quality *de novo* transcriptome.

Bakke, F.K.[1]; Stead, D.A.[2] Macqueen, D.J.[3]; Dooley, H.[4]

[1]*University of Aberdeen, Scotland, UK.*

2 Aberdeen Proteomics, Institute of Medical Sciences, University of Aberdeen, Scotland, UK.

[3] *Roslin Institute, University of Edinburgh, Scotland, UK.*

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Abstract: In the past the study of immune systems in comparative models has been hampered by a paucity of tools, such as monoclonal antibodies, that enable reliable protein identification and tracking. Work is currently restricted to the study of individual or small groups of proteins, limiting our understanding of the complex interactions between disparate immune components. Attempting to address these issues we applied a multi-omic approach. Hybrid second and third generation sequencing technologies were used to assemble and annotate a comprehensive, multi-tissue *de novo* transcriptome for the nurse shark. This transcriptome was used as a reference database for high-throughput proteomic analyses, aimed at characterizing changes in the nurse shark plasma proteome over a 49-day post-immunization time course. We reliably identified 474 plasma proteins in these animals, a number inconceivable using previously available tools. Further, 31 plasma proteins showed significantly different response trajectories in the immunized sharks compared with an unimmunized individual, indicating these proteins may play key roles at different stages of the immune response. This group includes at least one, as-yet, completely uncharacterized shark protein, with orthologs in multiple species across phylogeny, that likely would have been missed using other approaches. This work has dramatically increased our understanding regarding the nature and timing of humoral immune responses in the nurse shark. We predict that high throughput proteomic technologies will enable equally rapid advances in the study of other comparative models.

The exterior coating of telemetry tags influences the chronic inflammation observed in rainbow trout following surgical implantation.

Semple S.L.[1], Rodríguez-Ramos T.[1], Heath G.[1], Mulder I.M.[1], Harrison P.M.[1], Power M.[1], Dixon B.[1]

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Abstract: Telemetry transmitters are frequently used in studies of wild fish migration and behavior. Although the effects of surgically implanted transmitters on survival, tag retention, healing and growth have been studied, there has been minimal research regarding the potential immune response induced by these tags. It was previously shown by our group that surgical implantation of acoustic tags induced a significant increase pro-inflammatory cytokine gene expression at day 70. It was hypothesized that this response mimicked the foreign body response as the tags were often encapsulated at this later timepoint. In the current study, telemetry tags with different exterior coatings were used to determine whether a less bioactive substance could lessen the chronic inflammation previously observed. Fish were surgically implanted with regular tags (epoxy exterior), silicone coated tags, or ceramic coated tags. Alternatively, control fish received a sham surgical procedure. These fish were then sampled over a 10-week period so that pro-inflammatory cytokine expression in the spleen, peritoneal lavage and muscle at the surgical site could be assessed. Peritoneal lavage supernatants were also collected so that total protein levels and IL-1β protein could be examined. Externally, fish appeared to heal at similar rates regardless of the presence or absence of the transmitter, but the tag itself was prone to varying levels of encapsulation which appeared to be influenced by the exterior coating. This research could aid in the development of improved telemetry tags that are more innocuous, economic and better able to track fish behavior/movement.

Transcriptomic analysis of the duck TRIM repertoire finds tissue specific patterning of 51 TRIM or TRIM-like genes

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Abstract: Tripartite motif (TRIM) genes are an ancient family of genes which has greatly expanded throughout vertebrate evolution. TRIM proteins have various functions from controlling general cellular processes to antiviral inhibition, including restricting influenza A virus. Ducks are the natural host and reservoir of Influenza A virus, and as such likely have evolved many strategies to control the virus. It is currently unknown which TRIM genes are present in the duck TRIM repertoire, and if any of these genes are involved in influenza A resistance or restriction. To determine which TRIM genes are present in the duck the NCBI SRA database was mined for RNA sequencing projects and a *de novo* assembly of the duck transcriptome was performed. From this transcriptome, 51 TRIM or TRIM-like genes were found to be present in the duck. Seven of these genes appear to be specific to avian lineages and are not present in mammals. RNA seq data from various organs (spleens, lungs, intestines, fibroblasts, testis, ovaries, brains, livers, muscle and adipose tissue) were aligned to these TRIM genes to determine if any TRIM genes have tissue specific patterning of expression. While many TRIM genes demonstrate ubiquitous expression, some were limited to specific tissues. Interestingly, immune relevant tissues such as lung, intestine and spleen predominantly expressed TRIM genes which arose later in evolution. Immune privileged sites such as the brain or gonads predominantly expressed more evolutionarily ancient TRIM genes. By examining the TRIM gene repertoire in ducks, we can gain insight into the evolution of TRIM genes in vertebrates and find candidate genes for influenza A restriction in its natural host and reservoir, the duck.

Species-specific regulation of RIG-I by influenza A viruses

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Abstract: Influenza A viruses are a major cause of human and animal disease worldwide. Wild waterfowl, particularly dabbling ducks, are the primordial hosts of influenza A viruses and remain an important ecological reservoir. Mallard ducks are uniquely resistant to influenza disease compared to other birds, animals, and humans. However, relatively little is known about the host-pathogen interactions that may be contributing to disease resistance in this host species. The non-structural protein 1 (NS1) of influenza A viruses is an important virulence factor that regulates viral replication and controls host cell immune responses. In human cells, NS1 proteins inhibit the induction of innate immune signalling and type-I interferon by preventing the activation of the RIG-I receptor by the ubiquitin ligase TRIM25. It is unclear whether the inhibition of human TRIM25 is a universal function of all influenza A NS1 proteins or is strain-dependent. It is also unclear if NS1 proteins similarly target TRIM25 and the RIG-I pathway of mallard ducks. To answer these questions, I compared the ability of five different NS1 proteins to interact with human and duck TRIM25 and the consequences this had on RIG-I ubiquitination and signalling in both species. NS1 proteins from two low-pathogenic and two highly pathogenic avian influenza viruses efficiently inhibited RIG-I ubiquitination and interferon induction in human cells, while NS1 from a mouse-adapted strain did not. In contrast, none of the NS1 proteins reduced duck RIG-I ubiquitination, despite associating with duck TRIM25 in cells. Uninhibited RIG-I signalling may contribute to mallard ducks' resistance to influenza virus disease.

SKIN INNATE IMMUNITY IS ESSENTIAL FOR LUNGFISH TERRESTRIALIZATION

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Abstract: Conquest of terrestrial life was a pivotal point in evolution that allowed for the progression and diversification of jawed vertebrates. Terrestrialization held many novel stressors that were not encountered by ancestral vertebrates. To compensate for these new challenges, terrestrializing vertebrates underwent drastic physiological and morphological changes. However, how the immune system helped facilitate this transition is largely unknown. The extant relative to all tetrapods, the African lungfish (*Protopterus sp.*), is capable of living in both water and land. Terrestrialization involves formation of a mucus cocoon in response to unfavorable conditions. Lungfish possess unusually large reserves of granulocytes in the gonads, gut and kidney with unknown function. We hypothesize that successful terrestrialization in *Protopterus* requires mobilization of granulocytes from reservoirs to the skin and the formation of a highly antimicrobial barrier that protects the animal against infection during the terrestrialized phase. Transcriptomic profiling of terrestrialized lungfish skin shows a global pro-inflammatory state with increased expression of granulocyte markers. At the same time, egression of granulocytes from reservoirs and homing of granulocytes to the integument is observed in terrestrialized animals. In the skin, recruited granulocytes are capable of producing extracellular traps. In support, elimination of extracellular DNA in vivo results in defective cocoon formation, pathogen invasion and septicemia. Finally, elevated expression of antimicrobial peptide genes is a feature of the terrestrialized skin. These results indicate that lungfish utilize unique innate immune defenses in the skin for successful terrestrialization and that the ancient and evolutionary conserved process of ETosis is essential for lungfish to survive pathogen attack in land.

Discovery of granulocyte-lineage cells in the skin of the amphibian *Xenopus laevis*

Kelsey Hauser[1*], Milan Popovic[1*], Amulya Yaparla[1], Daphne Koubourli[1], Phillip Reeves[2,#], Aashish Batheja[2,#], Rose Webb[3], Maria Forzan[4], Leon Grayfer[1,¶]

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Abstract: The ranavirus Frog Virus 3 (FV3) and the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) are significant contributors to the global amphibian declines and both pathogens target the amphibian skin. In turn, our past work indicates that tadpoles and adults of the anuran *Xenopus laevis* express notable levels of granulocyte chemokines (*cxcl8a* and *cxcl8b*) within their skin and possess skin-resident granulocytes, which are possibly recruited/retained there by these chemo-attractants. In the present work, we show that healthy tadpole and adult *X. laevis* indeed possess granulocyte-lineage cells within their skin epidermis, which appear to be distinct from tadpole and adult mast cells, found predominantly in lower dermal layers. These specific esterase-positive cells responded to rCXCL8a and rCXCL8b in a concentration- and CXCR1/CXCR2-dependent manner, possessed polymorphonuclear granulocyte morphology, granulocyte marker surface staining and exhibited distinct immune gene expression compared to conventional tadpole and adult frog granulocytes. Our past work indicates that CXCL8b recruits immunosuppressive granulocytes and here we demonstrated that enriching esterase-positive skin granulocytes with rCXCL8b (but not rCXCL8a) increased tadpole susceptibility to FV3 and adult frog susceptibility to *Bd*. Conversely, pharmacological depletion of skin-resident granulocytes also resulted in increased tadpole susceptibility to FV3 but did not affect adult *X. laevis Bd*-susceptibility. Together, these data provide new insights into the composition and roles of immune cells within the amphibian skin, which is a critical barrier against pathogenic contributors to the amphibian declines.

Olfactory sensory neurons regulate mucosal antiviral immune responses

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Abstract: Neuroimmune interactions are essential to maintaining homeostasis and host defense during pathogenic invasion. The olfactory bulb (OB) is the nexus between the pathogen exposed olfactory epithelium (OE) and the vulnerable central nervous system (CNS), and, therefore is a likely a hotspot for cooperation between neurons and immune cells. In bony fish, we have previously identified a direct interaction between an aquatic rhabdovirus and olfactory sensory neurons (OSNs) and the onset of antiviral immune responses in the OE and OB. However, the individual contributions of neurons and leucocytes in the OB against peripherally detected viruses are unknown. **The goal of this project is to understand the effects of peripheral viral detection in zebrafish neuroimmune responses in the OB.** Preliminary data demonstrates viral-specific neuronal activation of OSNs projecting into the OB, suggesting that OSNs are electrically activated by the rhabdovirus. Further, behavioral changes can be seen in both adult and larval zebrafish immediately after viral exposure. Single cell (sc)RNA-Seq of the OB reveals the onset of a protective state where both microglia and neurons downregulate sensory differentiation factors and innate immune response mediators (cytokines/complement), in the absence of viral translocation. Further studies seek to identify whether this shift is created by an increased wave of infiltrating neural progenitors from the SVZ or a pause in differentiation of neurons already present. This study will reveal a novel pathway by which neurons arm the OB against potential invasion by a neurotropic pathogen.

CXCL12 mediates the homing/retention of *Xenopus laevis* macrophage-granulocyte precursors to the bone marrow

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Abstract: Across vertebrates, hematopoiesis takes place within designated tissues, wherein committed myeloid progenitors further differentiate towards megakaryocyte/erythroid progenitors (MEPs) or granulocyte/macrophage progenitors (GMPs). While the liver periphery (LP) of the amphibian *Xenopus laevis* functions as a principal site of hematopoiesis and contains MEPs, cells with GMP potential are instead segregated to the bone marrow (BM) of this animal. Through gene expression and western blot analyses of blood cell lineage-specific transcription factors, we confirmed that while the *X. laevis* LP hosts hematopoietic stem cells and MEPs, their BM contains GMPs. In support of our hypothesis that cells bearing GMP potential originate from the frog LP and migrate through blood circulation to the BM in response to chemical cues, we demonstrate that medium conditioned by the *X. laevis* BM is chemoattractive to LP and peripheral blood cells. Compared to LP and using a comprehensive panel of chemokine genes, we demonstrate that the *X. laevis* BM possess greater expression of a single chemokine, CXCL12, the recombinant form of which is likewise chemoattractive to LP and peripheral blood cells and appears to be a major chemotactic component within BM-conditioned medium. In confirmation of the hepatic origin of the cells that give rise to frog GMPs, we also demonstrate that the *X. laevis* BM supports the growth of their LP-derived cells.

Role of Fever in Tissue Repair

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Abstract: Tissue repair is a complex biological process accomplished through acute inflammation and cellular proliferation. Our lab has established an important role of behavioral fever in the induction and resolution of inflammation. The aim of this study is to investigate the role of behavioral fever in the healing of infected wounds in goldfish. Goldfish infected *in vivo* with *Aeromonas veronii* (Gram-negative bacterium) were assigned to three distinct temperature categories: 1) static 16˚C; 2) static 26˚C; and 3) Dynamic temperature (allowing the fish to swim freely through a stable thermal gradient) for 14 days. Video monitoring showed clear thermoregulation for fish in dynamic group. Macroscopic analysis revealed that wound closure was faster in dynamic compared to 16˚C group. This was associated with more rapid leukocyte recruitment and its control based on histopathological analyses. Quantitative PCR analyses also revealed a significantly higher expression of tissue repair related genes. Fish in the 26˚C static temperature group also showed some of the same benefits relative to the static 16˚C group. However, this did not include collagen deposition nor efficient epidermal repair in the wound area. In conclusion, we propose that fever has a potential role in restoration of homeostasis after infection by activation of tissue repair mechanisms. Mechanical increases of temperature offer some benefits but only partially recapitulate those offered through dynamic thermoregulation.

Alternative splicing contributes to diversification of leukocyte immune-type receptors (CaLITRs) of the goldfish (*Carassius auratus* L.)

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Abstract: Leukocyte immune-type receptors (LITRs) are a multigene family of teleost immunoregulatory proteins that share structural, phylogenetic, and likely functional relationships with important innate immune receptor proteins in mammals. Originally discovered in channel catfish (*Ictalurus punctatus*), representative IpLITR-types regulate diverse innate immune cell effector responses (i.e., degranulation, cytokine secretion, and phagocytosis). Although representative IpLITR-types have been functionally characterized using heterologous expression systems, there are still many unanswered questions regarding their precise roles in the regulation of fish immunity. Here, we report on the identification and characterization of five prototypic goldfish (*Carassius auratus*) CaLITRs and their variants. Putative CaLITR sequences were initially identified by searching the goldfish reference RNA database using a truncated CaLITR-like goldfish transcriptomic sequence as a template; subsequently, reverse-transcriptase (RT)-PCR was performed to verify the expression of the predicted CaLITR sequences in goldfish tissues and primary myeloid cell cultures. CaLITR transcripts were detected in all goldfish tissues, and in primary macrophage and neutrophil goldfish cell cultures. Representative expressed products were then cloned, sequenced, and characterized based on their predicted structures. Specifically, CaLITR1 is a functionally ambiguous receptor-type with no charged amino acids in its transmembrane (TM) segment and a short cytoplasmic tail (CYT) region devoid of tyrosine-based signaling motifs. CaLITR2 and CaLITR3 are putative activating receptor-types containing immunotyrosine-based activation motif (ITAM)s within their CYT regions and positively charged TM segments, respectively. CaLITR4 and CaLITR5 resemble receptor-types with diverse signaling capabilities since they contain a variety of immunoregulatory signaling motifs (i.e., putative Nck recruitment, STAT recruitment, ITAM-like, and ITIM motifs). For the first time, alternative splicing events in CYT regions of CaLITRs leading to variable tyrosine-based motif combinations were observed. These findings set the stage for exploring how alternative splicing of CaLITR tyrosine-containing CYT regions leads to the potential diversification of their immunoregulatory potentials.

Thyroid Disrupting Chemicals Perturb Thymocyte Development in *Xenopus laevis* tadpoles

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Abstract: Thyroid disrupting chemicals are ubiquitous water contaminants that cause varied health effects on humans and wildlife. Dysregulation of thyroid hormone signaling during perinatal development can weaken T cell function in maturity, raising the question of whether thyroid disrupting chemicals can perturb thymocyte development acutely. Using *Xenopus laevis* tadpoles as an exploratory model organism, we determined thyroid disrupting effects and thymocyte alterations following exposure to a mixture of thyroid disrupting chemicals used in unconventional oil and gas extraction (UOG) at concentrations well below those found in contaminated water. To assess thyroid disrupting activity, we determined antagonist effects on thyroid receptor gene expression in the tadpole brain and thymus, as well as hypertrophy-like pathology in the thyroid gland. Notably, exposure to UOG chemicals reduced the number of CD8+ single positive thymocytes as well as double positive thymocytes, and co-exposure to UOG and exogenous thyroid hormones nullified the effect. These results suggest that UOG chemicals can perturb thymocyte development through the thyroid hormone pathway. Future studies will investigate UOG effects on thymocyte apoptosis during selection and long-term effects on T cell mediated immunity following metamorphosis.

Antigen presentation genes in Gadoid species (haddock: *Melanogrammus aeglefinus* and Atlantic cod: *Gadus morhua*) raise questions about cross-presentation pathways and glycosylated beta-2-microglobulin.

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Abstract: The Atlantic cod immune system deviates from antigen presentation processes seen in other vertebrates in that they lack the necessary genes for exogenous antigen presentation (i.e. MH-II, li, CD4), while possessing an expanded repertoire of the MH-I genes that facilitate endogenous antigen presentation. These observations, combined with the identification of putative endosomal sorting signals in MH-I cytoplasmic tails, have led to speculation that cod rely on cross-presentation of exogenous antigens to elicit cytotoxic T-lymphocyte responses against extracellular threats. In light of this suggestion, we investigated MH-I genes in a closely related gadoid species, haddock. Analysis of transcripts from one individual revealed expression of 13 unique MH-I molecules, including two non-classical molecules as determined by the level of conservation at the peptide anchoring sites, suggesting that haddock MH-I repertoire has expanded similarly to cod. Analysis of haddock MH-I cytoplasmic tail sequences revealed that the dileucine- and tyrosine-based intracellular signaling motifs, suggested to facilitate cross-presentation in cod, were absent. Closer examination of the cod signaling motifs, including their relative position in the cytoplasmic tail region, indicates these motifs might be non-functional, further supporting the need for functional studies to assess cross-presentation. Finally, *in silico* analysis and *in vitro* N-type de-glycosylation experiments demonstrate that haddock and cod beta-2-microglobulin (β2M) are glycosylated at the same NQT sequence. Though the exact significance of β2M glycosylation has yet to be elucidated, phylogenetic comparisons predict that the same NQT glycosylation sequence occurs in 13 additional species comprising four different orders of Actinopterygii (Gymnotiformes, Esociformes, Beryciformes, Perciformes), suggesting either that this feature has arisen independently in multiple lineages or that it comes from a common ancestor and has been lost or modified in many species. Together, the analysis of gadoid MH-I genes and β2M molecules highlight the challenges in generalizing immune system paradigms across the most diverse vertebrate lineage (i.e., fish) and between fish and more well-studied mammals.

Discovery of rainbow trout nasal innate lymphocytes

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Abstract: Cytotoxic T cells are responsible for killing cancer cells and virally infected cells. Previously, we have described a CD8α^+TCR$\alpha\beta^+$IFNγ^{lo} T cell subset that rapidly infiltrates the nasal mucosa of rainbow trout in response to nasal rhabdovirus delivery. This influx is also observed in response to nasal delivery of live or dead bacteria but not in response to PAMPs. This ultra-rapid and broad local response led us to hypothesize that these T cells are innate lymphocytes, likely natural killer (NK) cells. In support, increased mRNA expression of NK cell markers such as *nccrp1* and *nitr2* but not nitr1, is observed in the trout olfactory organ (OO) following nasal rhabdovirus delivery. TCRα repertoire analyses of sorted CD8$^+$ lymphocytes indicate a polyclonal antiviral nasal response. Preliminary single cell RNA-Seq of sorted OO CD8$^+$ lymphocytes reveal the presence of eight CD8α^+ subsets in controls and seven in virally treated fish. Subsets expresses markers corresponding to all stages of NK cell differentiation including immature progenitors, early effectors and mature effectors. Wide expression of *nccrp1,* the natural killer enhancing factor (*nkef*) and the G-protein coupled receptor *gpr183* is detected in all subsets implying a cytotoxic function as well as tissue positioning guided by oxysterol-sensing. In conclusion, our results indicate the presence of several innate CD8$^+$ lymphocyte subsets in the rainbow trout nasal mucosa that respond quickly to nasal viruses. Future studies will determine the stress-derived ligands recognized by these innate lymphocytes and how neurons regulate their activity.

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Characterization of iT cells interacting with the MHC class I-like XNC4 during mycobacteria infection

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Abstract: Non-tuberculosis mycobacteria (NTM) have emerged as opportunistic pathogens in the last few decades. They not only pose an ecological threat to wide variety of amphibians and fish species but can also cause severe infections in humans if local or systemic host defenses are compromised. Patients suffering from immune disorders, like HIV, are highly susceptible to these infections. A major hurdle in dealing with mycobacterial infections is the lack of full understanding of mycobacteria-host interactions. Sub-populations of innate like T cells (innate Natural Killer T Cells and Mucosal Associated Innate T cells) have been implicated as early responders in human and rodent mycobacterial infections but their specific roles in the immune response have not been characterized clearly. Low frequency of iT cells in current mammalian models poses further hindrance in deciphering the functions of these cells and their interaction. Recent characterization of iT cells in the amphibian *Xenopus laevis* has opened avenues to study mycobacterial infection in an alternate model. Using this model, we have identified that the *Xenopus* non-polymorphic MHC like XNC4 gene is critical for imparting resistance against *Mycobacterium marinum (Mm) to X. laevis* tadpoles. With the use of MHC tetramer technology, flow cytometry and genetic screens, we are currently investigating the interactions and functions of XNC4 interacting iT cells. Preliminary results indicate that XNC4 tetramers synthesized by co-culture in insect cell lines with *Mm* recognize different iT cell populations in infected liver than when synthesized without them. The iT cell population in infected liver also differs from cells in uninfected spleen suggesting an activation and/or selection of a particular subset of iT cells as a response to *Mm* infection. Further characterization of T cell receptor repertoire of these iT cell subsets is underway.

Characterization of MHC class I-like XNC4 function in host resistance against *Mycobacterium marinum*

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Abstract: Tuberculosis and non-tuberculosis mycobacteria are major health concern in human because of the lack of effective treatments. In fact, new therapeutic strategies have been limited by the lack of knowledge on the (1) interaction between mycobacteria and the host immune system as well as (2) the underlying protective immunity of the host. MHC-I-like restricted innate-like (i)T cells (CD1d-restricted iNKT and MR1-restricted MAIT) are involved in the early immune response against *Mycobacterium tuberculosis* in human and mice. Nevertheless, their immune role remains to be fully characterized. For the first time in a non-mammalian species, iT cells have been characterized in the amphibian *Xenopus*. Notably, reverse genetic approaches have revealed that MHC-like XNC4-restricted iVα45 T cells expressing invariant Vα45-Jα1.14 TCR have a critical non-redundant role in tadpoles' resistance against *Mycobacterium marinum* (*Mm*). To characterize cell expressing surface XNC4 during mycobacteria infection, we have screened and obtained a camelid derived specific nanobody (NB5) from a synthetic yeast library using recombinant XNC4. We generated XNC4-deficient *Xenopus* stably transfected cell lines to confirm the specificity on the NB5 nanobody. Following inoculation of DsRed fluorescent *Mm,* recruitment of XNC4^{high+} MHC-II^{high+} myeloid cells together with iVα45 T cell can be detected by flow cytometry at the site of infection (*i.e.*, peritoneal cavity and liver), both in tadpoles and frogs. Interestingly, in tadpoles but not in frogs of a transgenic strain expressing *gfp* under the control of the *mpeg* macrophage-specific promoter, *mpeg*+/XNC4^{high+} peritoneal leucocytes were preferentially infected. Whether macrophages expressing XNC4 are more permissive to Mm infection and whether they can present Mm-derived ligands to activate iT cells remains to be investigated.

The Holostean Major Histocompatibility Complex

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Abstract: The major histocompatibility complex (MHC) is a cluster of genes that are responsible for antigen processing and display. Throughout tetrapods and cartilaginous fish, MHC class I and class II genes are tightly linked. This linkage of class I and class II genes is not typically observed in teleost species and is believed to be a result of the teleost whole genome duplication (tWGD). Holosteans, such as bowfin and gar, are ray-finned fishes that are the closest relatives of teleosts that did not undergo the tWGD. Here, we report our analysis of the genome sequencing and annotation of the MHC locus in representative holosteans. Our findings indicate the overall organization of MHC regions in bowfin are similar to tetrapods and cartilaginous fish, supporting the model that the loss of linkage between class I and class II genes in teleosts occurred after their divergence from holostean fish and is likely associated with the whole genome duplication of teleosts. We highlight our analysis of the PSMB8 gene, which is part of the inducible proteasome complex and is responsible for systematically degrading proteins into short polypeptides for loading and display on MHC proteins. Within a given species, alternative haplotypes for these genes have been described. For example, in numerous teleosts species, the 31st amino acid residue of the mature PSMB8 protein is found to contain either an alanine or a phenylalanine (PSMB8A or PSMB8F, respectively). These trans-species polymorphisms may lead to functional changes in the types of proteins degraded and thus the antigens displayed. We found the holostean PSMB8 gene, while highly conserved with other fish species, encodes novel alleles containing either a threonine or serine at the 31st residue. Many additional polymorphisms were also identified in the neighboring genes, suggesting holosteans experienced unique co-evolution of these genes at this locus likely resulting in functional differences of antigen presentation.

Primordial B Cell Selection Sites in the Nurse Shark Spleen

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Abstract: B cell selection and affinity maturation are immune processes which, in mammals, occur in germinal centers (GCs), specialized structures within secondary lymphoid organs. While sharks, members of the oldest extant taxonomic group (Chondrichthyes) to have immunoglobulin-based adaptive immunity, lack true GCs, they can affinity mature their B cell repertoires. Thus, we hypothesized that sharks possess structures that are the evolutionary foundation of GCs in mammals. To identify these sites, nurse sharks (*Ginglymostoma cirratum*) were immunized with the fluorescent antigen phycoerythrin (PE) to visualize antigen trafficking in situ. Spleen samples were collected and analyzed using immunofluorescent microscopy (IF) and RNA fluorescent in situ hybridization (RNA FISH). IF experiments showed antigen is clustered in the center of IgNAR+ B cell follicles at 40 days post-immunization. RNA FISH experiments showed aggregates of T cells interacting with the borders of B cell follicles, and AID expression appears to be upregulated in IgNAR+ B cells at the periphery of the follicles. We believe we have identified primordial sites of B cell selection in the nurse shark spleen and that these are the evolutionary precursors to canonical GCs. Future work will focus on elucidating the chemokines and cytokines that regulate this process in the nurse shark.

Characterization of immunoglobulins and B lymphocytes in the olfactory epithelium and nasal mucus of the Nurse shark (*Ginglymostoma cirratum*)

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Abstract: Five hundred million years ago, the adaptive immune system based on B and T lymphocytes emerged in cartilaginous fish. The olfactory organ of sharks is a large mucosal epithelium that is in constant contact with the outside environment making this an easy point of entry into the body by invading pathogens. The identification of nasopharynx-associated lymphoid tissue (NALT) in bony fish indicated the presence of abundant B lymphocytes in the olfactory epithelium. We hypothesized that sharks also have a NALT rich in B cells and that shark nasal mucus contains secreted immunoglobulins (Igs). Immunofluorescence staining of nurse shark olfactory organ showed presence of scattered B cells with IgM>IgW>IgNAR and no evidence of an organized NALT structure. IgW^+ B lymphocytes were observed adjacent to or in the lamina propria suggesting that IgW provides protection to the neuronal and vascularized tissue. IgM^+ B lymphocytes, in turn, were located apically in the epithelium where direct secretion of IgM into the nasal cavity was observed. Western blotting analyses confirmed the presence of IgW, IgNAR as well as low and high molecular weight IgM in nasal mucus, gut mucus and serum. Based on these findings, we propose that IgM^+ B lymphocytes are acting as a first line of defense in by transcytosing Igs into the nasal mucosal layer. IgW^+ B lymphocytes may be acting as a second line of defense after pathogen have entered the olfactory organ. Our findings suggest NALT emerged at least 500 million years ago and that nasal Ig secretion may occur via direct secretion in cartilaginous fish.

Antibody affinity modification in fish

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Abstract: Antibody affinity maturation is the process to improve antibody affinity for an antigen during a humoral immune response; it is mediated by the immunoglobulin (Ig) mutator enzyme activation-induced cytidine deaminase (Aicda). In homeotherms, this process occurs in distinct structures called germinal centers (GCs). Fish were thought to lack affinity maturation because they lack histologically distinct GCs. However, a functional homologue of Aicda has been identified in fish, where Aicda expressing cells co-exist with a population of pigmented cells called melano-macrophages (MMΦs). These aggregates are associated with the essential components of GCs. We hypothesized that these clusters are functionally analogous to GCs. To test our hypothesis, we generated whole VDJ repertoires for melano-macrophage clusters (MMΦCs) from vaccinated and unvaccinated zebrafish. Our results have shown that each cluster is nucleated initially by two clonally expanding B-cells. Later 2-3 activated B-cells enter, proliferate and acquire mutations.

To determine if there is an active selection process within MMΦCs, we examined the distribution of replacement vs. silent mutations on CDRs and framework regions (FRs) within the variable domain of the Ig. CDRs are the sites that determine the Ag binding affinity while FRs provide the structural backbone of the Ig. Therefore, we generally expect to see a higher ratio of R to S mutations on CDRs but not on FRs if there is a mechanism for selection. Using SHazaM, our results show that CDRs significantly accumulate more R mutations than FRs, which is consistent with the presence of selection processes. Also, using Hill numbers we found low clonal diversity and more related clones within MMΦCs, which could indicate the presence of an effective recruitment mechanism within these clusters, where few B-cells are recruited and diversified.

These results provide strong evidence of affinity modification occurring in organized cellular structures in fish much as GCs in mammals.

ENHANCING INOSINE MONOPHOSPHATE DELIVERY USING NOVEL PHYTOGLYCOGEN-BASED NANOPARTICLES IN BOVINE AND RAINBOW TROUT MODELS

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Recently, there has been interest in the use of dietary nucleotides in industrial feeds, with inosine monophosphate (IMP) showing beneficial effects on growth and immunity. Rainbow trout and Alberta cattle are two farmed animals of economical importance to Canada and enhancing their growth rates and immunity would further improve their value. The current project focused on using a novel phytoglycogen nanoparticle (NP) as a carrier for IMP to test whether it enhanced IMP's positive effects on growth and immunity. The impacts of IMP, the phytoglycogen-based nanoparticle (NP) and inosine monophosphate cationically bound to the phytoglycogen-based nanoparticle (IMP-NP) were characterized *in vitro* in rainbow trout (RTgutGC) and bovine (BT-IMF) cell lines and *in vivo* in rainbow trout. In order to study the effects of IMP-NP in bovine *in vitro*, a novel bovine cell line was first characterized. The novel bovine intestinal cell line isolated from a fetal bovine intestine was successfully maintained in culture for over 20 passages. At passage 11, the cell line was positive for vimentin and smooth muscle actin (α-SMA) and negative for pancytokeratin suggesting the cells are myofibroblast in type. Thus, the cell line was named BT-IMF (*Bos taurus* intestinal myofibroblast) and the optimal media composition for the cell line was elucidated. Stimulation with IMP-NP proved to enhance proliferation of BT-IMF. In rainbow trout *in vitro*, IMP-NP significantly increased metabolism rates in RTgutGC at the highest dose tested (0.14 mg/mL IMP bound to 6.25 mg/mL NP). *In vivo*, rainbow trout that were fed an IMP-NP containing feed had significantly higher levels of lysozyme compared to control groups, and while not statistically significant demonstrated a trend towards higher weight gains. Thus, the use of IMP-NP should be further examined in order to enhance growth performance in Canada's rainbow trout and cattle.

Antigen trapping by cells in the putative germinal centre of fish

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Fish were originally thought to lack antibody affinity maturation in part due to absence of high affinity antibodies in blood following immunization. However, recent work in our lab identified the presence of the mutator enzyme activation-induced cytidine deaminase (Aicda) in catfish, which is required for somatic hypermutation (SHM) of immunoglobulin genes. In mammals, Aicda initiates 'random' point mutations in the V(D)J exons encoding the antigen recognition site of the antibody. These mutated B-cells then compete for antigen trapped on the surface of Follicular dendritic cells (FDCs) within the light zone of germinal centre (GC). Successful B-cells receive signal to proliferate and differentiate into antibody secreting plasma or long-lived memory cells while the unsuccessful B-cells undergoes apoptosis.

In catfish, the AICDA expressing cells were in close proximity to auto-fluorescent melanomacrophages (MMs) and surrounded by reticular cells, the latter of which are known to have linage relationship with FDCs. Considering these we hypothesized that either MMs or reticular cells could be functionally analogous to FDCs.

Goldfish were immunized with protein molecules (Bovine serum albumin & Keyhole limpet hemocyanin) conjugated to fluorescent tag Alexa fluor 647 and leukocytes were isolated from the spleen and kidney of these fish followed by imaging. To differentiate surface trapping of antigen from phagocytosis beads conjugated to specific antibodies (anti-BSA or anti-KLH) were used. Preliminary results suggested that MM were involved in antigen trapping. Co-immunoprecipitation of MM cell surface receptor followed by mass spectrometry will also be performed to identify the receptor involved in antigen trapping.

Previous work has already shown that the melanomacrophage clusters (MMCs) in fish are involved in long-term antigen retention, and through this work we are attempting to identify the cellular and molecular context for retention. My findings are consistent with another line of work showing evidence of SHM within MMCs in zebrafish. These finding may inform decisions on how better to vaccinate fish in aquaculture. Funded by NSERC.