INSECT BIOTECH CONFERENCE

Annual Scientific Meeting

June 1 - 3, 2022

Schedule and Contributed Abstracts

Hilton Garden Inn, Niagara-on-the-Lake, ON

Sponsored by:

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FroggaBio scientific solutions

Conference Schedule

The Meeting Takes Place in the Gardenview Room
(All sessions, Coffee Breaks and Meals)

Wednesday Evening – June 1

6:00 pm **Registration**

7:00 pm **Plenary Talk**

TRACKING THE INVASION OF THE ASIAN LONGHORNED BEETLE (CERAMBYCIDAE: *ANOPLPHORA GLABRIPENNSIS* MOTSCHULSKY): SURPRISING POPULATION STRUCTURE, INVASION HISTORY, AND SIGNATURES OF FUNCTIONAL ADAPTATION.
[Page 10]
Amanda Roe,
Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, ON, Canada.

7:45 – 10:00 pm **Reception**: (Pizza, Veggies and Beverages)

Thursday, June 2

7:45 – 9:10 am **Breakfast (Gardenview Room)**

9:10 am **Opening Remarks: Andrew Donini**

Long Talk Session [15 minutes Total]: Session Chair: Farwa Sajadi

9:15 am **GLIAL EXPRESSION OF VOLTAGE-GATED K+ CHANNELS MODULATES THE *DROSOPHILA* CRITICAL THERMAL MINIMUM** ***[Page 11]***
Andersen, M.K., and MacMillan, H.A.
Department of Biology, Carleton University, Ottawa, ON, Canada.

9:30 am **LIFETIME MACRONUTRIENT INTAKE OF *GRYLLODES SIGILLATUS*: WHICH PROTEIN TO CARBOHYDRATE RATIO MAXIMIZES YIELD?** ***[Page 12]***
Muzzatti, M., S.J. Harrison, C.T. Brittain, H. Brzezinski, C.C. Stabile, S.M. Bertram, and MacMillan, H.A.
Department of Biology, Carleton University, Ottawa, Ontario, Canada

9:45 am **EVIDENCE FOR THE UPTAKE AND BREAKDOWN OF MICROPLASTICS BY FIELD CRICKETS IN AN AGRICULTURAL ENVIRONMENT.** ***[Page 13]***
McColville, E.[1], Ritchie, M.[2], Allison, J.[2], Lara Pineda, R.[1], Vermaire, J.[1], Provencher, J.[2,3], MacMillan, H.A.[2]
[1] Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Canada
[2] Department of Biology, Carleton University, Ottawa, Canada.
[3] Environment and Climate Change Canada, Government of Canada

10:00 am **THE DIGESTIVE SYSTEM OF A CRICKET PULVERIZES POLYETHYLENE MICROPLASTIC BEADS** ***[Page 14]***
Ritchie, M.W.[1], Provencher, J.F.[2], Allison, J.E.[3], Muzzatti, M.J[1], MacMillan, H.A.[1]
[1] Department of Biology, Carleton University, Ottawa, Ontario, Canada
[2] National Wildlife Research Centre, Environment Canada, Ottawa, Ontario, Canada
[3] University of Ottawa, Department of Biology, 30 Marie Curie, Ottawa, Ontario, Canada

10:15 am **THE IMPACT OF MICROPLASTIC INGESTION ON TISSUE-SPECIFIC GENE EXPRESSION IN THE TROPICAL HOUSE CRICKET, *G. SIGILLATUS*. *[Page 15]***
Cheslock, A.[1], Provencher, J.[1,2], and MacMillan, H.A.[1]
[1] Department of Biology, Carleton University, Ottawam, Ontario, Canada
[2] Environment and Climate Change Canada, Ottawa, Ontario, Canada

10:30 – 11.00 am **Coffee Break**

Long Talk Session [15 minutes Total]: Session Chair:Britney Picinic

11:00 am **OCTOPAMINE AND TYRAMINE RECEPTORS IN THE DENGUE VECTOR *AEDES AEGYPTI*. *[Page 16]***
Finetti, L. [1], Paluzzi, J.P. [2], Orchard, I. [1], and Lange, A.B. [1]
[1] Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.
[2] Department of Biology, York University, Toronto, Ontario, Canada.

11:15 am **ECDYSTEROID-SIGNALING AND REPRODUCTION IN *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS DISEASE. *[Page 17]***
Benrabaa, S., Orchard, I., Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.

11:30 am **GONADULIN: A NOVEL INSULIN-LIKE PEPTIDE INVOLVED IN OVIPOSITION IN *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS DISEASE. *[Page 18]***
Leyria, J., Orchard, I., and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.

11:45 am **EXPLORING THE ROLE OF GLYCOPROTEIN HORMONE GPA2/GPB5 IN THE MEDICALLY IMPORTANT INSECT, *RHODNIUS PROLIXUS*. *[Page 19]***
Al-Dailami, A.N., Leyria, J., Orchard, I., and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

12:00 pm **MOLECULAR CHARACTERIZATION AND IMMUNOLOCALIZATION OF ITP AND ITP-L IN THE MOSQUITO, *AEDES AEGYPTI*. *[Page 20]***
Sajadi, F., and Paluzzi, J.P.
Department of Biology, York University, Toronto, Canada

12:15 – 1:30 pm **Lunch Break**

Long Talk Session [15 minutes Total]: Session Chair: Mads Andersen

1:30 pm **A GONAD-SPECIFIC HEAT SHOCK PROTEIN IN THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERNANA*. *[Page 21]***
Fick, W.E., and Quan, G.
Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Ontario

1:45 pm **MULTIPLE FLAVIVIRUS INFECTION: DYNAMICS IN HOST AND VECTOR CELLS. *[Page 22]***
Shivafard, S., Garrido de Castro, M., and Hunter, F.
Department of Biological Sciences, Brock University, St Catharines, Ontario, Canada

2:00 pm **VIRAL INTERACTIONS OF ZIKA VIRUS AND MAYARO VIRUS IN MAMMALIAN CELLS.** ***[Page 23]***
Garrido de Castro, M., Williams, A., and Hunter, F.
Department of Biological Sciences, Brock University, St. Catharines, ON, Canada.

2:15 pm **CELLULAR MECHANISMS INVOLVED IN HIGHER LONGEVITY DRIVEN BY FLUCTUATING THERMAL REGIMES IN *DROSOPHILA MELANOGASTER*. *[Page 24]***
Hunter-Manseau, F., Cormier, J., and Pichaud, N.
Department of Chemistry and Biochemistry, Université de Moncton, Moncton, New Brunswick, Canada

2:30 pm **INVESTIGATING THE ROLE OF PKG IN THE SIGNALING CASCADE OF A CAPA NEUROPEPTIDE IN THE MALPIGHIAN TUBULES OF *DROSOPHILA MELANOGASTER*. *[Page 25]***
Snan, L., Sajadi, F., and Paluzzi, J.P.
Department of Biology, York University, Toronto, Ontario, Canada

2:45 – 3:15 pm **Coffee Break**

Long Talk Session [15 minutes Total]: Session Chair: Luca Finetti

3:15 pm **A PUTATIVE ROLE OF RENAL (MALPIGHIAN) TUBULES IN REGULATING THE CALCIUM HOMEOSTASIS OF *AEDES AEGYPTI* MOSQUITO AFTER BLOOD FEEDING.** ***[Page 26]***
Li, Y. and Piermarini, P.
Department of Entomology, The Ohio State University, Wooster, Ohio, USA

3:30 pm **DEVELOPMENT OF DIAGNOSTIC TOOLS FOR AGROCHEMICAL EXPOSURE IN THE WESTERN HONEY BEE (*APIS MELLIFERA*) USING TRANSCRIPTOMICS.**
[Page 27]
Jamieson, A., Pepinelli, M., Newburn, L.R., and Zayed, A.
Department of Biology, York University, Toronto, Ontario, Canada

3:45 pm **INVESTIGATING MECHANISMS THROUGH WHICH PROCTOLIN MODULATES CONTRACTION IN *DROSOPHILA*. *[Page 28]***
Jung, J. and Mercier, J.A.
Dept. of Biological Sciences, Brock University, St. Catharines, Ontario, Canada.

4:00 pm **EXAMINING THE PHYSIOLOGICAL ROLE OF TACHYKININS ON THE *DROSOPHILA MELANOGASTER* MALPIGHIAN 'RENAL' TUBULES.** ***[Page 29]***
Agard, M., and Paluzzi, J.P.
Department of Biology, York University, Toronto, Ontario, Canada.

4:15 pm **ASSESSING THE EFFECTS OF THE HUMAN CONTRACEPTIVE PILL ON AEDES AEGYPTI FECUNDITY.** ***[Page 30]***

K.A. Hunt[1], I. Drahun[2], B.J. Cassone[1]
[1] Department of Biology, Brandon University, Brandon, Manitoba, Canada
[2] Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada

SHORT EXPOSURES (5 MINUTE TALK and QUESTIONS)

Session Chair: Andrew Donini

4:30 pm **METABOLIC PROFILING OF DENGUE VIRUS CHALLENGED *AEDES AEGPYTI* THAT ARE REFRACTORY TO VIRUS.** ***[Page 31]***

Elliott, K., Umaña Diaz, C., Giron Ceron, D., and Lowemberger, C.
Centre for Cell Biology, Development and Disease, Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada.

4:40 pm **IDENTIFICATION OF DENGUE VIRUS RESTRICTION AND DEPENDENCY FACTORS IN *AEDES AEGYPTI.*** ***[Page 32]***

Umaña Diaz, C., Eliott, K., Giron Ceron, D., and Lowemberger, C.
Centre for Cell Biology, Development and Disease, Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada.

4:50 pm **THE POTENTIAL EFFECT OF ISVS ON EGG LAYING CAPABILITIES IN *CULEX PIPIENS* (CULICIDAE)**. ***[Page 33]***

Benton, N., Drahun, I., Williams, A., Eicken, A., Brundula, I., El Khal, A., Hunter, F.F., and Patterson, I.
Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada.

5:00 pm **End of Session**

6:30 to 10:00 pm **Chateau des Charmes, 1025 York Road, St. Davids, ON L0S 1P0**

Map and Directions for Banquet at:

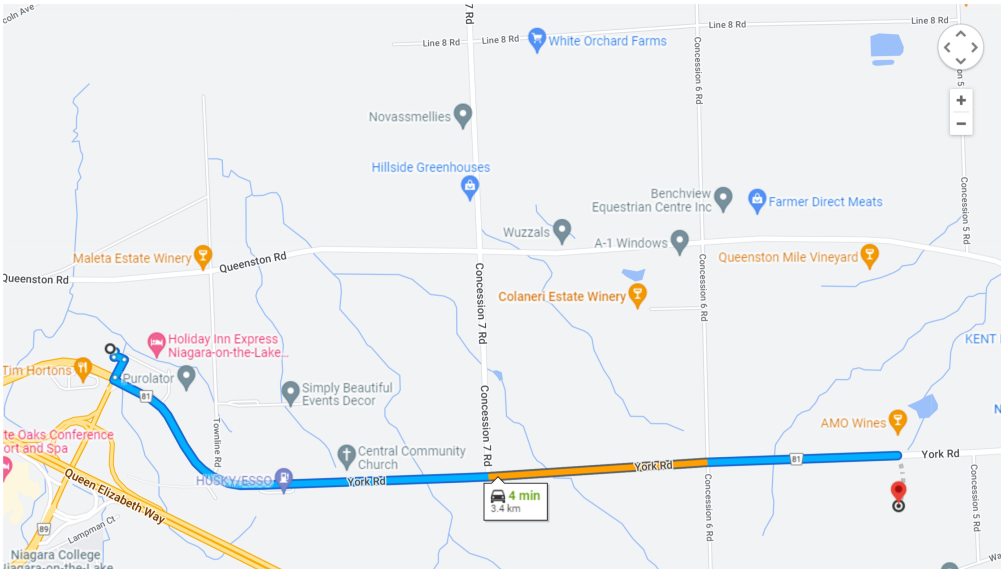
Chateau des Charmes (1025 York Road, St. Davids, ON, L0S1P0)

Estimated travel time ~4 min (3.5 km) via the following driving directions:

Hilton Garden Inn Niagara-on-the-Lake
500 York Rd, Niagara-on-the-Lake, ON L0S 1J0

1. Head south toward Glendale Ave — 22 m
2. Turn left toward Glendale Ave — 42 m
3. Turn right onto Glendale Ave — 77 m
4. Turn left at the 1st cross street onto York Rd/Niagara Regional Rd 81 — 3.2 km

Château des Charmes
1025 York Rd, St. Davids, ON L0S 1P0



Friday, June 3

7:45 – 9:15 am **Breakfast**

Long Talk Session [15 minutes Total]: Session Chair: Heath MacMillan

9:15 am **THE EFFECT OF DIET ON AQUAPORIN ABUNDANCE AND LOCALIZATION IN THE DISEASE VECTOR MOSQUITO, *AEDES AEGYPTI*. *[Page 34]***
Picinic, B.N., Paluzzi, J.P. and Donini, A.
Department of Biology, York University, Toronto, Ontario, Canada

9:30 am **INVESTIGATING THE MODULATION OF DROSOPHILA MELANOGASTER BODY-WALL MUSCLE CONTRACTION BY THE NEUROPEPTIDE DPKQDFMRFAMIDE. *[Page 35]***
Wasilewicz, L.J., and Mercier, A.J.
Department of Biology, Brock University, St.Catharines, Ontario, Canada.

9:45 am **DETERMINING THE ROLE OF RYAMIDES IN THE DISEASE VECTOR, *AEDES AEGYPTI. [Page 36]***
Luong, T., and Paluzzi, J.P.
Department of Biology, York University, Toronto, ON, Canada

10:00 am **CHARACTERIZATION AND INSIGHT INTO THE PHYSIOLOGICAL ROLE OF THE CCHAMIDES IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*. *[Page 37]***
Tan, J., and Paluzzi, J.P.
Department of Biology, York University, Toronto, Ontario, Canada

10:15 – 10:45 am **Coffee Break**

SHORT EXPOSURES (5 MINUTE TALK and QUESTIONS)

Session Chair: Jean-Paul Paluzzi

10:45 am **EXAMINING DIRECT ACTIONS OF A NEUROPEPTIDE ON POSTSYNAPTIC MUSCLE CELLS**. ***[Page 38]***
Gagnon, Z., and Mercier, J.A.
Department of Biological Sciences, Brock University, St. Catharines, ON, Canada

10:55 am **ELUCIDATING THE ROLE OF TACHYKININS IN THE MOSQUITO AEDES AEGYPTI USING CRISPR/CAS9**. ***[Page 39]***
Kohandel, Z, and Paluzzi, J.P.
Department of Biology, York University, Toronto, Ontario, Canada

11:05 am **ENERGY METABOLISM OF MOUNTAIN PINE BEETLES AND EMERALD ASH BORER DURING DIAPAUSE**. ***[Page 40]***
Haider, F., and MacMillan, H.
Department of Biology, Carleton University, Ottawa, Ontario, Canada.

11:15 pm	**Closing Remarks (**Jean-Paul Paluzzi and Andrew Donini)
11:25 - 12:00 noon	**Check out of hotel rooms**
11:45 am – 1:15 pm	**Lunch**

TRACKING THE INVASION OF THE ASIAN LONGHORNED BEETLE (CERAMBYCIDAE: ANOPLPHORA GLABRIPENNSIS MOTSCHULSKY): SURPRISING POPULATION STRUCTURE, INVASION HISTORY, AND SIGNATURES OF FUNCTIONAL ADAPTATION.

Roe, A.
Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada

Asian longhorned beetle (*Anoplophora glabripennis* – ALB), is a native forest pest in China and the Korean peninsula. This species has successfully invaded and spread to hardwood forests in North America and Europe. ALB mines the heartwood of a range of tree species and poses a significant threat to invaded forest ecosystems. Tracking and responding to the ALB invasion requires knowledge of its native population structure and the ability to mount a rapid response to new infestations. We used genomic approaches to characterize the native population structure and trace the North American invasion of ALB. Our results showed clear differences between ALB populations in China and South Korea, as well as pronounced population structure within China. Signatures of selection were detected in a number of SNPs, including a missense variant in glycerol kinase (GLK3), a critical enzyme in the production of the glycerol, which is used as a cryoprotectant to survive low winter temperatures. A latitudinal cline in allele frequencies within GLK3 hint at regional adaptations to different winter conditions. The results provide a foundation for mapping the global invasive history of ALB.

GLIAL EXPRESSION OF VOLTAGE-GATED K+ CHANNELS MODULATES THE *DROSOPHILA* CRITICAL THERMAL MINIMUM.

Andersen, M.K., and MacMillan, H.A.
Department of Biology, Carleton University, Ottawa, ON, Canada.

At the critical thermal limits, animals experience a general loss of function which often manifests as a paralytic or coma-like phenotype. In insects, this has been attributed to a loss of central nervous function caused by a spreading depolarization (SD) event which is characterized by a rapid surge in extracellular K+ concentration. This neurophysiological limit to performance is, however, not static and most insects are capable of altering the SD-inducing temperature through acclimation. Here we use Drosophila melanogaster to investigate how acclimation alters the temperature leading to cold-induced SD and whether this plasticity relates to expression voltage-gated K+ channels in the brain. After confirming that cold-acclimated flies experience SD at lower temperatures, we investigated the role of voltage-gated K+ channels in driving the difference between acclimation groups by injecting the inhibitor 4-aminopyridine and compared this to the effect of a general K+ channel inhibitor (tetraethylammonium). Blocking voltage-gated K+ channels increased the SD temperature in both cold- and warm-acclimated flies. This effect was smaller in warm-acclimated flies, but in both groups it represented a large proportion of the effect of general K+ channel blockade, indicating that the acclimation-induced differences in SD temperature were largely related to voltage-gated channels. This is supported by a UAS-Gal4-mediated glial knock-down screen showing that knockdown of channels encoded by the seizure and shaw genes had similar, albeit smaller, effects compared to the pharmacological blockade. Thus, differential expression of voltage-gated K+ channels represents a key mechanism by which flies are able to alter their thermal tolerance to match the environment.

Acknowledgements: This research was supported by NSERC and the Carlsberg Foundation

ME MACRONUTRIENT INTAKE OF *GRYLLODES SIGILLATUS*: WHICH PROTEIN TO CARBOHYDRATE RATIO MAXIMIZES YIELD? *[Page 12]*

Muzzatti, M., S.J. Harrison, C.T. Brittain, H. Brzezinski, C.C. Stabile, S.M. Bertram, and MacMillan, H.A.
Department of Biology, Carleton University, Ottawa, Ontario, Canada

Mass rearing of insects for feed and food is a rapidly developing industry and crickets, such as Gryllodes sigillatus, are one of only a few species that are currently being farmed in Canada. A primary goal of agricultural research is to increase yield, but cricket farms struggle with how best to do this at a scale of billions of crickets that are required to make a profit. Diet is known to strongly impact cricket life history traits. For example, the relative availability of dietary protein and carbohydrates influences weight gain, growth rates, adult body size, and survival. To date, however, most cricket farms do not carefully consider the nutrient balance in the diet they feed their crickets, opting for a generic feed formulation instead. While a protein:carbohydrate (P:C) ratio of 3:1 is known to maximize weight gain in Gryllus veletis, nutrient requirements in insects are species-specific. It is therefore unclear what P:C ratio maximizes yield in Gryllodes sigillatus. To test this, we individually reared hundreds of G. sigillatus from hatch to adulthood on seven diets with different P:C ratios (8:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:8) while simultaneously measuring survival, time to adulthood, adult body size, and lifetime food consumption. We used a nutritional geometry framework to determine how lifetime protein and carbohydrate intake affected G. sigillatus' life-history traits. Our results will contribute towards determining the optimal standard feed formulation that is required to maximize G. sigillatus yield in farming production.

This research was supported by Entomo Farms, the province of Ontario, NSERC, and the Canada Foundation for Innovation.

EVIDENCE FOR THE UPTAKE AND BREAKDOWN OF MICROPLASTICS BY FIELD CRICKETS IN AN AGRICULTURAL ENVIRONMENT.

McColville, E.[1], Ritchie, M.[2], Allison, J.[2], Lara Pineda, R.[1], Vermaire, J.[1], Provencher, J.[2,3], MacMillan, H.A.[2]

[1] Institute of Environmental and Interdisciplinary Science, Carleton University, Ottawa, Canada
[2] Department of Biology, Carleton University, Ottawa, Canada.
[3] Environment and Climate Change Canada, Government of Canada

Since the beginning of large-scale plastic production in the 1950s, plastic has become a ubiquitous environmental contaminant. While terrestrial environments experience the greatest quantity of plastic deposition, they are often regarded merely as transport pathways to the marine environment, and their capacity to become a long-term plastic sink is overlooked. Microplastics (plastics < 5mm) are becoming a leading environmental concern due to their impacts on soil physio-chemistry, general cytotoxicity, incorporation into agroecosystems (e.g. via sludge from wastewater treatment), and suitability for ingestion by biota. In laboratory settings, crickets will uptake microplastic when given no alternative, but no previous experiment has examined the environmental relevance of plastic ingestion by these generalist omnivores in an agricultural setting. To determine whether crickets willfully uptake microplastics in the environment, the gut tracts of 50 wild field crickets originating from a sludge-treated field in Winchester, Ontario, were dissected and analyzed. Over half of the crickets (52%) contained some microplastics within their alimentary canal; in total, 89 microplastics were found, 63 being microfragments, and 26 being microfibers. The notably high concentrations of similar-looking fragments observed within individual crickets suggests plastic transformation after ingestion, raising concern that crickets may contribute to both plastic transport and transformation in an agricultural environment.

This research was supported NSERC, CFI, and ECCC.

THE DIGESTIVE SYSTEM OF A CRICKET PULVERIZES POLYETHYLENE MICROPLASTIC BEADS.

Ritchie, M.W.[1], Provencher, J.F.[2], Allison, J.E.[3], Muzzatti, M.J[1], MacMillan, H.A.[1]
[1] Department of Biology, Carleton University, Ottawa, Ontario, Canada
[2] National Wildlife Research Centre, Environment Canada, Ottawa, Ontario, Canada
[3] University of Ottawa, Department of Biology, 30 Marie Curie, Ottawa, Ontario, Canada

Microplastics (MPs; plastics smaller than 5 mm in size) are a growing environmental concern but a poorly understood threat to biota. In recent years, there has been a spike in research on MPs, but most of this work has focused on marine systems. The potential interactions terrestrial organisms (e.g., insects) have with MPs have been understudied, especially with an estimated 4900 megatons of plastics to date being directed to terrestrial systems and some plastic specifically added to the soil in agricultural systems. We used a generalist insect (a cricket; Gryllodes sigillatus) to examine whether individuals would ingest and transform MPs in their food to explore the fate of the MPs. We fed crickets fluorescent MPs mixed into a standard diet (0, 2.5, 5, and 10% w/w) and dissected the major gut regions to isolate the MPs within. By comparing plastic content and fragment size within regions of the gut, we sought to identify whether and where crickets can fragment ingested MP particles. Given the digestive tract morphology of this species, we expected that the crickets would both ingest and egest the MPs. We also predicted that the MPs would be fragmented into smaller pieces during this digestive process. We found that G. sigillatus can indeed ingest MPs, and we found that when fed 100 µm MPs, individuals egested much smaller pieces (equal to or smaller than 2 µm). The bulk of this fragmentation of the MP occurs early in the digestive process of this insect (e.g. during mastication). These findings suggest that when generalist insects encounter MPs, they can serve as agents of plastic transformation in their environment.

This research was supported by ECCC and NSERC.

THE IMPACT OF MICROPLASTIC INGESTION ON TISSUE-SPECIFIC GENE EXPRESSION IN THE TROPICAL HOUSE CRICKET, *G. SIGILLATUS*.

Cheslock, A.[1], Provencher, J.[1,2], and MacMillan, H.A.[1]
[1] Department of Biology, Carleton University, Ottawam, Ontario, Canada
[2] Environment and Climate Change Canada, Ottawa, Ontario, Canada

Microplastics are omnipresent in our environment; animals are exposed to them every day via food, water, and air. Despite most plastics being used and produced on land, microplastics research has been centered around aquatic environments and mismanaged waste entering the ocean. Various physiological effects due to microplastic ingestion have been recorded in aquatic animals (e.g., immunotoxicity, mitochondrial dysfunction), however, consequences to terrestrial animals, such as insects, remain unclear. It is crucial we advance our understanding of how insects respond to microplastic consumption at the physiological and genetic levels, and how these changes impact fitness-related traits. Here, we reveal tissue-specific genetic responses to ingested microplastics in the model cricket species Gryllodes sigillatus, using next generation sequencing (RNA-Seq). Crickets are an ideal model system for microplastics (e.g., readily consume them, survive long-term and acute exposures, are easily reared), thus we also intend to further establish G. sigillatus as a model species by generating a de novo transcriptome using Trinity, as there is no genome established for this species to date. Using this de novo transcriptome, we evaluate gene expression in the midgut and hindgut (organs directly in contact with ingested microplastics), fatbody (important to energy supply and immunity) and ovaries (important to reproductive success), providing a comprehensive view of how dietary plastics influence cricket physiology. We hypothesize microplastic ingestion will primarily impact gene expression involved in the digestive and immune systems, as this has been previously reported in aquatic model systems. In this talk, preliminary results will be shared on how microplastic ingestion impacts gene expression, as well as the quality of our de novo transcriptome assembly for this species.

This research was supported by NSERC.

OCTOPAMINE AND TYRAMINE RECEPTORS IN THE DENGUE VECTOR *AEDES AEGYPTI*.

Finetti, L. [1], Paluzzi, J.P.[2], Orchard, I. [1], and Lange, A.B. [1]
[1]Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.
[2]Department of Biology, York University, Toronto, Ontario, Canada.

In insects, the biogenic amines octopamine (OA) and tyramine (TA) are involved in controlling several physiological and behavioural processes. OA and TA act as neurotransmitters, neuromodulators or neurohormones performing their functions by binding to specific receptors belonging to the G protein-coupled receptor (GPCR) superfamily. Numerous studies have shown that the GPCRs for OA and TA are involved in reproduction, smell perception, metabolism, and homeostasis. Moreover, they are targets for insecticides, such as amitraz.

With regard to the Dengue vector *Aedes aegypti*, no research has been previously reported on their OA or TA receptors. Here, we identify and characterize the OA and TA receptors in *A. aegypti*. Bioinformatics tools have been adopted to identify four OA and three TA receptors in the genome of A. aegypti. The seven receptors are expressed in all developmental stages of *A. aegypti*; however, their highest transcript abundance is observed in the adult compared to the larval stages. Among several adult *A. aegypti* tissues/organs (central nervous system, antennae and rostrum, midgut, Malpighian tubules, ovaries, or testes), the type 2 TA receptor (TAR2) transcript is most abundant in the ovaries and the type 3 TA receptor (TAR3) is enriched in the Malpighian tubules, leading us to hypothesize a putative role for these receptors in reproduction and diuresis, respectively. Furthermore, the blood meal influenced OA and TA receptor expression patterns in adult female tissues/organs at several time points post blood meal, suggesting physiological implications for each receptor.

The present work provides information for better understanding the physiological roles of OA, TA, and their receptors in *A. aegypti*, and additionally, may help in the development of novel strategies for the control of these human disease vectors.

This work was supported by grants to ABL and IO from NSERC.

ECDYSTEROID-SIGNALING AND REPRODUCTION IN *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS DISEASE.

Benrabaa, S., Orchard, I. and Lange, A.B.
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.

Rhodnius prolixus is a blood-gorging insect which is medically important since it transmits Chagas disease via feces and urine containing *Trypanosoma cruzi*. In adult females, ecdysteroid hormone (20-hydroxyecdysone) is involved in the growth of the ovary and development of eggs post blood meal (PBM). Halloween genes are necessary for ecdysteroid synthesis since they code for cytochrome P450 enzymes in the ecdysteroidogenic pathway. The ecdysteroid receptor (EcR/USP) binds 20-hydroxyecdysone, resulting in activation of ecdysone-responsive genes. We have identified and characterized the Halloween genes, Neverland, CYP18a1, and eight ecdysone-responsive genes in the *R. prolixus* ovary using transcriptomic data. We used BLAST to compare transcriptome sequences with other arthropod sequences to identify similar transcripts. Our results indicate that the Halloween genes and ecdysone-responsive genes are present in the ovary of *R. prolixus*. We have quantified by qPCR. Halloween gene transcript expression and ecdysone-responsive gene transcript expression in the ovary following a blood meal. Most of the Halloween genes and ecdysone-responsive gene transcripts are up regulated by a blood meal during the first three days PBM. Knockdown of EcR, USP and shade result in a significant reduction in the number of eggs produced and a severe reduction in egg-laying and hatching rate. Furthermore, silencing the ecdysone receptor and shade altered the expression of the chorion genes transcripts Rp30 and Rp54 at day 3 and 6 PBM. Future work will measure the level of vitellogenin yolk proteins in the hemolymph following a blood meal.

Supported by NSERC Discovery Grants to IO and ABL.

GONADULIN: A NOVEL INSULIN-LIKE PEPTIDE INVOLVED IN OVIPOSITION IN *RHODNIUS PROLIXUS*, A VECTOR OF CHAGAS DISEASE.

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Insulin-like peptides (ILPs) are vital hormones involved in a wide range of physiological processes in all organisms. In insects, insulin signalling has been reported to be involved mainly in detecting and interpreting nutrient levels for egg production. Based on publicly available transcriptomes, a new ILP named gonadulin has been reported and suggested to be expressed by the gonads (hence its name). Although the identification of gonadulin establishes its existence, its physiological relevance remains poorly understood. Rhodnius prolixus is an obligate hematophagous insect and a primary vector of Trypanosoma cruzi, the etiological agent of Chagas disease. In this study on R. prolixus we report for the first time the participation of gonadulin in reproductive performance of an hemipteran. By RT-qPCR, we find that the R. prolixus gonadulin (Rhopr-gonadulin) transcript is highly expressed in the reproductive system, particularly in the calyx, a structure through which eggs pass into the lumen of the lateral oviducts. Using Fluorescence in situ Hybridization (FISH) we confirm the location of the Rhopr-gonadulin transcript in the calyx. Interestingly, when the Rhopr-gonadulin signaling cascade is impaired using RNA interference (RNAi), eggs are retained primarily in the ovarioles and calyx, and thus oviposition is inhibited. Overall, the results support the hypothesis of Rhopr-gonadulin acting in an autocrine or paracrine way, in order to enable oviposition. Understanding the physiological mechanisms of reproductive performance in R. prolixus can shed light on potential targets for effective production of biopesticides by translational research, thereby controlling insect populations and transmission of the disease.

Supported by the Natural Sciences and Engineering Research Council of Canada

EXPLORING THE ROLE OF GLYCOPROTEIN HORMONE GPA2/GPB5 IN THE MEDICALLY IMPORTANT INSECT, *RHODNIUS PROLIXUS*.

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Glycoprotein hormones are formed by the heterodimerization of alpha and beta subunits. In vertebrates, there are five glycoprotein hormones, four of which have a common alpha subunit (GPA1) bound to a specific beta subunit (GPB1, GPB2, GPB3, or GPB4), and the fifth, thyrostimulin, is formed by the dimerization of GPA2 and GPB5 subunits. These hormones mediate physiological events such as development, metabolism, and reproduction. Recent reports in invertebrates suggest that GPA2/GPB5 plays a critical role in development, diuresis, and reproduction. In this study, we characterize the transcripts for the glycoprotein hormone GPA2/GPB5 and its receptor (LGR1) in fifth instar *Rhodnius prolixus*, a vector of Chagas disease. Sequence analyses reveal considerable identity and similarity between GPA2/GPB5 and LGR1 and those reported in other arthropod species. qPCR shows that both subunit transcripts, GPA2 and GPB5, and LGR1 transcripts are present in a variety of tissues, with greatest expression of the subunits in the central nervous system (CNS) and highest LGR1 expression in the Malpighian tubules (MT). A reduction in LGR1 transcript expression (via RNA interference) led to greater weight loss in unfed insects and increased mortality rate in both unfed and fed insects. Using immunohistochemistry, GPB5 is found expressed throughout the CNS and is present in neurosecretory cells in the brain and abdominal neuromeres and their neurohemal organs, indicating a neurohormonal role. In addition, in the adult female GPB5-like axonal projections are present in the trunk nerves extending to the oviduct indicating direct neural control. A unique signal is also observed in the tropharium of the ovary and LGR1 transcript expression increases in the adult female reproductive system post-feeding, adding further support to a role in reproduction. Overall, the results suggest that the GPA2/GPB5 signaling may play roles during a prolonged unfed state, in feeding-related events, and in reproduction in the adult female *R. prolixus*.

This research was supported by NSERC.

MOLECULAR CHARACTERIZATION AND IMMUNOLOCALIZATION OF ITP AND ITP-L IN THE MOSQUITO, *AEDES AEGYPTI*.

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The insect ion transport peptide (ITP) and its alternatively spliced variant, ITP-like or ITP-long (ITPL) belong to the crustacean hyperglycemic hormone family of peptides and are widely conserved among insect species. While limited, various studies have characterized the ITP/ITPL signaling systems within many insect species, and putative functions including regulation of ion and fluid transport, ovarian maturation, thirst/excretion, and clock neuron modulation have been proposed. However, to date, the expression pattern, tissue distribution, and putative physiological function of either ITP or ITPL has not been determined in the mosquito, *Aedes aegypti*. Here, we aim to molecularly investigate ITP and ITPL expression profiles in *A. aegypti*, and examine peptide immunolocalization and distribution within the adult tissues. Thus far, transcript expression profiles of both ITP and ITPL reveal enrichment in males, with ITP exclusively expressed in the adult brain, and ITPL found predominantly in the abdominal ganglia. Using whole mount immunohistochemistry, the central nervous system from adult mosquitoes revealed ITP-like immunostaining in one pair of lateral neurosecretory cells in the posterior region of each brain hemisphere whereas ITPL-like immunostaining was observed in one neurosecretory cell located medioposteriorly and ventrally on abdominal ganglia 1 through 7, with nerve processes migrating anteriorly from each neurosecretory cell and emanating laterally through projections to potential putative neurohaemal sites. Due to the suggested anti-diuretic role of ITP, further examining the distribution and physiological roles of both ITP and ITPL may uncover possible osmoregulatory mechanisms within the adult *A. aegypti* mosquito.

Acknowledgements: Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an NSERC Discovery Grant (JPP) as well as NSERC CGS-D (FS)

A GONAD-SPECIFIC HEAT SHOCK PROTEIN IN THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERNANA*.

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The effect of heat shock on chromosomes was first reported 60 years ago. Some years later, the elevated production of certain proteins was noted after heat shock and this led to their designation as heat shock proteins (HSPs). Since this early work on *Drosophila*, HSPs have been found in all living organisms studied, including both prokaryotes and eukaryotes. Additionally, their upregulation has been shown to be stimulated by various stressors other than heat and they appear to have functions beyond the role of mitigating the effects of adverse conditions. Previously, we reported the existence of 15 small HSPs in the spruce budworm, *Choristoneura fumiferana*, a major destructive pest in the boreal forests of North America. Only nine of these sHSPs are upregulated after heat shock. Today, we share new findings on one of these, CfHSP20.2. Whereas both the transcript of CfHSP20.2 and its protein are present in various tissues after heat shock, they are exclusively found in the gonads under normal rearing conditions. High expression is concurrent with known times for gametogenesis in males and females of this insect, suggesting that this protein may play roles in both reproductive health and overall protection against extreme temperatures.

This research was funded by Natural Resources Canada.

MULTIPLE FLAVIVIRUS INFECTION: DYNAMICS IN HOST AND VECTOR CELLS.

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West Nile virus (WNV) and Zika virus (ZIKV) are examples of disease-causing arboviruses that can have severe outcomes. Arboviruses are transmitted by arthropods, and *Aedes albopictus* mosquitoes have been implicated as a vector shared by WNV, ZIKV and many others. The geographical range of Ae. albopictus mosquitoes is continuously expanding, mainly due to climate change, and starting in 2016, they have been found in Southern Ontario. With the arrival of this new vector in Canada, there is a chance of infection of mosquitoes with multiple viruses. In this study, we aim to investigate the dynamics of WNV and ZIKV coinfection and superinfection in mammalian and insect cell lines to determine the possibility of viral interference. Both Vero E6 cells, derived from the African green monkey, and C6/36 cells, derived from *Aedes albopictus* larva, were infected with WNV (WN-NY99 strain) and ZIKV (PRVABC59 strain). For coinfection experiments (WNV-ZIKV CI), cells were infected with both viruses simultaneously, in a 1:1 ratio, at a final MOI of 0.01. For the superinfection experiments, an initial infection was performed with one of the viruses, followed by the second virus 24 hours later (WNV/ZIKV SI and ZIKV/WNV SI). Once the cells were infected, culture supernatant was collected every day for 5 days. RNA was extracted from the samples and submitted to RT-qPCR for viral detection. All supernatants were tested for the presence of both WNV and ZIKV RNA. A standard curve was used to calculate log starting quantity (LSQ) values from cycle threshold (CT) values. WNV had consistently higher LSQ values in Vero cells in both the coinfection and WNV/ZIKV SI. No significant difference was seen on day 5 for ZIKV/WNV SI. No difference was seen in C6/36 cells for coinfection, but the order of viruses had a significant impact on LSQ in C6/36 superinfection experiments. Multiple infections affect the dynamics of WNV and ZIKV infections in Vero and C6/36 cells.

VIRAL INTERACTIONS OF ZIKA VIRUS AND MAYARO VIRUS IN MAMMALIAN CELLS.

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Arboviruses cocirculating in similar geographic areas with the same arthropod vectors pose a risk to public health as they can cause simultaneous infections in humans. Understanding how these viruses interact within host cells can help predict their impacts on human health, specifically by deducing whether there is viral interference or enhancement of either virus during multiple infections. Zika virus (ZIKV) and Mayaro virus (MAYV) are mosquito-borne pathogens co-circulating in South America and the Caribbean. Both have the capacity to be transmitted by the urban vectors Aedes aegypti and Aedes albopictus. The objective of this research is to determine the effects of coinfection and sequential infection (superinfection) on ZIKV and MAYV replication in mammalian cells. Vero E6 cells were used in this investigation to mimic mammalian host cell behaviour. The conditions studied were coinfection (CI), 1-hour superinfections (1h SI), and 24-hour superinfections (24h SI), in which all SI experiments tested each virus as the primary and secondary infection. Supernatant was collected over six days and viral RNA was extracted for analysis by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). The log starting quantity (LSQ) of each virus was calculated using standard curves derived from known titers of each virus. Our results indicate that the presence of MAYV caused viral interference of ZIKV while the presence of ZIKV, regardless of the order of SI infection, did not affect the replication of MAYV. Additionally, the order of viruses during SI was important in the extent of viral interference of ZIKV. We also show that 1h SI results were not different from CI results in most cases. These results give insight into the interactions of these viruses within areas that they co-circulate, and further investigation will reveal the effects of these conditions on viral titer and cell viability.

CELLULAR MECHANISMS INVOLVED IN HIGHER LONGEVITY DRIVEN BY FLUCTUATING THERMAL REGIMES IN *DROSOPHILA MELANOGASTER*.

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Ectotherms exposed to constant low temperature suffer from physiological injuries that could potentially compromise their survival. In contrast, fluctuating thermal regimes (FTR) has been shown to increase *Drosophila melanogaster* longevity. However, cellular mechanism allowing for this increase longevity is not yet known. The mitochondrial unfolding protein response (UPRmt) could be involved in this process. Indeed, this mechanism is activated following misfolded protein build-up in mitochondria to restore homeostasis, and thus improve survival. After a stress occurs, the UPRmt promotes expression of heat shock protein genes, antioxidant activity, mitophagy and mito-biogenesis. In this study, we aim to characterize the cellular response following a mild thermal stress and establish if the UPRmt is involved in the increase of longevity that we observed. Therefore, *Drosophila* raised for five days on a constant temperature of 24 °C were either subjected to another five days on a constant 24 °C (controls) or five days of a FTR of 24 °C/15 °C day/night. Our results suggest that *Drosophila* exposed to FTR have less cold and heat tolerance, as well as a significantly higher mitochondrial oxygen consumption for CI-LEAK and complex IV compared to *Drosophila* maintained at 24 °C. On the other hand, no significant difference was observed for the expression of genes involved in the UPRmt. This shows that more study is needed to characterize cellular mechanisms involved in the higher longevity following thermal stress.

This study was supported by NSERC and NBIF.

INVESTIGATING THE ROLE OF PKG IN THE SIGNALING CASCADE OF A CAPA NEUROPEPTIDE IN THE MALPIGHIAN TUBULES OF *DROSOPHILA MELANOGASTER*.

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In the fruit fly, *Drosophila melanogaster*, the renal component of the excretory system consists of two pairs of Malpighian tubules (MTs), an anterior and posterior pair, which function to maintain internal homeostasis and regulate fluid secretion. In *Drosophila*, the neuronally-derived CAPA peptides have been shown to exhibit diuretic and anti-diuretic effects depending on hormone concentration through the NOS/cGMP/PKG pathway. However, the specific downstream cellular targets involved in the signaling pathway remain unclear. The objectives of this study were to further elucidate the secretory role of a CAPA neuropeptide in *D. melanogaster*, unravel the specific protein kinase G (PKG) enzymes activated through CAPA signaling, and explore whether CAPA exhibits differential activity on the anterior and posterior tubules. Femtomolar range DrosCAPA-2 was found to elicit the most potent anti-diuretic effect in both anterior and posterior tubules of one- and four-day old female MTs. Moreover, using pharmacological blockers against nitric oxide synthase (NOS) and PKG abolished the anti-diuretic effects of this neurohormone, confirming the importance of NOS/cGMP/PKG pathway in CAPA activity. Finally, knockdown of PKG1 using GAL4/UAS bipartite system abolished the anti-diuretic effects of CAPA in both anterior and posterior MTs, suggesting the involvement of this specific PKG enzyme in CAPA-mediated inhibition in both tubule pairs. Future studies will examine of other PKG isoforms, including PKG2 (i.e., DG2P1 and DG2P2) plays a role in the anti-diuretic activity of CAPA on the MTs. These results will advance our understanding of the roles and effects of this neurohormone including the signaling components involved in the control of the insect excretory system.

This research was funded by: NSERC Discovery Grant (JPP) and Ontario Ministry of Research & Innovation Early Researcher Award (JPP).

A PUTATIVE ROLE OF RENAL (MALPIGHIAN) TUBULES IN REGULATING THE CALCIUM HOMEOSTASIS OF *AEDES AEGYPTI* MOSQUITO AFTER BLOOD FEEDING.

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In flesh flies and fruit flies, the renal Malpighian tubules (MTs) play a key role in regulating extracellular fluid (hemolymph) Ca2+ homeostasis. However, the renal regulation of Ca2+ balance in hematophagous flies, such as mosquitoes, has not been extensively examined, especially in the context of hematophagy. Given that blood feeding is an elemental behavior that is involved with reproduction and disease transmission in female Aedes aegypti, the objective of our study was to understand how MTs might contribute to mosquito Ca2+ homeostasis during blood meal digestion. We first conducted experiments to examine the chronological changes in Ca2+ content in adult female Ae. aegypti after blood feeding. Female mosquitoes were fed blood one week after emergence and then either frozen or dissected at several time points post blood meal; non-blood fed mosquitoes served as controls. For each time point, the Ca2+ content was measured in homogenates of whole bodies and isolated MTs using a Ca2+-selective sensor. The results suggest that no significant changes occurred in the Ca2+ content of mosquito whole bodies after blood feeding. However, we found dynamic changes of the Ca2+ content in MTs after blood feeding. To generate molecular insights into a transport mechanism potentially responsible for the changes in Ca2+ content, we characterized the mRNA expression of a putative plasma membrane Ca2+-ATPase (PMCA) in isolated mosquito MTs before and after blood feeding using reverse transcriptase quantitative PCR (RT-qPCR). We found that PMCA mRNA was differentially expressed in MTs during time periods when MT Ca2+ content was changing. Taken together, our results suggest that MTs undergo dynamic changes in Ca2+ content after blood feeding that may in part be mediated by changes in abundance of PMCA. The physiological significance of the changes in Ca2+ content remains under investigation.

This research is partly supported by Sigma Xi Grant G20211001-003, the Ohio Mosquito and Vector Control Association Grant in Aid program, as well as the CFAES Internal Grants Program.

DEVELOPMENT OF DIAGNOSTIC TOOLS FOR AGROCHEMICAL EXPOSURE IN THE WESTERN HONEY BEE (*APIS MELLIFERA*) USING TRANSCRIPTOMICS.

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Pollination is vital in agriculture as it is a key process in the reproduction of flowering plants. The eusocial western honey bee, Apis mellifera, is the only honey bee in North America and is the most commonly used managed pollinator in the world. In Canada, honey bees contribute $3.97 to $5.5 billion to the national economy each year via their pollination services. Unfortunately, honey bees are susceptible to a wide range of interacting stressors, including chemicals used in agriculture. These agrochemicals are used for a wide range of purposes and can include insecticides, herbicides, and fungicides. Many of which are systemic in the environment and can affect off-target organisms. This has resulted in increasing rates of overwintering mortality worldwide. In particular, Canadian beekeepers have been losing more than a quarter of their colonies annually since 2006. Neonicotinoids are a specific class of insecticides that have been linked to decline in pollinator health. Exposure results in neural dysfunction leading to changes in behavior, altered development, or death. It can be difficult to disentangle and identify specific stressors that affect a particular colony. Our research aims to solve this problem by conducting field and laboratory studies to develop biomarkers specific to common agrochemicals based on differential gene expression in response to exposure.

Acknowledgements: This research was supported by GenomeCanada, Ontario Genomics, Genome British Colombia, and GenomeQuebec

INVESTIGATING MECHANISMS THROUGH WHICH PROCTOLIN MODULATES CONTRACTION IN *DROSOPHILA*.

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We are examining mechanisms through which proctolin modulates muscle contractions elicited by L-glutamate (L-Glu) in body wall muscles of Drosophila 3rd instar larvae. Proctolin increased the amplitude of contractions evoked by bath application of L-Glu. Using a transgenic fly line expressing the calcium-binding fluorophore, GCaMP6, in body wall muscles, we find that calcium signals are enhanced by applying proctolin simultaneously with L-Glu. This suggests that proctolin contributes to increase cytoplasmic calcium as an underlying mechanism.
Contraction requires both calcium influx and release of calcium from the sarcoplasmic reticulum (SR). Calmodulin is reported to mediate inactivation of L-type calcium channels and should inhibit calcium influx across the plasma membrane. Calmodulin also inhibits calcium release from the SR by inactivating ryanodine receptors (RYR) at high cytoplasmic calcium concentration. We, therefore, tested the hypothesis that proctolin may enhance Glu-evoked contractions by inhibiting calmodulin. At 2-30 µM, the calmodulin inhibitor, W7, induced contractions, both in the presence and absence of extracellular calcium. W7 also enhanced Glu-induced contractions in calcium-containing saline. Proctolin enhanced W7-induced contractions, but W7 did not occlude proctolin's ability to enhance Glu-evoked contractions. This suggests that proctolin and W7 modulate contractions by different mechanism. We also examined the possible involvement of the calcium/calmodulin-dependent protein kinase enzyme (CaMKII). The selective CaMKII antagonist, KN93, did not alter proctolin's ability to enlarge Glu-induced contractions. This finding suggests that CaMKII is not necessary for the peptide to modulate contractions. Taken together, our results suggest that activating the proctolin receptor in Drosophila enhances contractions via mechanisms that increase cytosolic calcium.

Funded by NSERC

EXAMINING THE PHYSIOLOGICAL ROLE OF TACHYKININS ON THE *DROSOPHILA MELANOGASTER* MALPIGHIAN 'RENAL' TUBULES.

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In the fruit fly, *Drosophila melanogaster*, the excretory organs rely on the neuroendocrine system to modulate the process of diuresis to maintain haemolymph homeostasis. The excretory system consists of the hindgut and Malpighian tubules (MTs) that function to maintain ion and water balance. The MTs are involved in primary urine formation and are composed primarily of two cell types, the principal and stellate cells, that are regulated by circulating hormones including neuropeptides. One group of neuropeptides are the *Drosophila* tachykinins (DTKs) that function by binding to their cognate G-protein-coupled receptor, the *Drosophila* tachykinin receptor (DTKR). However, there has been no research that established the physiological effects of DTKs on the *D. melanogaster* MTs. In the present study, we examine DTKs and study their physiological role in secretion by the adult fruit fly MTs. We attempt to localize cells expressing the DTKR in the MTs by using the bipartite GAL4-UAS system to knockdown DTKR gene expression in a cell-specific manner using RNAi. So far, data from RT-qPCR analysis suggests that DTKR is localized in the stellate cells due to greater knockdown in DTKR-RNAi flies driven by the stellate cell-specific GAL4 driver. The physiological role of DTKs is also validated using the Ramsay bioassay to measure the secretion rates from MTs isolated from DTKR knockdown and wild-type flies. Results indicate DTK acts as a diuretic leading to a significant increase in fluid secretion from MTs isolated from wild-type flies. However, MTs isolated from DTKR knockdown flies displayed lower secretion rates when treated with DTK. Future studies will help elucidate the physiological role of DTK as a diuretic hormone in the fruit fly and determine the mechanism of action and cellular target in the MTs.

Acknowledgements: Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an NSERC Discovery Grant (JPP).

ASSESSING THE EFFECTS OF THE HUMAN CONTRACEPTIVE PILL ON AEDES AEGYPTI FECUNDITY.

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Arthropod-borne viruses transmitted by mosquitoes (Culicidae) remain a constant risk to more than 3.9 billion people in over 129 countries. Aedes aegypti is an important global vector efficient at transmitting dengue virus, chikungunya virus and Zika virus. With insecticide resistance becoming a more prevalent issue combined with few alternative mosquito control methods, novel approaches that lessen the burden of mosquito-borne diseases are needed. Interference with mosquito reproductive capabilities continues to be heavily researched in the way of male sterility; however, field-based applications remain difficult to implement. The human birth control component, 17α-Ethinyl estradiol (EE2), belongs to the increasing list of Endocrine Disruptors Chemicals (EDCs) capable of interfering with the endocrine system in both vertebrates and invertebrates. Despite its dispersion throughout the environment, not much is known about its mode of action and harmful effects on invertebrates. To assess the potential effects of EE2 on mosquito fecundity, we administered human birth control pills containing EE2 into larval water and adult sugar wicks. We then measured the results using female fecundity parameters such as the ability to lay eggs, the number of eggs laid, and offspring viability. We found that exposing Ae. aegypti to the human contraceptive pill negatively impacted female fecundity. There was a significant difference in oviposition success in females exposed to EE2. There was also a significant difference in the number of eggs laid between control to experimental groups. However, there was no significant difference in the other parameters, suggesting an all-or-nothing effect. The goal of this study was to determine the extent to which EE2 could potentially disrupt reproductive success in Ae. aegypti, and further understand the implications of ECDs and their potential application in mosquitoes.

This research was supported by NSERC and Canada Foundation for Innovation.

METABOLIC PROFILING OF DENGUE VIRUS CHALLENGED *AEDES AEGPYTI* THAT ARE REFRACTORY TO VIRUS.

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Nearly half the world's population lives in areas with circulating dengue virus (DENV), producing 400 million infections each year. Developing novel viral control measures is paramount to global health efforts and requires a thorough understanding of host-virus interactions. Some naturally occurring populations of *Aedes aegpyti* eliminate DENV and do not transmit the virus. However, the mechanisms governing resistance to DENV infection remain elusive. Our group has isolated two populations with hyper-susceptible and refractory characteristics, which act as a model system for investigating host-virus interactions. Previously, we have utilized genomics, transcriptomics, and microbiome sequencing of these populations to understand DENV susceptibility and uncovered pathways contributing to phenotypic differences. However, despite significant progress, we have been unable to recapitulate a refractory phenotype. Therefore, we sequenced refractory or susceptible insects' metabolomes to determine biochemical pathways perturbed by the DENV challenge. We used highly sensitive chemical isotope labelling liquid chromatography-mass spectrometry (CIL LC-MS) metabolomics to survey metabolites present in the midguts of refractory and susceptible insects with and without DENV challenge at various times post-infection. Metabolomics has been employed in mosquitoes to elucidate small molecules that impact viral replication but has never been performed in naturally occurring resistant and susceptible populations. Our objective is to determine small molecules that influence viral replication with the hopes that these compounds help discover new targets for mitigating viral spread.

IDENTIFICATION OF DENGUE VIRUS RESTRICTION AND DEPENDENCY FACTORS IN *AEDES AEGYPTI.*

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Dengue viruses (DENv) infect 50-400 million people annually and can lead to severe morbidity and mortality. There are no effective vaccines or treatment for this disease. Dengue is transmitted by mosquitoes, primarily *Aedes aegypti*, and most Dengue management programs try to reduce or eliminate the vector population. These strategies involve: 1) Physical controls (insecticides and larvicides), and 2) Biological controls including mosquito biomanipulation or genetic modification techniques to induce sterility, decrease lifespan, or reduce vector competence. Even though these control approaches have shown great promise in developing new and effective vector control strategies, the idea of integrating foreign genes or organisms into a wild species is very controversial.
With colleagues in Colombia we have identified naturally refractory mosquitoes (Cali-R) that co-exist with susceptible mosquitoes (Cali-S) in areas of endemic transmission. Using a combination of molecular biology and bioinformatic analyses we have identified pro-Dengue genes in the Cali-S strain; these are mosquito genes that are essential for DENv to enter and replicate within insect cells. We are using RNAi techniques to confirm the transcriptomic data and are editing these genes in the genome of *Ae. aegypti* to engineer a genetically stable dengue-refractory strain. In addition we are developing a novel system to deliver constructs in an efficient manner to specific tissues to improve the efficiency of generating transgenic mosquitoes compared with the currently used, highlu inefficient, embryo injection system. This will generate another tool to develop ecologically sound, self-sustainable and safe vector control strategies to dampen dengue transmission.

THE POTENTIAL EFFECT OF ISVS ON EGG LAYING CAPABILITIES IN *CULEX PIPIENS* (CULICIDAE)

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Culex pipiens has been implicated as a major vector of West Nile virus and continues to be a bridge vector for the virus from birds to humans. While many focus on the mosquitoes transmitting viruses with major medical importance, it is important to understand the effects that other viruses may or may not have on these vectors. Insect-specific viruses (ISVs) are viruses that are only transmitted from insects to other insects but have no known effect on humans. ISVs are common in many different groups of insects and are prevalent in many wild mosquito taxa. Despite this, little is known about the effects of certain ISVs on different major vector species. We've exposed the larval forms of Cx. pipiens to three ISVs simultaneously, Negev virus, cell fusing agent virus, and Phasi Charoen-like virus, to see their infectivity and transmission in the mosquito. The adult F0 and F1 mosquitoes were collected and will be assayed for presence of each ISV. The experimental infection has had a major effect on fecundity. 50% of ISV-exposed, blood-fed *Cx. pipiens* failed to produce any eggs. In some cases of those that managed to produce eggs, some females failed to create the characteristic egg rafts that are typically seen in *Cx. pipiens*. We will continue to look at the rates of horizontal and vertical transmission in *Cx. pipiens*, as well as explore the effects of ISV infection on *Cx. pipiens* oviposition behavior and egg mortality, which could lead to potential pathogen control methods.

Research was supported by NSERC

THE EFFECT OF DIET ON AQUAPORIN ABUNDANCE AND LOCALIZATION IN THE DISEASE VECTOR MOSQUITO, *AEDES AEGYPTI*.

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The disease vector mosquito, *Aedes aegypti*, is located in sub-tropical and tropical regions around the globe. Adult mosquitoes acquire a nectar meal from a variety of plants to obtain nutrients necessary for regular bodily functions. Unique to females is their ability to acquire a blood meal from vertebrate hosts, which is required to provide the necessary proteins to produce mature and viable eggs. However, both a blood meal in female A. aegypti and a nectar meal in male *A. aegypti* pose an osmoregulatory challenge to the animal. Collectively, the Malpighian tubules (MTs) and hindgut are responsible for the production and alteration of urine in *A. aegypti* and, in turn, are responsible for dealing osmoregulatory challenges associated with a blood or nectar meal. Following feeding, *A. aegypti* undergo an increase in ions and water in their haemolymph. The presence of aquaporins (AQPs) in the MTs allow for the transport of excess water by osmosis into the tubule lumen, which follows the movement of ions driven by the apical V-type H+-ATPase. There have been six AQPs identified in *A. aegypti*, where AaAQP1, 2, and 6 are identified as water specific, and AaAQP4 and 5 are identified as entomoglyceroporins, able to transport other solutes as well as water. In this study, AaAQP1, 4, and 5 have been localized in isolated MTs of female *A. aegypti* in non-fed and blood fed animals. In addition, AaAQP1, 4, and 5 have been localized in whole body male *A. aegypti*, in sugar fed and starved animals. To further identify if diet has an effect on AQPs in *A. aegypti*, western blot has also been completed for both females and males. Results suggest that feeding, whether a blood meal in females or nectar meal in males, does affect AQP abundance in the mosquito, *A. aegypti*.

This research was supported by NSERC.

INVESTIGATING THE MODULATION OF DROSOPHILA MELANOGASTER BODY-WALL MUSCLE CONTRACTION BY THE NEUROPEPTIDE DPKQDFMRFAMIDE.

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The neuropeptide, DPKQDFMRFamide, is found in neurosecretory cells in *Drosophila melanogaster* and is thought to be released as a hormone to modulate neuromuscular junctions. This neuropeptide has previously been shown to enhance excitatory junctional potentials (EJPs) elicited by specific neurons, to enhance nerve-evoked contractions, and to induce contractions directly in *Drosophila* 3rd instar larval body wall muscles. We investigated how DPKQDFMRFamide modulates muscle contractions elicited by the excitatory transmitter, L-glutamate, in D. melanogaster 3rd instar larvae. Effects were assessed by co-applying peptide with L-glutamate after removing the central nervous system. DPKQDFMRFamide enhanced glutamate-evoked contractions in a dose-dependent manner, and there was synergy between the effects of L-glutamate and DPKQDFMRFamide on muscle contraction. Although DPKQDFMRFamide does not depolarize muscle cells by itself, it increased membrane depolarization elicited by glutamate, indicating some effect at the plasma membrane. The enhanced depolarization was blocked by nifedipine, suggesting the involvement of an L-type calcium channel. The peptide also enhanced contractions induced by caffeine in the absence of extracellular calcium. Thus, the peptide also appears to act downstream of the cell membrane, possibly by increasing calcium release from the sarcoplasmic reticulum (SR). The effects of DPKQDFMRFamide do not appear to involve the calcium/calmodulin-dependent protein kinase enzyme, CaMKII. These results suggest that this neuropeptide may act at multiple sites within postsynaptic cells and may help shed light on physiological functions of this peptide hormone.

This research was supported by NSERC

DETERMINING THE ROLE OF RYAMIDES IN THE DISEASE VECTOR, *AEDES AEGYPTI*

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Insects can utilize neuropeptides in order to fine-tune their various physiological systems, including tissues and organs involved in excretion, respiration, reproduction, and metabolism. The focus of this study is on members of the RYamide neuropeptide family and their potential role as regulators of the excretory system in the disease vector, *Aedes aegypti*. Thus far, distribution of RYamide-1 (RYa-1) has been determined using immunohistochemistry, with primary antibodies specific to the *A. aegypti* RYa-1 peptide sequence. RYa-1 localization was found in the terminal abdominal ganglia of the nervous system, which innervates the rectum of the adult mosquito. Furthermore, RYa-1 immunoreactivity was observed in fine processes associated with the rectal pads of the hindgut, regions linked to reabsorption. Utilizing a cell-based functional assay, the endogenous RYamide receptor (RYa-R) was deorphanized and shown to have high specificity to the two mosquito RYamide peptides, with little to no activation by structurally-distinct neuropeptides. In addition, real time PCR (RT-qPCR) was used to determine developmental and tissue expression profiles for the RYamide and RYa-R transcripts. Soon, the role of RYamide in regulating ion transport across the hindgut epithelia, particularly over the rectal pads, will be examined in order to determine the physiological relevance of this neuropeptide family in the context of the excretory system. Together, this study hopes to establish the groundwork needed for future work on these understudied neuropeptides.

This research was supported by NSERC and the Ontario Graduate Scholarship program.

CHARACTERIZATION AND INSIGHT INTO THE PHYSIOLOGICAL ROLE OF THE CCHAMIDES IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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As a widely distributed anthropophilic mosquito species, *Aedes aegypti* is able to transmit various pathogens leading to human diseases. Studying the neuroendocrine system of mosquitoes allows us to better understand their physiology. The neuropeptides CCHamide1 (CCHa1) and CCHamide2 (CCHa2) and their associated G protein-coupled receptors (CCHa1R and CCHa2R) were recently identified across insects. In the fruit fly *Drosophila melanogaster*, CCHa2 is a sugar-responsive peptide hormone synthesized primarily in the central nervous system, fat body and the gut that stimulates insulin-producing cells in the brain and regulates growth and feeding-related activities. However, expression profiles and physiological roles of CCHa1, CCHa2 and CCHa2R in other insects, including *A. aegypti*, remains unclear. This research aims to quantify and localize expression of CCHa1, CCHa2 and CCHa2R to help elucidate their physiological function in the yellow fever mosquito. To date, RT-qPCR data demonstrates that the transcript abundance of CCHa1, CCHa2 and CCHa2R changes over development with the highest expression in four-day old male, one-day old male and late-stage pupa, respectively. Differential expression of CCHa2 and CCHa2R transcript was also observed in tissues/organs of adult mosquito indicating the CCHa2 transcript is most highly enriched in the midgut while CCHa2R is expressed across various tissues. Further, CCHa2 was immunolocalized in neurons in the ventral nerve cord and endocrine cells in the posterior midgut adjacent to the midgut-hindgut junction corroborating the transcript expression profile. A heterologous expression system was used to confirm the specificity and sensitivity of the CCHa2 receptor by assessing the activity of diverse peptidergic ligands, which revealed CCHa2 exhibited the strongest response. Future research will aim to deorphanize CCHa1R and employ various bioassays and reverse genetic approaches (i.e. RNAi) to elucidate the physiological roles of CCHa2 in this important human-disease vector.

Acknowledgements: This research was Funding: Supported by an NSERC Discovery Grant (JPP), Ontario Ministry of Research & Innovation Early Researcher Award (JPP)

EXAMINING DIRECT ACTIONS OF A NEUROPEPTIDE ON POSTSYNAPTIC MUSCLE CELLS

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Understanding the mechanisms by which neuropeptides function is key to understanding how cells function on a larger scale, such as through involvement in complex cellular processes, or disruptions to communication which may result in disease forms. The insect neuropeptide, DPKQDFMRFamide, is known to be present in neurosecretory cells of *Drosophila melanogaster* and is thought to act as a neurohormone at neuromuscular junctions of 3rd instar larvae. Knock-down experiments reported previously show that this peptide can act on receptors in presynaptic terminals to increase release of neurotransmitters and on postsynaptic receptors to induce muscle contraction when applied in bathing solution. Recent work shows that DPKQDFMRFamide increases contractions induced in larval muscles by either glutamate or caffeine, and this modulatory effect would presumably reflect a postsynaptic action. The presence of both presynaptic and postsynaptic receptors, however, poses the question of whether the peptide might enlarge these contractions by increasing spontaneous release of either glutamate or perhaps co-transmitters from the nerve terminals. This question might be addressed by determining whether the peptide alters contractions when transmitter is released. We use larvae expressing the temperature-sensitive, dynamin-mutant, shibire, in which neurotransmitter release ceases at elevated temperature. Co-application of glutamate or caffeine with DPKQDFMRFamide at high temperature will allow us to determine whether or not the neuropeptide can enhance contractions while neurotransmitter release is prevented. This approach will provide a better understanding of the mechanisms of action of DPKQDFMRFamide, and it could be used to distinguish presynaptic and postsynaptic actions of other substances.

Supported by NSERC.

ELUCIDATING THE ROLE OF TACHYKININS IN THE MOSQUITO AEDES AEGYPTI USING CRISPR/CAS9

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The mosquito *Ae. aegypti* is one of the most widely distributed disease vectors on the planet, posing important medical concerns owing to its transmission of the dengue and yellow fever viruses. As a result, research aimed at better understanding its biology has received great attention. The aim of this study is to determine the functions of tachykinin-related neuropeptides in the mosquito *Ae. aegypti*, which will contribute towards a more comprehensive understanding of this organism. Specifically, using CRISPR-Cas9 technology, this research will develop a tachykinin knockout to elucidate the role of this neuropeptide family in Ae. aegypti. Performing CRISPR-Cas9 system in *Ae. aegypti* has been routinely successful via embryonic injection but also using an alternate technique recently described called Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control), which utilizes a peptide fragment (P2C) of yolk precursor proteins that are taken up by the ovaries during vitellogenesis in females. To date, our studies have optimized the induction and purification of the recombinant proteins, namely P2C-Cas9 and P2C-EGFP-Cas9, which will be used for site-specific genome engineering. After the P2C-EGFP-Cas9 was injected intrathoracically into vitellogenic females at different times post blood feeding, EGFP was observed throughout developing oocytes. Lastly, several single-guide RNA (sgRNA) targeting the Ae. aegypti tachykinin gene were designed and ongoing experiments will involve injection into the haemolymph of vitellogenic female mosquitoes along with P2C-Cas9 to induce CRISPR-Cas9 site specific editing of the tachykinin gene. Tachykinin neuropeptides have not been studied in *Ae. aegypti* and thus their functions remain unknown; this study will advance knowledge of this neuropeptide family in this important disease-vector mosquito.

ENERGY METABOLISM OF MOUNTAIN PINE BEETLES AND EMERALD ASH BORER DURING DIAPAUSE

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Diapause, an essential strategy to withstand harsh environments, is often coupled with food cessation, metabolic depression, and conservation of energy reserves. And yet, basic maintenance during diapause still requires energy, and post-diapause recovery, growth and reproduction are all energy demanding processes. Different macromolecular energy sources (protein, carbohydrate, lipid) could be used to fuel these processes using different metabolic pathways. Wood boring insects, such as mountain pine beetles (MPB, *Dendroctonus ponderosae*) and Emerald Ash Borer (EAB, *Agrilus planipennis*), enter diapause in the larval or pupal stage, passing the winter in their host tree and reemerging as adults to reproduce in spring. In this process they damage their host trees and attack new ones; causing economic losses in the wood industry. What metabolic pathways help ensure their survival is still unclear, so to better understand these processes, we will assess energy metabolism in MPB and EAB during their diapause. Fourth instar MPB and pupal EAB in diapause will be exposed to step-wise decreasing temperatures that these freeze avoidant species can tolerate (0, -5, -10, and -15°C). To examine the macromolecule usage variation at these temperatures, subsets of beetles will be extracted to measure energy stores. We hypothesize that MPB and EAB both primarily use carbohydrates during diapause and preferentially retain protein and lipid for later use in recovery and metamorphosis, respectively. These data will be used to better understand the mechanisms of overwintering survival in these beetles, and hopefully inform better models of beetle survival in their native and invasive ranges.

This research was supported by Genome-Canada.