

10<sup>th</sup> International Symposium on the  
Environmental Physiology of  
Ectotherms and Plants

Vancouver, British Columbia, Canada

July 14 – 18, 2025



## Contents

Table of contents .....	<b>Error! Bookmark not defined.</b>
Welcome .....	3
Sponsors.....	4
Schedule-at-a-Glance.....	5
Venue Information.....	7
Campus Map .....	10
Excursions .....	11
Intertidal Excursion: .....	11
Whale-watching and Cannery Tour .....	11
Plenary Talks .....	13
Tuesday 15 <sup>th</sup> Morning: Plenary .....	13
Thursday 17 <sup>th</sup> Morning: Plenary.....	13
Friday 18 <sup>th</sup> Morning: Plenary .....	14
Oral Presentations .....	15
Tuesday 15 <sup>th</sup> Morning: Beating the Heat.....	15
Tuesday 15 <sup>th</sup> Early Afternoon: Surviving the Cold .....	19
Tuesday 15 <sup>th</sup> Late Afternoon: Energetics & metabolism .....	23
Thursday 17 <sup>th</sup> Morning: Behavioural and life history responses to stress.....	27
Thursday 17 <sup>th</sup> Early Afternoon: Freeze Tolerance .....	30
Thursday 17 <sup>th</sup> Late Afternoon: Coping with salinity challenges.....	33
Friday 18 <sup>th</sup> Morning: Molecular mechanisms of life history transitions.....	36
Friday 18 <sup>th</sup> Early Afternoon: Seasonal responses to cold .....	40
Friday 18 <sup>th</sup> Late Afternoon: Thermal Plasticity .....	44
Poster Session Thursday 17 <sup>th</sup> Evening: .....	47

## Welcome

On behalf of the ISEPEP10 organizing committee, we are delighted to welcome you to beautiful Vancouver, Canada, for the tenth edition of the International Symposium of the Physiology of Ectotherms and Plants (ISEPEP10). This milestone edition is being held at the University of British Columbia (UBC), nestled between ocean and mountains on Canada's west coast.

We acknowledge that UBC is located on the traditional, ancestral, and unceded territory of the Musqueam people. We are deeply grateful to be able to gather and share knowledge on this land, which has been stewarded by the Musqueam community for countless generations.

ISEPEP remains one of the few dedicated forums for researchers focused on the environmental physiology of ectotherms, often with a strong emphasis on invertebrate ecophysiology. As the challenges posed by environmental change and human activities continue to grow, this field plays a critical role in understanding the physiological responses of organisms to their environments—insights essential for both science and conservation.

This year's four-day conference will feature keynote lectures from world-renowned experts, a rich program of scientific talks and posters, and a chance to reconnect and exchange ideas in person with colleagues from around the world. Our conference will conclude with a truly memorable excursion: a whale watching expedition along the stunning Pacific coastline—an opportunity to connect with nature and reflect on the broader ecosystems our research helps to understand and protect. We are honored to host this international gathering of scientists and students from more than 10 countries.

We extend our sincere thanks to all the volunteers who have made this event possible. We gratefully acknowledge the generous support of our sponsors. Our beautiful logo was designed by Sylvia Heredia.

We hope you enjoy a stimulating symposium and an unforgettable experience in Vancouver.

Warm regards,  
The ISEPEP10 Organizing Committee

Dr. Katie Marshall, Dr. Benjamin Matthews, & Dr. Philip Matthews

## Sponsors

We gratefully thank the financial sponsors of this meeting:

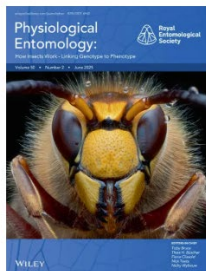


THE UNIVERSITY OF BRITISH COLUMBIA  
**Faculty of Science**



DEPARTMENT OF ZOOLOGY  
UBC

ISEPEP-Program-6.30.pdf  
178 KB • 24 minutes ago



### Physiological Entomology

Edited By: Toby Bruce, Thies H. Büscher, Fiona Clissold, Nick Teets and Nicky Wybouw

Print ISSN: 0307-6962 | Online ISSN: 1365-3032 | Impact Factor: 1.6

## Schedule-at-a-Glance



### 10th International Symposium on the Environmental Physiology of Ectotherms and Plants (ISEPEP 2025)

July 14-18, 2025

#### Program

Monday, July 14, 2025		
13:00 – 15:00	Intertidal Excursion (Optional)	Meeting Point: East Mall and Walter Gage Road
15:00 – 17:00	Registration and Welcome Reception	Brock Commons South Foyer
Tuesday, July 15, 2025		
8:15 – 8:45	Registration and Coffee	Brock Commons South Foyer
8:45 – 9:00	Welcome and Logistics	Brock Commons South, Room 1030
9:00 – 10:00	Plenary 1: Raymond Huey	Brock Commons South, Room 1030
10:00 – 10:30	Coffee Break	Brock Commons South Foyer
10:30 – 11:45	Talks: Nicole Bison Marcella Cross Camille Morgaint Jon Harrison Cecilia Hodson	Brock Commons South, Room 1030
11:45 – 13:30	Lunch	Brock Commons South Foyer
13:30 – 14:45	Talks: Samuel Robichaud Panalee Petcharat Jan Lubawy Heath MacMillan Mahmoud El-Saadi	Brock Commons South, Room 1030
14:45 – 15:15	Coffee Break	Brock Commons South Foyer
15:15 – 16:45	Talks: Fievet Manon Lauren Buckley Simran Bawa Charles Darveau Lea Herpe Alexander Coverley	Brock Commons South, Room 1030
Wednesday, July 16, 2025		
07:00 – 17:00	Marine Life in BC Excursion (Optional)	Shuttle Bus Pick Up: UBC Walter Gage Residence (5959 Student Union Blvd loading area)
Thursday, July 17, 2025		
8:30 – 9:00	Coffee	Brock Commons South Foyer
9:00 – 10:00	Plenary 2: Andrea Durant	Brock Commons South, Room 1030
10:00 – 10:30	Coffee Break	Brock Commons South Foyer
10:30 – 11:30	Talks: Luke Crosby Gang Ma Jacinta Kong Antoine Gekiere	Brock Commons South, Room 1030
11:30 – 13:30	Lunch	Brock Commons South Foyer
13:30 – 14:45	Talks: Brent Sinclair	Brock Commons South, Room 1030

	Stefane Saruhashi Jantina Toxopeus Nick Teets Milos Simovic	
14:45 – 15:15	Coffee Break	Brock Commons South Foyer
15:15 – 16:30	Talks: Alice Dennis Britney Picinic Amber Reinsborough Jonathan Chiang Elissa Khodikian	Brock Commons South, Room 1030
17:00 – 19:15	Poster Session	Brock Commons South Foyer
<b>Friday, July 18, 2025</b>		
8:30 – 9:00	Coffee	Brock Commons South Foyer
9:00 – 10:00	Plenary 3: Sylvain Pincebourde	Brock Commons South, Room 1030
10:00 – 10:30	Coffee Break	Brock Commons South Foyer
10:30 – 11:45	Talks: Caroline Williams Lourenço Martins Tomas Diaz Willem Wildering Farzaneh Ehtemam	Brock Commons South, Room 1030
11:45 – 13:30	Lunch	Brock Commons South Foyer
13:30 – 14:45	Talks: Quentin Willot Ellie McCabe Tomáš Štětina Jona Lopez Pedersen Emily Black	Brock Commons South, Room 1030
14:45 – 15:15	Coffee Break	Brock Commons South Foyer
15:15 – 16:30	Talks: Saskia Delac Sean Michaletz Jackie Lebenzon Greg Ragland Laura Unfried	Brock Commons South, Room 1030
18:00 – 20:15	Banquet	<a href="#">Alumni Centre, Jack Poole Hall</a>

## Venue Information

We look forward to hosting you at the University of British Columbia (UBC) Vancouver campus, which we respectfully acknowledge is situated within the traditional, ancestral and unceded territory of the x<sup>w</sup>məθk<sup>w</sup>əyəm (Musqueam).

The 10th International Symposium on the Environmental Physiology of Ectotherms and Plants (ISEPEP 2025) will take place at the **Brock Commons South Building**, 6180 Walter Gage Road, with accommodations available at the Walter Gage Residence on the UBC campus for those who are traveling to the event. The banquet will be held at **Jack Poole Hall** in the **Alumni Centre**.

### CONFERENCE MAPS

Conference Map: The main conference map attached highlights the locations of conference events.

More complete maps of UBC can be found at <https://www.maps.ubc.ca/> (interactive map)

### REGISTRATION DESK INFORMATION

You may pick up your delegate badge at the ISEPEP Registration Desk during the time outlined below. The registration desk will be located at the Brock Commons South Building.

- July 14: 3 – 5pm
- July 15: 8 – 2pm

### CONFERENCE FOOD & BEVERAGE

There will be refreshment breaks and lunch provided during the conference, as well as a welcome reception on July 14<sup>th</sup> and banquet on July 18<sup>th</sup>. Any allergies mentioned during your registration have been taken into consideration although there may still be a risk of cross contamination. If you have severe allergies, do let us know or carry appropriate medicines.

### ACCESSIBILITY

Brock Commons South Building is wheelchair accessible.

### ACCOMMODATION DETAILS

If you made accommodation arrangements through the conference block, below are the details:

**Walter Gage Residence**  
5959 Student Union Boulevard  
Vancouver BC  
V6T 1K2 CANADA

All rooms are designated non-smoking. No pets allowed (except service animals).

**Check In:** Your room will be ready for check in from 3:00 PM onward.

**Check Out:** Check out time is anytime before 11 AM.

**Hotel Parking at Gage Residence:** Temporary parking is available at time of check-in in front of the Walter Gage Residence at 5959 Student Union Boulevard. A front desk agent will direct you to the parkade. Parking is available at a daily rate of \$18.00 plus tax per day.

## **OTHER UBC PARKING**

For conference attendees staying off-campus, we recommend parking in the lot closest to Brock Commons located at the North Parkade, 6115 Student Union Boulevard, Zone ID 5667 on the HONK mobile parking app. You may view all UBC parking lots online: <https://parking.ubc.ca/map>.

## **VANCOUVER PUBLIC TRANSIT**

The main bus terminal on campus is located on Wesbrook Mall and only a short walk south from the Walter Gage Residence. Buses run frequently to downtown Vancouver with connections to attractions in Vancouver. For further information, schedules and maps please visit [www.translink.ca](http://www.translink.ca).

## **TAXI**

The Vancouver International Airport is 15 km (9.5 miles) from the UBC Campus. Taxi fare out of the airport is charged by zone. UBC is charged at \$46 for a one-way trip. Zone fare information is found at <https://www.yvr.ca/en/passengers/transportation/taxis>.

## **CURRENCY EXCHANGE**

Banks with currency exchange capabilities are located in the University Village Marketplace. Automated Teller Machines dispensing Canadian funds are located in the main lobbies of Walter Gage Residence.

## **INTERNET ACCESS**

A complimentary *ubcvisitor* network is available for use around Campus. If you booked your accommodation at UBC, the hotel rooms are equipped with complimentary high-speed internet access in each suite.

## **FOOD SERVICES**

There are many food venues located in The Nest, the University Village Marketplace, and other various outlets around campus. Please refer to the [Food, Amenities & Attractions at UBC](#) for locations.

## **OTHER SERVICES**

A post-office, pharmacy, mini-mart, travel agent and banks are located in the University Village, within a 10-minute walking distance from the Walter Gage Residence.

## **BUSINESS CENTRE**

There is no designated Business Centre for the conference. However, there is a **Staples** at 2135 Allison Rd. Tel 604-221-4780, which is conveniently located in the University Village.

## **CAMPUS ACTIVITIES**

If you are like us and enjoy fitness and nature, UBC campus offers no shortage of walking paths and trails for running in Pacific Spirit Regional Park that surrounds the campus. Make time to visit the UBC

Botanical Garden and add the Forest Canopy Walk. Finally, stop at the UBC Rose Garden at sunset for an unforgettable view overlooking the water.

For some down time, follow the [UBC campus self-guided walking tour](http://bit.ly/4eGhnAM) (<http://bit.ly/4eGhnAM>).

Finally, UBC has a number of additional campus highlights to explore:

**Beaty Biodiversity Museum**

**UBC Museum of Anthropology**

**Nitobe Japanese Garden**

**UBC Bookstore** - The bookstore sells more than just textbooks! Pay them a visit and shop for much more - including clothes and souvenirs. Use CON15 to receive a 15% discount on clothing & giftware. Please show your ISEPEP badge at check out.

For a full list of attractions, visit our website at <https://hostatubc.com/about-us/campus-amenities/>

**TOURISM LINKS:** [www.ubc.ca](http://www.ubc.ca), [www.tourismvancouver.com](http://www.tourismvancouver.com), [www.travel.bc.ca](http://www.travel.bc.ca)

We sincerely look forward to welcoming you to the 10th International Symposium on the Environmental Physiology of Ectotherms and Plants in a few days!

Kind regards,

ISEPEP Conference Secretariat

**CONTACT INFORMATION:**

Secretariat, 10th International Symposium on the Environmental Physiology of Ectotherms and Plants  
Conferences and Accommodation at UBC

Phone Number: 604-822-1050 (Mon - Fri 9am – 4pm)

Email: [registration@housing.ubc.ca](mailto:registration@housing.ubc.ca)



## Excursions

### Intertidal Excursion:

July 14<sup>th</sup>, 1 – 3pm

Come join us for a free guided excursion to the intertidal zone on UBC's campus. We will meet at the corner of East Mall and Walter Gage Road at 1pm, then walk down Trail 3 through temperate rainforest to Tower Beach. There we will see the very scenic Vancouver's North Shore, historic WW2 artillery towers, and a bevy of invertebrates revealed by low tide (predicted lowest point at 1:50 pm). Graduate students from UBC's Zoology program will guide you to the shoreline, show the critters, and guide you back to the conference opening reception.

#### Notes:

1. Tower Beach is clothing-optional. Please be respectful of the bathers, and be careful that any photographs do not include them.
2. Be prepared to manage steep stairs down to the beach and back up. Unfortunately, there is no other way to access that beach point, but we would be glad to suggest other easier access points in other parts of the city.
3. To access the beach, there will be a short scramble over logs. Be sure to wear sensible footwear.
4. More information about this beach can be accessed here: [https://en.wikipedia.org/wiki/Wreck\\_Beach#Acadia\\_Beach\\_-\\_Tower\\_Beach\\_-\\_Point\\_Grey](https://en.wikipedia.org/wiki/Wreck_Beach#Acadia_Beach_-_Tower_Beach_-_Point_Grey) and here: <https://hr.ubc.ca/sites/default/files/wp-content/blogs.dir/39/files/Tower-Beach-Trail.pdf>

### Whale-watching and Cannery Tour

July 16, 8 – 5pm

Join professional Vancouver Whale Watching guides for a 3 to 5 hour whale watching tour, starting in the picturesque West Coast fishing village of historic Steveston and traveling through the spectacular Gulf Islands and the Strait of Georgia. Their extensive whale spotting network has given them a 98% sighting success rate, with a focus on magnificent orcas (killer whales) and majestic humpback whales, and other marine wildlife such as porpoises, sea lions, seals, and bald eagles.

More details at: <https://vancouverwhalewatch.com/whale-watching-tours/>

After the whale watching, you can either explore Steveston, or join us on a private tour of the Gulf of Georgia Cannery National Historic Site. Built in 1894, the Cannery was once the largest of its kind, and was known as the "Monster Cannery". The exhibit showcases the history of Canada's West Coast fishing industry.

More details at: <https://gulfofgeorgiacannery.org/visit/>

At 4pm, we'll board the shuttle once more and head back to UBC where you can relax for the rest of the evening.

Price includes all tour entrance fees, shuttle to and from UBC, and bagged lunch on the whale watching tour. Limited Capacity. Shuttle Bus Pick Up: UBC Walter Gage Residence (*5959 Student Union Blvd loading area*).

**What to wear:**

Please note that it is up to 10 °C cooler and windy on the water. We recommend dressing warmly and bringing some layers with a wind breaker/jacket, hat, gloves, and closed-toed shoes. It is best to be prepared with sunglasses and sunscreen, and please feel free to bring a camera.

## Plenary Talks

Tuesday 15<sup>th</sup> Morning: Plenary

### **Ups and downs of thermal performance curves**

Huey, Raymond B.\*

Department of Biology, University of Washington, Seattle, WA USA

For well over a century, physiologists have published curves depicting the impact of the body temperature on the relative activities of diverse physiological traits of ectotherms. But in the 1970s, I argued that the resulting "thermal performance curves" -- specifically when motivated by ecological questions -- should be based the thermal dependence of whole-organism traits (e.g., locomotion, digestion, or growth) rather than on lower-level traits (e.g., enzyme activity, twitch-tension of muscles). The latter are more appropriate to analyses of underlying mechanisms. TPCs, when weighted by the distribution of body temperatures experienced in nature, inform patterns of integrated performance or fitness over time or in different habitats. TPCs have since become a basic tool for forecasting impacts of climate change. Nevertheless, the use of TPCs in forecasting is fraught with issues that lead to uncertainty of understanding. These issues include shifts in TPCs with ontogeny and acclimation, non-congruence for various performance traits, sensitivity to interactions of temperature with other factors, and duration of exposure time. Exploring such challenges will require the integration of novel mathematical and empirical approaches.

Thursday 17<sup>th</sup> Morning: Plenary

### **What we know so far about how some dipteran larvae have solved the physiological problems of osmoregulating in seawater**

Durant, A.<sup>1\*</sup>, Khodikian, E.<sup>1</sup>, Prabhakaran, R.<sup>1</sup>

<sup>1</sup>Department of Biology, University of Washington, WA, USA

Insects comprise the majority of animal species on Earth but have generally failed to conquer marine systems. Among flies, less than 5% of species of mosquitoes and midges are associated with the sea and whereby the larval stages are salinity tolerant and can develop in seawater. Similarly to marine teleost fish, larvae drink copious amounts of seawater to achieve water balance and actively excrete ions via the gill-like organs to counteract a significant salt load in the hemolymph. Among mosquitoes, saline tolerance arose independently across multiple genera, but in each instance, the selective pressures of salinity have landed on the excretory organs of these larvae. The recta of saline-tolerant larvae have distinct morphological and physiological departures from freshwater-restricted species. Using marine mosquito and midge representatives from tidal marshlands and supratidal rock pool habitats, this talk will review recent progress (from our lab and others) in our understanding of the cellular and systemic mechanisms that enable larvae to maintain, very narrowly, hemolymph osmotic pressure in the face of wide fluctuations in salinity and temperature. We explore some of the first data from marine midges

indicating a generality across dipteran taxa for the involvement of the rectum in salinity tolerance and we also suggest that a once overlooked organ—the anal gills—are just as critical as the rectum for life in seawater.

## Friday 18<sup>th</sup> Morning: Plenary

### **Will microhabitats turn into heat traps for insects under climate change?**

Pincebourde, S.<sup>1\*</sup>, Leclerc, M.<sup>1</sup>, Gibernau, M.<sup>2</sup>

<sup>1</sup>Insect Biology Research Institute, CNRS—University of Tours, Tours, France. <sup>2</sup>Laboratory Sciences for the Environment, CNRS—University of Corsica, Ajaccio, France

As insects are facing depressing declines worldwide, engaged research is ongoing to inform on the mechanisms at play. Here, instead of listing the multiple treats to insects, I will focus on an important mechanism through which insects may escape the heat: the use of microhabitats that can buffer warming. We spent years to measure microhabitat temperatures and insects' thermal limits with the view that vegetation cover or other aspects produce buffered microclimate to the benefit of insect survival. However, many microhabitats, related to vegetation, do not buffer amplitude of warming and by contrast, they push insects to their own thermal limits even faster. I will exemplify this process by focusing on plant-pollinator interactions. We integrated various ecophysiological traits of a thermogenic plant (temperate *Arum* species) and of its main pollinator (moth-flies in the genus *Psychoda*), acquired at high resolution and combining field and laboratories experiments, to unravel when and how the flower exposes its own pollinator to challenging thermal conditions. The flowering period is rather long in this *Arum* species and we found that the moth-fly is constrained by different ecophysiological traits early (when climate still is cold) versus late (when the air gets warm) in the flowering period. We suggest that the flower can become a heat trap, not necessarily by exposing flies to lethal temperature but by modulating its life history traits such that the fly cannot accomplish the pollination service.

## Oral Presentations

Tuesday 15<sup>th</sup> Morning: Beating the Heat

### **The kinetic basis of photosynthetic heat tolerance**

Bison, N.<sup>1\*‡</sup>, Michaletz, S.<sup>1</sup>

<sup>1</sup>Botany Department, UBC, Vancouver, BC, Canada

*nicole.bison@ubc.ca*

Life on earth operates within thermal limits above which critical functions fail. For terrestrial vegetation, this upper limit is characterized as photosynthetic heat tolerance – the temperature above which photosynthetic machinery is permanently damaged and primary production ceases. However, little is known about the mechanistic drivers of heat tolerance for plants, limiting our ability to predict how vegetation will respond to extreme high temperatures. We propose a new, kinetic framework for quantifying photosynthetic heat tolerance that provides mechanistic insights into the stability of photosynthetic machinery and reflects tolerance to both the magnitude and duration of high temperature. We parameterize this model with photosynthetic heat tolerance data from 188 plant species representing 157 families in a common garden. From this theory, we derive a prediction from first principles for an absolute thermal limit for vegetation, above which the cellular machinery that drives primary production cannot persist. We test this prediction with compiled literature data for 1088 species. In doing so, we test for the first time whether a universal mechanism underlies photosynthetic heat tolerance at a macroecological level, and propose a biophysical explanation for the absolute upper limit of plant heat tolerance.

### **Decoupling of photosynthesis and evapotranspiration in heatwaves: implications for carbon – water trade-offs**

Cross, M.<sup>1\*‡</sup>, Knox S.<sup>2</sup>, Black, T.A.<sup>3</sup>, Jassal, P.<sup>3</sup>, Moore, P.<sup>2</sup>, Nescic, Z.<sup>3</sup>, Michaletz, S.<sup>1</sup>

<sup>1</sup>University of British Columbia, Department of Botany and Biodiversity Research Center. <sup>2</sup>McGill University, Department of Geography. <sup>3</sup>University of British Columbia, Department of Land and Food Systems

*marcella.cross@botany.ubc.ca*

Stomatal optimization models hypothesize that plants regulate stomatal conductance to maximize carbon gain relative to water loss. These models predict that at high temperatures and/or vapor pressure deficits, stomata will close to minimize water loss via transpiration, thereby limiting photosynthesis. However, emerging data and theory suggest that at extreme stomatal conductance and transpiration rates may instead increase despite declining photosynthesis rates. This indicates that stomatal regulation may not always prioritize short-term carbon gain but instead be a mechanism for leaf cooling to avoid heat stress and necrosis. We use Ameriflux eddy covariance data from the 2021 North American heat dome to explore the impacts of extreme heat on carbon and water fluxes in

forested, agricultural, and bog sites. During the heat dome, air temperatures exceeded long-term averages by more than 10 °C, reaching a record high of 49.6 °C. Across all site types, we found strong evidence of decoupling above temperatures of 30-35 °C, where carbon assimilation declined to near-zero while evapotranspiration continued. We also explore variable dependencies and how fluxes vary with environmental and physiological variables. These findings have important implications for land surface models that largely assume tight coupling between photosynthesis and transpiration. Incorporating this mechanism may improve predictions of ecosystem functioning in response to future heatwave events. More broadly, our results challenge conventional assumptions about carbon-water trade-offs by highlighting the critical role of transpiration in preventing heat injury and necrosis at extreme temperatures.

### **How will terrestrial isopods cope with heat and desiccation?**

Morgaint, C.<sup>1\*‡</sup>, Rault, C.<sup>1</sup>, Grève, P.<sup>1</sup>, Marcadé, I.<sup>1</sup>

<sup>1</sup> Université de Poitiers, Laboratoire Écologie et Biologie des Interactions, UMR CNRS 7267 Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France

*camille.morgaint@univ-poitiers.fr*

Climate change is leading to raising average temperatures and decreasing local precipitations, increasing the number and intensity of heatwaves and droughts. Temperature and humidity are crucial for the survival of organisms, especially terrestrial isopods as ectotherms and crustaceans. A key adaptation to terrestrial life in these crustaceans is the diversity of their aerial respiratory system which includes primitive gill-like structure, or pseudotracheae (pleopodal lungs) providing a higher respiratory performance. In this study, heat and desiccation tolerance were investigated in five terrestrial isopod species according to their respiratory system characteristics. We hypothesized that both heat and desiccation tolerance of a species would increase with the number of pseudotracheae (0, 2, or 5 pairs). To assess heat tolerance, individuals were exposed to four constant temperatures ranging between 34-44°C, at saturated humidity, and time to death was recorded for six hours. We used the Thermal Death Time method to analyze the exponential relationship between temperature and the time to heat failure. For desiccation tolerance, individual survival was monitored at 34°C under 20, 50, and 80% relative humidity for six hours. As expected, species with pseudotracheae showed higher heat tolerance. Yet, under low humidity, the 5-pair species was the most sensitive, while 2-pair species were most tolerant, suggesting other factors like cuticle thickness or conglobating behavior also impact desiccation tolerance. In a context of climate change, these findings highlight the role of morpho-anatomical traits in shaping stress tolerance which has led to terrestrial isopod diversity.

### **Negative Effects of Heat Waves on Colony Thermoregulation and Population Dynamics in Honey Bees**

Harrison, J.F.<sup>1\*†</sup>, Chen, J.<sup>1</sup>, Fisher, A.<sup>1</sup>, Ozturk, C.,<sup>1</sup> DeGrandi-Hoffman, G.<sup>2</sup>, Smith, B.<sup>1</sup>, Fewell, J.<sup>1</sup>, Kang, Y.<sup>1</sup>, Maxwell, K.<sup>1</sup>, Overcash, K.<sup>1</sup>, Chahal, K.<sup>1</sup>

<sup>1</sup>School of Life Sciences, Arizona State University, Tempe, AZ, USA

*j.harrison@asu.edu*

Honey bees have well-documented mechanisms to cope with heat exposure that could help them adapt to extreme heat. However, there have been no studies to date that have assessed how natural heat waves affect the capacity of *A. mellifera* colonies to thermoregulate and grow. To test the hypothesis that excessive heat impairs honey bee colony growth by exceeding the thermoregulatory capacity, we studied how variation in summer temperatures affected hive temperature regulation and colony growth during an Arizona desert summer in which maximal shaded air temperatures intermittently exceeded 40°C. We monitored the growth of nine colonies biweekly and thermal conditions at the brood center, brood edge, and outer hive edge. As suggested by prior studies, average temperatures in the brood center and edge were quite stable and within the optimal range of 34-36°C necessary for healthy brood development throughout the summer. However, all hive locations exhibited cyclic, diurnal thermal fluctuations, and brood experienced considerable portions of each day (14% for the brood center, 33% for the brood edge) above and below the optimal range. Higher maximal air temperatures and greater temperature fluctuations within the hive were negatively associated with colony population growth. These findings indicate that excessive with maximal temperatures over 40°C can reduce colony populations, likely at least partially by impairing the thermoregulation of brood or by exposing adults to temperatures that shorten lifespans. If excessive heat periods occur more frequently as predicted due to climate change, this could limit regions where honey bee colonies can successfully survive the summer.

### **Crab-solutely Resilient: Unraveling the Genomic Basis of Heat and pH Tolerance of Two Porcelain Crabs**

Hodson, C.<sup>1\*‡</sup>, Stillmann, J.<sup>1</sup>

<sup>1</sup>Department of Biology, San Francisco State University

*chodson@sfsu.edu*

Climate change poses a growing threat to marine life, underscoring the need to understand genomic mechanisms behind key adaptations, such as thermal and pH tolerance. The recently published genomes of two porcelain crab species, *Petrolisthes cinctipes* and *Petrolisthes manimaculis*, offer a unique opportunity to explore how genome structure influences physiological responses to environmental stress. These crabs exhibit distinct physiological responses to thermal and pH stress, likely due to adaptations to their respective habitats: *P. cinctipes* inhabits the upper intertidal zone and is exposed to regular intervals of emersion and thermal variability, while *P. manimaculis* is restricted to the subtidal zone, experiencing more stable conditions.

My research leverages previous RNA-seq data and the newly available genomes to investigate evolutionary shifts linking genome structure to physiological adaptations. I will identify differentially expressed genes, compare them between species, and functionally annotate the targeted genes to analyze important gene families and functions to the physiologic responses to environmental stressors. This approach aims to uncover the genomic basis of heat and pH tolerance in a diverse group of

crustaceans, improving our understanding of how genomes drive physiological adaptations and providing critical insights into how marine organisms may respond to changing ocean conditions.

**Cold-case: proteostasis in seasonal phenotypes of *Drosophila suzukii***

Robichaud, S.<sup>1\*‡</sup>, Colinet, H.<sup>2</sup>, Lamarre, S.<sup>3</sup>, Pichaud, N.<sup>1</sup>

<sup>1</sup>Département de chimie et biochimie, UdeM, Moncton, NB. <sup>2</sup>CNRS ECOBIO, Université de Rennes, France. <sup>3</sup>Département de biologie, UdeM, Moncton, NB

*esr4633@umoncton.ca*

*Drosophila suzukii* originally a tropical species and now a threat to agricultural productivity, has expanded its range to temperate regions worldwide. This distribution is associated with its ability to survive cold climates, demonstrating clear phenotypic plasticity between summer and winter flies. While the transcriptomic, lipidomic and metabolic modifications have been studied, adjustments of protein metabolism and proteostasis in this species remains unknown. Protein synthesis, degradation and maintenance are key, energetically costly processes required for acclimation, and may play a role in this phenotypic switch. This project aims to explore the impact of cold acclimation on the protein metabolism of *D. suzukii*. For this purpose, we raised two cohorts of *D. suzukii* one at 25°C (summer phenotype), and the other at 10°C (winter phenotype). Winter flies showed an increase in polyubiquitinated proteins targeted to proteasomal degradation, along with a decrease in proteasome 20S activity. The increase in polyubiquitinated proteins does not appear to be caused by an increased oxidative stress during winter acclimation since we observed no differences in in-vivo mitochondrial ROS production, SOD2 and CAT activity, or protein carbonyl content. The rate of protein synthesis was decreased in winter flies, indicating a potentially limited energy supply or production. The adenylate energy charge, an indicator of cellular energy homeostasis, was decreased in winter flies, supporting the possibility of a mismatch between ATP production and demand. In summary, winter acclimation seems to induce an energy shortage, which is compensated by inhibition of energy consuming processes.

**Diapause and cold acclimation stimulate remodeling of phospholipid composition in membranes of subarctic *Drosophila lummei***

Petcharat, P.<sup>1,2\*‡</sup>, Rozsypal, J.<sup>1</sup>, Berkova, P.<sup>1</sup>, Kostal, V.<sup>1</sup>

<sup>1</sup>Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic. <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

*panalee.zoology@gmail.com*

Most ectotherms have been shown to alter the lipid composition of biological membranes during cold acclimation to prevent damage to the phospholipid bilayer at low environmental temperatures. Cold-induced phospholipid remodeling has also been studied in tropical *D. melanogaster* and subtropical *D. suzukii*, where relatively moderate changes were observed. Here, we investigated phospholipidomic changes in whole body (WB) and indirect flight muscle (DLM) tissues of the subarctic *Drosophila lummei* to gain insight into how this fly maintains membrane integrity and function during the long and severe boreal winter. We observed relatively deep changes in the molar proportions of different fatty acids

(with four major species accounting for >90%). The responses in WB and DLM were very similar and consisted of three sequential steps: (i) photoperiodic entry into diapause was associated with an increase in the relative proportion of palmitoleic acid (C16:1) at the expense of palmitic acid (C16:0), early steps of cold acclimation caused an increase in the proportion of oleic acid (C18:1), while later steps caused an increase in the proportion of linoleic acid (C18:2). Overall, cold acclimation resulted in an almost twofold increase in the ratio of unsaturated to saturated fatty acids, which was countered by a significant increase in the average fatty acyl chain length. We discuss how these changes may contribute to bilayer order/fluidity, functionality of embedded proteins, and vital maintenance of membrane potential.

### **Neuropeptide-mediated cold stress response in *Tenebrio molitor*: Insights from neuroendocrine regulation**

Natalia Konopińska<sup>1</sup>, Ewelina Paluch-Lubawa<sup>2</sup>, Arkadiusz Urbański<sup>1</sup>, Jan Lubawy<sup>1\*</sup>

<sup>1</sup>Department of Animal Physiology and Developmental Biology, Adam Mickiewicz University, Poznań, Poland. <sup>2</sup>Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6 Str., 61-614 Poznań, Poland

*j.lubawy@amu.edu.pl*

Through neuroendocrine integration, the nervous and endocrine systems coordinate to regulate physiological processes and maintain homeostasis, both under normal and stressful conditions such as cold exposure. Cold stress significantly impacts insect physiology. Thus neuropeptides—key mediators of the neuroendocrine system— which regulate physiological and behavioral responses, may therefore influence thermal tolerance.

We aimed to investigate the role of the insect neuroendocrine system and its peptides in response to cold stress. Transcriptomic analyses of isolated brains and ventral nerve cords of *Tenebrio molitor* identified neuropeptides whose expression levels change during cold stress exposure (-5°C for 1 hour). We then examined the effects of two selected peptides—Tenmo-MIP5 (allatostatin) and Tenmo-TRP7 (tachykinin)—through injection and inhibition experiments on various physiological parameters. Injection of Tenmo-MIP5 led to an increase in chill coma recovery time (CCRT), activation of the immune system, and higher post-cold stress mortality. Similarly, Tenmo-TRP7 injection increased CCRT. However, silencing TRP receptor significantly reduced CCRT, raised the supercooling point, and decreased survival following cold stress.

Our findings demonstrate that neuropeptides play significant roles in modulating cold stress responses in *T. molitor*, affecting both thermal tolerance and survival. Targeting specific neuropeptides may offer novel strategies for enhancing cold tolerance in insects, with potential applications in pest management and insect physiology research.

This work was financed by grant no 2019/35/D/NZ4/02731 from National Science Center, Poland

### **Thermal profiling of novel and known miRNAs during cold acclimation in the mosquito, *Aedes aegypti***

De Nicola, E.H.M.<sup>1</sup>, Biggar, K.K.<sup>2</sup>, Hahn, D.A.<sup>3</sup>, MacMillan H.A.<sup>1,2\*</sup>

<sup>1</sup>Department of Biology, Carleton University, Ottawa, Canada, <sup>2</sup>Institute of Biochemistry, Carleton University, Ottawa, Canada, <sup>3</sup>Department of Entomology and Nematology, University of Florida, Gainesville, USA

*heathmacmillan@cunet.carleton.ca*

Many insects can better survive low temperature exposure following cold acclimation, and such plasticity may be contributing to recent range expansion in *Aedes aegypti*. MicroRNAs (miRNAs) are known to regulate thermal plasticity in other animals. We therefore sought to identify miRNAs involved in thermal plasticity in this species and reveal physiological systems impacted by these miRNAs. We used a bioinformatic approach to identify three novel miRNAs in *Ae. aegypti*. After acclimating adult female *Ae. aegypti* to cold (15°C) and warm (25°C) conditions, we performed differential expression analysis on these novel miRNAs and four other miRNAs downregulated during diapause in *Ae. albopictus*. Two novel miRNAs were upregulated in cold-acclimated individuals, while two described miRNAs associated with lipid metabolism and reproduction were downregulated in the cold-acclimated group. Temperature alone can alter miRNA target genes, and the thermodynamic parameters used to predict miRNA targets are typically established assuming body temperatures relevant to endotherms, rather than those applying to poikilothermic ectotherms. Here, we instead tested for cold-influenced targets of miRNAs of interest, predicted to only become thermodynamically stable at 15°C. This approach provides insight into the putative roles that these novel miRNAs could play in seasonal phenotypes. Many predicted targets logically relate to insect low temperature survival, like maintenance of ion homeostasis and membrane stability, while others suggest roles for understudied processes. Our results suggest that miRNAs play a regulatory role in mosquito cold acclimation that deserves further attention."

### **Exploring the phenomenon of latent chilling injuries in a chill-susceptible insect**

El-Saadi, M.I.<sup>1\*‡</sup>, MacMillan, H.A.<sup>1</sup>

<sup>1</sup>Department of Biology, Carleton University, Ottawa, ON, Canada

*mahmoudelsaadi@cmail.carleton.ca*

Chill-susceptible insects suffer negative effects of chilling at low temperatures above the freezing point of their body fluids. At the organismal level, chilling injuries commonly manifest as a loss of neuromuscular function that affects an insect's ability to stand, walk, or fly. These injury phenotypes can be evident quickly, or with some delay, following removal of the insect from the cold. We have characterized how and when "latent" chilling injuries appear in *Drosophila melanogaster* in the hours and days following a cold stress, but their underlying mechanisms remain unclear. Here, we show that latent injuries are female-specific, temperature-dependent (significantly slowed injury progression at lower rearing temperatures), and mitigated by prior cold acclimation. The latent injuries that manifest in female flies are observed after either acute (-2°C for 3 h) or chronic (0°C for 15 h) chilling, and the same mitigation is observed following cold acclimation in either cold condition. We further show that supplementing female flies with an antioxidant (ascorbic acid) significantly mitigated latent chilling injuries, while flies that lack a functional Toll immune pathway suffer from additional latent injury.

Together, these results suggest that subsequent injury following a cold stress involves an interplay between oxidative stress and immune activation.

**Echoes of the Wild: divergent responses to a neonicotinoid in wild and commercial bumblebees**

Manon FIEVET<sup>1,2\*</sup>, Denis MICHEZ<sup>2</sup>, Kevin TOUGERON<sup>1</sup>

<sup>1</sup>Ecology of Global Change and Interactions, University of Mons, 20 Place du Parc, 7000, Belgium.

<sup>2</sup>Laboratory of Zoology, University of Mons, 20 Place du Parc, 7000, Belgium

*Manon.FIEVET2@umons.ac.be*

Bees (Hymenoptera: Anthophila) provide essential ecosystem services by significantly contributing to agricultural productivity, biodiversity maintenance, and human health. Nevertheless, they face multiple threats contributing to their decline, including the massive use of pesticides. Historically, the European honey bee, *Apis mellifera*, has been widely used as a model species in ecotoxicological studies. However, in recent years, the bumblebee *Bombus terrestris* has emerged as a complementary model, notably due to the availability of commercially reared colonies. As a result, many studies rely on these commercial bees, while colonies initiated from wild-caught queens remain unstudied — potentially overlooking intraspecific variation in pesticide sensitivity. This research aims to characterize the sensitivity of wild vs. commercial *B. terrestris* to acute oral pesticide exposure of the neonicotinoid Acetamiprid. To this end, we compared the lethal response of wild and commercial bees by measuring their oral LD50s. Sublethal effects were subsequently assessed on both strains by measuring metabolic activities, flight behavior and cognition, using an environmental dose and the LD10 of wild *B. terrestris*. First results show that wild bumblebees exhibit a greater sensitivity than their commercial counterparts.

**Climate change induced selection on and evolution of insect thermal sensitivity**

Hatcher, T.M.<sup>1</sup>, Shlichta, J.G.<sup>2</sup>, Kingsolver, J.G.<sup>3</sup>, and Buckley, L.B.<sup>1\*</sup>

<sup>1</sup>Department of Biology, University of Washington, Seattle, WA, USA. <sup>2</sup>Department of Biology, Edmonds College, Edmonds, WA, USA. <sup>3</sup>Department of Biology, University of North Carolina, Chapel Hill, NC, USA

*lbuckley@uw.edu*

How have recent changes in temperature means and variability altered selection on and evolution of the thermal sensitivity of insect growth? We address this question for cabbage white butterflies, *Pieris rapae*, by repeating a 1999 study examining how the temperature sensitivity of caterpillar short-term growth rate determined survival, development time, and final body size in an experimental garden. We detected evolution of thermal performance curves over 25 years such that caterpillars now grow faster at intermediate (23°C) temperatures at the cost of growing more slowly at hot temperatures (35°C). The shift corresponds to directional selection documented in 1999 to grow faster at temperatures up to 23°C but slower at warmer temperatures. This evolution of thermal sensitivity coupled with accelerating warming has resulted in shifts in selection over 25 years: selection remains similar at lower temperatures, but has reversed to favor increased growth at 35°C. This reversal is consistent with hot temperatures increasing in incidence since the initial studies. Our study demonstrates the potential for rapid evolution of thermal sensitivity in response to climate change in an agricultural pest. However,

selection to initially capitalize on warming temperatures can ultimately decrease fitness as warming temperatures move into a stressful range, driving reversals in the direction of selection.

### **Variation in energetics along an elevational gradient in a grasshopper community**

Bawa, S.J.<sup>1\*‡</sup>, Sheffer, M.M.<sup>1</sup>, Buckley, L.<sup>2</sup>, Schoville, S.<sup>3</sup>, Nufio.C.<sup>4</sup>, Williams, C.<sup>1</sup>

<sup>1</sup>Integrative Biology, UCB, Berkeley, California, USA. <sup>2</sup>Department of Biology, UW, Seattle, Washington, USA. <sup>3</sup>Entomology Department, UWM, Madison, Wisconsin, USA. <sup>4</sup>HHMI, DC, USA

*bawa@berkeley.edu*

Anthropogenic climate change is raising average temperatures and increasing the frequency of extreme events. These fluctuations pose significant threats to locally adapted populations, particularly thermally specialized ectotherms, by increasing mortality risks and raising energy costs. Predicting the effects of climate change therefore requires understanding how changes in both mean temperatures and extremes influence organismal energetics across environmental gradients. Since many ectotherms are primary herbivores in food webs, understanding their physiological constraints is essential to predicting species persistence in changing environments. This research investigates how energetic trade-offs shape organismal performance and broader demographic processes by studying both a thermal specialist (*Melanoplus boulderensis*) and thermal generalist (*Melanoplus sanguinipes*) grasshopper species. Grasshoppers, as dominant herbivores in grassland ecosystems, are central to ecosystem stability making their physiological responses to environmental change ecologically consequential. We assessed metabolic rates and lipid content of grasshoppers reciprocally transplanted along an elevation gradient and wild counterparts. *M. boulderensis* grasshoppers from higher elevations showed elevated metabolic rates in both transplanted and wild-caught individuals. Comparing *M. boulderensis* to *M. sanguinipes* across an elevational gradient will help clarify how environmental constraints influence energy allocation and resilience.

### **Bumblebee queens' overwintering metabolism prepares them to face flooding challenges**

Darveau, C.-A.\*<sup>1</sup>, Rondot, A.<sup>1</sup>, Rojas, S.<sup>1</sup>, and Rondeau, S.<sup>1</sup>

<sup>1</sup>Department of Biology, University of Ottawa, Ottawa, Canada

*cdarveau@uottawa.ca*

Overwintering bumblebee queens enter a state of diapause, remaining buried underground until spring. The common eastern bumblebee queen was recently shown to survive underwater for up to a week. Queens exhibit a profound metabolic depression, accompanied by minimal metabolic reorganization in tissues such as the flight muscle. Their diapause metabolic rate indicates that queens cannot survive with onboard oxygen stores. We therefore tested the hypothesis that submerged queens undergo aquatic respiration and/or switch to anaerobic metabolism to survive flooding events. We measured the resting metabolic rates of queens in lab-induced diapause for five to six months and continuously monitored them while simulating a flooding event. A large burst of CO<sub>2</sub> was measured during submergence, followed by a decrease in metabolic rate compared with the resting rate in air. We kept

the queens underwater for eight days and measured their CO<sub>2</sub> production rate on days four and eight of submersion. Even when submerged and immobile, the queens exhibited increased CO<sub>2</sub> production when disturbed. Metabolic rates obtained from carbon dioxide measurements at the air interface were validated by monitoring the decline in water oxygen levels. Submerged bees were also shown to accumulate lactate as an anaerobic end-product. The recovery from submergence exhibits an immediate and large increase in metabolic rate, followed by a gradual decline to pre-submergence levels over a week, accompanied by changes in breathing patterns. This study reveals the remarkable ability of bumblebee queens to survive and recover from prolonged flooding, partly by breathing underwater, assisted by anaerobic metabolism.

### **A not so 'alternative' mitochondrial complex: The mitochondrial G3PDH**

Herpe L.<sup>1,2\*</sup>‡, Aminot M.<sup>1,2</sup>, Pichaud N.<sup>1,2</sup>

<sup>1</sup>New Brunswick Centre of Precision Medicine, Moncton, NB, Canada. <sup>2</sup>Department of Chemistry and Biochemistry, Université de Moncton, Moncton, NB, Canada

*elh0926@umoncton.ca*

Recent studies have shown that *Drosophila melanogaster* exposed to high-fat or high-sugar diets display mitochondrial inflexibility characterized by an impairment of the complex I of the electron transport system (ETS). This results in decreased oxygen consumption, reduced ATP levels, and increased reactive oxygen species (ROS) production. Interestingly, this dysfunction appears to be partially compensated by the mitochondrial glycerol-3-phosphate dehydrogenase (mtG3PDH), an understudied mitochondrial complex often described as 'alternative'. This study aimed to determine the role of mtG3PDH in mitochondrial flexibility during nutritional stress. To do so, male and female *Drosophila melanogaster* knock-down lines for mtG3PDH (Gpo1) were used and mitochondrial respiration, ATP synthesis, and ROS production were assessed on mitochondria isolated from thoraxes after five days on high-fat (HFD) or high-sugar (HSD) diets. Surprisingly, a higher mortality in the Gpo1 groups was observed, as well as a 'lethargic' behavior, compared to the control group, even on the standard diet. This phenotype was mainly due to a ~60% decrease in ATP production rather than elevated ROS. In fact, Gpo1 flies produced ~70% less ROS than controls, likely due to the reduced direct and reverse electron transfer-related ROS production from mtG3PDH. Moreover, sex differences were mainly detected for the mitochondrial efficiency (ATP/O) with male showing reduced mitochondrial efficiency compared to controls. These results support a key role for mtG3PDH in metabolic flexibility and suggest sex-specific differences in its contribution. Analysis on HFD and HSD are currently ongoing and will be presented."

### **Does warming winters and acclimation to thermal variability impact overwintering energetic drain and southern range margins?**

Coverley, A.<sup>1\*</sup>‡, Roe, A.<sup>2</sup> Marshall, K.<sup>1</sup>

<sup>1</sup>Zoology Department, UBC, Vancouver, BC, Canada. <sup>2</sup>NRCAN, Canada

*coverley@zoology.ubc.ca*

The spruce budworm (*Choristoneura fumiferana*) is a defoliating Lepidopteran pest found across North America. It overwinters for 7+ months as a 2nd instar larva without feeding, therefore energetic drain through the winter may be an important component of population persistence. Most studies of species range focus on the effects of mean temperatures, yet temperature varies in the wild. How these thermal fluctuations impact energy drain and subsequent effects on populations is poorly understood.

We tested whether acclimation to thermal variability impacts overwintering energy use in spruce budworm in a laboratory experiment, and coupled that to a biophysical model to determine whether the rate of energy depletion and balance differs across latitudes and among populations.

We predicted that at the southern range margin a) acclimation reduces energy expenditure and increase survival relative to constant temperatures and b) that overwintering energetic drain can explain reduced survival relative to more northerly populations

We acclimated overwintering L2 larvae to constant 2°C, or daily cycles of 2±8°C or 2±12°C. Through the winter, we repeatedly measured thermal sensitivity of metabolic rate and 24 hour metabolic rate, and energetic reserve assays.

Preliminary analysis shows a slight reduction in thermal sensitivity of metabolic rate in spruce budworm acclimated to thermal variability and metabolic rate increases exponentially above 6°C; suggesting warmer winters increase energetic drain over specific thresholds. Taken together, our study incorporates a novel application of biophysical modelling to predict overwintering energy drain and how warming winters reduce fitness.

### **Cool Under Pressure: Can Microclimates Help Butterflies Beat the Heat?**

Crosby, L.<sup>1\*</sup>‡ and Iossa, G.<sup>2</sup>, School of Natural Sciences, University of Lincoln, UK

<sup>1</sup>School of Natural Sciences, University of Lincoln, UK.

*19698651@students.lincoln.ac.uk*

Extreme temperatures, such as heatwaves, may damage individual fertility more than average increases. Lab studies show heightened thermal sensitivity of male gametes than female gametes in a number of taxa including Lepidoptera, especially during gonad development and gametogenesis at the pupal life-stage. How exposure to these events can impact populations and their long-term persistence in natural environments is unclear. The climate that insects experience – the microclimate – could buffer some of this exposure and damage. In this study, we investigate the buffering ability of microclimates on insect fertility in nature. By simulating the effects of heatwaves on the large white butterfly *Pieris brassicae* in outdoor enclosures during the peak window of thermal sensitivity of fertility, we explore the role of fine-scale microclimate - i.e., temperature at the level of the plant - in buffering from heatwaves. We report on the impact of elevated temperatures to gamete quality, reproductive output, fertilisation success, and adult body size and survival.

### **Fine-scale behavioral thermoregulation of small insects under climate warming: individual responses, demographic rates and cascading consequences**

Gang Ma<sup>\*1</sup>, Xue Bai<sup>1</sup>, Xing Nie<sup>1</sup>, Chun-Sen Ma<sup>1</sup>

<sup>1</sup>Climate Change Biology Research Group, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

*magang@caas.cn*

Climate warming has led to substantial increases in intensity, frequency and duration of extreme high temperatures, challenging organismal persistence across the globe. Behavior stands the first line for animals to buffer the impacts of thermal extremes. Many ectotherms, including insects, can use behavioral thermoregulation to avoid overheating via exploiting diverse microclimates within their habitats. However, small insects are usually confined into limited space to test their thermal performances, overlooking the key role of behavioral thermoregulation in buffering against extreme temperatures. Aphids are sap-sucking herbivorous insects with body sizes less than 5 mm. Thermoregulatory behaviors and the ecological consequences of such small insects have rarely been studied. In field conditions, the dense crop canopy and farmland irrigation provide the grain aphid *Sitobion avenae* with a thermal gradient across the plant canopy. Our laboratory simulated studies showed that, when encountering high ambient temperatures, the aphids could behaviorally avoid heat stress by transferring onto soil surface from the host plants. This led to changes in their redistribution within and across the host plants. Importantly, fine-scale behavioral thermoregulation within heterogenous microclimates facilitated their demographic rates and population growth under heat

waves and thus supported the persistence of biological control functions of predatory insects. These findings highlight the importance of behavioral thermoregulation even in small insects in predicting the effects of climate warming on population dynamics and trophic cascades.

### **Temperature-dependence of life history in an edible cricket: Implications for optimising mass rearing**

Kong, J.D.<sup>1\*</sup>, Vadboncoeur, É.<sup>1</sup>, Bertram, S.M.<sup>1</sup>, MacMillan, H.A.<sup>1</sup>

<sup>1</sup>Department of Biology, Carleton University, Ottawa, ON, Canada

*jacintakong@cunet.carleton.ca*

Optimization of life history and organismal performance underlies success in insect mass-rearing. Rearing schedules, resource use and production yield depend on many aspects of insect fitness and performance within and across generations, such as growth, development, longevity, and fecundity, which are all temperature dependent. Despite this general understanding, we often lack species-specific information needed to make informed decisions about manipulating rearing temperatures to optimise insect growth and development. Here, we characterise the effects of rearing temperature on nymph to adult development and lifespan (20 – 38°C), and reproductive output (30 – 38°C) in a farmed cricket (*Gryllobates sigillatus*). Crickets grew larger and reached adulthood sooner at higher developmental temperatures at the expense of longevity. Reproductive output was similar across a range of temperatures but decreased at 38°C. Therefore, while temperature control is necessary to maximize production rates, temperature is unlikely to affect production yield in a fixed harvest cycle provided it is maintained within the narrow range enabling both fast growth and stable reproduction (32 – 36°C). Our study provides a fundamental basis for further optimisation of insect rearing operations and a deeper understanding of the thermal biology of this commonly farmed species.

### **Brood safety first: bumble bees prioritise metal avoidance over sugar intake only in the presence of larvae**

Gekière, A.<sup>1\*</sup>, Snowden, T.<sup>2</sup>, Masson, T.<sup>2</sup>, Brown M.J.F.<sup>3</sup>, Tougeron, K.<sup>2</sup>

<sup>1</sup>Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium. <sup>2</sup>Ecology of Interactions and Global Change, Research Institute in Biosciences, University of Mons, Mons, Belgium.

<sup>3</sup>Department of Biological Sciences, Centre for Ecology, Evolution, and Behaviour, School of Life Sciences and the Environment, Royal Holloway University of London, Egham, Surrey, TW20 0EX, United Kingdom

*antoine.gekiere@umons.ac.be*

Metal pollution poses a growing threat to wildlife, including bees, which play a crucial role in pollination. While the toxic effects of metals on bees are well documented, their ability to avoid contaminated food sources, and whether this behaviour is shaped by brood presence, remains unclear. Besides, studies often investigated such avoidance abilities through no-choice assays, which do not mirror field conditions. Using the buff-tailed bumble bee and two metals, copper (i.e., essential metal) and cadmium (i.e., non-essential metal), we assessed workers' avoidance of metal-laced 50% sucrose solutions when given a choice with an uncontaminated alternative. We also introduced a trade-off by reducing sucrose

concentration to 20% in the untreated solution. To test the influence of parental care, workers were either provided with larval siblings or kept without brood. When both treated and untreated solutions contained 50% sucrose, workers consistently avoided the metal-contaminated solution, regardless of brood presence. However, when sucrose concentration was reduced in the uncontaminated solution, workers preferred the contaminated option, but only in the absence of brood. In the presence of brood, workers favoured the metal-free but sucrose-poor solution, suggesting adaptive provisioning to protect larvae. This study provides the first evidence that bumble bee workers not only avoid metal-contaminated solutions but also adjust foraging strategies based on social context.

## Thursday 17<sup>th</sup> Early Afternoon: Freeze Tolerance

### **When, how, and why did ice get nice?**

Sinclair, B.J.

Department of Entomology, Cornell University, Ithaca, NY, USA

*bjs299@cornell.edu*

Eastern North America as a natural laboratory for studying insect cold tolerance We have known for more than 200 years that some insects can survive internal ice formation. We've identified plenty of biochemicals and processes that are correlated with freeze tolerance, and we can speculate about the pressures leading to the evolution of freeze tolerance, but we're a long way from unravelling the causative mechanisms and their history. I will summarize the background of speculation about how freeze tolerance evolved, and then introduce well-studied freeze-tolerant species in North America (the goldenrod gall fly *Eurosta solidaginis* and the spring field cricket *Gryllus veletis*) that I think might provide opportunities to explore the relationships environment, evolution, and cold tolerance strategy, and facilitate identifying factors that are necessary and sufficient to become freeze tolerant.

### **When ice is nice: osmotic balance and mitochondrial protection in freeze tolerant cricket**

Saruhashi, S.<sup>1\*‡</sup>, Coulson, S.Z.<sup>2</sup>, Staples, J.F.<sup>3</sup>, Martin Moos<sup>4</sup>, Sinclair, B.J.<sup>1</sup>

<sup>1</sup>Entomology Department, Cornell University, NY, USA. <sup>2</sup>College of Health Sciences, University of Memphis, TN, USA. <sup>3</sup>Department of Biology, Western University, ON, Canada. <sup>4</sup>Institute of Entomology, České Budějovice, Czech Republic

*ss4346@cornell.edu*

The mechanisms underlying insect freeze-tolerance remain unclear. We investigated how Malpighian tubules support freeze tolerance in *Gryllus veletis*, which transition from freeze-intolerant (FI) to freeze-tolerant (FT) under laboratory conditions. Since Malpighian tubules regulate ion and water balance, we first characterised how freezing and thawing affects ion and water balance. Freezing disrupted ion-osmotic balance in FI crickets, but FT crickets maintained their homeostasis. The Malpighian tubules of FI crickets lost pumping activity after being frozen, while Malpighian tubules remained functional in FT, but with lower activity. Because maintaining ion balance is energetically expensive, we then tested whether FT insects protect their mitochondria from freezing damage or repair freeze damage post-thaw. Using high-resolution respirometry, transmission electron microscopy, and lipidomics, we assessed mitochondrial function and morphology before and after chilling and freezing. FT mitochondria were rounder and larger than FI mitochondria but had lower cristae density. Despite these structural differences, mitochondrial function remained unchanged in FT crickets. Freezing also damaged FI mitochondria, reducing function and cristae integrity, whereas FT maintained their function and morphology, supporting the protection hypothesis. There was no evidence of damage repair in either FI or FT mitochondria, contradicting the recovery hypothesis. Cold exposure alone altered FT but not FI

mitochondrial shape, suggesting FT crickets relax their mitochondrial structure at low temperatures. Here we highlight the role of mitochondrial plasticity as a key adaptation to extreme cold.

### **Supervillin: the hero of insect freeze tolerance?**

Toxopeus, J.<sup>1\*</sup>, Joshi, A., Vestby, B.<sup>1</sup>, Nobbe, A.<sup>1</sup>

<sup>1</sup>Biology Department, St. Francis Xavier University, Antigonish, NS, Canada

*jtoxopeu@stfx.ca*

Some insect species are freeze tolerant, including the spring field cricket *Gryllus veletis*. However, the physiological mechanisms that support freeze tolerance are still under active investigation. The spring field cricket is a good model organism for studying freeze tolerance mechanisms as it is easy to rear and manipulate in laboratory settings. Previous work on this species has shown that freeze-tolerant spring field crickets increase the abundance of F-actin (cytoskeletal microfilaments) in their fat body tissue, and have the ability to maintain their F-actin structure following a survivable freeze treatment. Other previous work has shown that freeze-tolerant crickets have a relatively high mRNA abundance of the actin-binding protein Supervillin (yes, that is how it's spelled!) in their fat body tissue. The current study tested the hypothesis that the Supervillin facilitates the F-actin reorganization seen in freeze-tolerant crickets. First, we confirmed that Supervillin protein accumulated in freeze-tolerant crickets relative to freeze-intolerant crickets. Then we used RNA interference (RNAi) to knock down Supervillin mRNA, but our protocol was not successful at reducing the abundance of Supervillin protein or F-actin. Future work will further optimize the RNAi protocol to test our hypothesis, and determine whether Supervillin-driven F-actin reorganization is important for freeze tolerance.

### **Integrating multiple abiotic stressors through cross tolerance in an Antarctic insect**

Lima, C.L.<sup>1,2</sup>, Kawarasaki, Y.<sup>3</sup>, Gantz, J.D.<sup>4</sup>, Colinet, H.<sup>5</sup>, Michel, A.P.<sup>6</sup>, Convey, P.<sup>7</sup>, Hayward, S.A.L.<sup>8</sup>, Teets, N.M.<sup>1\*</sup>

<sup>1</sup>Department of Entomology, University of Kentucky, Lexington, KY; <sup>2</sup> Department of Biology, University of Kentucky, Lexington, KY; <sup>3</sup>Department of Biology, Gustavus Adolphus College, St. Peter, MN;

<sup>4</sup>Department of Biology and Health Sciences, Hendrix College, Conway, AR; <sup>5</sup> Ecobio, Universite de Rennes, Rennes, France; <sup>6</sup>Department of Entomology, The Ohio State University, Wooster, OH; <sup>7</sup>British Antarctic Survey, Cambridge, UK; School of Biosciences, University of Birmingham, Birmingham, UK

*n.teets@uky.edu*

While Antarctica is notorious for being cold, terrestrial organisms residing there experience a variety of abiotic stressors, including cold, desiccating conditions, high UV, and inundation with meltwater and seawater. The Antarctic midge, *Belgica antarctica*, can enhance its freeze-tolerance through cross-tolerance triggered by mild exposure to unrelated stressors, but whether this cross-tolerance extends to other stressors is unclear. In this study, we tested the hypothesis that mild exposure to abiotic stress elicits a generalized protective response that enhances tolerance to unrelated stressors. Larvae were exposed to a variety of mild pretreatments (heat, cold, dehydration, hyperosmotic conditions, and

hypoosmotic conditions) for 7 d, followed by a 24 h exposure to a harsh stressor that was normally lethal. While exposure to every pretreatment enhanced freeze-tolerance, this cross-protection was not reciprocal, and few pretreatments enhanced tolerance to other stressors. Thus, larvae were finely tuned to enhance freeze-tolerance in response to a variety of environmental signals, but tolerance to other stressors was less plastic. To identify mechanisms associated with cross-tolerance, we are using a multi-omics approach to quantify transcripts, proteins, and metabolites after each of the pretreatments. At the transcript level, few genes respond the same to every treatment, suggesting multiple gene expression states enhance freeze-tolerance. However, the genes responding various treatments do share some similar functions, and ongoing proteomic and metabolomic analyses will determine the extent these treatments converge on similar physiological and biochemical states.

### **Acquisitive fine root strategy could predispose yellow-cedar to climate change induced decline**

Milos Simovic<sup>1,2\*‡</sup>, Sean T. Michaletz<sup>1,2</sup>

<sup>1</sup>Department of Botany, The University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada; <sup>2</sup>Biodiversity Research Centre, The University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

*milos.simovic@botany.ubc.ca*

Yellow-cedar (*Callitropsis nootkatensis*) is a hardy, long-lived, and slow-growing conifer native to the Pacific Northwest of North America. Over the past century, *C. nootkatensis* has suffered extensive and widespread mortality, impacting approximately 4,000 sq. km of stands across Southern Alaska, the North and Central Coast regions of British Columbia, and the Haida Gwaii archipelago. Recent evidence indicates that this decline is primarily driven by fine root freeze-thaw injuries due to climate change-induced loss of winter snow cover. Interestingly, sympatric tree species are not experiencing similar symptoms, suggesting a species-specific vulnerability.

We hypothesize that *C. nootkatensis* employs a highly acquisitive fine root strategy which, while enabling the species to compete with sympatric conifers, renders it vulnerable to freeze-thaw injuries. Results from our experiment are consistent with our primary hypothesis. *C. nootkatensis* fine roots have significantly higher specific length and lower diameter compared to sympatric conifer species. The cortical cells of *C. nootkatensis* fine roots are characterized by thin walls with centrally aligned lignified bands known as phi thickenings. In contrast, the fine roots of sympatric species belonging to the *Tsuga* genus have significantly lower specific length and higher diameter, with thickened and fully lignified cortical cell walls. These morphological and anatomical differences led to significant differences in cortical cell injuries sustained during extreme freeze-thaw cycles induced during the experiment, translating to near-total loss of water uptake ability in the most damaged *C. nootkatensis* roots.

**The impact of salinity on cold-shock induced gene expression in an estuarine snail**

Alice B. Dennis\*<sup>1,2</sup> and Thomas Inaebnit <sup>2</sup>

<sup>1</sup> URBE & ILEE, University of Namur, Namur, Belgium, <sup>2</sup> Unit of Evolutionary Biology/ Systematic Zoology, Institute of Biochemistry and Biology, University of Postdam, Potsdam, Germany

*alice.dennis@unamur.be*

Melampus is a genus of freeze tolerant snails occurring in estuarine habitats on the Atlantic coast of North America. Genetic work has found that this is a cryptic complex of three species, occupying overlapping ranges with different latitudinal extents. Ecological niche modelling previously indicated that salinity and temperature differ subtly in the ranges occupied by each species. To explore the impact and interaction of these stressors, we acclimated *M. jaumei* to different salinities (5, 20, and 35ppT) followed by an acute cold shock. Both transcriptomic and metabolomic comparisons suggest that low salinity exposure markedly changes their cold response. Furthermore, examination of genes upregulated in the cold has identified a small group of candidate genes that might act as an ice nucleator.

**A Prip-like aquaporin (CrAQP2) in the larval midge *Chironomus riparius* and its role in osmoregulation in response to road de-icers**

Picinic, B.<sup>1\*‡</sup>, Reinsborough, A.<sup>1</sup>, Jonusaite, S.<sup>2</sup>, Paluzzi, J.P.V.<sup>1</sup>, Donini, A.<sup>1</sup>

<sup>1</sup> Biology Department, York University, Toronto ON, Canada, <sup>2</sup> Health and Natural Sciences: Biological Sciences, The University of Tulsa, Tulsa OK, USA

*bpicinic@yorku.ca*

The freshwater midge *Chironomus riparius* is found widely distributed throughout the Northern hemisphere. The larvae serve important ecological functions as food for fish while recycling nutrients by consuming detritus and decaying plant material. They face dilution of body fluids and osmoregulate by the coordinated transport of ions and water across the epithelia of osmoregulatory organs. In their natural environment, snowfall during winter months causes hazardous roadways which results in the application of de-icers, such as road salt (NaCl). Melting snow into nearby water causes freshwater salinization. Recently, “eco-friendly” alternatives to road salt including brine beet juice de-icer (BBJD) have been implemented, although, their effects on freshwater organisms have not been tested. This study aimed to identify an aquaporin (AQP) gene in the osmoregulatory organs of *C. riparius* larvae and study its role in response to exposure to NaCl and BBJD. AQPs are water channel proteins that permit the movement of water across cell membranes. We identified an AQP gene, CrAQP2, in larval *C. riparius*, which is a likely homolog of the *Aedes aegypti* AaAQP2 and *Drosophila melanogaster* PRIP. We found expression of CrAQP2 in all of the osmoregulatory organs in the midge, with highest expression in the Malpighian tubules. We observed changes in CrAQP2 transcript abundance in the organs of BBJD and NaCl-treated midge larvae. Knockdown of CrAQP2 reduced body moisture in freshwater and after acute

exposure to road de-icers. Knockdown of CrAQP2 coupled to long term exposure to BBJD and NaCl caused lethality in larval midges, highlighting a role for CrAQP2 in acclimation to salinity.

### **Implications of salinization from de-icers on key ion-regulating organs in *Chironomus riparius***

Reinsborough, A.<sup>1\*‡</sup>, Donini, A.<sup>2</sup>

<sup>1</sup> Biology Department, York University, Toronto, ON, Canada

*amber121@my.yorku.ca*

The *Chironomus riparius* is a chironomid species residing in freshwater ecosystems which contributes to its environment by feeding on organic matter and acting as a crucial food source for fishes. The application of road de-icers leads to salinizing these habitats. This includes common de-icers such as NaCl which has negative effects on freshwater fauna and their osmoregulation. Organic de-icers such as beet juice brine (BBJD) have been implemented to aid in controlling for the effects of traditional de-icing agents. Although, BBJD contains salt-brines, and high [K<sup>+</sup>], which poses new risks to freshwater environments. This study examined the effects of a 24hr exposure to NaCl and BBJD on the osmoregulation of *C. riparius* larvae, focusing on the anal papillae (AP) and Malpighian tubules (MTs). It was hypothesized that the high [K<sup>+</sup>] in BBJD would cause osmoregulatory stress to the larvae, so exposure to NaCl/KCl and KCl treatments at levels matching those in BBJD were also evaluated. Hemolymph [Na<sup>+</sup>] increased in NaCl and BBJD exposed larvae, and BBJD exposed larvae had lower body moisture. At the AP, BBJD exposure decreased absorption of Na<sup>+</sup> and K<sup>+</sup> ions and NaCl exposure resulted in Na<sup>+</sup> secretion and decreased K<sup>+</sup> absorption. MTs of BBJD exposed larvae had higher rates of fluid secretion and secreted fluid had higher [K<sup>+</sup>]. The NaCl/KCl and KCl treated larvae had similar results as found for BBJD treated larvae. This suggests that the high levels of K<sup>+</sup> in BBJD is driving the observed changes in osmoregulatory physiology of the larvae. Furthermore, the data suggests changes made at the AP mitigate effects of NaCl, and MTs may play a greater role in adjusting to BBJD exposure.

### **De novo genome assembly and annotation of the saline tolerant coastal rockpool mosquito, *Aedes togoi***

Chiang, J.<sup>1\*‡</sup>, Matthews, B.J.<sup>2</sup>

<sup>1</sup>Genome Science and Technology, UBC, Vancouver, BC, Canada. <sup>2</sup>Zoology Department, UBC, Vancouver, BC, Canada

*jonathan.chiang@ubc.ca*

The coastal rock pool mosquito, *Aedes togoi*, has the rare capability to tolerate high salinity water, whereas most mosquito species require freshwater to breed. This extreme physiological adaptation makes *Ae. togoi* an ideal organism to study the sensory and ion regulatory systems of mosquitoes, which are involved in oviposition decision making and larval-stage survival. Highlighting significant adaptations within these systems in mosquitoes is crucial to understanding mosquito breeding site preferences, physiological tolerances, and geographical distributions—all key factors that inform potential vector control strategies. However, to study this species in sufficient detail, genomic resources are first needed.

Here, we present the first genome assembly for *Ae. togoi*, generated de novo utilizing PacBio HiFi and ONT long-read sequencing and Hi-C data. The genome assembly has a total length of 667.414 Mb, scaffold N50 of 226.308 Mb, contig N50 of 2.186 Mb, and a (BUSCO) completeness score of 97.2%. Transcriptomics data were collected from female and male adult mosquitoes to support gene annotations driven by BRAKER3. Additional transcriptomics data comparing *Ae. togoi* larvae in freshwater versus saltwater conditions reveal differential expression of transmembrane ion transporter activity. These resources and initial findings lay groundwork to further develop *Ae. togoi* as a model organism, characterize the specific elements driving *Ae. togoi*'s rare physiological tolerances, and open new avenues for comparative analyses that could improve our understanding of sensory and ion regulatory systems across mosquito species.

### **Impact of insecticide resistance on ion regulation of aquatic mosquito larvae**

Khodikian, E.<sup>1\*‡</sup>, Darkwah S.O.<sup>1</sup>, Durant, A.<sup>1</sup>

<sup>1</sup>Department of Biology, University of Washington, Seattle, WA, USA

*elissakhodikian@hotmail.com*

Pyrethroids are a class of insecticides that kill insects by binding to voltage-gated sodium channels of neurons and prolonging action potentials, causing paralysis and death. The ubiquitous use of pyrethroids for vector control due to high mosquito disease burdens (e.g. Zika and dengue viruses) has led to pyrethroid resistance—mutations in pyrethroid binding sites of voltage-gated sodium channels—within mosquito populations. It has been recently demonstrated that the aquatic larvae of a pyrethroid-resistant strain of *Aedes aegypti* mosquito have enhanced thermal tolerances but not salinity tolerances. Therefore, we asked whether a consequence of insecticide resistance is an adjustment to systemic ion regulation mediated in large part by the renal Malpighian tubules. To test this, *Aedes aegypti* larvae from three different populations: Liverpool (pyrethroid susceptible), Rockefeller (pyrethroid susceptible), and Puerto Rico (pyrethroid resistant), were reared in freshwater at 24 and 30°C and the secretion rates and ionic composition of primary urine from the renal Malpighian tubules were determined using ion-selective micro-electrodes. We found that the resistant larvae produce more urine but secrete significantly less sodium in the urine across the Malpighian tubule epithelium compared to susceptible strains. This highlights potentially negative trade-offs of sodium regulation that cause larvae to be less tolerant of external salinity. Impacts on potassium flux and basal metabolic rates of larvae will also be discussed. Understanding these mechanisms can aid in the development of targeted mosquito control strategies for insecticide-resistant populations of mosquitoes.

**Testing the threshold trait model to predict plasticity of wing dimorphism in *Gryllus lineaticeps* field crickets**

Lourenço M. A. Martins<sup>1\*‡</sup>, Lily J. Donzeiser<sup>1</sup>, Olivia M. Ruffins<sup>1</sup>, Jacqueline E. Lebenzon<sup>2</sup>, Lisa A. Treidel<sup>3</sup>, Abigail Keller<sup>1</sup>, Kristi Montooth<sup>4</sup>, Colin Meiklejohn<sup>4</sup>, Caroline M. Williams<sup>1</sup>

<sup>1</sup>University of California, Berkeley. <sup>2</sup>University of Calgary. <sup>3</sup>College of William & Mary. <sup>4</sup>University of Nebraska, Lincoln.

*lmartins@berkeley.edu*

Threshold traits are widespread quantitative traits, wherein an unobservable continuous liability is mapped to a discrete, dimorphic phenotype by a threshold. These traits are often shaped by multiple genes and environmental factors, complicating predictions of how they respond to joint variation in both. The threshold trait model predicts that phenotypic plasticity only arises when an individual's liability crosses the threshold and is therefore expected to be cryptic if genotypes are far from the threshold. We tested this prediction in the wing-dimorphic field cricket *Gryllus lineaticeps*, in which wing length is a heritable and plastic threshold trait with a well-characterized endocrine basis. We established the sensitive period for wing morph determination and validated an endocrine proxy for liability. Then we generated a range of genetic variation in liability through quantitative genetic crosses and artificial selection and measured plasticity at the morphological and physiological levels. As predicted, lines with the most extreme genetic liabilities showed reduced plasticity at the morphological level. Physiological measures revealed parallel plastic responses, supporting the endocrine basis of liability. Our results show that the threshold trait model can robustly predict phenotypic outcomes under joint genetic and environmental variation. Our findings underscore the importance of developmental timing and endocrine proxies for understanding how complex life-history traits arise from genotype-environment interactions. This work provides a generalizable framework for studying plasticity in other threshold traits, including those relevant to health and evolution.

**Mechanisms of flight muscle histolysis in *Gryllus lineaticeps* crickets**

Williams, C.M.<sup>1\*</sup>, Diaz, T.<sup>1</sup>, Menze, M.<sup>2</sup>, Vázquez Medina, J.P.<sup>1</sup>, Treidel, L.A.<sup>3</sup>, Laturney, M.<sup>1</sup>, Lebenzon, J.<sup>4</sup>

<sup>1</sup>Department of Integrative Biology, UC Berkeley USA. <sup>2</sup>Department of Biology, University of Louisville USA. <sup>3</sup>Department of Biology, William and Mary, USA. <sup>4</sup>Department of Biological Sciences, University of Calgary, Canada.

*cmw@berkeley.edu*

Insect flight muscle histolysis—the targeted breakdown of flight muscles, primarily the dorsolongitudinal muscles (DLMs)—is an adaptive strategy to conserve energy and reallocate resources. Despite its prevalence and importance to life history tradeoffs in insects, the cellular mechanisms driving this remodeling remain poorly understood. In the California variable field cricket (*Gryllus lineaticeps*), long-winged individuals histolyze their DLMs prior to reproduction. We investigated the structural and

functional changes underlying this process using transmission electron microscopy, qPCR, and bioenergetic analyses. We then used RNAi and pharmacological approaches to test mechanisms governing DLM breakdown and maintenance of the adjacent dorsoventral muscle (DVM). We hypothesized that autophagy, mitophagy, and iron buffering regulate muscle remodeling. During histolysis, autophagosomes form as DLM myofibrils and mitochondria degrade and respiration declines. Autophagy- and mitophagy-related genes are upregulated prior to breakdown, and RNAi targeting beclin (an autophagy-related gene) delays degradation. The DVM remains largely intact, showing limited mitochondrial loss and a smaller drop in respiration, along with upregulation of mTOR signaling—consistent with a protective role. Rapamycin, an mTOR inhibitor, reverses DVM protection. Meanwhile, increased expression of iron transporters accompanies extensive iron release from degrading DLMs, limiting oxidative damage. These findings reveal that autophagy, mitophagy, and iron homeostasis are tightly coordinated to regulate DLM histolysis. Ongoing work explores how nutrition and reproductive timing influence this process.

### **Metal links: Iron transport as a connection between muscle breakdown and oogenesis in *Gryllus lineaticeps***

Diaz, T.<sup>1\*‡</sup>, Laturney, M.<sup>1</sup>, Treidel, L.<sup>2</sup>, Menze, M.<sup>3</sup>, Vazquez-Medina, J.<sup>1</sup>, Lebenzon, J.<sup>1,4</sup>, Williams, C.<sup>1</sup>

<sup>1</sup>Integrative Biology Department, UC Berkeley, Berkeley, CA, USA. <sup>2</sup>Biology Department, College of William and Mary, Williamsburg, Virginia, USA. <sup>3</sup>Department of Biology, University of Louisville, Louisville, Kentucky, USA. <sup>4</sup>Department of Biological Sciences, UCalgary, Calgary, Alberta, Canada

[tomasdiaz@berkeley.edu](mailto:tomasdiaz@berkeley.edu)

To modulate the energy costs of flight, many insects break down their flight muscle in a process known as histolysis. Flight muscle histolysis reduces the energetic costs of muscle maintenance and recycles nutrients, but the molecular mechanisms underlying this remodelling remain unclear. The variable field cricket, *Gryllus lineaticeps*, histolyzes flight muscles when transitioning from dispersal flight to reproduction. We found that autophagy selectively degrades flight muscle mitochondria and myofibrils, causing massive iron release, which is buffered by upregulation of the iron transporter transferrin. Given that iron is an essential micronutrient for reproduction in many insects as well as a potent pro-oxidant, we hypothesized that the need for iron may drive flight muscle breakdown and that coordinated iron transport from the flight muscle to the ovaries facilitates reproduction and avoids oxidative stress during histolysis. We manipulated dietary iron alone and with RNAi knockdown of the iron transporter transferrin and measured the impacts on flight muscle histolysis, oxidative damage, oogenesis, and fecundity. We have found that females fed low-iron diets exhibit increased rates of histolysis and larger ovary masses compared to those fed high-iron diets. In contrast, females fed high-iron diets have heavier flight muscles but smaller ovaries. With transferrin knockdown, females on a low-iron diet have reduced ovary mass. This finding suggests that iron released and transported from flight muscle supports reproduction and buffers dietary iron deficiencies. Flight muscle histolysis can elucidate how tissue remodeling is coordinated to balance life history allocation

### ***Lymnaea stagnalis*: lessons on aging from a model for integrative molecule-to-behavior studies of nervous system function and dysfunction**

Wildering, W.<sup>1\*</sup>, Lee, J.<sup>1</sup>, McFadden, E.<sup>1</sup>, Watson, S.<sup>1</sup>, Ehtemam, F.<sup>1</sup>, Hermann, P.<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Faculty of Science, University of Calgary, Calgary, Alberta, Canada

*wilderin@ucalgary.ca*

*Lymnaea stagnalis* (L.), commonly known as the great pond snail, is becoming an increasingly popular model organism in various scientific research fields. These include comparative and evolutionary neuroscience, studies on neural regeneration, neuroendocrine control of complex behaviors, comparative genomics, environmental toxicology, parasitology, and human disease transmission. Like other gastropod mollusks, this freshwater gastropod features a relatively simple central nervous system (CNS) with many identified and behaviorally characterized neurons and neural circuits. As a result, *Lymnaea stagnalis* is particularly well-suited for integrative molecule-to-behavior studies of learning and memory, and disruptors thereof. In this presentation I introduce *Lymnaea stagnalis* as a model for aging studies, summarize the neurobiological foundations of learning and memory, and present evidence implicating lipid peroxidation-associated activation of phospholipase A2 (PLA2) activation as pivotal factor in the characteristic age-related neurophysiological decline and associative learning and memory impairment in this model. Furthermore, I will present surprising evidence suggesting that *Lymnaea's* organismal survival is a particularly sensitive function of the serviceability of a notably non-redundant neural control system involved in the regulation of body water content and cast these findings in the context of biological robustness, reliability theory, and control system architecture.

### **Mitochondrial Dynamics Imbalance as a Driver of Neuronal and Cognitive Decline During Healthy Brain Aging**

Ehtemam, F.<sup>1\*‡</sup>, Hermann, P.M.<sup>1</sup> and Wildering, W.C.<sup>1</sup>

<sup>1</sup>Biological department, University of Calgary, Alberta, Canada

*farzaneh.ehtemam@ucalgary.ca*

Aging is characterized by a progressive decline in multiple physiological functions, including learning and memory, ultimately leading to increased mortality and reduced fertility. Neurons undergo structural and functional deterioration with age, contributing to cognitive decline. Mitochondria, the energy-producing organelles of eukaryotic cells, play a key role in maintaining neuronal function through ATP production, calcium buffering, and redox regulation. These organelles are highly dynamic, undergoing continuous fission and fusion to preserve mitochondrial integrity and distribution. Aging likely disrupts this dynamic balance and impairs mitochondrial transport, and are associated with neuronal dysfunction.

Neurons are high-energy demand cells using most of their energy to power the electrical and synaptic signaling functions underpinning their information processing functions. Disruptions in mitochondrial function, mobility or network architecture are therefore likely to lead to impaired neuronal activity and cognitive deficits. Using *Lymnaea stagnalis* as a model of normal aging and age-associated learning and memory impairment, we investigated the effects of experimentally modulating mitochondrial dynamics on mitochondrial structure and function. Through combined NADH/FAD autofluorescence imaging and electrophysiological recordings, we demonstrate that altering the fission-fusion balance impacts mitochondrial architecture, membrane potential, and redox state in aged neurons thus underscoring the

critical role of mitochondrial dynamics in maintaining neuronal and behavioral functions across this gastropod's life cycle.

Friday 18<sup>th</sup> Early Afternoon: Seasonal responses to cold

**Winter is coming: Circa-annual cycles and obligate diapause drive endogenous cold hardening in temperate ants**

Quentin Willot<sup>1,2\*</sup>, Alice B. Dennis<sup>1</sup>, Vladimír Košťál<sup>3</sup>, Johannes Overgaard<sup>2</sup>

<sup>1</sup>Institute of Life, Earth & Environment, Research Unit of Environmental and Evolutionary Biology, University of Namur, Namur 5000, Belgium. <sup>2</sup>Department of Biology, Section for Zoophysiology, Aarhus University, Aarhus-C 8000, Denmark. <sup>3</sup>Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, 37005 Czech Republic

*quentin.willot@unamur.be*

The ability to acquire cold tolerance and cope with seasonality are among the most fundamental traits for temperate ectotherms. Cold-adapted ants, which are ecologically successful insects in these climates, rely on a complex annual life cycle to survive multiple consecutive winters. Following winter reactivation, colonies enter a fixed phase of development in spring whose duration is controlled by Kipyatkov's "sand-glass device." This device will ultimately enforce a new dormancy period for colonies after a set period of time, even when colonies are maintained under permissive developmental conditions (e.g., warm temperatures and long days). Here, we show that colony-level dormancy in temperate ants, triggered by Kipyatkov's sand-glass device, is associated with endogenous diapause and shifts in worker cold tolerance even without prior exposure to low temperatures. The onset of this "winter phenotype" (i) lowers the minimal temperature for worker neuromuscular activity (CT<sub>min</sub>) and enhances responsiveness of CT<sub>min</sub> to subsequent cold acclimation. It also (ii) increases acute cold-stress survival (LTe<sub>50</sub>) in most species. Our work also explores the metabolomic reorganization linked to cold acclimation in workers, where we highlight the accumulation of key molecules putatively involved in cold tolerance (e.g., trehalose, inositol). To our knowledge, this is the first evidence linking a circa-annual cycle to the acquisition of cold tolerance in social insects. Our results show that endogenous cold hardening can occur independently of environmental cues in ants, offering new insight into how this ecologically successful group of insects adapted to highly seasonal environments.

**Thermal tolerance is shaped by rapid adaptive tracking in the invasive fly *Drosophila suzukii***

Ellie McCabe<sup>1\*‡</sup>, Nicholas Teets<sup>1</sup>

<sup>1</sup>Entomology Department University of Kentucky

*ellieanmccabe@gmail.com*

Adaptive tracking is the rapid oscillation of allele frequencies in short-lived organisms in response to fluctuating environmental conditions. While genomic shifts consistent with adaptive tracking have been documented in several multivoltine species, the extent to which these changes optimize performance in seasonal performance is less clear. In *Drosophila melanogaster*, alleles associated with thermal tolerance predictably fluctuate on seasonal time scales, and outdoor caged populations undergo evolved changes in thermal tolerance across seasons. However, the generality of adaptive tracking is

unknown, and the extent to which phenotypes rapidly evolve in wild populations. We characterized changes in thermal tolerance of *D. suzukii* collected three times per year for four consecutive years in central Kentucky. Flies were phenotyped immediately after field collection and in isofemale lines following four generations of laboratory rearing, to tease apart environmental and genetic drivers of phenotypic shifts. The critical thermal minimum (CT<sub>min</sub>) predictably fluctuated on an annual cycle, with the CT<sub>min</sub> being lowest in flies collected early in the season. Thus, flies descending from the overwintering generation had the highest cold tolerance. Further, phenotypic oscillations tended to be more pronounced in the F4 generation, indicating a strong genetic component to the changes. We also used pool-seq to characterize genetic shifts and results are consistent with adaptive genetic variation contributing to these phenotypic changes. Together, our results indicate that adaptive tracking allows temperate populations of *D. suzukii* to optimize performance in seasonal environments.

### **Massive accumulation of myo-inositol in overwintering boreal fly, *Drosophila lummei*: Is myo-inositol a cryoprotectant?**

Štětina, T.<sup>1\*</sup>, Kučera, L.<sup>2</sup>, Moos, M.<sup>1</sup>, Košťál, V.<sup>1</sup>

<sup>1</sup>Institute of Entomology, Biology Centre, Czech Academy of Sciences, 37005 České Budějovice, Czech Republic. <sup>2</sup>Czech Centre of Phenogenomics, Institute of Molecular Genetics, Czech Academy of Sciences, 25250 Vestec, Czech Republic

[tomas.stetina@entu.cas.cz](mailto:tomas.stetina@entu.cas.cz)

Species of the genus *Drosophila* that inhabit the boreal taiga had to evolve mechanisms to survive the long and harsh northern winters. In this study, we investigated cold hardiness in *Drosophila lummei*, conducting a detailed analysis of its enhancement during laboratory cold acclimation designed to simulate the onset of the boreal winter. *D. lummei*, a member of the montana group with a Holarctic distribution, enters a photoperiodic winter diapause during which it increases cold tolerance and accumulates high concentrations of myo-inositol. A similar correlation or coincidence between elevated cold hardiness and the accumulation of potentially cryoprotective substances has been reported in a large number of insect species. Our goal was to support this correlative evidence with direct proof. To achieve this, we artificially increased myo-inositol levels in pre-acclimated flies to match those observed in cold-acclimated individuals. However, this increase had no measurable effect on any parameter of cold hardiness. We concluded that our methods of artificial augmentation (microinjection into the hemolymph and dietary enrichment) were ineffective in achieving precise tissue-specific localization of myo-inositol, which may be critical for its protective role.

### **Temperature-dependent gene expression during early diapause of the eastern spruce budworm**

Lopez Pedersen, J.<sup>1\*†</sup>, Ojukwu, K.C.<sup>1</sup>, Roe, A.D.<sup>2</sup>, Marshall, K.E.<sup>3</sup>, Toxopeus, J.<sup>1</sup>

<sup>1</sup>Biology Department, St. Francis Xavier University, Antigonish, NS, Canada. <sup>2</sup>Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada. <sup>3</sup>Zoology Department, University of British Columbia, Vancouver, BC, Canada

*jpederse@stfx.ca*

The eastern spruce budworm (Lepidoptera: Tortricidae: *Choristoneura fumiferana*) overwinters as a second-instar larva. In early fall, the larva enters a dormant state known as diapause and remains dormant during winter. Yet, we know little about the physiological processes governing their diapause development. Roe et al. (2024) found that short exposures to warm temperatures in early diapause increased overwintering survival, while longer (but not short) exposures to cool temperatures also increased survival. This suggests that temperature in early diapause affects the spruce budworm's ability to complete diapause during winter. In this study, we tested the hypothesis that exposure to warmer temperatures accelerates early diapause development. We predicted that developmental traits in early diapause would be differentially expressed between budworm exposed to cool or warm temperatures. To investigate the effects of temperature on early diapause, we exposed pre-diapause larvae to cool (10 °C) or warm (20 °C) temperatures for up to 10 weeks. Larvae were sampled at 8 time points during these 10 weeks to measure gene expression (via transcriptomics) and survival (motility). Larval motility decreased over time, especially at warm temperatures. We will also share RNAseq results about how gene expression changed over time, and how this differed at the cool and warm temperatures. Finally, from the transcriptomic data we plan to identify candidate genes that can be key "markers" for developmental transitions during early diapause, and whether those markers are expressed in a temperature-dependent manner.

### **Integrating climate, physiology, and species distributions to improve model performance of a Canadian boreal forest pest**

Black, E.N.<sup>1,3\*‡</sup>, Pureswaran, D.<sup>2</sup>, Roe, A.<sup>3</sup>, Marshall, K.E.<sup>1</sup>

<sup>1</sup>Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada.

<sup>2</sup>Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, 1350, Regent Street, Fredericton, NB E3C 2G6, Canada. <sup>3</sup>Natural Resources Canada, Great Lakes Forestry Centre, 1219, Queen Street East, Sault Ste. Marie, ON P6A 2E5, Canada

*emily@zoology.ubc.ca*

Species are expected to shift their ranges due to climate change in order to track their thermal niches. By exploring the environmental and physiological factors which influence these shifts, we can make better predictions of future distributions. Most species distribution models (SDMs) rely on macroclimatic variables such as temperature and lack physiological grounding which would increase their reliability, especially under novel conditions. However, models which combine macroclimatic and physiological predictors (also known as hybrid models) are still being developed. The spruce budworm (*Choristoneura fumiferana*) is a model species for forest entomology which can be used to investigate hybrid models. As a major Lepidopteran defoliator of Canada's boreal forest, its physiology is well-characterized, and aerial records of its outbreaks have been used to build SDMs to evaluate how climate influences defoliation and predict range shifts. Thus, we aim to understand how physiology and the environment influence spruce budworm defoliation through space and time, and if hybrid modelling can improve predictions of defoliation under climate change. First, we investigated how incorporating temperature fluctuations (which impacts spruce budworm overwintering fitness) affects model performance and influences

future defoliation predictions. Second, we examine how including population genomic structure in a hybrid model may reveal regional differences in outbreak patterns and how populations will respond to climate shifts. Overall, our work demonstrates the value of hybrid models which integrate many streams of data to better understand the drivers of species distributions.

**Aminergic regulation of honeybee flight muscles in the context of thermoregulation**

Delac, S.<sup>1\*‡</sup>, Ankenbrand, M.<sup>2</sup>, Thamm, M.<sup>1</sup>

<sup>1</sup>Chair of Behavioral Physiology & Sociobiology, University of Würzburg, Bavaria, Germany. <sup>2</sup>Center for Computational and Theoretical Biology (CCTB), University of Würzburg, Bavaria, Germany

*saskia.delac@uni-wuerzburg.de*

As climate change intensifies, causing more extreme temperature fluctuations, understanding thermoregulation in ectotherms becomes essential for studying their survival. As an ectotherm, the western honeybee (*Apis mellifera*) exhibits remarkable adaptability through specialized thermoregulation strategies, making it an ideal model for studying these mechanisms. They enable honeybees to maintain optimal temperatures at both individual and colony level. Here, the flight muscles are crucial – they generate heat via shivering thermogenesis, power flight to collect water for evaporative cooling, and dissipate excess heat through fanning. The flexibility to switch between these functions is crucial for maintaining thermal homeostasis. The objective of our research is to elucidate how thermal stress alters the biogenic amine system, which are key regulators of muscle function, to understand the implications for thermoregulation and survival. Initially, we measured biogenic amine levels via HPLC to assess the impact of heat and cold stress on the flight muscles. Subsequently, we proceeded to analyze gene expression changes related to biogenic amines, utilizing RNA-seq. Finally, we explored how these transcriptional changes relate to thermogenesis performance, which is essential for the survival of honeybees, using thermographic imaging to quantify heat production in stressed individuals. Our findings indicate a complex interaction between thermal stress, gene regulation and flight muscle performance.

**Climate acclimation (not traits or phylogeny) drives variation in photosynthesis temperature response**

Michaletz, S.T.<sup>1\*</sup>, Garen, J.C.<sup>1</sup>

<sup>1</sup>Department of Botany, The University of British Columbia, Vancouver, BC, Canada

*sean.michaletz@ubc.ca*

Understanding variation in plant assimilation-temperature (AT) responses is essential for improving forecasts of climate change feedbacks and their impacts on the biosphere. Previous studies have focused on acclimation to weather or adaptation to climate of origin, but little attention has been paid to AT relationships with leaf functional traits or phylogeny. To evaluate the influence of climate, traits, and phylogeny on AT response, we used the new Fast Assimilation-Temperature Response (FAsTeR) gas exchange method to measure AT response in 102 species from 96 families in a common garden. We also measured climate variables, saturating light intensity, and key leaf functional traits. Local environmental conditions were the strongest predictors of AT response parameters. Optimal temperature for photosynthesis (Topt) responded positively to recent air temperature and light exposure, and was best predicted by mean air temperature on the day of measurement. Temperature response parameters

showed no phylogenetic structure, and only modest variation with leaf functional traits or climate of origin. Plant AT response was primarily driven by physiological acclimation to local climate variables, rather than by adaptation to climate of origin. Thermal acclimation of photosynthesis occurred on much shorter timescales than expected.

### **Activation of the chaperone response, not cryoprotection, drives cold tolerance in diapausing Colorado potato beetles**

Lebenzon, J.E.

Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

*jackie.lebenzon@ucalgary.ca*

Temperate insects spend over half their lives overwintering, during which they experience sub-zero temperatures. As ectotherms, they risk freezing of body fluids—a state which most insects cannot survive. Instead, most insects are freeze-avoidant and rely on remaining unfrozen, surviving temperatures at or above their supercooling point. In this temperature range, proteins are particularly vulnerable, and cold-induced protein unfolding can lead to failure of vital physiological processes and ultimately death. Many overwintering insects express heat shock proteins (HSPs), thought to help protect against cold-induced protein damage. However, their functional role in cold tolerance remains unclear, with most studies focusing only on patterns of HSP expression. Using Colorado potato beetles, we investigated protein protection mechanisms that may contribute to cold tolerance in freeze-avoidant insects. By exposing diapausing beetles to a fluctuating thermal regime, we induced a “cold-tolerant” phenotype which improved sub-zero temperature survival. We then used RNA-seq to explore gene expression associated with protein protection. Transcriptomic differences between cold-tolerant and diapausing beetles were driven by increased expression of chaperone-related transcripts in the fat body, notably HSP68 and its co-chaperone dnaJ. These changes were functionally relevant: cold-tolerant beetles showed reduced fat body protein ubiquitination after cold shock, suggesting enhanced protein repair capacity. Our findings support a mechanistic link between chaperone-mediated protein protection and improved insect cold tolerance.

### **Low evolutionary conservation of thermal, transcriptional plasticity across temperate and tropical *Drosophila* lineages, with a few intriguing exceptions**

Gregory Ragland\*<sup>1</sup> and James deMayo<sup>1</sup>

<sup>1</sup>University of Colorado, Denver, USA

*gregory.ragland@ucdenver.edu*

An accumulating body of literature suggests that evolution in relatively novel environments may often reflect initial suppression of maladaptive plasticity rather than enhancement of beneficial plasticity. This pattern seems to be particularly consistent for transcriptional, plastic responses that serve as a snapshot of tissue-wide or organism-wide physiological change across environments. Less is known about how transcriptional plasticity may evolve as species diversify across environmental space over

macroevolutionary timescales. We performed experiments on the plasticity of transcriptional responses to acute cold stress across eight species in the *Drosophila* genus diversified into tropical and temperate environments to test several hypotheses about macroevolutionary trends in plasticity evolution. We find that long-term evolution in cold environments does not consistently enhance or repress transcriptional responses to cold – in other words, cold-adapted species are not necessarily more or less transcriptionally plastic than warm-adapted species. Rather, divergence in transcriptional plasticity is best predicted by the degree of evolutionary conservation of differentially expressed genes – plastic transcripts tend to map to genes that have diversified via gene gain/loss across species lineages. There are a (very) few exceptions to this rule, and this talk will also discuss a few, remarkably evolutionarily conserved responses to cold that include possible links to the circadian clock.

### **Multigenerational rearing of *Drosophila melanogaster* populations in variable thermal environments has limited influence on reproductive capacity**

Unfried, L.N.<sup>1\*‡</sup> and Teets, N.M.<sup>1</sup>

<sup>1</sup> University of Kentucky

*[laura.unfried@uky.edu](mailto:laura.unfried@uky.edu)*

The seasonal variability hypothesis predicts that exposure of organisms to a wider range of temperatures will increase thermal tolerance breadth. Thus, environments with a broader thermal range should select for organisms with higher acclimation capacity. This project tested whether we could target specific types of acclimation capacity by rearing populations of *Drosophila melanogaster* in five different thermal environments for 21 generations. One environment was a stable temperature (ST), and populations from the remaining four variable environments were compared to populations from the stable environment. The remaining environments were variable adult environment (VAE), variable developmental environment (VDE), daily heat shock (DHS) and daily cold shock (DCS). We hypothesized that populations from these thermal environments would show higher reproductive capacity following exposure to fluctuating temperatures compared to flies kept at a stable temperature. Reproductive capacity was determined by counting total eggs laid in a 24-hr period and assessing egg-to-adult viability. Overall, both egg counts and egg viability were largely unaffected by rearing thermal environment. DCS flies did have higher egg counts than ST flies following cold exposure, and VAE flies had a higher viability rate compared to ST flies following exposure to low. However, VAE flies had lower total egg counts than ST flies at all tested temperatures. Therefore, rearing *Drosophila melanogaster* populations in variable thermal environments has limited influence on reproductive capacity in fluctuating temperatures.

## Poster Session Thursday 17<sup>th</sup> Evening:

### **Quantitative analysis of the aminergic neurohormonal response to abiotic stressors in honeybees (*Apis mellifera*)**

Gabrielė Gurinskaitė\*, Markus Thamm, Ricarda Scheiner

Behavioral Physiology & Sociobiology (Zoology II), Julius-Maximilians-University of Würzburg, Würzburg, Germany

*gabriele.gurinskaite@stud-mail.uni-wuerzburg.de*

Honeybees are vital pollinators and play a critical role in natural ecosystems and agriculture.

Despite their importance, the molecular mechanisms underlying their general stress responses remain poorly understood. It is hypothesized that honeybees respond to stressors through physiological and behavioral adaptations mediated by biogenic amines. However, this assumption is lacking systematic research to be substantiated.

We systematically investigated the impact of heat and desiccation stress on the time-dependent dynamics of biogenic amine provision and release in honeybees. We simultaneously quantified the individual concentrations of biogenic amines in the brain and hemolymph of stressed and non-stressed individual honeybees using high-performance liquid chromatography with electrochemical detection. Interestingly, only the concentrations of octopamine and tyramine exhibited distinct responses to the stressors, whereas serotonin and dopamine did not show stressor-dependent variations. In the brain, octopamine concentrations showed a notable decrease to prolonged heat and desiccation stress, while in the hemolymph, the responses of octopamine and tyramine varied depending on the type of stressor. Additionally, a correlation between brain and hemolymph octopamine concentrations was observed under heat stress, suggesting a complex interplay between these two amines in response to stress.

This suggests that octopamine might have an important role in response to both stressors in the brain, while in the hemolymph, biogenic amines responses were specific to the stressor.

### **Exploring the metabolic costs of acute and chronic bacterial infection in *Drosophila melanogaster***

Gray, E.M.<sup>1\*</sup>, Smee, M.R.<sup>1</sup>, Lazzaro, B.P.<sup>1</sup>, Sinclair, B.J.<sup>1</sup>

<sup>1</sup>Department of Entomology, Cornell University, Ithaca, NY 14853, USA

*emg268@cornell.edu*

*D. melanogaster* that survive acute bacterial infection often experience lifelong chronic infections that may impose metabolic and other health costs. The energetic burden of chronic infection manifests as reduced starvation resistance, for example. While it is hypothesized that immune activity resulting from chronic infection drives increased metabolic costs, other associated symptoms such as changes in behavior may also be involved. This study explores instantaneous metabolic rate (MR) and activity in fruit flies during acute and chronic infection phases to determine how infection alters MR, and to what

extent this is due to physical activity (“sickness behavior”). We infected female *D. melanogaster* with a natural opportunistic bacterial pathogen (*Providencia rettgeri* Dmel), then measured rate of CO<sub>2</sub> production (VCO<sub>2</sub>) and activity for 18 hours immediately following infection (acute) as well as 5 days post-infection (chronic). Bacterial loads at 18h and 5 days were quantified in whole flies immediately after metabolic measurements. We found that VCO<sub>2</sub> increased during the acute phase of infection and remained high relative to controls at 5 days post-infection. Activity levels were also higher in infected flies relative to PBS-injected and anesthetized controls. These data establish first steps toward understanding of how host metabolism regulates and is altered by physiological responses to acute and chronic opportunistic infections.

### **Is the muscarinic system important in insect metabolism regulation as it is in vertebrates?**

Chowański, S.<sup>1\*</sup>, Walkowiak-Nowicka, K.<sup>1</sup>, Urbański, A.<sup>1</sup>, Marciniak, P.<sup>1</sup>, Pacholska-Bogalska, J.<sup>1</sup>

<sup>1</sup>Department of Animal Physiology and Developmental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

*szyymon@amu.edu.pl*

The insect fat body is a main hub for insect metabolic processes, playing roles in nutrient storage, energy conversion, detoxification, immunity, and reproduction. Its activity is regulated through complex neuronal and non-neuronal mechanisms. One key regulatory system appears to be the cholinergic system, mediated by muscarinic acetylcholine receptors (mAChRs).

mAChRs are metabotropic receptors that transmit signals in the nervous system and facilitate communication between neuronal and non-neuronal tissues. These receptors are widely expressed across various cell types. In insects, three types of mAChRs have been identified in contrast to vertebrates where only one type is presented. Existing data indicate their involvement in regulating muscle activity, behaviour, memory, and learning. Our research demonstrates that mAChRs also influence insect fat body metabolism.

We investigated how mAChRs modulate fat body metabolic activity, focusing on whether this regulation is direct or indirect. Using specific agonists (carbachol, pilocarpine) and antagonists (atropine, scopolamine), we observed that lipid metabolism is significantly altered by mAChR activation or inhibition. Changes occurred at multiple levels: enzyme activity, enzyme quantity, and gene expression, specifically in enzymes of  $\beta$ -oxidation, glycolysis and Krebs cycle. We also noted alterations in carbohydrate, amino acid and lipid composition in both fat body and haemolymph.

To determine whether these effects were direct, we examined the expression of insulin-like peptide genes. Our findings suggest that mAChRs regulate fat body metabolism through both direct and indirect pathways.

### **Repellent activity and impact of volatile organic compounds on the survival and reproductive performance of the pest *Tenebrio molitor***

Chowański, Sz.<sup>1</sup>, Marciniak, P., Słocińska, M.<sup>1</sup>

<sup>1</sup>Department of Animal Physiology and Developmental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland

*karolina.walkowiak@amu.edu.pl*

Soil pollution, the accumulation of harmful substances, and unintended environmental impacts are many consequences of modern agricultural practices. To mitigate these negative effects, scientists have long been engaged in intensive research to develop alternatives to conventional insecticides. One promising approach involves the use of natural compounds—specifically, plant secondary metabolites—due to their diverse biological activities. These substances can interfere with pests by reducing food intake, inhibiting growth, or disrupting reproductive processes in insects and nematodes.

In our research, we focused on four plant-derived secondary metabolites classified as volatile organic compounds (VOCs): (E)-2-decenal, furfural, 2-undecanone, and (E,E)-2,4-decadienal, each applied at a concentration of 10<sup>-5</sup> M. We examined their effects on survival and reproductive parameters in the *Tenebrio molitor* beetle, including the number of eggs laid, egg morphology, and larval hatchability following direct injection into the insects. We also assessed these compounds' repellent properties using a choice test.

Our findings indicate that the tested VOCs exhibit repellent activity, as the beetles actively avoided treated surfaces or food. Furthermore, the compounds reduced survival rates and adversely affected reproductive outcomes by decreasing the number of eggs laid and reducing larval hatch rates.

Given these results, along with economic considerations and the feasibility of sourcing these VOCs from plant residues such as citrus peels, leaves, stems, or corn cobs, it can be concluded that these compounds hold potential as sustainable, natural alternatives for plant protection.

### **Insect egg traits: ecological and evolutionary drivers shaping their diversity**

Iossa, G.<sup>1\*</sup>, de Jong, T.<sup>2,3</sup>, Greenberg, L.O.<sup>3</sup>, Catley, E.<sup>1</sup>, Fatouros, N.<sup>3</sup>

<sup>1</sup>School of Natural Sciences, University of Lincoln, UK. <sup>2</sup>University of Bristol, UK. <sup>3</sup>Biosystematics Group, Wageningen University & Research, The Netherlands

*giossa@lincoln.ac.uk*

Immobile life stages, such as embryos, are bottlenecks for insect populations. Determining what shapes this key life stage is crucial. One trait that is highly variable and underexplored is the insect eggshell and its pores. Once fertilised and laid, eggs must face a delicate trade-off between water and gas exchange to allow the survival of the insect embryo in the outside environment. Recent studies demonstrated that oviposition ecology is important in shaping egg size across insects, but how does the egg achieve this? Egg pores are likely key to this. Here, we first explore the ecological and evolutionary function of fertilisation pores, then we consider within- and between-populations egg pore differences in three closely related butterflies (Pieridae). We sampled *Pieris napi* over a latitudinal gradient of over 4,000km and used a common garden experiment to investigate variation in egg pores linked to respiration and reproduction under lab conditions. We found that: 1) even in identical greenhouse conditions, egg pore traits remained highly variable. Remarkably, the number and size of egg pores differed more between P.

napi populations from divergent climates than between sympatric *Pieris* species. 2) Egg pores linked to respiration and reproduction were strongly correlated with climatic variables, rather than with egg size. 3) We observed that reproductive pores gradually seal within hours of oviposition, suggesting a role in reducing environmental exposure post-fertilization. These results reveal that insect egg pores are not merely structural features, but dynamic, climate-sensitive traits that may shape reproductive success in a rapidly changing world.

### **Development under predation risk increases serotonin-signaling, variability of turning behavior and survival in adult fruit flies *Drosophila melanogaster***

Krams, I.<sup>1\*</sup>, Krams, R.<sup>1</sup>, Popovs, S.<sup>1</sup>, Krama, T.<sup>1</sup>

<sup>1</sup>Latvian Biomedical Research and Study Centre, Riga, Latvia

*indrikis.krams@proton.me*

The development of high-throughput behavioral assays, where numerous individual animals can be analyzed in various experimental conditions, has facilitated the study of animal personality. Previous research showed that isogenic *Drosophila melanogaster* flies exhibit striking individual non-heritable locomotor handedness. The variability of this trait, i.e., the predictability of left-right turn biases, varies across genotypes and under the influence of neural activity in specific circuits. This suggests that the brain can dynamically regulate the extent of animal personality. It has been recently shown that predators can induce changes in prey phenotypes via lethal or non-lethal effects affecting the serotonergic signaling system. In this study, we tested whether fruit flies grown with predators exhibit higher variability/lower predictability in their turning behavior and higher survival than those grown with no predators in their environment. We confirmed these predictions and found that both effects were blocked when flies were fed an inhibitor ( $\alpha$ MW) of serotonin synthesis. The results of this study demonstrate a negative association between the unpredictability of turning behavior of fruit flies and the hunting success of their predators. We also show that the neurotransmitter serotonin controls predator-induced changes in the turning variability of fruit flies, regulating the dynamic control of behavioral predictability.

### **A diabetes-like biochemical and behavioural phenotype of *Drosophila* induced by predator stress**

Krama, T.<sup>1\*</sup>, Popovs, S.<sup>1</sup>, Krams, R.<sup>1</sup>, Krams, I.<sup>1</sup>

<sup>1</sup>Latvian Biomedical Research and Study Centre, Riga, Latvia

*tatjana.krama@gmail.com*

Predation can have both lethal and non-lethal effects on prey. The non-lethal effects of predation can instil changes in prey life history, behaviour, morphology and physiology, causing adaptive evolution. The chronic stress caused by sustained predation on prey is comparable to chronic stress conditions in humans. Conditions like anxiety, depression, and post-traumatic stress syndrome have also been implicated in the development of metabolic disorders such as obesity and diabetes. In this study, we found that predator stress induced during larval development in fruit flies *Drosophila melanogaster*

impairs carbohydrate metabolism by systemic inhibition of Akt protein kinase, which is a central regulator of glucose uptake. However, *Drosophila* grown with predators survived better under direct spider predation in the adult phase. Administration of metformin and 5-hydroxytryptophan (5-HTP), a precursor of the neurotransmitter serotonin, reversed these effects. Our results demonstrate a direct link between predator stress and metabolic impairment, suggesting that a diabetes-like biochemical phenotype may be adaptive in terms of survival and reproductive success. We provide a novel animal model to explore the mechanisms responsible for the onset of these metabolic disorders, which are highly prevalent in human populations.

### **Effects of fall and winter warming on cold tolerance of eastern spruce budworm (*Choristoneura fumiferana*)**

Ojukwu, K.C.<sup>1,2\*‡</sup> and Toxopeus, J.<sup>1</sup>

<sup>1</sup>Department of Biology, St. Francis Xavier University, Antigonish, NS, Canada. <sup>2</sup>Department of Biology, Memorial University of Newfoundland, NL, Canada

[kojukwu@stfx.ca](mailto:kojukwu@stfx.ca)

Winter and fall temperatures are increasing, with potential effects on overwintering insects. Here, we assessed the effects of fall and winter warming on cold tolerance of eastern spruce budworm. The spruce budworm is a key insect pest of the boreal forest of North America that overwinters as a second instar larva. Diapausing second instar larvae were exposed to warm fall conditions of 20°C for 2 weeks (short fall) or 5 weeks (long fall), followed by simulated winter conditions of 2°C for 6 weeks. Budworm were then exposed to a single (10°C for 60 h) or repeated (10°C for 12 h in 5 cycles) winter warming treatment, or maintained in winter conditions (control, not warmed). Following warming, budworm were exposed to an additional 14 weeks at 2°C, followed by measurements of cold tolerance. These measurements included supercooling point (SCP) and survival after 4 h at -20°C. Energy stores (glycogen, lipids) and cryoprotectants (glycerol) were quantified at several time points during the simulated winter. We found no significant difference in SCP among winter warming treatments following short fall conditions. Following a long fall, the budworm that experienced a single warming event had a slightly lower SCP (-34°C) than not warmed controls (-33°C). We found no significant effect of warm fall duration or winter warming treatment on survival after exposure to -20°C for 4 h. Our study suggests a surprisingly limited effect of warming during fall and winter on the budworm, with implications for their population dynamics in coming years.

### **Fall Temperature Impacts on Spruce Budworm (*Choristoneura fumiferana*) Diapause, Cold Tolerance, and Fitness**

Laura-Anne Browning<sup>1\*‡</sup>, Amanda D. Roe<sup>2</sup>, Jantina Toxopeus<sup>3</sup>, and Katie E. Marshall<sup>1</sup>

<sup>1</sup>University of British Columbia | Department of Zoology. <sup>2</sup>Natural Resources Canada | Canadian Forest Service. <sup>3</sup>St. Francis Xavier University | Department of Biology

*lbrowning@zoology.ubc.ca*

Diapause is a programmed state of slowed development and is a common strategy used by insects to conserve energy throughout winter. Curiously, diapause onset often occurs well before stressful winter conditions when resources are still available. The effects of environmental conditions in this early stage of diapause have been relatively understudied compared to later stages. Here we examined how metabolic suppression during diapause onset, cold tolerance, and post-diapause fitness of the eastern spruce budworm *Choristoneura fumiferana* (Clemens, Lepidoptera: Tortricidae) change after experiencing different fall conditions. We hypothesized that hotter and longer falls will cause diapause onset to proceed more quickly and we predicted this will affect all above aspects of *C. fumiferana* physiology and cause them to be unable to survive diapause. We placed larvae into two different fall temperature treatments (10°C and 20°C) for five different fall durations (1, 2, 4, 5, and 7 weeks) and measured metabolic rate at the end. We then placed larvae into 2°C for 19 weeks to simulate winter conditions. We measured cold tolerance during the winter simulation and recorded time to pupation after diapause. We found no evidence of metabolic suppression during fall, indicating it might occur earlier in the *C. fumiferana* life cycle than previously thought. Longer fall exposures resulted in larvae surviving lower temperatures during the winter. Longer and warmer falls lead to larvae with increased time to pupation.

#### **Assessing non-invasive methods for determining larval instars in the buff-tailed bumble bee**

Fievet, M. <sup>1,2\*</sup>, Gekière, A. <sup>2\*</sup>, Michez, D. <sup>2</sup>, Tougeron, K. <sup>1</sup>

<sup>1</sup>Ecology of Interactions and Global Change, Research Institute in Biosciences, University of Mons, Mons, Belgium. <sup>2</sup>Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium

*antoine.gekiere@umons.ac.be*

Bees are critical for pollination services but are threatened by numerous anthropogenic stressors, including agrochemical exposure. While the impacts of these stressors on adult bees have been extensively studied, their effects on bee larvae remain poorly understood. So far, the only known method to measure larval stage (i.e., head capsule measurement) is invasive and increases the risk of larval mortality. To address this limitation, we evaluated three non-invasive methods to determine larval instar (i.e., measuring larval body mass, measuring larval body area, and extrapolating instars from larvae of a same batch). However, neither larval body mass nor larval body area appeared to cluster larvae into distinct instar stages. Additionally, larvae from the same batch did not consistently exhibit uniform instars, highlighting the risks of batch- based extrapolation. Although no fully reliable non-invasive methods were established, we encourage future research to include larval body mass or larval body area in their analyses.

#### **Heat Shock in the Cold: Investigating the functional role and metabolic cost of the chaperone response in overwintering Colorado potato beetles**

van Oirschot, M, L. <sup>1\*</sup>, Lebenzon, J, E. <sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Calgary, Calgary, Canada

*maranda.vanoirschot@ucalgary.ca*

Overwintering insects, as ectotherms, risk sub-zero temperature-induced protein damage. Many insects that survive low temperatures express chaperone proteins, including heat shock proteins (HSPs), which could mitigate protein damage via ATP-dependent protein refolding. However, many overwintering insects suppress their metabolic rate when they enter diapause, a form of dormancy. The question remains whether diapausing insects achieve costly HSP-mediated protein repair in a hypometabolic state. The Colorado potato beetle (*Leptinotarsa decemlineata*, CPB), a potato pest, overwinters in diapause as a freeze-avoidant adult. Previously we found that cold-tolerant, diapausing CPB increased expression of HSP-68 and its co-factor DnaJ in their fat body, a metabolically important insect tissue, suggesting they require protein protection during the winter. The goal of this project is to determine if expression of cellular chaperones HSP-60 and DnaJ protect CPB against protein damage and assess the metabolic cost of this repair during cold shock recovery. We will compare protein damage after cold shock and recovery in two winter phenotypes: Cold-tolerant (CT) and diapausing (D) CPB. CT beetles can survive lower temperatures and express HSPs to a greater extent compared to D beetles. We predict that CT beetles have higher metabolic rates and less protein damage than D beetles following the cold shock due to increased expression and activation of HSP-60 and DnaJ. This research will improve predictions of insect responses to climate change, inform pest control strategies, and provide insights into chaperone response regulation.

### **Metabolic recovery from single and repeated heat exposures in an intertidal barnacle**

Josh C. C. Yang<sup>1\*‡</sup>, Benny K. K. Chan<sup>1</sup>, Jeffrey G. Richards<sup>2</sup>, Katie E. Marshall<sup>2</sup>

<sup>1</sup>Biodiversity Research Center, Academia Sinica, Taipei, Taiwan. <sup>2</sup>Zoology, UBC, Vancouver, BC, Canada

*josh.yang@ubc.ca*

Heat waves can severely impact intertidal animals as they contend with intense fluctuations in their environment during tidal cycles. Heat stress is especially high during emersion at low tides and repeated exposures can occur over successive low tides during prolonged heat waves. Currently, most studies focus only on single heat events and those examining repeated heat stress often do not control for total stress duration. The energetic costs after thermal stress may reflect organismal fitness and future resilience to a warming world. To compare the relative energetic costs of repeated and single exposures, we held the total period of heat stress constant. Using intermittent flow respirometry in water, we measured oxygen consumption rate (proxy for metabolic rate) of the barnacle, *Fistulobalanus albicostatus*, from mangrove forests of Taiwan before and after single or repeated heat exposures during emersion. Barnacle MO<sub>2</sub> after single and repeated heat stress was initially depressed but gradually increased during recovery. Recovery was faster after a single heat stress exposure. By contrast, after emersion without heat stress, there was minimal change in MO<sub>2</sub>. The initial depression in MO<sub>2</sub> after heat stress may be a transient metabolic suppression to limit oxidative damage. The gradual increase in aerobic metabolism may reflect a transition from metabolic suppression to energetically costly repair. However, anaerobic metabolism was unaccounted for and we plan to use NMR

metabolomics to identify anaerobic by-products to obtain a holistic view of metabolism during stress and recovery to understand energy allocation during and recovery from thermal stress.