## THURSDAY, OCTOBER 25TH, 2018

### Plenary Lecture

**1.0 PLENARY LECTURE**  
*SPONSORED BY THE JOURNAL OF EXPERIMENTAL BIOLOGY*

**Thur., 5:00 - 6:00 PM, Astor Ballroom**

- **5:00 PM**  
  **1.1** Tesla Valves and Other Fluidic Devices in Reptile Lungs  
  **CG Farmer. Univ. of Utah**

- **6:00 PM**  
  **OPENING RECEPTION**  
  *Thur., 6:00 - 8:00 PM, Grand Gallery*

## FRIDAY, OCTOBER 26TH, 2018

### Oral

**2.0 HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION – 1**

**Fri., 9:00 - 10:30 AM, Astor Ballroom I & II**

**Chair:**  
**Frank van Breukelen. Univ. of Nevada**

- **9:00 AM**  
  **2.1** Extreme Physiological Plasticity in a Hibernating Basoendothermic Mammal, *Tenrec Ecaudatus*  
  **Frank van Breukelen. Univ. of Nevada**

- **9:30 AM**  
  **2.2** Hibernation and Daily Torpor in *Neotamias Cinereicollis*  
  **Ana Fabio Braga. Northern Arizona Univ.**

- **9:45 AM**  
  **2.3** Biologging and Endocrinology: Tools to Understand the Physiological Limits of Free-Living Arctic Ground Squirrels  
  **Victor Zhang. Northern Arizona Univ.**

- **10:00 AM**  
  **2.4** Anoxia-Reoxygenation Does Not Alter Mitochondrial Function in Ground Squirrels During Hibernation  
  **Leah Hayward. Univ. of Western Ontario**

- **10:15 AM**  
  **2.5** Effects of PH and Temperature on Blood Oxygen Transport in Hibernating and Non-Hibernating Rodents  
  **Anne B. Kim. Univ. of British Columbia**

### Oral

**3.0 CARDIOVASCULAR: ABSTRACT DRIVEN SESSION – 1**

**Fri., 9:00 - 10:30 AM, Astor Ballroom III**

**Chair:**  
**Todd Gillis. Univ. of Guelph**

- **9:00 AM**  
  **3.1** Powering a Zombie Heart: Metabolic Fuel Utilization in the Excised Hagfish Heart During Anoxia Exposure  
  **Todd Gillis. Univ. of Guelph**
9:30 AM  3.2  The Functional Significance of Plasma-Accessible Carbonic Anhydrase for Cardiovascular Oxygen Transport in Teleosts  
T. S. Harter.  Univ. of British Columbia

9:45 AM  3.3  Convergent Evolution of Reduced Temperature Dependent Hemoglobin-Oxygen Affinity in Regionally Endothermic Fishes  
Phillip R. Morrison.  Univ. of British Columbia

10:00 AM  3.4  Hemoglobin Adaptations to High Altitude Augment Arterial O₂ Saturation in Hypoxia but not Aerobic Capacity in Deer Mice (Peromyscus Maniculatus)  
Oliver Wearing.  McMaster Univ.

10:15 AM  3.5  Cardiac Performance of Juvenile Red Drum (Sciaenops Ocellatus) During Acute Hypoxia and the Effect Following Crude Oil Exposure.  
Derek Nelson.  Univ. of North Texas

Oral
4.0  OSMOREGULATION: ABSTRACT DRIVEN SESSION – 1

Fri., 9:00 - 10:30 AM, Toulouse A & B

Chair:  Ana Lyons.  Univ. of California, Berkeley

9:00 AM  4.1  Role of the Aquaporin Gene Family in Conferring Tolerance to Multiple Environmental Stressors in Tardigrades  
Ana Lyons.  Univ. of California, Berkeley

9:15 AM  4.2  The Septate Junction Protein Mesh Is Required for the Form and Function of Drosophila Malpighian Tubule  
Sima Jonusaite.  Univ. of Utah

9:30 AM  4.3  The Impact of Salt Contaminated Freshwater on the Physiology of the Rectum and Malpighian Tubules of Mayfly (Hexagenia Rigida) Nymphs  
Fargol Nowghani.  York Univ.

9:45 AM  4.4  A Novel Technique for Measuring Hindgut Reabsorption in Drosophila Reveals Adaptive Differences Between Species with Different Thermal Tolerance  
Mads Kuhlmann Andersen.  Aarhus Univ.

Oral
5.0  DEVELOPMENTAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION

Fri., 9:00 - 10:30 AM, St. Charles Ballroom

Chair:  Jason Breves.  Skidmore College

9:00 AM  5.1  Cortisol and Estrogenic Compounds Modulate Insulin-Like Growth-Factor Binding Protein Gene Expression During Vulnerable Life Stages of Atlantic Salmon  
Jason Breves.  Skidmore College

9:30 AM  5.2  Intestinal Hydrolase Transcriptional Responses During Rapid Diet Adjustment in Nestling House Sparrows (Passer Domesticus)  
William Karasov.  Univ. of Wisconsin-Madison
<table>
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<tr>
<th>Time</th>
<th>Session</th>
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<th>Presenter</th>
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<tbody>
<tr>
<td>9:45 AM</td>
<td>5.3</td>
<td>Developmental Variation in Embryos of Two Killifish Populations</td>
<td>Lindsey Daniel</td>
<td>Univ. of North Texas</td>
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<td>10:00 AM</td>
<td>5.4</td>
<td>Transgenerational Epigenetic Inheritance Induced by the Combined Exposure to Crude Oil and Hypoxia in the Zebrafish</td>
<td>Naim Martinez</td>
<td>Univ. of North Texas</td>
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<td>10:15 AM</td>
<td>5.5</td>
<td>Hypoxic Incubation Has No Effect on Permeabilized Cardiac Muscle Mitochondrial Oxygen Flux or ROS Production in the American Alligator</td>
<td>Edward Dzialowski</td>
<td>Univ. of North Texas</td>
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<td>10:30 AM</td>
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<td>COFFEE BREAK</td>
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<td>11:00 AM</td>
<td>6.0</td>
<td>CONNECTING GENOMES TO PHENOMES TO POPULATIONS</td>
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<td>COSPONSORED BY THE AMERICAN PHYSIOLOGICAL SOCIETY AND THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY</td>
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<td>Fri., 11:00 AM -1:00 PM, Astor Ballroom I &amp; II</td>
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<td></td>
<td>Chair: Allyson Hindle. Massachusetts General Hospital</td>
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<td>11:00 AM</td>
<td>6.1</td>
<td>Sex Dependent Phenological Plasticity in an Arctic Hibernator</td>
<td>C. Loren Buck</td>
<td>Northern Arizona Univ.</td>
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<td>11:30 AM</td>
<td>6.2</td>
<td>Insights Into Mutational Pathways of Biochemical Adaptation Using Ancestral Protein Resurrection</td>
<td>Jay Storz</td>
<td>Univ. of Nebraska</td>
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<td>12:00 PM</td>
<td>6.3</td>
<td>The Genomic and Physiological Basis of Rapid Adaptation to Temperature in a Globally Invasive Crab</td>
<td>Carolyn Tepolt</td>
<td>Woods Hole Oceanographic Institution</td>
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<td>12:30 PM</td>
<td>6.4</td>
<td>Modeling Photoperiodism in Subterranean Rodents</td>
<td>Gisele Oda</td>
<td>Universidade de São Paulo, Instituto de Biociências</td>
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<td>11:00 AM</td>
<td>7.0</td>
<td>VERTEBRATE ENERGETICS: ABSTRACT DRIVEN SESSION</td>
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<td>Fri., 11:00 AM-12:30 PM, St. Charles Ballroom</td>
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<td>Chair: Matthew Pamenter. Univ. of Ottawa</td>
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<td>11:00 AM</td>
<td>7.1</td>
<td>Digging Up the Evolutionary Origins of Hypoxia-Tolerance: Physiological Adaptations to Acute Hypoxia in 9 Species of African Mole Rats</td>
<td>Matthew Pamenter</td>
<td>Univ. of Ottawa</td>
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<td>12:00 PM</td>
<td>7.3</td>
<td>The Cost of Good Parenting: Altered Maternal Care in High Altitude Deer Mice, <em>Peromyscus Maniculatus</em></td>
<td>Cayleigh Robertson</td>
<td>McMaster University</td>
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DAILY SCHEDULE

12:15 PM 7.4 Colonizing High Altitude Hypoxic Environments: Strategies to Deal with Metabolic Needs
Christian Arias-Reyes. Institut universitaire de cardiologie et de pneumologie de Québec, Centre Hospitalier Universitaire de Québec (CHUQ), Faculty of Medicine, Université Laval

Oral

8.0 VENTILATORY FUNCTION: ABSTRACT DRIVEN SESSION

Fri., 11:00 AM-1:00 PM, Toulouse A & B

Chair: Mark Bayley. Aarhus Univ.

11:00 AM 8.1 Learning to Air Breathe; the First Steps
Mark Bayley. Aarhus Univ.

11:30 AM 8.2 Characterizing the Branchial Hypercarbia Recovery Mechanisms Following Extreme Hypercapnia in the Highly CO₂ Tolerant Hagfish
Greg Goss. Univ. of Alberta

12:00 PM 8.3 Developmental Changes in the Ventilatory Response to CO₂ in Semi-Fossorial Mammals
Ryan Sprenger. Univ. of British Columbia

12:15 PM 8.4 Haemoglobin Adaptations to High Altitude Alter Breathing Pattern in Deer Mice (Peromyscus Maniculatus)
Catherine Ivy. McMaster Univ.

12:30 PM 8.5 The Effect of Chronic Hypercapnic Incubation on Breathing Patterns in American Alligator (Alligator Mississipiensis)
Justin Conner. Univ. of North Texas

12:45 PM 8.6 Effects of Gravidity on Grasshopper Oxygen Delivery
Scott Kirkton. Union College

Oral

9.0 THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION -1

Fri., 11:00 AM-12:45 PM, Astor Ballroom III

Chair: John VandenBrooks. Midwestern Univ.

11:00 AM 9.1 Oxygen Limitation of Thermal Tolerance Varies Depending on the Life Stage and Behavior of Terrestrial Organisms
John VandenBrooks. Midwestern Univ.

11:30 AM 9.2 Ultra-Violet B Radiation, the Often Neglected Ubiquitous Environmental Stressor in Aquatic Environments
Craig Franklin. The Univ. of Queensland

12:00 PM 9.3 Understanding the Effects of Food Availability, Thermal Tolerance, and Sirtuin Activity on the Feeding Physiology of Mytilus Californianus
Melissa May. California Polytechnic State Univ.

12:15 PM 9.4 A Distal Bat Wing Muscle Operates at Low Temperature in Vivo, and Has Low Thermal Sensitivity of Contractile Properties
Andrea Rummel. Brown Univ.
12:30 PM  9.5  Assessing Summertime Thermoregulatory Properties Across the Pelage Molt in a Polar Pinniped: The Weddell Seal  
Skyla Walcott. Univ. of Alaska, Anchorage

1:00 PM  LUNCH ON YOUR OWN

10.0  CANCELED

Concurrent  
11.0  COMPARATIVE INSIGHTS INTO ANIMAL RESPONSES TO HYPOXIA AND ANOXIA

Fri., 2:00 – 4:00 PM, Astor Ballroom I & II  
Chair: Jon Harrison. Arizona State Univ.

2:00 PM  11.1  Role of the Mitochondrion in Low Oxygen Signalling in the Painted Turtle. Leslie Buck. Univ. of Toronto

2:30 PM  11.2  Re-Oxygenation Resilience - The Other Aspect of the Crucian Carp's Anoxia Tolerance Sjannie Lefevre. Univ. of Oslo

3:00 PM  11.3  Learning from the Experts: How Marine and Freshwater Bivalves Cope with Anoxic Transgression Doris Abele. Alfred Wegener Institute for Polar and Marine Research

3:30 PM  11.4  Flies Are Not Turtles or Carp: Non-Conventional Anoxia Tolerance Jacob Campbell. USDA-ARS

Concurrent  
12.0  MICRORNAS IN COMPARATIVE AND EVOLUTIONARY PHYSIOLOGY

Fri., 2:00 – 4:00 PM, St. Charles Ballroom  
Chair: Julie Reynolds. Ohio State Univ.

2:00 PM  12.1  For Everything There Is a Season: MicroRNA Regulation of Insect Diapause Julie Reynolds. Ohio State Univ.

2:15 PM  12.2  The Role of MiRNA Regulation on Phenotypic Responses to Environmental Stressors in Fish Paul Craig. Univ. of Waterloo

2:30 PM  12.3  A Cool Story: Non-Coding RNAs in Natural Models of Cold Adaptation Pier Jr Morin. Université de Moncton

3:00 PM  12.4  Regulation of MicroRNA Activity to Promote Multipotent Cell Fate During Dauer Diapause Xantha Karp. Central Michigan Univ.


Concurrent  
13.0  THE ROLE OF GASOTRANSMITTERS IN HYPOXIC AND CHALLENGING ENVIRONMENTS

Fri., 2:00 – 4:00 PM, Toulouse A & B  
Chair: Michael Tift. Univ. of California, San Diego
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<th>Time</th>
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<tr>
<td>2:00 PM</td>
<td>13.1</td>
<td>Introduction to Gasotransmitters and the Role of Carbon Monoxide (CO) in Hypoxia-Tolerant Species</td>
<td><strong>Anthony Signore.</strong> <em>Univ. of Nebraska, Lincoln</em></td>
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<td>2:30 PM</td>
<td>13.2</td>
<td>Roles of NO and H₂S Signaling in Hibernators</td>
<td><strong>Angela Fago.</strong> <em>Aarhus Univ.</em></td>
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<td>3:00 PM</td>
<td>13.3</td>
<td>Carbon Monoxide Signaling in the Control of Breathing and Impacts for High-Altitude Adaptation</td>
<td><strong>Erica Heinrich.</strong> <em>Univ. of California, San Diego</em></td>
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<td>3:30 PM</td>
<td>13.4</td>
<td>Hydrogen Sulfide and Oxygen Sensing: From Evolution to Function</td>
<td><strong>Kenneth Olson.</strong> <em>Indiana U Sch Medicine South Bend</em></td>
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**Concurrent 14.0**

**THE ROLE OF THERMAL PERFORMANCE CURVES IN PHYSIOLOGY, ECOLOGY AND CONSERVATION**

*Sponsored by the Society of Experimental Biology*

Fri., 2:00 – 4:00 PM, Astor Ballroom III

**Chair:** **Johannes Overgaard.** *Aarhus Univ.*

- **2:00 PM 14.1** The Thermal Performance Curve: Mechanisms, Applications, and Pitfalls for a Concept that Just Turned 40 (Happy Birthday!)
  - **Michael Angilletta.** *Arizona State Univ.*

- **2:30 PM 14.2** Translating Physiology to Fitness Using Thermal Performance Curves
  - **Timothy Clark.** *Deakin Univ.*

- **3:00 PM 14.3** Assessing the Role of Acclimation and Adaptation in Thermal Performance Curves
  - **Johannes Overgaard.** *Aarhus Univ.*

- **3:30 PM 14.4** Comparing Thermal Performance Curves Across Traits: How Consistent Are They?
  - **Vanessa Kellermann.** *Monash Univ.*

**Poster 15.0**

**POSTER SESSION 1: ODD NUMBERED POSTER PRESENTATIONS**

Fri., 4:00 – 6:00 PM, Grand Ballroom

**Board #**

- **1 15.1** Mapping Key-Words in Our Journal to Predict the Future of Comparative Physiology
  - **Tobias Wang, Jens Peter Andersen, Flemming Skov.** *Aarhus Univ.*

- **3 15.2** Mechanisms Underlying Forelimb Vs. Hindlimb Function During Terrestrial Locomotion in Juvenile Alligators
  - **Adrien A. Arias, Emanuel Azizi.** *Univ. of California, Irvine*

- **5 15.3** Effect of PIT Tagging on Aerobic Metabolism and Growth of the Gulf Killifish, Fundulus Grandis
  - **Jessica Reemeyer, Brennalyn LeMaire, Bernard Rees.** *Univ. of New Orleans*

- **7 15.4** The Effects of Body Mass on Immune Cell Concentrations of Terrestrial Mammals
  - **Cynthia Downs, Ned Dochtermann, Ray Ball, Kirk Klasing, Lynn Martin.** *Hamilton College; North Dakota State Univ.; Zoo Tampa; Univ. of California, Davis; Univ. of South Florida*
15.5 Sequence Analysis, Expression, and Preliminary Functional Characterization of Aedes Aegypti Sodium-Dependent Cation-Chloride Cotransporters
Christopher M. Gillen, Grace F. Riley, John C. Crow, Adrienne C. DeBrosse, Mary E. Sawyer, Megha Kalsi, Peter M. Piermarini. Kenyon College; The Ohio State Univ.

15.6 Hypoxia Avoidance Behavior in Two Air-Breathing Fishes
Corey Jew. Univ. of California Irvine

15.7 Parasitic Infection-Associated Resemblance Between Locomotor Muscles of Dragonflies and Obese Vertebrates
Rudolf Schilder. Penn State Univ.

15.8 Beneficial Effects of Fluctuating Thermal Regimes in the Alfalfa Leafcutting Bee, Megachile Rotundata
Kendra Greenlee, George Yocum, Joseph Rinehart, Julia Bowsher. North Dakota State Univ.; USDA-ARS Red River Valley Agricultural Research Center

15.9 Intraspecific Variation in Thermal, Hypoxia and Acute High PH Tolerance in Rainbow Trout
Nicholas Strowbridge, Patricia Schulte. Univ. of British Columbia

15.10 Adenosine A1 Receptor Agonist-Induced Hibernation: Effects of Agonist and Seasons on Neuronal Pathways
Carla Frare, Mackenzie Jenkins, Kelly Drew. Univ. of Alaska, Fairbanks

15.11 Characterization of the HIF-1 Pathway in Response to an Acute Heat Stress in Antarctic Notothenioid Fishes
Anna Rix, Kristin O'Brien. Univ. of Alaska, Fairbanks

15.12 Variation in Thermoregulation and Linking Whole Organism Behavior to Thermosensory Neurophysiology in the Porcelain Crab, Petrolisthes Cinctipes
Emily Lam, Alex Gunderson, Brian Tsukimura, Jonathon Stillman. Univ. of California, Berkeley; Tulane Univ.; California State Univ., Fresno; San Francisco State Univ.

15.13 Bone Composition of an Elite Mammalian Diver, the Weddell Seal: Implications for the Use of Bone as a Buffer
Katrina Theiss, Allyson Hindle, Daniel Warren. St. Louis Univ.; Massachusetts General Hospital, Harvard Medical School

15.14 Identification and Characterization of a Sodium/Hydrogen Exchanger in Coral: A Potential Role in Biomineralization
Mikayla Ortega, Angus Thies, Martin Tresguerres. Scripps Institution of Oceanography, Univ. of California, San Diego

15.15 Cortisol Mediates Claudin-28b Abundance and Its Contribution to Model Gill Epithelium Barrier Properties Via the Mineralocorticoid Receptor
Dennis Kolosov, Scott Kelly. York Univ.

15.16 Osmotic Activation of Motility and Expression of Aquaporin Proteins in Sperm from the Gray Treefrog Dryophytes Chryoscelis
Deja Miller, David Goldstein, James Frisbie. Wright State Univ.

15.17 Does the Capacity for Seasonal Plasticity Differ Between Aquatic and Terrestrial Life-History Stages in the Eastern Newt (Notophthalmus Viridescens)?
Patrick Mineo, Roxanne Siuda. Elmhurst College
Plastic Plasticity: Phenotypic Plasticity at One Time Scale Changes Plasticity at Another Time Scale in Tigriopus Californicus

Timothy Healy, Ronald Burton. Univ. of California San Diego

Diversification of Characteristics Related to Endothermy in Thunnus Tunas

Barbara Block, Adam Ciezarek, Owen Osborne, Oliver N. Shipley, Edward J. Brooks, Sean Tracey, Jaime McAllister, Luke Gardner, Michael J.E. Sternberg, Vincent Savolainen. Stanford Univ.; Imperial College London; The Cape Elethera Institute; Univ. of Tasmania

Hypoxia-Induced Oxidative Stress in Fundulid Killifish

Ryan Hoffman, Brittney Borowiec, Chelsea Hess, Graham Scott, Fernando Galvez. Louisiana State Univ.; Mcmaster Univ.

Integration of Endocrinology, Behavior and Body Temperature of the South-American Tegu Lizard Salvator Merianae

Lucas Zena, Danielle Dillon, Kathleen Hunt, Carlos Navas, Kênia Bicego, C. Loren Buck. Northern Arizona Univ.; Univ. of Sao Paulo

Hot and Dry: Effects of Heat Waves and Water Limitation on Metabolic and Evaporative Water Loss Rates

Jordan Glass, Sugjit Singh, Zachary Stahlschmidt. Univ. of the Pacific

Skeletal Muscle Thermoregulation and Metabolic Control in Hibernating Arctic Ground Squirrels

Moriah Hunstiger, Jishnu Krishnan, Jace Rogers, S. Ryan Oliver. Univ. of Alaska, Fairbanks

Fat to the High-Altitude Fire: Thermoregulation in Deer Mice

Sulayman Lyons, Grant McClelland. McMaster Univ.

Modeling Energy Use of Overwintering Hatchling Turtles Using Over a Decade of Nest Temperatures

Tim Muir, Dat Tran, Lawrence Catalan, Marguerite Bednarek, Andrew Sward. Augusta College

Regulation of Muscle Pyruvate Dehydrogenase in High Altitude Deer Mice

Soren Coulson, Grant McClelland. McMaster Univ.

d-Amphetamine Exposure to Early Embryonic Zebrafish Reveal Neural and Developmental Consequences

Lisa Ganser, Brad Serpa, Jenn Bullard. Kennesaw State Univ.

Developmental Oxygen Preconditions Cardiovascular Response to Acute Hypoxic Exposure and Maximal B-Adrenergic Stimulation of Anesthetized Juvenile American Alligators (Alligator Mississippiensis)

Brando Smith, Janna Crossley, Ruth Elsey, James Hicks, Dane Crossley. Univ. of North Texas; Louisiana Wildlife & Fisheries; Univ. of California, Irvine

Chronic Crude Oil Exposure Affects Physiology and Sexual Differentiation to Zebrafish (Danio Rerio)

Kareem Vazquez Roman, Naim Bautista, Amelie Crespel, Warren Burggren. Univ. of North Texas; Univ. of Glasgow

Development and Characterization of a Primary Cultured Model of the Larval Sea Lamprey (Petromyzon Marinus) Gills

63 15.32 Participation of Orexin Receptor-1 in the Modulation of Respiratory Motor Activity in the Bullfrog (Lithobates Catebeianus)
- Centre de Recherche de l’Institut Universitaire De Cardiologie et Pneumologie de Québec; Univ. of São Paulo State

65 15.33 Does the Spotted Gar, Lepisosteus Oculatus, Express a Functional Endothelial Nitric Oxide Synthase?
Melissa Cameron, Shigehiro Karaku, Susumu Hyodo, John Donald. The Univ. of Sydney; RIKEN Center for Biosystems Dynamics Research (BDR); The Univ. of Tokyo; Deakin Univ.

67 15.34 Toxicity of Crude Oil Extracts in Chicken Embryos
Lara Amaral-Silva, Maria Rojas-Antich, Benjamin Dubanski, Hiroshi Tazawa, Warren Burggren. Univ. of North Texas

69 15.35 Cardiac Proteome Changes in the Western Painted Turtle in Response to Cold Acclimation and Anoxia
Claire Riggs, Daniel Warren. Saint Louis Univ.

71 15.36 Biotinylation of Elephant Seal Blood to Determine RBC Lifespan and Total Blood Volume
Robby Boparai, Christina Blaul, Daniel Crocker, Judy St. Leger, Todd Schmitt, Scott Johns, Mark Fuster, Tatum Simonson, Michael Tift. Univ. of California, San Diego; Sonoma State Univ.; SeaWorld

73 15.37 Linking Genotypes to Phenotypes Reveals the Underlying Mechanisms of Intestinal Brushborder Remodeling in Snakes
Stephen Secor, Blair Perry, Todd Castoe. Univ. of Alabama; Univ. of Texas, Arlington

75 15.38 Rapid Evolution of Starvation Resistance in Drosophila: Physiological and Molecular Mechanisms
Austin J. McKenna, Alaric Smith, Allen G. Gibbs. Univ. of Nevada, Las Vegas

77 15.39 Testing the Functional Consequences of Genetic Variation in Insulin-Like Growth Factor 1 (IGF1) in Lizards Via Primary Culture Experiments
Amanda Clark, Abby Beatty, Tonia Schwartz. Auburn Univ.

79 15.40 Modelling Human APOL1 Variant Related Kidney Dysfunction in Guinea Pigs
Kolawole Ajiboye, William Nabofa. Babcock Univ.

81 15.41 Metabolomic Profiles Reveal That Upregulation of Protein Degradation and Nicotinamide Pathways Are Linked with Successful Pregnancy in Weddell Seals
Michelle Shero, Amy Kirkham, Gregg Adams, Robert McCorkell, Jennifer Burns. Woods Hole Oceanographic Institution; Univ. of Alaska Anchorage; Univ. of Saskatchewan; Univ. of Calgary

83 15.42 Evolution of Thermal Tolerance in Pumpkinseed Sunfish (Lepomis Gibbosus)
Brittney Borowiec, Reem Hashem, Derek Campos, Anna Rooke, Michael Fox, Vera Almeida-Val, Graham Scott. McMaster Univ.; National Institute for Research of the Amazon; Trent Univ.

85 15.43 Calcium Transport Across the Placenta in a Placentotrophic Lizard: New Insights About Gestation
Yurany Nathaly Hernández Díaz, Francisca Leal, Martha Patricia Ramírez-Pinila. Universidad Industrial de Santander; Univ. of Florida

87 15.44 A Role for Kisspeptin Receptor in the Pituitary Gonadotroph in Male Mice
Olubusayo Awe, Yaping Ma, Sheng Wu, Andrew Wolfe. Johns Hopkins School of Medicine
### SATURDAY, OCTOBER 27TH, 2018

#### Oral 16.0 HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION – 2

**Sat., 9:00-10:30 AM, Astor Ballroom I & II**

**Chair:** [Sylvain Giroud](http://example.com) [Univ. of Veterinary Medicine Vienna](http://example.com)

- **9:00 AM** 16.1 The Effect of Lipids on Hibernation and Cardiac Function  
  **Sylvain Giroud [Univ. of Veterinary Medicine Vienna](http://example.com)**

- **9:30 AM** 16.2 A Systems Level Approach Reveals Incomplete Caspase Cascade Function During Mammalian Hibernation  
  **Michael Treat [Univ. of Nevada](http://example.com)**

- **9:45 AM** 16.3 Changes in Protein Phosphorylation and Acetylation Correspond with Suppression of Mitochondrial Metabolism During Mammalian Hibernation  
  **Katherine Mathers [Univ. of Western Ontario](http://example.com)**

- **10:00 AM** 16.4 Perineuronal Nets Cover Parvalbumin-Positive Neurons in Ground Squirrel Cerebral Cortex  
  **Christine Schwartz [Univ. of Wisconsin-La Crosse](http://example.com)**

- **10:15 AM** 16.5 The Impacts of Snow Cover Variation Across Elevation on Overwintering Montane Insects  
  **Kevin T. Roberts [Univ. of California, Berkeley](http://example.com)**

#### Oral 17.0 MITOCHONDRIAL BIOLOGY: ABSTRACT DRIVEN SESSION

**Sat., 9:00-10:30 AM, Toulouse A & B**

**Chair:** [Daniel Munro](http://example.com) [Univ. of Ottawa](http://example.com)

- **9:00 AM** 17.1 Digging Up the Mitochondrial Origins of Hypoxia-Tolerance in African Mole Rats  
  **Daniel Munro [Univ. of Ottawa](http://example.com)**

- **9:30 AM** 17.2 A Comparative Analysis of Mitochondrial Supercomplexes in Vertebrates  
  **Amanda Bundgaard [Aarhus Univ.](http://example.com)**

- **9:45 AM** 17.3 The Influence of Thyroid Hormone Manipulation on Cardiac Muscle Mitochondrial Function in Developing Chickens  
  **Jessica Rippamonti [Univ. of North Texas](http://example.com)**

- **10:00 AM** 17.4 Metabolic Underpinnings of Life History Allocations: Mitochondrial Function Is Fine-Tuned to Meet Divergent Energetic Demands in Two Species of Wing-Polymorphic Crickets  
  **Lisa A. Treidel [Univ. of California, Berkeley](http://example.com)**

- **10:15 AM** 17.5 Cellular Metabolism and Oxidative Stress as a Possible Determinant for Longevity in Small Breed and Large Breed Dogs  
  **Ana Jimenez [Colgate Univ.](http://example.com)**

#### Oral 18.0 OMIC RESPONSES TO STRESS: ABSTRACT DRIVEN SESSION

**Sat., 9:00-10:30 AM, St. Charles Ballroom**

**Chair:** [Wes Dowd](http://example.com) [Washington State Univ.](http://example.com)
9:00 AM 18.1  Physiological Mean-Variance Relationships Among Intertidal Mussels Depend on Environmental Context  
**Wes Dowd. Washington State Univ.**

9:30 AM 18.2  Transcriptomic Responses to Low Salinity Among Locally Adapted Populations of Olympia Oyster, an Estuarine Foundation Species  
**Tyler Evans. California State Univ. East Bay**

9:45 AM 18.3  Unexpected Natural Modification of Mt-DNA Alters Centenarian Bivalve Physiology and Ecology  
**Doris Abele. Alfred Wegener Institute for Polar and Marine Research**

10:00 AM 18.4  Integrating the Effects of Food Availability and Sirtuins on Stress Tolerance to Multiple Levels of Biological Organization  
**Lars Tomanek. California Polytechnic State Univ.**

10:15 AM 18.5  Evolution of Higher Rate of Living Leads to Enhanced Inducibility of Gene Expression: Evidence from Heat Shock Genes in Drosophila  
**Josh Alpern. Queens Univ.**

**Oral**

**19.0  ENERGETICS: ABSTRACT DRIVEN SESSION**

**Sat., 9:00-10:30 AM, Astor Ballroom III**

**Chair:**  **Timothy Healy. Univ. of California San Diego**

9:00 AM 19.1  Polygenic Mapping Reveals Genetic Associations with Variation in Routine Metabolic Rate in Fundulus Heteroclitus  
**Timothy Healy. Univ. of California San Diego**

9:30 AM 19.2  Evolutionary Variation in Hypoxia Tolerance in Fundulidae Killifishes  
**Brittney Borowiec. McMaster Univ.**

9:45 AM 19.3  Protein Turnover: A Biochemical Basis for Endogenous Variation in Growth and Energy Metabolism  
**Scott L. Applebaum. Univ. of Southern California**

10:00 AM 19.4  Bioenergetics of Protein Metabolism Under Experimental Environmental Change  
**Francis Pan Univ. of Southern California**

10:15 AM 19.5  Dietary Antioxidants and Flight Exercise Affect the Extent to Which Antioxidants are Delivered to the Mitochondria and How Female Birds Allocate Nutrients to Eggs  
**Scott McWilliams. Univ. of Rhode Island**

10:30 AM  **COFFEE BREAK**

**Concurrent**

**20.0  EVOLUTION OF PHENOTYPIC PLASTICITY IN PHYSIOLOGICAL SYSTEMS**  
**SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY**

**Sat., 11:00 AM-1:00 PM, Astor Ballroom I & II**

**Chairs:**  **Graham Scott. McMaster Univ.**  
**Anne Dalziel. Université Laval**
11:00 AM  20.1  Mechanisms Underlying Thermal Acclimation and Their Evolution
Frank Seebacher.  Univ. of Sydney

11:30 AM  20.2  Evolution of the Acclimation Responses to Hypoxia and Cold in Deer Mice Native to High Altitudes
Graham Scott.  McMaster Univ.

12:00 PM  20.3  Cold Adaptation Drives Evolution of Metabolic Plasticity in Drosophila Melanogaster
Caroline Williams.  Univ. of California, Berkeley

12:30 PM  20.4  Evolution of Osmoregulatory Flexibility During Transitions Between Marine and Freshwater Habitats in Fishes
Andrew Whitehead.  Univ. of California Davis

Oral
21.0  CARDIOVASCULAR: ABSTRACT DRIVEN SESSION – 2

Sat., 11:00 AM-1:00 PM, Astor Ballroom III

Chair:  Hans Malte.  Aarhus Univ.

11:00 AM  21.1  Assessing the Full Significance of the Bohr/Haldane Effect for Gas Exchange in the Tissues
Hans Malte.  Aarhus Univ.

11:30 AM  21.2  Weddell Seals Selectively Limit Guanylyl Cyclase-Mediated Vasodilation: Implications for Perfusion of the Brain During Diving
Allyson Hindle.  Massachusetts General Hospital

12:00 PM  21.3  Developmental Differences in Anoxia-Induced Gene Expression in the Heart of the Painted Turtle
Cornelia Fanter.  St. Louis Univ.

12:15 PM  21.4  The Influence of Cellular Stretch on Extracellular Connective Tissue Deposition in Cultured Trout Cardiac Fibroblasts
Elizabeth Johnston.  Univ. of Guelph

12:30 PM  21.5  Re-Assessment of the Biochemistry of Metabolic Acidosis Using Metabolite and Reaction H⁺ Coefficients Computed from Multiple Competitive Cation Binding
Robert Robergs.  Queensland Univ. of Technology

Oral
22.0  MAMMALIAN MOLECULAR PHYSIOLOGY: ABSTRACT DRIVEN SESSION

Sat., 11:00 AM-12:30 PM, Toulouse A & B

Chair:  Jane Khudyakov.  Univ. of the Pacific

11:00 AM  22.1  Obesity-Related Gene Expression During Fasting in a Naturally Obese Marine Mammal
Jane Khudyakov.  Univ. of the Pacific

11:30 AM  22.2  The Weddell Seal Skin Transcriptome Reflects Local Mechanisms in Endocrine Regulation of Molt
Amy Kirkham.  Univ. of Alaska, Fairbanks

12:00 PM  22.3  Development of a Biomarker Panel of Chronic Stress in Free-Ranging Marine Mammals
Laura Pujade Busqueta.  Univ. of the Pacific
DAILY SCHEDULE

  Jenna Monroy. Claremont Colleges

Oral

23.0 THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION – 2

Sat., 11:00 AM-1:00 PM, St. Charles Ballroom

Chair: Suzanne Currie. Acadia Univ.

11:00 AM 23.1 Social Cues Can Push Amphibious Fish to Their Thermal Limits
  Suzanne Currie. Acadia Univ.

11:30 AM 23.2 Developmental Changes in Oxygen Consumption and Hypoxia Tolerance in the Heat- and Hypoxia-Adapted Tabasco Line of the Nile Tilapia (Oreochromis Niloticus)
  Warren Burggren. Univ. of North Texas

12:15 PM 23.4 Acute Thermal Tolerance, Not Hypoxia Tolerance, Affects the Temperature Sensitivity of Hypoxia Tolerance in Marine Fishes
  Derek Somo. The Univ. of British Columbia

12:30 PM 23.5 Critical Windows in Rainbow Trout Embryos: Effects of Thermal Shifts on Survival, Growth and Oxygen Consumption
  Christopher Melendez. California State Univ. San Marcos

1:00 PM LUNCH ON YOUR OWN

Concurrent

24.0 COMPARATIVE ASPECTS OF ACID-BASE REGULATION

Sat., 2:00 – 4:00 PM, St. Charles Ballroom

Chair: Colin Brauner. Univ. of British Columbia

2:00 PM 24.1 Evolutionary Patterns of Acid-Base Regulation in Vertebrates
  Colin Brauner. Univ. of British Columbia

2:30 PM 24.2 Evolutionarily Conserved Mechanisms for Acid-Base Sensing
  Martin Tresguerres. Univ. of California San Diego

3:00 PM 24.3 Bicarbonate-Sensing Soluble Adenylyl Cyclase in Fishes
  Jinae Roa. Univ. of British Columbia

3:30 PM 24.4 The Alkaline Tide: Acid-Base Regulation During Digestion
  Tobias Wang. Aarhus Univ.

Concurrent

25.0 EVOLUTION OF METABOLIC PROTEINS

Sat., 2:00 – 4:00 PM, Astor Ballroom III

Chairs: Chris Moyes. Queen’s Univ.
  Jeffrey Richards. Univ. of British Columbia
2:00 PM 25.1 Molecular Evolution of Cytochrome C Oxidase in Hypoxia Tolerant Fish
Gigi Lau. Univ. of Oslo

2:30 PM 25.2 Function and Evolution of Cellulase and Hemicellulase Enzymes Within Invertebrates That Do Not Consume Significant Amounts of Plant Cellulose
Stuart Linton. Deakin Univ.

3:00 PM 25.3 Evolutionary Phylogenomics of UCP1 and Sarcolipin: Key Players Underlying Adaptive Thermogenesis Across Eutheria?
Kevin L. Campbell. Univ. of Manitoba

3:30 PM 25.4 Evolution of Metabolic Proteins: Pyruvate Dehydrogenase in Anaerobiosis
Michael Berenbrink. Liverpool Univ.

Concurrent

26.0 HARNESSING NATURALLY EVOLVED TORPOR TO BENEFIT HUMAN SPACEFLIGHT
CO-SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

Sat., 2:00 – 4:00 PM, Astor Ballroom I & II

Chairs: Hannah Carey. Univ. of Wisconsin-Madison
Matthew Regan. Univ. of Wisconsin-Madison

2:00 PM 26.1 Enhancing Metabolic Flexibility in Humans: Insights from Hibernation to Benefit Spaceflight
Hannah V. Carey. Univ. of Wisconsin-Madison

2:30 PM 26.2 Central Mechanisms of Torpor Induction
Matteo Cerri. Univ. of Bologna

3:00 PM 26.3 The Relationship Between Sleep and Torpor
Vladyslav Vyazovskiy. Univ. of Oxford

3:30 PM 26.4 The Spaceflight Environment
Jessica Meir. NASA

Concurrent

27.0 PHYSIOLOGY FROM THE NEOTROPICS: RHYTHMS, TEMPERATURE AND SEASON

Sat., 2:00 – 4:00 PM, Toulouse A & B

Chairs: Kenia Cardoso-Bicego. Sao Paulo State Univ.
Luciane Gargaglioni. Sao Paulo State Univ.

2:00 PM 27.1 Seasonal Physiology of a Hibernating and Facultative Endothermic Lizard
Kenia Bicego. Sao Paulo State Univ.

2:30 PM 27.2 Orexin in Ectotherms: Modulatory Role on Ventilation
Luciane H Gargaglioni. Sao Paulo State Univ.

3:00 PM 27.3 Temperature Effects on Cardiorespiratory Function in Amphibians and the Aplication of a Non-Invasive Methodology
Lucas Zena. Univ. of São Paulo

3:30 PM 27.4 Plasticity of 24h Body Temperature Rhythms in a South American Subterranean Rodent
Patricia Tachinardi. Univ. of São Paulo
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<thead>
<tr>
<th>Poster</th>
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<tr>
<td>28.0</td>
<td><strong>POSTER SESSION 2: EVEN NUMBERED POSTER PRESENTATIONS</strong></td>
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<td><strong>Sat., 4:00 – 6:00 PM, Grand Ballroom</strong></td>
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<td>2</td>
<td>28.1 On the Dynamics of Actomyosin Binding</td>
<td>Dean Culver. <em>Army Research Laboratory</em></td>
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<td>4</td>
<td>28.2 Effect of Substrate Compliance on Coordinated Landing in Rhinella Marina</td>
<td>Alex Duman, Emanuel Azizi. <em>Univ. of California, Irvine</em></td>
</tr>
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<td>6</td>
<td>28.3 The Interaction of Incompressible Fluid and Extracellular Connective Tissues in Lobster Muscle</td>
<td>David Sleboda, Caroline Wolek, Thomas Roberts. <em>Brown Univ.</em></td>
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<td>8</td>
<td>28.4 Effects of Passive Integrated Transponder Tagging on Cortisol Release by the Gulf Killifish Fundulus Grandis</td>
<td>Jasmine Harris, Ariel Hernandez, Bernard Rees. <em>Univ. of New Orleans</em></td>
</tr>
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<td>10</td>
<td>28.5 On the Role of the Visual and Vestibular Systems in Stabilising Perching in Zebra Finches</td>
<td>Natalia Perez-Campanero, David Perkel, Graham Taylor. <em>Univ. of Oxford; Univ. of Washington</em></td>
</tr>
<tr>
<td>14</td>
<td>28.7 A Comparison of Thermal Performance Among Latitudinally Separated Populations of the Intertidal Barnacle Balanus Glandula</td>
<td>Sarah Gilman, Gordon Ober, Rhianon Rognstad, Maddy Bunnenberg-Ross, Juanita Man. <em>Scripps College; Claremont McKenna College; Pitzer College</em></td>
</tr>
<tr>
<td>16</td>
<td>28.8 Active and Passive Energetics of Thermoregulation from Thermoconformity to Partial Thermoregulation</td>
<td>Adam Parlin, Asgeir Bjarnason, Paul Schaeffer. <em>Miami Univ.; Star Oddi</em></td>
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<td>18</td>
<td>28.9 Clocks and Meals Keep Mice from Being Cool</td>
<td>Steven Swoap, Vincent Van der Vinne, Mark Bingaman, David Weaver. <em>Williams College; Univ. of Massachusetts Medical School</em></td>
</tr>
<tr>
<td>20</td>
<td>28.10 Expression of TRP Channels in Notothenioid Fish</td>
<td>Julia York, Harold Zakon. <em>Univ. of Texas at Austin</em></td>
</tr>
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<td>24</td>
<td>28.12 Stage-Specific Oxygen Limitation of Thermal Tolerance in Schistocerca Cancellata</td>
<td>Jacob P. Youngblood, John M. VandenBrooks, Michael J. Angilletta Jr. <em>Arizona State Univ.; Midwestern Univ</em></td>
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<tr>
<td>26</td>
<td>28.13 The Effect of Salinity on Expression of Aquaporins 1 and 5 in the Gastric Caeca of Aedes Aegypti Mosquito Larvae</td>
<td>Elia Grieco, Lidiya Misyura, Andrew Donini. <em>York Univ.</em></td>
</tr>
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</table>
28.14 Comparative and Functional Analysis of Na\(^+\)/Ca\(^{2+}\) Exchangers Across Nematodes

28.15 Identification of the First Member of the Gap Junction Protein Family in the Protozoa Trypanosoma Cruzi, the Etiological Agent of Chagas Disease
Juan Güiza, Iván Barria, Francisco Solis, Valeska Molina, Pedro Zamorano, Jorge González, Jonathan Canan, Romina Sepulveda, Fernando González-Nil, Juan Carlos Sáez, José Luis Vega. Antofagasta Univ.; Universidad Andrés Bello; Pontifica Universidad Católica de Chile

28.16 Trade-Offs in Reproduction and Regeneration in Anolis Lizards
Abby Beatty, David Mote, Tonia Schwartz. Auburn Univ.

28.17 The Effect of Food Availability, Temperature and Sirtuin Inhibition on the Metabolic Rate of California Mussel Gill Tissue
Chessie Cooley-Rieders, Amanda Frazier, Tinh Ton That, Sarah Nancollas, Melissa May, Maria Christina Vasquez, Erin Flynn, Lars Tomanek, Anne Todgham. Univ. of California, Davis; Cal Poly San Luis Obispo; Cal Poly San Luis Obispo.

28.18 The Importance of Tidal Acclimation in Assessing the Physiological Responses of the Intertidal Crab Carcinus Maenas to Emersion
Sarah Nancollas, Iain McGaw. Univ. of California, Davis; Memorial Univ. of Newfoundland

28.19 Hypoxia and the Metabolic Phenotype in Daphnia
Kurtis Westbury, William Nelson, Christopher Moyes. Queen's Univ.

28.20 Establishing an Index of Habitat Quality and Reproductive Success for the Northern Fur Seal
Gregory Merrill, Ward Testa, Jennifer Burns. Univ. of Alaska Anchorage; NMFS Alaska Fisheries Science Center

28.21 Hypoxia Induces Differential Changes in Thermoregulation and Metabolic Rate Base on Body Size in the Bumblebee Bombus Impatiens
Sara Wilmsen, Edward Dzialowski. Univ. of North Texas

28.22 Does Individual Variation in Heat Loss Influence Thyroid and Metabolic Responses to Cold?
François Vézina, Theunis Piersma, Olivier Chastel. Université du Québec à Rimouski; NIOZ Royal Netherlands Institute for Sea Research; Centre National de la Recherche Scientifique

28.23 Does the Risk of Overheating Limit Maximum Rates of Energy Expenditure in Breeding Birds?
Gary Burness, Simon Tapper, Joe Nocera. Trent Univ.; Univ. of New Brunswick

Emily Cornelius Ruhs, Theunis Piersma, Olivier Chastel, François Vézina. Université du Québec à Rimouski; NIOZ Royal Netherlands Institute for Sea Research; Centre National de la Recherche Scientifique

28.25 Withdrawn

28.26 Depressing Mitochondrial Function During Paradoxical Anaerobism Leads to an Alcoholic Fish
Stanley Hillyard, Martin Jastrowch, Frank van Breukelen. Univ. of Nevada Las Vegas; Stockholm Univ.; Univ. of Nevada

28.27 The Impact of Developmental Hypoxia on the Cardiovascular Chemoreflex in Embryonic Snapping Turtles (Chelydra Serpentina)
Kevin Tate, John Eme, Dane Crossley. Texas Lutheran Univ.; California State Univ. San Marcos; Univ. of North Texas
Scaling of Major Organs in Hatchling Female American Alligators (*Alligator mississippiensis*)
John Eme, Cassidy Cooper, Andrew Alvo, Juan Vasquez, Sara Muhtaseb, Thomas Schmoyer, Susan Rayman, Ruth Elsey. *California State Univ. San Marcos; Louisiana Department of Wildlife and Fisheries*

Effects of δ9-Tetrahydrocannabinol (THC) on Zebrafish Embryo Development

Evolution of the Development of Respiratory Physiology in Deer Mice Native to High Altitude
Catherine Ivy, Mary Greaves, Elizabeth Sangster, Graham Scott. *McMaster Univ.*

A Legged Limitation on Insect Size? Scaling of Tracheal Systems in Scarab Beetles
Jon Harrison, Meghan Duell, Julian Wagner, Jillian Ciarlarello, C. Jaco Klok, John Vandenbrooks, J. Jake Socha. *Arizona State Univ.; Midwestern Univ.; Virginia Tech*

The Effects of a Bacterial Endotoxin LPS: Neuromuscular Junction and Cardiac Function in Fruit Fly (*Drosophila melanogaster*) and Blowfly (*Phaenicia sericata*) Larvae
Robin Cooper, Micaiah McNabb, Ogechi Anyagaligbo, Abigail Greenhalgh. *Univ. of KY.*

Examination of Predicted Cardiac Parameters Based on Ventricle Wall Thickness in the Northern Bobwhite Quail, *Colinus virginianus*
Kevin Stewart, Janna Crossley, Brandt Smith, Dane Crossley. *Univ. of North Texas*

Heart Rate and Angiogenesis in Chicken Embryos Exposed to the Environmental Contaminant TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin)
Lara Amaral-Silva, Warren Burggren. *Univ. of North Texas*

Baroreflex Changes with Body Size in the Green Iguana
Renato Filogonio, Cléo Leite. *Federal Univ. of São Carlos*

Withdrawn

Changes in the Gut Microbiota Over the Course of Gestation in Oviparous Eastern Fence Lizards (*Sceloporus undulatus*)
Brian Trevelline, Kirsty MacLeod, Tracy Langkilde, Kevin Kohl. *Univ. of Pittsburgh; The Pennsylvania State Univ.*

Comparative Analyses of Gene Expression in Snakes Yields Insight Into Conserved Mechanisms Underlying Intestinal Regeneration
Blair Perry, Stephen Secor, Todd Castoe. *Univ. of Texas Arlington; Univ. of Alabama*

Cold-Inducible RNA-Binding Protein as a Potential Regulator of Embryonic Gonadogenesis in the Red-Eared Slider Turtle
Rosario A. Marroquin-Flores, Nathan T. Mortimer, Rachel M. Bowden. *Illinois State Univ.*

Determinants of Growth in Hybrid Sunfish: Asymmetries in Expression of Maternal and Paternal Myogenin Genes
Chris Moyes, Rachel Soon-Shiong, Zhilin Chen, Shawn Garner, Bryan Neff. *Queen’s Univ.; Western Univ.*

Hydrogen Sulfide Metabolites in Tissues of Normoxic and Anoxic Freshwater Turtles (*Trachemys scripta*)
Birgitte Jensen, Christopher Kevil, Angela Fago. *Aarhus Univ.; Louisiana State Univ. Health Sciences Center*
84 28.42 Effects of Hind Limb Immobilisation and Castration on [3H]ouabain Binding Site Content and Na⁺, K⁺-ATPase Isoform Abundances in Rat Soleus Muscle
Muath Altarawneh. The Institute for Health and Sport (IHES), Victoria Univ., Melbourne, Australia

86 28.43 Preliminary Survey of Homeodomains in Lumbriculus Variegatus
Kathy Gillen, Fielding Fischer, Liana Valin. Kenyon College

88 28.44 Whole Genome De Novo Sequencing of the Atlantic and Pacific Bluefin Tuna Genomes

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**SUNDAY, OCTOBER 28TH, 2018**

**Oral 29.0 CLINICAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION**

Sun., 9:00-10:30 AM, Astor Ballroom III

Chair: Mirit Eynan. Israel Naval Medical Institute

9:00 AM 29.1 Blood Glucose Levels and Hyperbaric Pressure in SOD2 Enzyme Knockdown Mice
Mirit Eynan. Israel Naval Medical Institute

9:30 AM 29.2 A Proposed Role for the Mammalian Dive Response in Sudden Unexpected Death in Epilepsy
Jose Vega. Novant Health, Forsyth Medical Center

9:45 AM 29.3 A Comparative Study of Pulmonary Slowly Adapting Receptors Between Rabbits and Rats
Ping Liu. Univ. of Louisville

10:00 AM 29.4 Contribution of Group II Metabotropic Glutamate Receptors in the Dorsal Medullary Neuronal Groups During Hypertension Development
Julia Chu-Ning Hsu. Graduate School of Agricultural and Life Sciences, The Univ. of Tokyo

10:15 AM 29.5 The Evolving Cholecystokinin 1 Receptor as a Unique G Protein-Coupled Receptor Permanently Activated by Singlet Oxygen (GPCR-PABSO)
Zong Jie Cui. Beijing Normal Univ.

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**Oral 30.0 OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION – 1**

Sun., 9:00-10:30 AM, St. Charles Ballroom

Chair: Alexander Clifford. Univ. of Alberta

9:00 AM 30.1 Going Against the Gradient: Active NH₄⁺ Excretion by the Ammonia Tolerant Hagfish (Eptatretus Stoutii)
Alexander Clifford. Univ. of Alberta

9:30 AM 30.2 Ammonia Transporter Expression and Distribution in Organs of Caribbean Subpopulations of the Mosquito, Aedes Aegypti, Collected from Freshwater and High Ammonia Habitats
Andrea Durant. York Univ.

9:45 AM 30.3 Potential Role of a Rh Channel in Delivery of Ammonium from Coral Host Cells to Their Endosymbiotic Algae
Angus Thies. Scripps Institution of Oceanography
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
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<tr>
<td>10:15 AM</td>
<td>30.5</td>
<td>Impact of Sugar Beet De-Icing Liquid on Salt and Water Balance in Mayfly Nymph, Hexagenia Limbata</td>
<td>Laura Ana Cuciureanu</td>
<td>York Univ.</td>
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<td>Oral</td>
<td>DIGESTIVE PHYSIOLOGY: ABSTRACT DRIVEN SESSION</td>
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<td>Sun., 9:00-10:30 AM</td>
<td>31.0</td>
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<td>Matthew Regan</td>
<td>Univ. of Wisconsin-Madison</td>
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<td>9:00 AM</td>
<td>31.1</td>
<td>Exploring How a Shifting Gut Microbiome May Influence the Hibernation Phenotype</td>
<td>Matthew Regan</td>
<td>Univ. of Wisconsin-Madison</td>
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<tr>
<td>9:15 AM</td>
<td>31.2</td>
<td>Unraveling the Complexity of Seasonal Phenotypic Flexibility in Small Birds Via Omics Integration</td>
<td>Bernard W.M. Wone</td>
<td>Univ. of South Dakota</td>
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<td>9:30 AM</td>
<td>31.3</td>
<td>Withdrawn</td>
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<tr>
<td>9:45 AM</td>
<td>31.4</td>
<td>The Role of Microbial Symbionts in Bonnethead Shark Seagrass Digestion</td>
<td>Samantha Leigh</td>
<td>Univ. of California-Irvine</td>
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<td>10:00 AM</td>
<td>31.5</td>
<td>Will Abalone Survive Climate Change? Comparative Digestive Physiology and the Effect of Temperature Stress on Abalone Across the Pacific Ocean</td>
<td>Alyssa Frederick</td>
<td>UC Irvine</td>
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<td>10:15 AM</td>
<td>31.6</td>
<td>Quickly Becoming an Omnivorous Lizard: Interactions of Diet, Physiology, and Ecology Lead to Dynamic Changes in a Rapidly Evolving System</td>
<td>Beck Wehrle</td>
<td>Univ. of California, Irvine</td>
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<td>10:30 AM</td>
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<td>COFFEE BREAK</td>
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<td>Concurrent</td>
<td>CONDUCTING MECHANISTIC INVESTIGATIONS IN COMPARATIVE PHYSIOLOGY USING IN VITRO AND EX VIVO SYSTEMS</td>
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<td>Sun., 11:00 AM-1:00 PM</td>
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<td>Jose Vazquez-Medina</td>
<td>Univ. of California, Berkeley</td>
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<td>11:00 AM</td>
<td>32.1</td>
<td>Induced Pluripotent Stem Cells from 13-Lined Ground Squirrels: To Learn “hibernation” in a Dish?</td>
<td>Jingxing Ou</td>
<td>National Institutes of Health</td>
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<td>11:30 AM</td>
<td>32.2</td>
<td>Molecular Manipulations: The Power of Cell Culture for Defining Mechanisms of Anoxia Tolerance</td>
<td>Sarah Milton</td>
<td>Florida Atlantic Univ.</td>
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<tr>
<td>12:00 PM</td>
<td>32.3</td>
<td>Identifying Anti-Inflammatory Properties of Serum That Could Protect the Lungs of Deep-Diving Seals</td>
<td>Allyson Hindle</td>
<td>Massachusetts General Hospital</td>
</tr>
</tbody>
</table>
12:30 PM 32.4 Studying Natural Tolerance to Ischemia/Reperfusion Using Endothelial Cells Derived from Seals
Kaitlin Allen. Univ. of California Berkeley

Concurrent

33.0 INTEGRATING PHENOTYPES AND FUNCTIONAL GENOMICS TO UNDERSTAND MECHANISMS OF REMODELING AND GROWTH
Sun., 11:00 AM-1:00 PM, Toulouse A & B

Chairs: Todd Castoe. Univ. of Texas Arlington
Stephen Secor. Univ. of Alabama

11:00 AM 33.1 A New Perspective from Snakes on Conserved Vertebrate Stress and Growth Pathways Underlying Intestinal Regeneration
Todd Castoe. Univ. of Texas Arlington

11:30 AM 33.2 Using Natural Genomic Variation and Experimental Approaches to Understand the Function and Evolution of the Insulin and Insulin-Like Signaling Network in Reptiles
Tonia Schwartz. Auburn Univ.

12:00 PM 33.3 Transcriptome Dynamics in Hibernation: Cause or Consequence of Physiology?
Sandra L. Martin. Univ. of Colorado School of Medicine

12:30 PM 33.4 Cardiomyocyte Polyploidization Creates a Barrier to Heart Regeneration in Zebrafish
Juan Manuel González-Rosa. Harvard Univ.

Oral

34.0 THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION – 3
Sun., 11:00 AM-1:00 PM, Astor Ballroom III

Chair: Michael Dillon. Univ. of Wyoming

11:00 AM 34.1 Geographic Variation in Bumblebee Thermal Tolerance: Implications for Past and Future Range Shifts
Michael Dillon. Univ. of Wyoming

11:30 AM 34.2 Defying the Temperature Size Rule in Flight: Bigger Bees Perform Better at Higher Temperatures
Meghan E. Duell. Arizona State Univ.

11:45 AM 34.3 One for All or All for One: Emergent Thermal Physiology of Ant Colonies Along Tropical Mountain Ranges
Kaitlin Baudier. Arizona State Univ.

12:00 PM 34.4 Simultaneous Stress: Effects of Hypoxia-Temperature Interactions on Mortality, Thermal Tolerance, and Transcriptome of Drosophila Melanogaster
Leigh Boardman. Univ. of Florida

12:15 PM 34.5 Why Insects Die at Low Temperature: Depolarization Mediated Ca^{2+} Overload Causes Cell Death in Locusta Migratoria
Jeppe Bayley. Aarhus Univ.

12:30 PM 34.6 How to Assess Drosophila Heat Tolerance: Unifying Static and Dynamic Tolerance Assays to Predict Heat Distribution Limits
Lisa Bjerregaard Jørgensen. Aarhus Univ.
12:45 PM 34.7 Evolution of Body Size Toward Temperature-Dependent Oxygen Conditions in 188 Rotifer Species
   Aleksandra Walczynska. Jagiellonian Univ.

Oral
35.0 OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION – 2

Sun., 11:00 AM-12:30 PM, St. Charles Ballroom

Chair: Carol Bucking. York Univ.

11:00 AM 35.1 The Role of the Pyloric Ceca in Ion Balance in Rainbow Trout: Integrating Across Techniques to Understand Active Calcium Transport
   Carol Bucking. York Univ.

11:30 AM 35.2 Cellular Mechanism for Teleost Otolith Calcification, and Their Responses to Acid-Base Disturbances
   Garfield Kwan. Scripps Institution of Oceanography

11:45 AM 35.3 No Water, No Problem: A Metabolomics Analysis of Desiccated Annual Killifish Embryos
   Daniel Zajic. Portland State Univ.

12:00 PM 35.4 Distinct Ion Transport Properties in Airways of the Marsh Rice Rat (Oryzomys palustris)
   Leah Reznikov. Univ. of Florida

1:00 PM LUNCH ON YOUR OWN

Concurrent
36.0 ANIMAL INTESTINAL MICROBIOMES: COMMUNITY DIVERSITY AND SERVICES PROVIDED TO THE HOST

Sun., 2:00 – 4:00 PM, Toulouse A & B

Chairs: Beck Wehrle. Univ. of California, Irvine
       Brian Trevelline. Univ. of Pittsburgh

2:00 PM 36.1 It’s Not Easy Eating Green: The Importance of the Gut Microbiome in Facilitating Herbivory
   Kevin Kohl. Univ. of Pittsburgh

2:30 PM 36.2 The Enteric Microbial Communities of Sharks, Fishes, Island-Dwelling Lizards, and Abalone: Dietary and Phylogenetic Considerations
   Donovan German. Univ. of California, Irvine

3:00 PM 36.3 Host Genetic Background Contributes to Resistance to Microbiota Disruption and Host Development in an Evolution Model Organism
   Kathryn Milligan-Myhre. Univ. of Alaska, Anchorage

3:30 PM 36.4 Gut Microbial Community Dynamics in Arctic Ground Squirrels: Microbially-Liberated Urea-Nitrogen Use Across the Annual Cycle of Hibernation and Activity
   Khrys Duddleston. Univ. of Alaska, Anchorage
### Concurrent 37.0
**COMPARATIVE PHYSIOMICS: SYSTEMS-LEVEL APPROACHES TO COMPARATIVE PHYSIOLOGY**

**SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY**

**Sun., 2:00 – 4:00 PM, Astor Ballroom I & II**

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<tr>
<td>2:00 PM</td>
<td>37.1</td>
<td>Species-Specific Responses of Juvenile Rockfish to Elevated PCO₂ and Hypoxia</td>
<td>Cheryl Logan</td>
<td>California State Univ. Monterey Bay</td>
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<tr>
<td>2:30 PM</td>
<td>37.2</td>
<td>The Role of Small Noncoding RNAs in the Regulation of Metabolic Dormancy and Extreme Stress Tolerance</td>
<td>Jason Podrabsky</td>
<td>Portland State Univ.</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>37.3</td>
<td>Using Proteomics to Investigate Regulation of Stress Tolerance by Sirtuins in Mytilus Mussel Congeners</td>
<td>M. Christina Vasquez</td>
<td>Loyola Marymount Univ.</td>
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<tr>
<td>3:15 PM</td>
<td>37.4</td>
<td>Metabolic Response to Stress in Marine Mammals</td>
<td>Cory Champagne</td>
<td>National Marine Mammal Foundation</td>
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<tr>
<td>3:30 PM</td>
<td>37.5</td>
<td>From Genome to Phenome: Exploiting 13-Lined Ground Squirrel &quot;Omics&quot; to Achieve a Deeper Understanding of Hibernation</td>
<td>Katharine Grabek</td>
<td>Stanford Univ.</td>
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### Concurrent 38.0
**MANAGING FUEL METABOLISM UNDER LIMITED OXYGEN AND ENERGY SUPPLY**

**SPONSORED BY THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY**

**Sun., 2:00 – 4:00 PM, Astor Ballroom III**

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<th>Institution</th>
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<tr>
<td>2:00 PM</td>
<td>38.1</td>
<td>Metabolic Suppression Mechanisms for Fasting and Hypoxia</td>
<td>Jean-Michel Weber</td>
<td>Univ. of Ottawa</td>
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<tr>
<td>2:30 PM</td>
<td>38.2</td>
<td>Now or Later: Differential Fates for Glucose and Fructose in a Nectarivore</td>
<td>Morag Dick</td>
<td>Univ. of Toronto</td>
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<tr>
<td>3:00 PM</td>
<td>38.3</td>
<td>Feeding the Machine at the Top of the Food Chain: A Carnivore Conundrum</td>
<td>Terrie Williams</td>
<td>Univ. of California - Santa Cruz</td>
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<tr>
<td>3:30 PM</td>
<td>38.4</td>
<td>Fuelling Locomotion and Thermogenesis in High Altitude Native Deer Mice</td>
<td>Grant McClelland</td>
<td>McMaster Univ.</td>
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### Concurrent 39.0
**MECHANISMS OF CHANGE, PHYSIOLOGICAL RESPONSE TO ENVIRONMENTAL STRESSORS**

**SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY**

**Sun., 2:00 – 4:00 PM, St. Charles Ballroom**

<table>
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<tr>
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<td></td>
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<td></td>
<td>Sarah Alderman</td>
<td>Univ. of Guelph</td>
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<td>Todd Gillis</td>
<td>Univ. of Guelph</td>
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<tr>
<td>Time</td>
<td>Session</td>
<td>Title</td>
<td>Speaker</td>
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<tr>
<td>2:00 PM</td>
<td>39.1</td>
<td>Sublethal Effects and Biomarkers of Crude Oil Exposure in Anadromous Fish</td>
<td>Sarah Alderman</td>
<td>Univ. of Guelph</td>
</tr>
<tr>
<td>2:30 PM</td>
<td>39.2</td>
<td>Physiological Responses to Social Stressors</td>
<td>Kathleen Gilmour</td>
<td>Univ. of Ottawa</td>
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<tr>
<td>3:00 PM</td>
<td>39.3</td>
<td>Exploring Thermal Physiology: Effects of Environmental Temperature in Embryonic to Larval Frogs and Juvenile to Adult Copepods</td>
<td>Casey A. Mueller</td>
<td>California State Univ. San Marcos</td>
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<td>3:15 PM</td>
<td>39.4</td>
<td>Effects of Acute and Chronic Thermal Exposure on the Swimming Performance and Aerobic Scope of Sheepshead Minnows (Cyprindon Variegatus)</td>
<td>Amanda Reynolds Kirby</td>
<td>Univ. of North Texas</td>
</tr>
<tr>
<td>3:30 PM</td>
<td>39.5</td>
<td>Hypoxia and Ammonia Exposures Have Differential, Developmental-Stage Specific, and Long-Term Consequences on the Stress Response in Zebrafish</td>
<td>Nicholas Bernier</td>
<td>Univ. of Guelph</td>
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**Oral 40.0 SCHOLANDER AWARD ORAL PRESENTATIONS**

**Sun., 4:00 – 6:00 PM, St. Charles Ballroom**

**Chair:** Lynn Hartzler. Wright State Univ.

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<td>4:00 PM</td>
<td>41.0</td>
<td>The Functional Significance of Plasma-Accessible Carbonic Anhydrase for Cardiovascular Oxygen Transport in Teleosts</td>
<td>T. S. Harter</td>
<td>Univ. of British Columbia (3.2)</td>
</tr>
<tr>
<td>4:15 PM</td>
<td>41.1</td>
<td>The Septate Junction Protein Mesh is Required for the Form and Function of Malpighian Tubule</td>
<td>Sima Jonusaite</td>
<td>Univ. of Utah (4.2)</td>
</tr>
<tr>
<td>4:30 PM</td>
<td>41.2</td>
<td>Polygenic Mapping Reveals Genetic Associations with Variation in Routine Metabolic Rate in Fundulus Heteroclitus</td>
<td>Timothy Healy</td>
<td>Univ. of California San Diego (19.1)</td>
</tr>
<tr>
<td>4:45 PM</td>
<td>41.3</td>
<td>Metabolic Underpinnings of Life History Allocations: Mitochondrial Function is Fine-Tuned to Meet Divergent Energetic Demands in Two Species of Wing-Polymorphic Crickets</td>
<td>Lisa A Treidel</td>
<td>Univ. of California, Berkeley (17.4)</td>
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<tr>
<td>5:00 PM</td>
<td>41.4</td>
<td>Going Against the Gradient: Active NH4 + Excretion by the Ammonia Tolerant Hagfish (Eptatretus Stoutii)</td>
<td>Alexander Clifford</td>
<td>Univ. of Alberta (30.1)</td>
</tr>
<tr>
<td>5:15 PM</td>
<td>41.5</td>
<td>Evolutionary Variation in Hypoxia Tolerance in Fundulidae Killifishes</td>
<td>Britney Borowiec</td>
<td>Univ. McMaster (19.2)</td>
</tr>
<tr>
<td>5:30 PM</td>
<td>41.6</td>
<td>Convergent Evolution of Reduced Temperature Dependent Hemoglobin-Oxygen Affinity in Regionally Endothermic Fishes</td>
<td>Phillip R. Morrison</td>
<td>Univ. of British Columbia (3.3)</td>
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<tr>
<td>5:45 PM</td>
<td>41.7</td>
<td>The Influence of Cellular Stretch on Extracellular Connective Tissue Deposition in Cultured Trout Cardiac Fibroblasts</td>
<td>Elizabeth Johnston</td>
<td>Univ. of Guelph (21.4)</td>
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AWARDS BANQUET  
Sun., 7:00 – 9:00 PM, Astor Ballroom

Plenary Lecture

41.0  PLENARY LECTURE

Sun., 8:00 – 9:00 PM, Astor Ballroom

8:00 PM  41.1  Ecophysiology: Physiology Can Inform Ecology, and Ecology Can Inform Physiology  
Raymond Huey. *Univ. of Washington, Seattle*
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41.0 Plenary Lecture: Ecophysiology: Physiology can Inform Ecology, and Ecology Can
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The vertebrate respiratory system is one of the most diverse organ systems, but the reasons for this diversity are poorly understood. Among the most striking features of the respiratory system is the variation that exists in the conducting airways and patterns of airflow within the amniotes, especially among reptiles. The Classes Reptilia and Mammalia form a monophyletic group, the Amniota. The vast majority (79%) of the diversity of amniotes is found within the Reptilia, with birds comprising about 10,600 species and non-avian reptiles about 9,600. In mammals, the broncho-alveolar lung appears to be quite similar across all species, with a conducting airway that arborizes and tidal airflow. In contrast, the conducting airways and the path of gases within the lungs of birds and non-avian reptiles shows much greater variation due to the presence of aerodynamic valves. The lungs of crocodilians and a wide range of species of lizards have now been found to contain aerodynamic valves and one-way airflow. A preliminary study of a turtle, the red-eared slider, has revealed a diode that operates much like a Tesla valve. Given how rich in species and enormously diverse reptiles are in body form and life history, it is quite possible their lungs contain other fluid dynamic devices waiting to be discovered.

NSF IOS 1055080

2: HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION - 1

2.1

Extreme physiological plasticity in a hibernating basoendothermic mammal, Tenrec ecaudatus
Frank van Breukelen, Michael Treat
School of Life Sciences, Univ. of Nevada
Malagasy common tenrecs, Tenrec ecaudatus, have many plesiomorphic traits and may represent a basal placental mammal. We established a laboratory population of T. ecaudatus. Tenrecs exhibit extreme plasticity in thermoregulation and metabolism, a novel hibernation form, variable annual timing, rapid growth independent of age, and remarkable reproductive biology. For instance, tenrec body temperature (Tb) may approximate ambient temperature to as low as 12°C even when tenrecs are fully active. Conversely, hibernating tenrecs may have Tb of 28°C. During the active season, oxygen consumption may vary 25-fold with little or no changes in Tb. During the Austral winter, tenrecs are consistently torpid but the depth of torpor may be variable. A righting assay revealed that Tb contributes to but does not dictate activity status. Homeostatic processes are not always linked e.g. a hibernating tenrec experienced a ~34% decrease in heart rate while maintaining constant body temperature and oxygen consumption rates. This species may have as many as 32 young in a litter. Tenrecs may possess indeterminate growth and growth rates vary but young may grow ~40-fold in the 5 weeks until weaning. Despite all of this profound plasticity, tenrecs are surprisingly intolerant to extremes in ambient temperature (<8 or >34°C). We contend that while plasticity may confer numerous energetic advantages in consistently moderate environments, environmental extremes may have limited the success and distribution of plastic mammals.

Hibernation and daily torpor in Neotamias cinereicollis
Ana Fabio Braga, Loren Buck
Biology, Northern Arizona Univ.
Animals living in natural environments face seasonal variation of temperature, humidity, light, and other environmental variables. Endothermic animals are capable of producing sufficient endogenous heat which allows for constant body temperature (Tb), allowing certain degree of independence from fluctuation in environmental temperature. Nevertheless, some endotherms regulate their Tb for several hours or even weeks at a time significantly below euthermic levels. Decreased Tb lasting for less than 24h is classified as daily torpor whereas episodes lasting for more than 24h are classified as multiday torpor or hibernation. Western chipmunks (Neotamias) are represented by 23 species that inhabit very specific niches. N. cinereicollis occurs in coniferous forests at elevations between 1,950 and 3,440 meters of central and eastern Arizona, and central and southwest New Mexico. Little is known of activity patterns for this species, and it is not clear whether they display hibernation or even torpor. The aim of this study was to investigate torpor and hibernation patterns displayed by wild caught N. cinereicollis (n=6) in semi-natural conditions and relate these patterns with ambient temperature. Animals were implanted with temperature loggers (iButtons DS1922; Maxim; Dallas-TX) and kept in outdoor enclosures with water and food ad libitum from November 2017 to May 2018. During this period several environmental variables were recorded, including ambient temperature (HOB0 U30; Onset; Bourne-MA). While not torpid, Tb=36.7±1.5°C; during hibernation minimum Tb=1.55°C. Half of the animals displayed bouts of hibernation and 33% displayed only daily torpor. A regression between Tb and ambient temperature was created for each individual. The slopes were analyzed in a t-test to access their deviation from 0. The obtained slopes for Tb and ambient temperature differed from 0.
animal behavior in the context of seasonal environmental changes. Seven male and six female arctic ground squirrels were fastened with collars affixed with accelerometers and light loggers, which quantified above-ground overall dynamic body acceleration (ODBA), an index of activity-specific energy expenditure. In addition, we measured fecal cortisol metabolites (FCM) to non-invasively assess adrenocortical activity of squirrels across their active season. Female FCM concentrations were highest during the mid-lactation interval and lowest during post-lactation, while male FCM did not vary across the active season. Overall, males had higher baseline FCM levels than females across the year (male LS-mean = 6.18, SE = 0.01; female LS-mean=5.73, SE=0.09). Levels of above-ground activity were consistent with expectations of reproductive demand; activity levels of males were higher than females during the mating period and lower than in females during the mid-lactation period. Cold and wet weather, which is known to adversely affect conditions of thermal exchange, were associated with higher FCM and lower above-ground activity levels in both sexes. Furthermore, when temperatures were lowest in the early season, weather had the greatest effect on FCM concentrations, possibly due to reduced forage availability associated with early springtime in the Arctic. Lastly, we show that increases in cortisol secretion is associated with decreased above-ground activity and time spent above-ground in female, but not male, arctic ground squirrels. Our results suggest that female squirrels may respond to unpredictable environmental conditions by employing a “sit-and-wait” strategy, whereas males may have required more intense and/or prolonged environmental stressors than those observed for specific behavioral responses to become apparent. Collectively, this study furthers our understanding of how sex, reproductive state and environmental conditions may interact to influence behavioral choices, stress and physiology in free-living mammals.

Funding: This research was supported by Dr. Loren Buck’s Northern Arizona Univ. start-up package.

References:

2.4
Anoxia-reoxygenation does not alter mitochondrial function in ground squirrels during hibernation
Leah Hayward1, Katherine Mathers2, James Staples3
1Biology, Univ. of Western Ontario, 2Physiology and Pharmacology, Univ. of Western Ontario

The hibernation phenotype is associated with tolerance to models of ischemia-reperfusion in tissues (specifically
liver\(^1\) and brain\(^\text{\textsuperscript{1}}\)). Interestingly, hibernators such as the thirteen-lined ground squirrel exhibit reversible mitochondrial metabolic suppression during torpor-arousal cycling, which is characteristic of anoxia-tolerant animals\(^2\). We hypothesized that these mechanisms that allow for metabolic flexibility during hibernation also confer mitochondrial anoxia tolerance, thereby providing a mechanism for tissue ischemia-reperfusion tolerance. We isolated liver mitochondria from adult ground squirrels during the hibernation season (in torpor or interbout euthermia; IBE) and summer, as well as rats (non-hibernators). Using high-resolution respirometry and TPP\(^+\) electrodes, we compared three performance metrics (membrane potential and state 3 and 4 respiration rates using both complex I and II-linked substrates) of mitochondria before and after 5 minutes of anoxic exposure. Anoxia decreased absolute state 3 respiration rates by ~30-50% in all groups following 5 minutes of anoxia. Decreases in state 3 are paralleled by decreases in \(V_{\text{max}}\) of complex I in summer squirrels and rats, and complex II in IBE squirrels and rats, but no such decreases were observed for torpid mitochondria. Absolute state 4 respiration rates increased in all groups following anoxic exposure; however, for any given initial state 4 respiration rate, final state 4 respiration was significantly higher in summer squirrels than in winter squirrels. Membrane potential in state 4 was maintained in winter squirrels, and depolarized in summer squirrels and rats following anoxic exposure. These data suggest greater anoxia tolerance in winter squirrels, especially in torpor. We will measure mitochondrial antioxidant capacity to elucidate mechanisms underlying these differences.


This research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (Canada) and the Faculty of Science, Univ. of Western Ontario (RGPIN-2014-04860).

2.5 Effects of pH and temperature on blood oxygen transport in hibernating and non-hibernating rodents

**Anne B. Kim\(^1\), Phillip R. Morrison\(^2\), William K. Milsom\(^1\)**

\(^1\)Zoology, Univ. of British Columbia

Hibernators typically lower their body temperature to 1-2 °C above ambient temperatures during the winter. This greatly increases solubility of CO\(_2\) in the blood. Furthermore, there is a mismatch between ventilation and metabolic rate suppression during entrance into torpor that leads to retention of CO\(_2\) (hypoventilation). Together these changes result in an altered acid-base state in steady-state torpor. These changes in acid-base state and \(T_b\) influence the oxygen binding affinity of hemoglobin due to thermal sensitivity and the Bohr effect. To quantify the effects of temperature, pH change and hibernation state on blood oxygen transport, we constructed oxygen equilibrium curves (OECs) across the physiologically relevant temperature and pH ranges experienced by three species with different thermal strategies. We constructed OECs from whole blood of an obligate hibernator (13-lined ground squirrels; *Liocimyds tridecemlineatus*) during euthermia and hibernation, facultative hibernator (Golden Syrian hamsters; *Mesocricetus auratus*) during euthermia and multi-day torpor bouts and a non-hibernating rodent (Sprague Dawley rats; *Rattus norvegicus*) within their thermoneutral zone. Preliminary data suggest that the oxygen binding affinity of hemoglobin is inherently less temperature sensitive in species capable of torpor, in both euthermia and torpor. On-going experiments will allow us to elucidate how the two opposing factors of lowered temperature and lowered pH on hemoglobin’s oxygen binding affinity contribute to metabolic rate suppression during torpor by reducing oxygen supply to the tissues. This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

3: **CARDIOVASCULAR:**

3.1 Powering a zombie heart: metabolic fuel utilization in the excised hagfish heart during anoxia exposure

**Todd Gillis\(^3\), Lauren Gatrell\(^4\)**

\(^3\)Integrative Biology, Univ. of Guelph

Pacific hagfish, *Eptatretus stoutii*, can recover from 36 hours of anoxia and the systemic hearts of these animals continue to work throughout the exposure. Recent work demonstrates that glycogen stores are utilized in the *E. stoutii* heart during anoxia but that these are not sufficient to support the measured rate of ATP production. One metabolic fuel that could supplement glycogen during anoxia is glycerol. This is because glycerol can be derived from lipid stores, stored in the
heart, or delivered via the blood. The purpose of this study was to determine if glycerol could be used to fuel the heart during anoxia exposure. When excised E. stoutii hearts, perfused with metabolite free saline (mf-saline), were exposed to anoxia for 12 hours, there was no difference in heart rate, pressure generation (max-dP), rate of contraction (max-dP/dt_{sys}), or rate of relaxation (max-dP/dt_{dias}) compared to hearts perfused with mf-saline in normoxia. However, hearts perfused with saline containing glycerol (gly-saline) in anoxia had higher max-dP, max-dP/dt_{sys}, and max-dP/dt_{dias} than hearts perfused with mf-saline in anoxia. When glycerol was added to the perfusate in normoxia, there were higher levels of tissue glycerol, however, there was no such increase when glycerol was added to the perfusate in anoxia. Anoxia exposure did not cause the release of fatty acids into the perfusate or affect the activities of triglyceride lipase, glycerol kinase, or glycerol-3-phosphate dehydrogenase. Together, these results suggest that glycerol is utilized by the hagfish heart during anoxia but it is not derived from stored lipids. This work was supported by a Discovery Grant, and a Discovery Accelerator Supplement, from the Natural Sciences and Engineering Research Council of Canada to T.E. Gillis.

3.2 The functional significance of plasma-accessible carbonic anhydrase for cardiovascular oxygen transport in teleosts


1Zoology, Univ. of British Columbia, 2Ocean Sciences, Memorial Univ. of Newfoundland, 3NEUROFARBA, Univ. of Florence

A novel mechanism has recently been discovered in rainbow trout that enables an elevated partial pressure of oxygen (PO₂) in the red muscle of this species (4). Here we show the importance of the mechanism for cardiovascular O₂ transport in vivo, in salmonids and perhaps all teleosts. Most teleost species can actively regulate the intracellular pH (pHi) of their red blood cells (RBC) by adrenergically stimulated sodium-proton exchangers (β-NHE) that create H⁺ gradients across the RBC membrane. These H⁺ gradients are short-circuited in the presence of plasma-accessible carbonic anhydrase (paCA) at the tissues, creating a large arterial-venous pH shift that greatly enhances O₂ unloading from pH-sensitive hemoglobin (Hb). Thus, we tested the hypothesis that teleosts increase the O₂ capacitance of their blood (β₁) by a metabolon of RBC pH regulation and a heterogeneous distribution of paCA, which has functional significance for O₂ transport in vivo.

After β-NHE short-circuiting at the tissues, RBC pH must recover during venous transit (30-90 s) to enable renewed O₂-loading at the gills, and only then can the system enhance β₁ on a systemic level. Therefore, the halftimes (t₁/₂) and magnitudes of β-NHE stimulation, short-circuiting with paCA and recovery of RBC pHᵢ were assessed in vitro, on rainbow trout whole blood. Results indicate that: i) the t₁/₂ of β-NHE short-circuiting is likely within the time of RBC capillary transit; ii) the t₁/₂ of RBC pHᵢ recovery is 17 s and within the time of RBC venous transit; and iii) after short-circuiting RBCs re-establish the initial H⁺ gradient across the membrane. These findings are in line with a system that can sustainably enhance β₁ with every pass through the circulation.

To validate the role of paCA in cardiovascular O₂ transport in vivo, Atlantic salmon swimming at a moderate speed (50% of maximal swimming speed) were injected with C18 (a membrane-impermeable CA inhibitor) while cardiac output (Q) and the rate of O₂ consumption (ṀO₂) were recorded. The inhibition of paCA in resting or swimming fish required a compensatory increase in Q of ~30% to maintain ṀO₂ and swimming speed. At faster swimming speeds (~75% of maximal) fish collapsed after the inhibition of paCA. Therefore, the exercise performances typically observed in Atlantic salmon are not, even remotely, possible without the enhancement of β₁ that is facilitated by paCA. Furthermore, the discovery of paCA in the heart lumen of coho salmon may indicate that β-NHE short-circuiting also facilitates cardiac O₂ supply in salmonids; a critical finding in species where reproductive success depends on the ability to increase Q during spawning migrations (2).

We show, for the first time, a functional link between β-NHE and paCA activities, and the resulting increase in β₁ that reduces the requirements on the salmonid heart by about a third; the system is recruited over a wide range of conditions, including rest, exercise, recovery from exercise, and was further enhanced following acclimation to hypoxia.

It appears that all salmonids and most teleosts meet the mechanistic requirements to enhance cardiovascular O₂ transport by β-NHE short-circuiting, namely: i) pH sensitive Hbs, ii) β-NHE activity, and iii) a heterogeneous distribution of paCA (1, 3, 5). Thus, actively creating and eliminating H⁺ gradients across the RBC membrane may be an integral part of the teleost mode of cardiovascular O₂ transport, with important implications for future work on the physiology, the conservation and the evolutionary history, of nearly half of all vertebrates.

Convergent evolution of reduced temperature dependent hemoglobin-oxygen affinity in regionally endothermic fishes

**3.3**

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Several lineages of pelagic predatory fishes have independently evolved the capacity for regional endothermy. Whereas most fishes lose metabolic heat to the environment when blood thermally equilibrates with water at the gills, endothermic fishes have evolved heat exchanging retia that cool venous blood before it reaches the gills, enabling localized retention of metabolic heat. This remarkable evolutionary convergence enables tuna, billfishes, Pacific smalleye opah (*Lampris incognitus*), lamnid sharks, and common thresher shark (*Alopias vulpinus*) to maintain select tissues warmer than ambient water, favouring physiological functions that may give these predators a competitive edge over their prey. Having warm tissues also causes blood temperature to rapidly change in regionally endothermic fishes, as much as 10°C or more between the gills and cranium of the swordfish (*Xiphias gladius*), which requires hemoglobin (Hb) to maintain function over a steep temperature gradient. Adaptive changes in Hb-O2 affinity and its rapid change between the respiratory surface and the metabolizing tissues ensure matching between O2 supply and demand. Because heme-oxygenation is exothermic, increasing temperature decreases Hb-O2 affinity in most vertebrates, which could potentially disrupt O2 transport and be maladaptive in regionally endothermic fishes. However, for endothermic tunas, lamnid sharks, and marlins, the effect of temperature on Hb-O2 affinity is greatly reduced, which reduces premature Hb-O2 offloading and may be associated with the evolution of heat exchanging retia. If this is correct, we propose that all regionally endothermic fishes should possess Hbs with reduced temperature sensitivities, but this has not been thoroughly investigated in swordfish, opah, or common thresher shark. To investigate this, we examined the effect of temperature on whole blood Hb-O2 affinity in these species. Opah and common thresher shark Hb-O2 affinities were independent of temperature, but the temperature dependence of swordfish Hb-O2 affinity was pH dependent. At low blood pH (< 7.4) temperature had a negligible effect on swordfish Hb-O2 affinity, whereas at higher blood pH there was no effect of temperature below 50% saturation but increasing temperature decreased Hb-O2 affinity above 50% saturation. A reduced effect of temperature on Hb-O2 affinity is mechanistically linked to oxygenation dependent release of allosteric effectors (i.e., hydrogen ions and organic phosphates), but the relative contributions of these effectors, and potentially the evolution of novel binding sites, differs among species. We have confirmed reduced effects of temperature on the Hb-O2 affinities of three additional regionally endothermic fishes. Thus, the repeated evolution of endothermy in fishes seems to be associated with a remarkable functional convergence for a reduced effect of temperature on Hb-O2 affinity, which may facilitate a heat exchanging function for retia.

**3.4**

Hemoglobin Adaptations to High Altitude Augment Arterial O2 Saturation in Hypoxia But Not Aerobic Capacity in Deer Mice (*Peromyscus maniculatus*)

**Oliver Wearing**, **Catherine Ivy**, **Zac Cheviron**, **Jay Storz**, **Graham Scott**

1Department of Biology, McMaster Univ., 2Division of Biological Sciences, Univ. of Montana, 3School of Biological Sciences, Univ. of Nebraska

Deer mice native to high altitude have evolved higher aerobic capacity (VO2max) in hypoxia than mice from low altitude, a trait that appears to be selectively advantageous in the cold hypoxic environment at high altitude. High-altitude deer mice also possess hemoglobin (Hb) adaptations that increase O2 affinity, which could foreseeably contribute to these adaptive increases in VO2max by supporting higher arterial O2 saturation (SaO2). However, this relationship has never been tested in a way that disentangles the benefits of Hb adaptations from evolved differences in other cardiorespiratory traits. We examined this issue by hybridizing wild deer mice from high and low altitudes to produce an F1 inter-crossed population, such that the effects of distinct globin haplotypes could be assessed on an admixed genetic background. Thermogenic VO2max and several traits that underlie it (breathing, pulmonary O2 extraction, SaO2, and heart rate at VO2max) were measured before and after acclimation to hypobaric hypoxia (12 kPa O2) for 6 weeks. As predicted, mice with highland globin haplotypes had higher blood-O2 affinity and higher SaO2 in hypoxia than those with lowland haplotypes, when comparing mice that were acclimated to normoxia. However, hypoxia acclimation reduced blood-O2 affinity in mice with highland globin haplotypes, such that SaO2 was then similar between haplotypes. Surprisingly, variation in SaO2 had no effect on VO2max, and VO2max was similar across haplotypes. This suggests that Hb adaptations to high altitude do not underlie adaptive increases in VO2max, or that their adaptive benefit is contingent upon the evolution of other cardiorespiratory traits. Supported by NSERC of Canada.
3.5
Cardiac Performance of Juvenile Red Drum (Sciaenops ocellatus) During Acute Hypoxia and the Effect Following Crude Oil Exposure.
Derek Nelson¹, Andrew Esbaugh², Dane Crossley¹
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Red drum (Sciaenops ocellatus) are a near shore fish species found in the Gulf of Mexico that encounter both environmental and anthropogenic stressors. Prior studies of crude oil exposure have shown detrimental effects on multiple organisms at the level of the cardiovascular system. However, questions remain regarding the combine action of oil exposure and environmental stressors such as hypoxia. We investigate how convective oxygen transport is impacted following crude oil exposure by measuring cardiac output indices in juvenile red drum during a stepwise acute hypoxia challenge, six steps from normoxia (19.5 kPa O₂) to severe hypoxia (4 kPa O₂). Our working hypothesis was crude oil and hypoxia will result in greater negative effects of cardiovascular function that would be predicted due to a sum of these stressors alone. Baseline (n=7) heart rates during normoxia was 62 ± 2 bpm with a stroke volume of 0.97 ± 0.06 ml·kg⁻¹ resulting in a cardiac output of 57.2 ± 8.3 ml·min⁻¹·kg⁻¹. Bradycardia (56 ± 1 bpm) was pronounced at moderate hypoxia (15.5 kPa O₂) with reduced cardiac output (47.1 ± 7.2 ml·min⁻¹·kg⁻¹) as both, heart rate and cardiac output, proceeded to decrease throughout the challenge until severe hypoxia (50 ± 2 bpm, 36.9 ± 5.7 ml·min⁻¹·kg⁻¹, respectively). However, stroke volume remained constant until severe hypoxia (0.89 ± 0.06 ml·kg⁻¹). This study is ongoing and supported by the GoMRI RECOVER Consortium to A.R and D.C.

4: OSMOREGULATION: ABSTRACT DRIVEN SESSION - 1

4.1
Role of the Aquaporin gene family in conferring tolerance to multiple environmental stressors in tardigrades
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Members of the phylum Tardigrada are well known for their ability to survive a wide range of environmental extremes, yet the evolutionary history, diversity, and functional roles of tardigrade aquaporins—especially in relationship to various environmental stressors—remain unknown. Previous literature computationally identified ~10-11 putative aquaporins in three Eutardigrade species, yet no experimental work confirmed these proteins’ function in vitro or in vivo. Here, we utilize additional computational and experimental methods to characterize an expanded number of tardigrade aquaporins and aquaporin-like proteins in Hypsibius dujardini, Ramazzottius varieornatus and Milnesium tardigradum. Tardigrade proteins with E-value of 1.0e-1 were aligned via MUSCLE and manually inspected for the presence of motifs important for known aquaporin function. Our computational analysis—alongside analysis of de novo modeling of predicted candidate aquaporin structures—suggests that there may be numerous additional unannotated and uncharacterized aquaporin-like proteins in these tardigrade species, spanning a variety of functional classes. Swelling assays of heterologously expressed tardigrade candidate aquaporins, conducted in Xenopus embryos, show patterns of permeability of these proteins. In vivo inhibition of candidate tardigrade aquaporins explores the importance of these proteins in conferring tolerance to various environmental stressors (desiccation, chill coma, osmotic stress), via a suite of stress-specific phenotypic assays. This study is among the first to examine a connection between a gene family (aquaporins) present in multiple species of Eutardigrades, alongside its functional role in promoting tolerance to multiple environmental stressors.

4.2
The septate junction protein mesh is required for the form and function of Drosophila Malpigghian tubule
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Background/objective: Septate junctions (SJs) are specialized occluding cell-cell junctions that form paracellular diffusion barriers in the epithelia of invertebrates. In arthropods, there are two types of SJs: pleated SJs (pSJs) and smooth SJs (sSJs), found in ectodermally and endodermally derived epithelia, respectively. SJs are composed of transmembrane and cytoplasmic scaffolding proteins. In Drosophila, a number of pSJ-associated proteins have been identified, characterized and shown to be involved in epithelial barrier function. However, proteins of sSJs have received considerably less attention and many questions about their role in maintaining epithelial integrity and regulating paracellular solute transport remain open. Recently, an integral protein, mesh, has been identified within the sSJs of Drosophila midgut and Malpighian tubule epithelia, and shown to be required for the barrier function of the midgut. Here, we studied mesh in adult fly Malpighian tubules, which rely on regulated transepithelial ion and water transport to maintain internal homeostasis.
Methods: The GAL4-UAS system and a temperature sensitive GAL4 repressor, tub-GAL80°C, were used to achieve mesh knockdown throughout development or
during adulthood. Expression and localization of mesh and a scaffolding SJ protein, discs large (Dlg), in control and mutant tubules were determined using immunohistochemistry. Tubule ultrastructure was examined using electron microscopy. Transepithelial fluid and K⁺ transport in the main segment of control and mesh knockdown tubules were measured using the Ramsay assay and K⁺-specific electrode.

**Results:** The Malpighian tubules of an adult fly have four major morphologically and functionally distinct segments and are made up of the larger principal cells and smaller intercalated stellate cells. We found that developmental mesh knockdown in the tubule principal cells was associated with early lethality in adult flies. The tubules of these flies revealed defects in epithelial architecture, SJ organization and reduced junctional expression of Dlg. Furthermore, main segment transepithelial fluid secretion and K⁺ flux was completely abolished in developmental principal cell mesh knockdown tubules and significantly reduced in tubules subjected to principal cell mesh knockdown in adulthood as compared to the control groups.

**Conclusions and significance:** The sSJ protein mesh is essential for the development and maintenance of a functional *Drosophila* Malpighian tubule epithelium. Loss of mesh has profound effects on both the tubule epithelial cell and SJ integrity and transepithelial ion and water transport. Although future studies will be required to unravel the functional role of mesh in regulating the paracellular permeability of *Drosophila* Malpighian tubules, our data support the notion that integral SJ proteins play an important role in insect ionoregulatory epithelia and homeostasis. Funding: NIH DK106350.

### 4.3

The impact of salt contaminated freshwater on the physiology of the rectum and malpighian tubules of mayfly (*hexagenia rigida*) nymphs

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Salt is a significant environmental contaminant of freshwater ecosystems and in northern temperate climates sodium chloride from winter road salting is a major contributor to this problem. This puts many freshwater (FW) animals at risk because they are normally osmoregulators that actively maintain solute levels in body fluids far in excess of their surrounding habitat. Our previous work has demonstrated functional changes at the gills of mayfly nymphs (*Hexagenia rigida*) in response to salt contaminated water (SCW). In this study the physiology and biochemistry of two other important osmoregulatory organs, the rectum and Malpighian tubules (MTs), were examined in nymphs exposed to SCW. Exposure to SCW elevated hemolymph ion levels and increased body water content, suggesting that nymphs are able to maintain ionoregulatory processes in SCW at a new steady state. In response to SCW, the rectum displayed a significant decline in K⁺ absorption relative to FW control nymphs. Na⁺-K⁺-ATPase (NKA) and V-type H⁺-ATPase (VA) activity in this region tended to decline in SCW but not significantly. Localization of basolateral NKA and apical VA along the MTs depicted a complex multicellular composition. MTs also exhibited a decline in K⁺ absorption in response to SCW as well as a significant decrease in NKA and VA activity. Data provide insight into how FW mayfly nymphs regulate salt and water balance in response to the environmental problem of FW salination.

### 4.4

A novel technique for measuring hindgut reabsorption in *Drosophila* reveals adaptive differences between species with different thermal tolerance

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It has become increasingly clear that the ability to maintain osmotic and ionic balance at low temperature dictates cold tolerance in many insects, *Drosophila* included. In insects, maintenance of osmotic and ionic balance is primarily achieved through the secretory actions of the Malpighian tubules and the reabsorptive nature of the hindgut which act in synchrony to regulate ion and fluid balance in the hemolymph. Previous studies have used Ramsay assays to investigate inter- and intraspecific differences in the capacity of Malpighian tubules to regulate secretion at low temperature in *Drosophila*. These studies have revealed adaptive differences that support variation in cold tolerance among and within species but little focus has been directed towards the capacity of the hindgut. This shortage is mainly due to the lack of methods to study fluid and ion transport in small insects. Here, we present and discuss the use of a novel assay that allows for simultaneous measurements of net ion (Na⁺ and K⁺) and fluid reabsorption of the drosophilid hindgut and employ it in a comparative study on the underlying mechanism of *Drosophila* cold tolerance. Specifically, we study fluid and ion transport at high and low temperature in three *Drosophila* species with marked differences in cold tolerance. Preliminary results indicate that fluid reabsorption is similar across three *Drosophila* species at benign temperature and that the main ion reabsorbed is Na⁺. In response to cold, we observed marked decreases in fluid reabsorption of all three species, but a larger degree of suppression in the least cold tolerant species. Interestingly, Na⁺ reabsorption was suppressed more in cold sensitive species and actually reversed (net Na⁺ secretion) whereas cold tolerant species were able to better maintain Na⁺ reabsorption while reducing K⁺ reabsorption. These results indicate that our newly
developed assay is capable of detecting small differences in fluid and ion flux across the *Drosophila* hindgut and our result help to explain previous observations showing superior homeostatic capacity of cold adapted species. From a methodological point of view this study introduces a novel assay that can deliver repeated measures of fluid and ion transport in the hindgut of small *Drosophila*. Further work is needed to optimize the protocol, but we are optimistic that this assay holds the potential to study reabsorptive processes in a similar manner as the Ramsay assay has been used in hundreds of studies to investigate the mechanisms of ion and fluid secretion in insects.

### ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS

**5: DEVELOPMENTAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION**

#### 5.1

Cortisol and estrogenic compounds modulate insulin-like growth-factor binding protein gene expression during vulnerable life stages of Atlantic salmon


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The growth hormone (Gh)/insulin-like growth-factor (Igf) system is a major controller of growth and development in vertebrates, including teleost fishes. The biological activities of IGFs are modified via their interactions with IGF binding proteins (IGFBPs). Anadromous Atlantic salmon (*Salmo salar*) express a broad suite of *igfbp* transcripts in key metabolic tissues throughout their early life history. The manner in which IGFBPs are modulated by extrinsic and intrinsic factors during their vulnerable early life-stages remains unresolved. In a series of experiments, we examined the effects of cortisol, 17α-ethinylestradiol (EE₂), 17β-estradiol (E₂) and 4-nonylphenol (NP) on various freshwater life-stages of juvenile salmon. Parr implanted with cortisol for 14 days exhibited a dose dependent reduction in growth rate. Cortisol lowered hepatic *igf1* mRNA levels in accord with diminishing the anabolic actions of Igf1. Cortisol simultaneously stimulated hepatic *igfbp1b1* and -1b2 mRNA levels as a means for their translated products to further attenuate the effects of Igf1. With respect to estrogenic compounds, exposures to EE₂ and NP for 21 days reduced *igf1* and -2 mRNA levels in fry. EE₂ and NP reduced hepatic *igfbp1b1*, -2a, -2b1, -4, -5b2 and -6b1, and stimulated *igfbp5a* mRNA levels. In smolts, a 4-day exposure to EE₂ diminished plasma Gh and Igf1 levels in parallel with a reduction in hepatic *igf1* mRNA.

Consistent with patterns observed in fry, EE₂ and E₂ diminished hepatic *igfbp1b1*, -4 and -6b1, and stimulated *igfbp5a* mRNA levels in smolts. Interestingly, while the *igfbp* system operated in a fashion to pause somatic growth when cortisol was elevated, the *igfbp* system seemingly acted in a fashion to counterbalance the catabolic effects of estrogenic compounds via their effects on *igf1/igf1*. Collectively, our experiments reveal that hepatic *igfbps* are key modulators of the Gh/igf system during the freshwater life-stages of Atlantic salmon.

#### 5.2

Intestinal hydrolase transcriptional responses during rapid diet adjustment in nesting house sparrows (*Passer domesticus*)


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Many bird species increase intestinal hydrolase activity when exposed to an increase of the specific substrate in the diet. To increase understanding of the underlying mechanism(s), we studied nesting house sparrows (*Passer domesticus*) switched between high protein diet (63% protein/5% starch) and high starch diet (30% protein/38% starch). In nature, the nestlings undergo a natural switch from high protein insects to starch-containing seed diet during development. Both intestinal α-glucosidase (AG; maltase and succrase) and peptidase (APN; aminopeptidase-N) activity, and their respective mRNA, were induced by their respective substrates within 24 h of a diet shift - consistent with the hypothesis that altered gene expression occurs in mature enterocytes on intestinal villi. For AG, evidence indicated that induction of transcription began >18 h after diet switch, and incorporation of new protein and activity in the apical brush border membrane occurred in ≤6 h after that. For APN, evidence indicated that induction of transcription began as fast or faster than for AG. For both AG and APN, downward modulation of mRNA and activity appeared to begin within 18 h of a large reduction in their respective inducing agent. Overall, changes in intestinal hydrolase activity are so fast (<1 d) that nestlings could potentially track daily changes in resource availability with fine-tuned changes in digestive performance. Supported by NSF IOS-1354893.
5.3 Developmental Variation in Embryos of Two Killfish Populations
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The first 4 hours post-fertilization represent a critical time point in the developmental trajectory of teleosts. It is an overlooked source of phenotypic variation seen later in development. Such critical developmental events may be unique to a species, with variation in early development even occurring among populations within a species. Critical developmental time points drive the development in embryos of the killfish Fundulus grandis, as for other teleosts. Cell division of the blastocyst of Fundulus is temperature sensitive, so temperature-induced variation in cleavage rate, cell morphology, and \( Q_{50} \) may vary between fish populations. This experiment targets cell stages in early embryos (fertilization to ~4 hours) from two distinct killfish populations. Patrick’s Bayou (TX) killfish reside in a polluted area within the Houston Ship Channel and are resistant to dioxin-like compounds. Populations outside the Houston Ship Channel are non-resistant – i.e. are affected during development by DLC’s (dioxin-like compounds). Accompanying these differences are a suite of biochemical and physiological differences, as well. To test the hypothesis that temperature influences critical time points of early killfish embryonic development and that the responses are population-dependent, fertilized killfish embryos from resistant and non-resistant populations were maintained in one of three different temperatures (22°C, 25°C, and 28°C). Repeated measures of each blastocyst cleavage per embryo tracked individual variation to the 64 cell stage.

Resistant populations of Gulf killfish at 22°C in non-polluted water showed a significantly (\( p<0.01 \)) higher development rate of 13.5 cleavages/hour compared to the non-resistant population’s cleavage rate of 13.2 cleavages/hour in non-polluted water, indicating intrinsic differences in cleavage rate associated with each population. Further, cleavage rate of the resistant population to 64 cells was significantly (\( p<0.01 \)) less temperature sensitive (\( Q_{50} = 1.8 \)) than that in the non-resistant population (\( Q_{50} = 2.8 \)). The difference in \( Q_{50} \) values indicates significant differences in the developmental trajectory of the resistant vs. non-resistant population, as expressed by cleavage time to 64 cells.

Alterations in physiological and morphological phenotypes associated with exposure to different temperatures indicate that temperature alters initial developmental trajectory, as expected. Unexpected was that this fundamental biological process would vary between different killfish populations. Additional experiments are underway to determine the mechanism(s) for these differences.

5.4 Transgenerational Epigenetic Inheritance Induced by the Combined Exposure to Crude Oil and Hypoxia in the Zebras
Naim Martinez1, Amelie Crespel2, Warren Burggren1
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Organisms face constant selection pressures imposed by the interaction of natural and anthropogenic factors, and respond with phenotypes that influence offspring survival and fitness. However, the majority of previous experimental designs have considered only a single factor at a time, and their analysis and interpretation embrace only the generation that has been directly exposed. Usually not investigated are the potential effects on offspring generations. In fish, especially in the Gulf of Mexico, hypoxic conditions and exposure to crude oil may co-occur. However, how the all-important interactions between these environmental stressors affects parental and offspring populations are still poorly understood. Consequently, a multi-scale approach employing the zebrafish (Danio rerio) was used to test the chronic responses over 5 weeks of exposure in four parental experimental groups: 1) Control (normoxia, control diet); 2) Hypoxia (~60% DO, control diet); 3) Oil (normoxia, oil-loaded diet) and; 4) Hypoxia-Oil (~60% DO, oil-loaded diet). Offspring from each parental group was exposed to conditions similar to the parental exposures and their survival and performance were determined. Survival (~95%), growth rate, hypoxia resistance (LOE), and heart rate (~240bpm) did not significantly differ among parental groups, indicating that the adult populations were not excessively stressed despite combinations of hypoxia and oil exposure. However, global DNA methylation in adults was significantly decreased in heart tissue (\( P<0.05 \)) but not in gonads. \( F_2 \) offspring obtained from parents exposed to hypoxia and oil conditions exhibited higher survival (up to 50%) compared with the control group when raised in hypoxia combined with crude oil conditions. However, regardless of parental exposure, \( F_2 \) exposed to oil and/or hypoxic conditions exhibited significant bradycardia in comparison with non-exposed larvae (~110 and ~180 bpm, respectively). Percentage of whole larvae Global DNA methylation was significantly decreased (\( P<0.05 \)) in larvae from hypoxic-oil exposed parents in comparison with control and any of the two one-factor exposed parents. Although chronic exposure to environmental stressors in parental populations might not elicit phenotypic modifications, they do prompt signals to offspring populations via transgenerational epigenetic inheritance, likely helping offspring populations to survive while facing persistent environmental conditions. Epigenetic inheritance plays an important role in shaping phenotypic responses in offspring populations facing...
changing environments. However, responses to specific factors may compromise individual performance when more than one stressor is present. Furthermore, considering the parental exposure-experience and also more than one factor in experimental designs will offer a more holistic approach to understand how organisms cope with environmental stressors.

5.5
Hypoxic incubation has no effect on permeabilized cardiac muscle mitochondrial oxygen flux or ROS production in the American alligator
Edward Dzialowski, Janna Crossley, Jessica Rippamonti, Dane Crossley

Alligator eggs developing in nest mounds have the potential to be exposed to hypoxic conditions during incubation. Here we investigated the effects of hypoxic incubation on development and cardiac muscle mitochondrial respiration and emission of reactive oxygen species in developing American alligator (Alligator mississippiensis). Alligator eggs were incubated in normoxia or 10% hypoxia at 30°C. One group of animals was examined at 90% of incubation and another group of animals was allowed to hatch and develop for a year in normoxia before measurements were made.

Mitochondrial respiration and ROS production under leak and oxidative phosphorylation states were measured in permeabilized cardiac muscle with high-resolution respirometry coupled with fluorometry. To examine the response of mitochondria to anoxia and reoxygenation, permeabilized cardiac muscles were exposed to 25 min of anoxia, followed by reoxygenation during measurement of mitochondria respiration and ROS production. Hypoxic incubation blunted growth of the embryos and these mass differences were maintained through the first year of life. In contrast, hypoxic incubation had no effect on mitochondria respiration or ROS production at either 90% of incubation or 1-year post hatching. After exposure to anoxia for 25 min, the rate of oxidative phosphorylation of permeabilized cardiac muscle returned to pre-anoxia levels. There was no change in ROS production observed upon reoxygenation of the permeabilized cardiac muscle.

Our results suggest that hypoxic incubation has little influence on cardiac myocyte mitochondrial physiology in the developing alligator. This study was funded in part by NSF grant IOS 1146758 to EMD.

6: CONNECTING GENOMES TO PHENOMES TO POPULATIONS
COSPONSORED BY THE AMERICAN PHYSIOLOGICAL SOCIETY AND THE SOCIETY OF INTEGRATIVE AND COMPARATIVE BIOLOGY DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY

6.1
Sex dependent phenological plasticity in an arctic hibernator
C. Loren Buck, Cory Williams

Sexual selection involves the production of offspring that outcompete conspecifics of both sexes for limited resources (Buck and Barnes, 1999, Williams et al. 2016). Aided by biologging devices, here we show that arctic ground squirrels exhibit sex-dependent plasticity in the physiology and phenology of hibernation in response to late spring snowstorms (Williams et al., 2017). Females and non-reproductive males responded to a >1 month delay in snow melt by extending heterothermy or re-entering hibernation after several days of euthermy, leading to a 2-3 weeklong delays in reproduction. Extended hibernation consisted of repeated 2-3 day torpor bouts with a slightly elevated minimum body temperature as compared to minimum body temperatures measured during torpor in mid-hibernation. In contrast, reproductive males did not extend or re-enter hibernation. It is likely that reproductively mature males are unable to re-enter a torpid state because androgen secretion associated with seasonal gonadal recrudescence inhibits expression of torpor. Our findings reveal intriguing differences in how...
males and females respond to climatic stressors, which may lead to phenological mismatches between the sexes. Funding was provided by the National Science Foundation, Integrative Organismal Systems under grant numbers 1558056 and 1147187 to CLB and CTW.

6.2 Insights into mutational pathways of biochemical adaptation using ancestral protein resurrection

Jay Storz1, Chandrasekhar Natarajan2, Anthony Signore1, Federico Hoffmann3, Roy Weber1, Angela Fago4

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The step-by-step evolution of novel phenotypes is central to several fundamental questions in biology. In studies of novel protein functions, the problem becomes experimentally tractable if it is possible to identify and functionally characterize the complete set of causative mutations. With such a system, it is possible to address key questions: Do novel functions evolve via the successive fixation of beneficial mutations that each produce an adaptive change in phenotype when they first arise? Alternatively, are evolutionary transitions in protein function facilitated by neutral mutations that produce no adaptive benefit when they first arise, but which potentiate the function-altering effects of subsequent mutations? By reconstructing all possible mutational pathways that connect ancestral and descendant proteins it is also possible to address fundamental questions about the roles of contingency and determinism in protein evolution. For example: Can novel functions evolve from any possible ancestral starting point, or are specific evolutionary outcomes contingent on prior history? We address these questions by experimentally dissecting the molecular basis of biochemical adaptation. Using ancestral protein resurrection in conjunction with a combinatorial protein engineering approach based on site-directed mutagenesis, we examine the effects of sequential mutational steps in the evolution of novel functional properties in avian and crocodilian hemoglobins.

6.3 The genomic and physiological basis of rapid adaptation to temperature in a globally invasive crab

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Marine invasive species are characterized by broad dispersal and an ability to thrive in a variety of novel environments over very short time scales. I hypothesize that this success is due both to phenotypic plasticity and to rapid genotypic selection, despite the limited adaptive potential suggested by high gene flow and population bottlenecks. Using the globally invasive European green crab (Carcinus maenas), I compared thermal adaptation in populations spanning thermal gradients in the species’ native and invasive ranges. Heat and cold tolerance (measured via cardiac photoplethysmography) differed significantly among populations even after one month of acclimation under common conditions. In addition, within a population, there were significant plastic differences in tolerance observed after acclimation to 5°C versus 25°C. Transcriptome sequencing revealed ten putatively-selected genomic regions closely associated with both temperature and population-level thermal tolerance. These associations were observed in the species’ native range and were recapitulated in under 200 years after introduction to North America. One such region represents a likely genomic island of divergence, and contains at least 18 distinct genes in very strong linkage disequilibrium. This region is significantly enriched for non-synonymous SNPs relative to the transcriptomic data as a whole. Four genomic regions, including the potential island of divergence, also showed a strong and significant association with winter temperature in an independent test of five invasive populations spanning >1,500 km, all of which were descended from a single, highly bottlenecked introduction in 1990. Overall, these results strongly indicate local adaptation in the species’ native range, and suggest a role for ongoing, rapid selection to temperature in the success of this widespread marine invader.

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6.4 Modeling photoperiodism in subterranean rodents

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Photoperiodism plays an important role in the synchronization of populational phenomena such as seasonal reproduction. Organisms that inhabit the extreme photic environment of the subterranean provide an opportunity to verify persistence of daily and seasonal biological rhythms, as well as the minimal photic input for 24h entrainment and photoperiodism. The genus Ctenomys of South American subterranean rodents, commonly known as tuco-tucos, has circa 60 described species, and can be found south of 12o5 Latitude. We obtained automated recordings of daily light exposure and activity patterns of individual tuco-tucos in the field, as well as preliminary data on their seasonal reproduction times. Laboratory experiments have shown that their circadian oscillators display “splitting” of locomotor activity under constant light conditions, which is a hallmark of a 2 coupled clock structure in epigeous
organisms. These 2 neuronal populations that comprise the circadian oscillator is associated to photoperiod decoding through mathematical modeling. We have developed modeling studies to approach photoperiodic time measurement in tuco-tucos, based on field data and laboratory experiments, aiming at integrating multilevel neuronal population phenomena to individual and populational, seasonal biological rhythms (FAPESP, CONICET, FONCyT).

7: VERTEBRATE ENERGETICS: ABSTRACT DRIVEN SESSION

7.1
Digging up the evolutionary origins of hypoxia-tolerance: physiological adaptations to acute hypoxia in 9 species of African mole rats
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Mammals rely on continuous O2 delivery for energy production but O2 availability is often limited by environmental factors. Some mammals have evolved to survive hypoxic environments; studying these animals may provide key insights into the evolution of adaptive mechanisms that support life in hypoxia. Of particular interest are African mole rats, which experience periods of hypoxia while sleeping and digging in poorly ventilated underground burrows. During a recent field trip to South Africa, we measured metabolic, ventilatory, and thermal responses to stepwise exposure to acute hypoxia (18 to 2% O2) in 9 species of African mole rats. These species spanned a range of magnitude in physical size and included animals that live solitary versus in complex social groups, and under varying soil particulate size and density. All species except Bathyergus suillus tolerated 3% O2, whereas Heliophobius argenteocinereus was the only species to tolerate 2% O2. All species exhibited robust and progressive reductions in metabolic rate with progressive hypoxia, small to moderate reductions in body temperature (in most cases to nearly ambient temperature), and a hypoxic ventilatory response that was characterized by increasing ventilation with concomitantly decreasing O2 levels, mediated primarily by increased tidal volume and a lesser contribution from breathing frequency. Hypoxia-tolerance did not correlate with body mass (B. suillus was the largest species, but H. argenteocinereus was the 2nd largest), degree of sociality, or soil density. We conclude that all African mole rat species are tolerant to environmental hypoxia and exhibit broadly similar physiological responses to acute hypoxic exposure.

7.2
Hummingbird daily energy expenditure allometry: Is bigger better?
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Allometric scaling relationships examine how the scaling of one trait (e.g. body size) correlates with the scaling of another trait (e.g. field metabolic rate, brain size). These scaling relationships have intrigued scientists for decades because there seem to be rules that govern how metabolic processes scale up with body size. In birds, field metabolic rate (FMR) scales with body mass (M) in the form FMR = aM0.68 (Anderson and Jetz 2005; Nagy 2005), where a is a constant. However, localized taxon-level patterns could be masked by looking only at the scaling exponent of all birds. Hummingbirds have among the highest mass-specific rates of all vertebrates, as well as a much higher wing aspect ratio than predicted for their size. Previous studies estimate the FMR-body mass slope for hummingbirds to be 1.21 (Nagy et al. 1999a), without accounting for the phylogeny. This would indicate that bigger hummingbirds are more metabolically inefficient than smaller birds. We collected FMR and mass data, which combined with published data, spanned 17 hummingbird species over a 6-fold size range (2.7 - 17.5 g), and eight of the nine hummingbird clades. After accounting for phylogenetic relatedness, field metabolic rate scales with body mass as FMR = 2.19*Mass0.89. This is a much lower slope than what has been predicted for hummingbirds previously. Further, since temperate hummingbirds tend to feed on densely clumped resources more, and are closer to their thermoneutral zone than tropical hummingbirds are, we expected tropical birds to have a higher FMR to body size exponent than temperate birds to support increased foraging effort. Contrary to the usual trend among birds and mammals, we find, both with and without adjusting for phylogenetic relatedness, that tropical hummingbirds have a higher FMR to mass slope on average than temperate birds.

7.3
The cost of good parenting: Altered maternal care in high altitude deer mice, Peromyscus maniculatus.
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1Biology, McMaster Univerity
Low oxygen and temperatures at high altitude (HA) are particularly challenging for small mammals due to the high energetic costs of aerobic thermogenesis. Altricial rodents are born without the capacity to independently
thermoregulate and instead rely exclusively on maternal care early in postnatal development. The onset of independent endothermy occurs in the first month of life, a period of high mortality and a potential selective window. Previously, we found that the development of thermo-effector organs and the onset of endothermy is delayed in HA compared to low altitude (LA) native deer mouse pups. HA pups likely rely more on their mothers during this sensitive window. Thus, we tested the hypothesis that high altitude adaptation includes enhanced maternal care, using lab-reared descendants of deer mice native to LA (400 m a.s.l)) and HA (4300 m a.s.l.). Mothers reared their litters postnatally under common garden warm + normoxia and under combined cold + hypoxia (5°C: 430mmHg). We characterized maternal energetic demands and quality of care. The metabolic cost of provisioning offspring in a cold/hypoxic environment is exceptionally high. We found that the resting metabolic rate of lactating HA females was 70% of their VO2 max while the cost for LA females was even higher (85%, VO2 max). Surprisingly, despite rearing/provisioning larger litters than their LA con-specifics, HA mothers spent considerably less time caring for their pups under any condition. Both populations altered their maternal behavior in response to cold/hypoxic conditions, however, LA pups were significantly developmentally delayed whereas HA pups developed normally. Our data suggest a conflict between offspring and maternal energetic demands at high altitude that manifests as a trade-off in the quality/efficiency of maternal care.

7.4 Colonizing high altitude hypoxic environments: strategies to deal with metabolic needs

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The aptitude of mammals to colonize high altitude (HA) environments is limited by their ability to tolerate decreased oxygen availability. While rats are not found under natural conditions above 2500 meters of altitude, mice are commonly found in such habitats. Previous research in our lab showed that rats and mice display divergent physiological and molecular responses after acute (short-term) exposure to hypoxia. It is well known that the response to hypoxia is linked with the regulation of metabolism, in consequence, we aimed to identify the strategies underlying the metabolic response of rats and mice to short- and long-term exposure to hypoxia.

Sprague – Dawley rats and FVB mice (all males) were exposed to hypoxia (12% O2) for short (6 hours and 24 hours) and long (7 days and 21 days) periods of time. During the last hour of the exposure, the metabolic rate (O2 consumption (V O2) and CO2 production (V CO2)) and minute ventilation (V E) were measured by indirect calorimetry and whole-body plethysmography methods. The hematological response was evaluated by the quantification of hematocrit and hemoglobin concentration. As the brain is particularly vulnerable to hypoxic conditions due to its high energy requirements, we assessed the mitochondrial respiration in permeabilized brain cortex samples using the high-resolution respirometer Oxygraph-2k (Oroboros Inc.). In comparison to normoxic controls, mice showed a higher metabolic rate (increased V O2 and V CO2) after 7 days of hypoxia. No change in rat’s metabolism was observed. In line with the increased V O2, mice showed a continuous augmentation in V E with a peak at 7 days of exposure. Rats showed a weaker rise in the V E at 6h with no further increase. At brain level, mice showed an increased mitochondrial respiration after 24 hours of hypoxia, while those exposed to 7 and 21 days were similar to the controls. Though in rats only weak changes in the ventilation and no response at brain-mitochondrial respiration were observed, they showed a strong hematological response. The hemoglobin concentration and hematocrit increased in a sustained way starting at 24 hours, reaching a plateau at 21 days of exposure to hypoxia. Hematological adjustments occurred in mice only after 21 days.

Altogether, our results suggest that mice privilege an increase of their ventilatory activity to cope with hypoxia resulting in an enhanced metabolic rate and preserved mitochondrial activity in brain cortex. In rats, whereas the ventilatory response is weak, they favour a rapid and sustained hematological response. We propose that the ability to tolerate hypoxia ultimately relies on the strategy to capture, distribute and use the available oxygen to cope with the metabolic needs during the process of acclimatization.


8: VENTILATORY FUNCTION: ABSTRACT DRIVEN SESSION

8.1 Learning to air breathe; the first steps
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Air-breathing in vertebrates has evolved many times amongst the bony fish whilst in water. Its appearance fundamentally impacted the regulation of respiratory gas exchange and acid base status with terrestrial vertebrates combining ventilatory control of both; something that is not available to the water-breathers. We review the physico-chemical constraints imposed by water and air on gas exchange, place the extant air-breathing fish into this framework and show how the advantages of combining control of ventilation and acid base status become clearly available in the most obligate of air-breathing fish. Presenting new data on acid base control across temperature in several teleosts, we argue that ventilatory control linked to internal (though not necessarily central) CO2/H+ sensing is probably widespread across the Actinopterygii, but normally hidden by the hypoxic ventilatory drive.

8.2 Characterizing the branchial hypercarbia recovery mechanisms following extreme hypercapnia in the highly CO2 tolerant hagfish
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As scavengers that feed on decaying carrion (e.g. fish, large marine mammals), hagfish (Eptatretus stoutii) can be subjected to noxious environmental stresses such as high ammonia, anoxia and hypercapnia. While the physiological impacts and the hagfishes’ tolerance of these stressors have been well characterized, little data exists on the mechanisms and strategies for recovery from these stressors that would be mandatory in the natural environment. Indeed, hagfish can tolerate extreme hypercapnia (>30 Torr; 72h) by building up plasma [HCO3−] over 24 – 48 h of exposure, attaining the highest plasma [HCO3−] levels ever observed in any organism (>70 mmol HCO3− L−1). The goal of this study was to characterize the hypercarbia recovery strategies of the highly CO2 tolerant following hypercapnia exposure at the whole animal and tissue (gill) levels. We exposed hagfish to hypercapnia (4% CO2) for 48 h to induce severe hypercarbia before being allowed to recover in normocapnic seawater. During this recovery period, measurements of blood acid/base status, plasma [Cl−] and net H+HCO3− flux were made to elucidate the recovery strategies of the hagfish. Upon reintroduction into normocapnic conditions, hagfish rapidly (<8h) offloaded the compensatory base load (65.8 ± 2.1 mmol HCO3− L−1) while sustaining an incredible blood alkalosis of ~0.8 pH units compared to post-exposure conditions, to a blood pH 8.67 ± 0.03 within 4h of recovery. During recovery, rates of HCO3− offloading paralleled rates of Cl– uptake. While increases in both whole-animal HCO3− excretion and glomerular filtration were observed throughout recovery (2-8 h), neither can account for the majority of the observed rates of whole-animal HCO3− loss, which peaked at ~3.5 mmol kg−1 h−1. inhibition of all carbonic anhydrase activity via infusion of acetazolamide revealed that restoration of plasma [HCO3−] from hypercapnia-induced hypercarbia is likely facilitated in a dualistic manner, initially relying on both carbonic anhydrase mediated CO2 offloading and secondarily, by increasing Cl–/HCO3− exchange processes, both of which are likely either upregulated or further activated as recovery progresses. Using isolated gills from hypercapnia-exposed hagfish allowed to recover for various amounts time (informed by whole-animal studies), we utilized a recently developed in situ hagfish dual gill perfusion/perfusion (extracellular aspect/water duct) technique along with a panel of permeant (Acetazolamide) and impermeant (C-18) CA pharmacological inhibitors, in an attempt to further characterize whether this CA-dependent HCO3−-equivalent mechanism is driven by intracellular and/or plasma-accessible carbonic anhydrase. Similarly, using both pharmacological profiling and Cl–-free artificial seawater/salines, we also evaluated the roles of Na+/HCO3−cotransporter and Cl-/HCO3−exchanger in gill in base-recovery in this highly CO2–tolerant organism.

8.3 Developmental Changes in the Ventilatory Response to CO2 in Semi-Fossorial Mammals.
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Adult semi-fossorial rodents such as the Golden-Syrian hamster (facultative hibernator) and the 13-Lined ground squirrel (seasonal hibernator) have a blunted ventilatory response to CO2 in comparison to adult non-fossorial rodents. Rats display a “sensitive period” during respiratory development where ventilatory responses to hypercapnia become blunted before rising to typical adult levels (a triphasic response). This study used pneumotachography and whole body plethysmography to examine the metabolic and ventilatory responses to CO2 of Golden-Syrian hamsters, 13-Lined ground squirrels, and Sprague-Dawley rats through neonatal development (P0-30) to determine whether the blunted adult response seen in semi-fossorial rodents is a retention of the transient blunted response seen in rats. Hypercarbia (1, 5, and 7% CO2) did not affect oxygen consumption (VO2, mL/min/kg) in any species but initially
elevated ventilation and the air convection requirement (mL/mLO₂) in all three species. Rats in our study exhibited the triphasic ventilatory response (when expressed as %Δ) to CO₂ previously described. Hamsters had an early (PO-2) robust response that progressively waned to a blunted adult response. Squirrels also exhibited an early, robust ventilatory response to CO₂ that decreased within days and remained attenuated through development to adulthood. Our study shows three distinct developmental patterns that appear to be shaped by lifestyle despite all animals being raised in normocarbic (non-fossorial) conditions. This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

8.4
Haemoglobin Adaptations to High Altitude Alter Breathing Pattern in Deer Mice (Peromyscus maniculatus)
Catherine Ivy¹, Oliver Wearing¹, Zachary Cheviron², Jay Storz³, Graham Scott¹
¹Department of Biology, McMaster Univ., ²Division of Biological Sciences, Univ. of Montana, ³School of Biological Sciences, Univ. of Nebraska
Hypoxia at high altitudes constrains O₂ supply to support activity and thermoregulation. Many highland taxa have adapted to these challenges by optimizing O₂ uptake, through changes in the pattern and/or control of breathing, enhancements in gas-exchange capacity of the lungs, and/or genetically based increases in haemoglobin-O₂ affinity. These evolved changes are believed to safeguard arterial O₂ saturation (SaO₂) in hypoxia to maintain O₂ supply to tissues. For example, deer mice native to high altitude possess haemoglobin (Hb) adaptations that augment blood-O₂ affinity, arising from genetic variants in the HbA and HbB gene cluster. Despite the common belief that haemoglobin plays no direct role in the control of breathing, we show that allelic variation in Hb function also contributes to changes in breathing pattern in high-altitude deer mice. We created an F2 inter-crossed population by hybridizing wild mice from high and low altitudes, which allowed us to compare mice with highland and lowland Hb genotypes on an admixed genetic background. The hypoxic ventilatory response was measured before and after hypoxia acclimation (12 kPa O₂ for 6-8 weeks). Hypoxia acclimation led to the expected increases in total ventilation, SaO₂ in hypoxia, and haematocrit. HbA genotype had a significant influence on breathing pattern, with mice possessing the highland genotype exhibiting deeper but less frequent breaths across a range of inspired O₂. This was not clearly associated with effects of HbA genotype on SaO₂, because differences in breathing persisted when mice breathed hyperoxic air. These findings suggest that the evolution of Hb genes may have pervasive effects across the O₂ transport cascade, and that Hb may contribute to environmental adaptation via physiological mechanisms that are not commonly ascribed to this protein.

8.5
The Effect of Chronic Hypercapnic Incubation on Breathing Patterns in American alligator (Alligator mississippiensis).
Justin Conner¹, Ruth Elsey², Dane Crossley¹
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During ontogeny phenotypic plasticity is a mechanism responsible for trait differences caused by the environment. This epigenetic regulation of phenotype may be particularly influential in embryonic reptiles that naturally experience fluctuations in environmental variables such as carbon dioxide, which could result in chronic acidosis (16-35 torr). However, the phenotypic outcomes of exposure to changes in CO₂ are relatively unknown in reptiles. Terrestrial vertebrates have two mechanisms for maintaining acid-base homeostasis: metabolic compensation and respiratory compensation. A prior study on America alligators demonstrated that hypercapnic incubation causes kidney enlargement suggesting a lasting impact of the incubation environment on the post hatched organism. Based on this we hypothesized that embryonic hypercapnia would decrease the sensitivity to acute hypercapnic exposures in juvenile American alligators (Alligator mississippiensis). To test this hypothesis, we monitored ventilation parameters and metabolism of juvenile alligators in response to hypoxic and hypercapnic gas PO₂=15,10,5,3 and PCO₂= 4,6,8,10 kPa. We found resting tidal volumes for animals incubated in hypercapnia were significantly lower (8.8mL/kg) when compared to control animals (14.7mL/kg). During hypoxic exposure oxygen consumption and the ratio of CO₂ produced relative to O₂ consumed or respiratory quotient (RQ) were constant for the control animals while hypercapnic incubated animals decreased oxygen consumption and increased RQ. Hypercapnic exposure did not affect oxygen consumption in either experimental group, while carbon dioxide production increased at 4 kPa for both groups. Carbon dioxide production was significantly lower in hypercapnic animals at 8 and 10 kPa when compared to normocapnic animals. Our initial findings support our hypothesis that developmental hypercapnia alters ventilatory function in juvenile American alligators.

8.6
Effects of Gravidity on Grasshopper Oxygen Delivery
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Although the insect tracheal system is extremely efficient, life history changes can reduce oxygen delivery. For example, intermolt growth results in compressed tracheae, lower tracheal volumes, and reduced femoral air sac ventilation rates. During gravidity, the egg mass of *Schistocerca americana* grasshoppers can reach up to 40% of their body mass. We investigated whether this increase in egg mass compressed tracheae, lowered tracheal volumes, and reduced femoral air sac ventilation rates in the abdomen and femur. Using micro-dissections and live video analysis, we examined the tracheal system of thirty-six female grasshoppers. We found that abdominal compression rates correlated with the inflation of proximal and distal femoral air sacs. In addition, abdominal compressibility decreased with gravidity, suggesting lower tidal volumes. However, there was a positive correlation between gravidity and tracheal compression rate. Current work is using micro-computed tomography to examine whether gravid females have reduced tracheal volumes. If so, gravid females may compensate for reduced tidal and tracheal volumes by increasing tracheal ventilation rates to improve oxygen delivery. This research was funded in part by NSF award 1531850 to SDK.

9: **THERMAL BIOLOGY:**
**ABSTRACT DRIVEN SESSION -1**

9.1 Oxygen limitation of thermal tolerance varies depending on the life stage and behavior of terrestrial organisms

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Oxygen availability and temperature are two of the most important environmental factors affecting all of animal life. However, the two are not independent of each other and may exert similar selective pressures on animals. Animals should be most susceptible to high temperatures and oxygen variation during times of high performance and during early developmental stages. Through a series of experiments on lizards, birds, and insects, we have begun to examine the interactive effect of oxygen and temperature on terrestrial animals exposed to hypoxia, high temperatures, or a combination of both during various life stages and levels of activity. The results of these experiments have been mixed in their support for the concept of an effect of oxygen during periods of thermal stress. Oxygen did limit thermal tolerance during embryonic development in lizards. However, while extreme hypoxia limits thermal tolerance in adult lizards, we have shown that ecologically relevant oxygen levels have no effect on voluntary maximum temperature. In adult flies from a variety of DGRP lines, there was a genetic correlation between flight performance during hypoxia and flight performance during high temperatures. However, while both hypoxia and high temperature have a detrimental effect on flight in adult Oregon-R flies, there was no interactive effect between temperature and oxygen during development. Lastly, we have begun to look at the effect of metabolic rate on thermal tolerance in quail eggs through the use of thyroid hormone manipulation. Based on these experiments, the effect of oxygen on thermal tolerance varies depending on the life stage and activity level of these organisms. However, more experiments under ecologically relevant conditions and behaviorally relevant activities need to be carried out to further test this hypothesis.

9.2 Ultra-violet B radiation, the often neglected ubiquitous environmental stressor in aquatic environments

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High energy, short wave ultra-violet B radiation (UVBR) is a powerful natural stressor emitted by the sun. Although the majority of solar UVBR reaching the outer atmosphere is absorbed by stratospheric ozone, a small amount does reach the Earth’s surface and can penetrate aquatic environments, especially in shallow water bodies or near the surface where fish and amphibian larvae often live. UVBR can interact with a range of biological molecules, and is capable of causing extensive cellular and molecular (DNA and proteins) damage as well as generating ROS. At the organismal level, UVBR exposure can adversely impact survival, immune function, growth rates, developmental trajectories and locomotion. UVBR can also interact with other environmental drivers within aquatic environments, such as temperature, hypoxia, and pH. Multi-factorial studies have been identified as a key area that is needed to disentangle the underlying mechanisms behind global amphibian declines. This presentation will examine the interaction of UVBR and temperature where the effects of UVBR are more pronounced at low temperatures. New data provide insight into the increased susceptibility of amphibians, both larvae and adults (via carry-over effects through metamorphosis) to UVBR at low temperatures which in turn may help to explain the predominance of amphibian declines in montane regions.

9.3 Understanding the effects of food availability, thermal tolerance, and sirtuin activity on the feeding physiology of *Mytilus californianus*

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The California mussel, *Mytilus californianus*, is a dominant member of coastal marine communities where they have a profound impact on intertidal and subtidal community structure. Mussels are regularly exposed to temperature extremes during periods of emersion and persistence of mussels along the coast depends, in part, on the ability of adult mussels to withstand environmental stress. Thermal tolerance in mussels is influenced by diet (i.e. phytoplankton composition) and mussels are known to modulate their feeding physiology in response to both environmental conditions and food availability. Previous studies in Mytilids suggest that sirtuins (a class of deacetylases that regulate numerous cellular processes through posttranslational modification) are activated during food limitation and act as a cellular energy sensor linking food availability and thermal tolerance in mussels. To investigate the mechanistic link between diet and temperature sensitivity, we conducted a large-scale experiment with high temporal resolution to evaluate the dynamics of the stress response in *M. californianus* across multiple levels of biological organization (i.e. cellular, organ, and organismal). As part of this study, we evaluated how acclimation to a combination of feeding regimes (low or high algal abundance) and thermal history (exposure to 20°C or 30°C maximum aerial temperature during each daytime low tide period) altered the feeding physiology of mussels, measured as variation in clearance rate, ciliary activity, and siphon opening during the last 48 h of the three-week acclimation period. Following acclimation, we chemically inhibited sirtuin activity in half of the mussels, exposed all mussels to acute heat shock (6 h heat ramp to 33°C), and measured how sirtuin inhibition and acute heat shock altered the feeding physiology over the course of 48 h in each of the treatment groups. Preliminary analysis of the data suggest that diet plays an important role in the feeding behavior of mussels and that both acute heat stress and sirtuin inhibition decrease ciliary activity, clearance rate, and siphon opening. However, the response of feeding physiology to heat stress and sirtuin inhibition is complex and depends on acclimation history and time of day. For instance, clearance rates were reduced in mussels exposed to acute heat stress and in those exposed to both sirtuin inhibitors and acute heat stress relative to our baseline measurements, but these effects were exacerbated during the day and in those fed the low algal food ration. This research is funded by the National Science Foundation (IOS-1057500 & 1557496).

9.4

A distal bat wing muscle operates at low temperature *in vivo*, and has low thermal sensitivity of contractile properties

**Andrea Rummel**, **Sharon Swartz**, **Richard Marsh**

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Flight in bats requires fast and coordinated movement of the forelimbs to maintain wing cycling frequencies of approximately 12 Hz or more, and involves contributions from muscles along the trunk, arm, and forearm. As nocturnal fliers, small bats likely incur substantial thermoregulatory cost from forced convection and radiative heat loss to the night sky; however bat wings are poorly-insulated, and muscles in the wing may operate at relatively low temperatures. Since rate-related processes in muscle slow at cooler temperatures, temperature effects in the wing muscles may impair flight performance. Here, we ask: (1) are bat wing muscles cold during flight, and (2) if so, how do bats compensate for effects of temperature on muscle contractile properties? We investigated these questions by measuring the temperature of flight muscles during wind tunnel flights in *Carollia perspicillata*, a small neotropical fruit bat, at an environmental temperature of 22°C; then by determining the temperature dependence of contractile properties in the extensor carpi radialis longus muscle (ECRL), a forearm extensor muscle. We compared bat ECRL properties to a distal but well-insulated limb muscle in the mouse, the extensor digitorum longus (EDL), at 22, 27, 32, 37, and 42°C. Our continuous measurements of muscle temperature during flight suggest a steep proximal-to-distal temperature gradient in the wings. Core body temperature ($T_b$) was maintained at approximately 39°C for all individuals. After commencement of flight in the wind tunnel, biceps and ECRL temperatures dropped substantially over the course of minutes-long flights to approximately 5°C and 12°C cooler than $T_b$, respectively. Instantaneous measurements immediately post-flight indicate that the deep pectoralis may operate at or several degrees above $T_b$ during flight. The thermal dependence of the bat ECRL is low relative to the mouse EDL for all isometric and isometric properties we studied, including shortening velocity ($Q_{10,ECRL} = 1.1$ vs $Q_{10,EDL} = 1.5$ from 32–37°C) and half-relaxation times for twitch and tetanus; the mouse EDL was stable *in vitro* to a higher maximal temperature (42°C). When we evaluate $Q_{10}$ for each muscle relative to maximum experimental temperature, the thermal performance curves are of the same shape, suggesting a shift in temperature optimum rather than an increase in performance breadth. Preliminary data for contractile properties of the pectoralis muscle in *C. perspicillata* indicates that it is more temperature sensitive than other studied skeletal muscles, including the bat ECRL and the mouse EDL. The high temperature
sensitivity of the pectoralis may relate to its proximal location and maintenance at or above \( T_e \) during rest and activity. This work was approved by the Brown Univ. IACUC and funded by NSF and AFOSR.

9.5
Assessing summertime thermoregulatory properties across the pelage molt in a polar pinniped: the Weddell seal

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Antarctic pinnipeds face complex thermoregulatory challenges, as they must conserve heat in both air and water. Primary adaptations to reduce heat loss, or flux, include a thick blubber layer and the ability to limit the amount of blood flow to the peripheries. These adaptations reduce heat flux by decreasing the temperature gradient between the animal and the environment. However, during the annual pelage molt, hair follicles may require more constant blood perfusion which would compromise heat conservation and result in higher rates of heat loss and energy expenditure. Thus, polar species such as the Antarctic Weddell seal (\textit{Leptonychotes weddellii}), may only have a short temporal window during which molting can be accomplished without incurring large energetic costs.

To determine how ambient conditions influence heat loss and energy expenditure, surface temperature (ST) and heat flux (HF) were directly measured in 77 adult female Weddell seals prior to, during, and following the molt. Seal ST was mean±SE 6.64°C ± 0.34, and 73% of ST variation could be explained by intrinsic (mass, surface area, blubber thickness and molt status) and extrinsic (ambient temperature, wind speed, relative humidity and solar radiation) factors measured at the time of animal handling (General Linear Model). Surface temperature increased most significantly with increased ambient temperature and decreased wind speed, which explained 47.8% and 46.0% of the variation respectively. Surface temperature was not related to molt status. In contrast, HF was most impacted by molt status, with molting seals having higher HF than those handled prior to the molt start. As HF is a direct proxy for energy expenditure, this suggests that perfusion is increased during molt to support hair growth despite the increased thermoregulatory costs.

Finally, to determine if seals offset the increased HF during the molt by reducing time in water (due to higher conductivity of water), we determined the time-activity budgets of 55 seals using time-depth recorders, and paired their haul-out behavior with information on ambient conditions. On average, seals spent 9.98 ± 0.16 SE hours per day hauled out, and the amount of time seals spent hauled out was independent molt status. Quantifying the contributions of behavior and intrinsic and extrinsic conditions will allow us to understand how energy requirements might be altered by changing environmental conditions.

11: COMPARATIVE INSIGHTS INTO ANIMAL RESPONSES TO HYPOXIA AND ANOXIA

11.1
Role of the Mitochondrion in Low Oxygen Signalling in the Painted Turtle.

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The western painted turtle is an anoxia-tolerant vertebrate that provides a useful model to study the natural cellular mechanisms that are protective of low oxygen stress. It wasn’t long ago that mitochondria were not considered capable of being low oxygen stress sensors; however, more recently that opinion has changed. We know in anoxic turtle brain that pyramidal neurons undergo: an increase in GABA receptor currents, a decrease in glutamate receptor currents, an increase in whole-cell conductance, a movement of membrane potential towards the Cl\(^-\) reversal potential, decrease in reactive oxygen species, and a depolarization of mitochondria membrane potential (MMP), and calcium release through mitochondrial K\(_{\text{ATP}}\) channels (mK\(_{\text{ATP}}\)). We know little about the role of mitochondria in non-excitable tissues such as liver; therefore, we used electrophysiological and fluorescent imaging techniques to examine membrane potential, whole-cell conductance, mitochondrial Ca\(^{2+}\) release and MMP in hepatocytes during a normoxic to anoxic transition. Unlike brain, hepatocyte membrane potential remains constant at -28 mV and conductance decreases. There was no anoxia-mediated increase in cytosolic Ca\(^{2+}\) levels but similar to brain MMP did decrease to a new steady state, and the addition of an uncoupler further decreased the MMP. An inhibitor of the mK\(_{\text{ATP}}\) channel partially reversed the decrease in MMP during anoxia and an activator decreased MMP during normoxia. While mitochondria in both tissues undergo depolarization with anoxia, this only leads to increases in Ca\(^{2+}\) levels in brain tissue. Therefore, in pyramidal neurons mitochondria can act as a low oxygen sensor but in hepatocytes it remains unclear.
11.2
Re-oxygenation resilience - the other aspect of the crucian carp’s anoxia tolerance

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The crucian carp Carassius carassius is capable of surviving without oxygen for several months, by constantly matching ATP supply and demand. It is aided by its unique ability to produce ethanol as an anaerobic end-product and undergo partial metabolic rate suppression, shutting down non-vital functions such as vision. In this presentation, however, I will focus more on another aspect of anoxia, namely the associated re-oxygenation, which in hypoxia- and anoxia-insensitive species leads to severe tissue damage and even death, but which the crucian carp tolerates when its habitat is re-oxygenated every spring. In addition to investigating the effect of anoxia and re-oxygenation on brain cell death, oxidative damage, and memory and learning ability, we have used next-generation sequencing followed by de-novo transcriptome assembly to identify mRNA sequences differentially regulated between normoxic, anoxic and re-oxygenated crucian carp, in order to find processes and pathways that may respond differently in crucian carp as compared to non-tolerant species.

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11.3
Learning from the experts: how marine and freshwater bivalves cope with anoxic transgression

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Maximum performance, such as highest respiration and heart rates, or pumping activities are often interpreted as marking the range of “optimal physiological performance” in marine species. Mollusks and especially some bivalves display, however, a hypoxia-adapted phenotype to the point where they maintain extremely low oxygen partial pressure (<4 kPa pO2) in shell water and hemolymph against normoxic (21 kPa) “outside” pO2 over periods that can last many hours. As their respiration is usually “oxygen-conforming” in that low pO2 range, “underperformance” is not necessarily a sign of physiological strain in these organisms, but essentially self-induced. When exposed to environmental oxygen shortage, these specialists withstand prolonged hypoxia for many days and up to weeks by reducing metabolism to extremely low rates of sometime less than 10% of normoxic performance. I will present new insight into the complex strategies employed by hypoxia-tolerant marine and freshwater bivalves, combining different organizational levels from the whole animal and its microenvironment to subcellular reorganization.

With help of their microbial biofilms and gut microbiome, bivalves create their own chemical microenvironment inside the closed shell. Within minutes of shell closure, the shell water pO2 becomes hypoxic and eventually anoxic. Using fluorescent dyes that react with reactive oxygen and nitrogen species, we showed how NO accumulates in the endothelial cells surrounding the blood vessel in Mytilus edulis gills as pO2 was lowered to 7kPa (30 % oxygen saturation), conferring relaxation of the endothelium and improving hemolymphatic flow to counteract the oxygen shortage. As pO2 declines further, facultative anaerobic nitrate respiring bacterial biofilms produce nitrite which accumulates and eventually disproportionate to nitrous oxide (N2O) and nitric oxide (NO) (Stief 2013). NO accumulating in shell water competitively inhibits cytochrome-c oxidase (CCO) activity in peripheral tissues (gills), slowing down gill respiration and supporting oxygen distribution to the central body.

Many bivalves have preserved special anaerobic mitochondrial pathways, inheritance from bacterial endosymbionts (Tielens et al. 2002), which support reduction of short-chained organic acids (fumarate) to succinate and from there to propionate for slightly better ATP yield compared to anaerobic glycolysis. Mitochondria that switch between aerobic and anaerobic electron transport are often also endowed with an alternative end-oxidases (AOX) that receives electrons directly from the ubiquinone pool, bypassing complexes III and CCO (complex IV). AOX has recently been sequenced in several sediment dwelling bivalves and we find it is strongly upregulated in hypoxia and anoxia exposed freshwater mussels (Yusseppone et al. 2018). As AOX is resistant to sulfide inhibition, it can stabilize mitochondrial electron flow and membrane potential under low oxygen and potentially under sulfidic conditions. Maintenance of low mitochondrial membrane potential lowers the risk of reactive oxygen species (ROS) production upon reoxygenation. Therefore, significant induction of antioxidants during hypoxic exposure is often not detected, and presumably not necessary in these bivalves. Furthermore, lower shell water pO2 enables constitutive protein levels of hypoxia-inducible transcription factor (HIF-α) in bivalve and gastropod tissues which reinforces expression of genes supporting anoxic survival. I will show bivalve examples in support of these low oxygen strategies in evolutionary early non-model organisms, which can be so different from all we perceive as “normal”.


Tielens et al. (2002) TRENDS in Biochemical Sciences, 27(11), 564-572.

11.4
Flies are not turtles or carp: non-conventional anoxia tolerance

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The ability to tolerate bouts of oxygen deprivation varies tremendously across the animal kingdom. Animal species and individuals from different regions show large variation in tolerance to hypoxia; additionally, it is widely known that neonatal mammals are much more tolerant to anoxia than their adult counterparts, including in humans. Furthermore, oxygen limitation plays a key role in many pathologies, and yet we still lack a fundamental understanding of the mechanisms responsible for intraspecific or interspecific variation in hypoxia/anoxia tolerance. Here, we use a variety of experiments designed to test hypotheses for how adult *Drosophila melanogaster* survive longer than larvae (LT50: 8 vs. 1 h). First, we tested the most common mechanism for anoxia tolerance in vertebrates: are more tolerant individuals better able to maintain ATP and thereby prevent ion disruption during anoxia? During the first two hours, larval ATP fell to <1% of normal and hemolymph [K⁺] rose by 50%; survival decreased with time, in strong correlation with the fall in ATP and rise in [K⁺]. During the same time period in adults, ATP also fell strongly, while hemolymph [K⁺] rose even more strongly than in larvae, but survival was 100%. During the next six hours, adults maintained high survival, while ATP was maintained at 2% of normal levels, and hemolymph [K⁺] continued to rise to 5x normal. After 8 h of anoxia, adult ATP levels decreased further and [K⁺] continued to rise; and over this time period, both of these variables correlated with decreased survival. The superior anoxia tolerance of adult *Drosophila* appears to be due to the capacity to maintain and tolerate very low ATP levels, and to the ability to tolerate high extracellular [K⁺]. This suggests that protective mechanisms downstream of ATP depletion and an ionic disruption may be important for surviving anoxic bouts. Next, we used a targeted H-NMR metabolomics approach to investigate three questions. Are developmental differences in anoxia tolerance associated with metabolic rate, anaerobic capacity, and/or protective metabolites? Despite the fact that metabolic rates were not different after 30 min of anoxia, adults did suppress metabolic rates to levels 40% lower than larvae during the first 30 min of anoxia, likely due to the paralysis of adults vs the escape locomotion of the larvae. Secondly, adults utilize alternate anaerobic end products (alanine, succinate and acetate) more so than larvae, likely attaining a better ATP/H⁺ ratio and redox balance. Lastly, adults have higher levels or increased concentrations of several putatively protective metabolites (i.e. polyols, β-alanine, taurine) that likely reduce cellular damage associated with osmotic or antioxidant stress during or after anoxia. In a third set of experiments, we use the *Drosophila* Genetic Reference Panel to assess genetic variation in anoxia tolerance. We show that anoxia tolerance is a highly variable trait, and that much of this variation is determined genetically. Male and female anoxia tolerance is tightly correlated, yet there is still a substantial amount of variation within some lines. Genome-wide association analyses for adult anoxia tolerance identified many genes with functions closely related to immune/inflammatory response, consistent with the strong up-regulation of immune genes after anoxic/hypoxic exposure. These data strongly suggest that genetically-based differences in immune function are key differentiators of anoxia-tolerance, but the mechanisms responsible remain elusive. GWA also identified multiple ion transport function genes whose allelic variation affected anoxia-tolerance in adults; examination of how these alleles affect ion disruption or tolerance of ion disruption may provide important insights into the maintenance of genetic variation in these ion transporters. Altogether, this study suggests that a new focus of research in anoxia-tolerance should be the mechanisms by which animals can survive and quickly recover from such hypo-energetic and  ionally-disrupted conditions. Supported by NSF IOS1256745.

12: MICRORNAS IN COMPARATIVE AND EVOLUTIONARY PHYSIOLOGY

12.1
For Everything There is a Season: microRNA Regulation of Insect Diapause

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Diapause is an endogenously regulated dormancy that provides insects, and other animals, a means to “escape” seasons of inimical conditions and to synchronize periods of growth and reproduction with seasons of abundant resources. Diapause is a complex, alternative phenotype, characterized by changes in developmental timing, metabolism, and stress tolerance, that is coordinated by molecular mechanisms that are not completely understood. MicroRNAs, small (18-25 nucleotide) non-coding RNAs) are emerging as components of a conserved “toolkit” of processes that regulate gene expression before, during, and after diapause in evolutionarily diverse insect species including flesh flies, mosquitoes, and moths. Combined RNA-seq and qRT-PCR studies have been used to identify differences in the abundance of miRNAs in diapausing insects relative to their nondiapause counterparts. Pupal diapause in the flesh fly, *Sarcophaga bullata* is characterized by changes in the abundance of at least ten conserved microRNAs¹. MiR-289-5p and miR-1-3p are increased by as much as 2-fold in diapausing pupae, while miR-9c-5p, miR-13b-3p, miR-289-5p and miR-1-3p are increased by as much as 2-fold in diapausing pupae, while miR-9c-5p, miR-13b-3p,
miR-31a-5p, miR-92b-3p, miR-275-3p, miR-276a-3p, miR-277-3p, and miR-305-5p are underexpressed in adult females of Culex pipiens mosquitoes that are programmed to enter diapause compared to females not programmed to enter diapause. Many of these (e.g., miR-13b-3p, miR-275-3p, miR-277-3p, and miR-305-5p) are also underexpressed in adult females of Culex pipiens and H. zea. In the moth, Helicoverpa zea, downregulation of miR-289-5p and miR-277-3p following termination of pupal diapause provide evidence these miRNAs regulate at least some of the diapause-relevant changes in Lepidoptera as well as in Diptera. The precise targets of these miRNAs are differentially regulated in S. bullata, Cx. pipiens, and H. zea, have yet to be identified in these species, but their functions, inferred from studies on Drosophila melanogaster and Aedes aegypti, suggest they regulate diapause-relevant processes including cell cycle progression, developmental timing, suppression of metabolism, and stress responses. Future challenges include integrating these control molecules into the “big picture” of previously identified signaling pathways and gene networks that mediate the diapause entry, maintenance, and termination. This work was funded by National Science Foundation Grants NSF IOS-1354377 and NSF IOS-1755318, and USDA/NIFA Grant 2015-67013-23416.  
2. Meuti et al., 2018. Submitted  
3. Reynolds et al., 2018. Submitted

12.2 The role of miRNA regulation on phenotypic responses to environmental stressors in fish.  
**Paul Craig**, 1 **Heather Ikert**, 1 **Ivan Cadonic**, 1 **Nathan Benoît** 1  
1Biology, Univ. of Waterloo  
MicroRNA (miRNA) are small (~21-22 nucleotides in length), non-coding RNA that can bind to multiple target transcripts, effectively reducing translation. This can ultimately result in decreased functionality and altered phenotype of a given protein or pathway. MicroRNA have been an influential tool in studying pathologies of numerous human related diseases, such as cancer and heart disease. However, less than 3% of comparative physiology approaches examine the functional consequences of miRNA, which is instrumental in the regulation of phenotypic plasticity. This presentation will provide an overview of the recent advancements in understanding microRNA regulation of phenotype from individual pathways all the way to whole animal impacts in fish. Emphasis will be placed on how the environment plays an essential role in mediating phenotypic response in teleosts, through changes in microRNA. Unique facets of microRNA will also be discussed, including the conservation of binding sites in key transcripts across millions of years of evolution, secretion and circulation of miRNAs following a stressful event, and microRNA regulation of energetics. A final discussion will focus on the use of microRNA shed into the water as a non-invasive marker for the identification of aquatic communities that are under stress. At the conclusion of this presentation, it is hoped that there is a greater appreciation and understanding of microRNA regulation of phenotypic responses related to environmental challenges in teleosts. This work is funded through NSERC Discovery.

12.3 A cool story: Non-coding RNAs in natural models of cold adaptation  
**Pier Jr Morin**, 1 **Mathieu D Morin**, 1 **Jacques J Frigault**, 1  
1Department of chemistry and biochemistry, Université de Moncton  
Characterization of the underlying molecular changes associated with cold adaptation has revealed insightful clues on how various models cope with low temperatures. Nevertheless, much remains to be accomplished in order to clarify the complete molecular picture linked with life at low temperatures and to translate this knowledge into practical applications. Several natural models of cold adaptation exist, such as mammalian hibernators and cold-hardy insects, and can provide an overview of the recent advancements in understanding microRNA regulation of phenotype from individual pathways all the way to whole animal impacts in fish. Emphasis will be placed on how the environment plays an essential role in mediating phenotypic response in teleosts, through changes in microRNA. Unique facets of microRNA will also be discussed, including the conservation of binding sites in key transcripts across millions of years of evolution, secretion and circulation of miRNAs following a stressful event, and microRNA regulation of energetics. A final discussion will focus on the use of microRNA shed into the water as a non-invasive marker for the identification of aquatic communities that are under stress. At the conclusion of this presentation, it is hoped that there is a greater appreciation and understanding of microRNA regulation of phenotypic responses related to environmental challenges in teleosts. This work is funded through NSERC Discovery.

**2018 APS INTERSOCIETY ABSTRACTS OF INVITED AND VOLUNTEERED PRESENTATIONS**
12.4

Regulation of microRNA activity to promote multipotent cell fate during dauer diapause

Xantha Karp1, Allison Cale2, Megan Wood2, Isaac Smith1, Amelia Alessi2, Mallory Freeberg2, John Kim2, Liberta Nika3, Kyal Lalk1, Mikayla Schmidt1, Anna Zinovyeva2, Alexis Santos1, Payton Salomon1

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Animal developmental programs are robust to the diverse environmental and physiological conditions experienced in nature. One mechanism used by multiple species to withstand adverse environmental conditions is entry into diapause, or developmental arrest. When conditions improve, development resumes and is completed normally. During diapause, multipotent stem and progenitor cells must retain the capacity to give rise to all appropriate cell types. The mechanisms that enable development to proceed normally after diapause are incompletely understood, and this question forms the basis of our work. In C. elegans, adverse conditions drive entry into dauer diapause midway through larval development [1]. If environmental conditions improve, dauer larvae recover and complete development normally. However, there are differences in the genetic network required to specify development after dauer [2].

One of the best understood examples of this phenomenon is the cell fate specification of lateral hypodermal seam cells, a C. elegans stem cell model. During larval development seam cells are multipotent and undergo self-renewing divisions to produce additional seam cells as well as differentiated cell types. Seam cell fate is specified by a network of “heterochronic” genes [3]. During early development, transcription factors and RNA-binding proteins specify early seam cell fates. Progression to later cell fates depends on the expression of microRNAs that downregulate the early-promoting genes [3]. Intriguingly, many heterochronic genes that are normally necessary for development are completely dispensable after dauer. Furthermore, seam cell fate appears to be re-set during dauer [2]. Using a combination of genetic mutants and gene expression data, we find that this dauer-specific developmental program involves modulation of microRNA activity. Mutants with compromised microRNA-induced silencing activity display penetrant phenotypes during non-dauer development but appear completely healthy after dauer [4]. During dauer, expression of heterochronic microRNAs is low. We find that a subset of the genes that control the decision to enter dauer diapause are also required to prevent inappropriate expression of heterochronic microRNAs during dauer, and thereby maintain multipotent cell fate. Surprisingly, these heterochronic microRNAs act through a novel pathway, distinct from the canonical pathway in which they act to promote developmental progression during non-dauer development. Thus, we have identified a new, microRNA-dependent mechanism that coordinates diapause with multipotent cell fate in C. elegans. Because the genes involved are highly conserved in animals, this mechanism is potentially relevant to the maintenance of multipotent stem cell fate outside of nematodes.


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12.5

A network of microRNAs and RNA binding proteins acts maternally to regulate sex determination in the C. elegans embryo.

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1NIDDK Intramural Research Program, National Institutes of Health

Although many roles of microRNAs in differentiated tissues have been described, relatively few microRNAs are known to function in early embryonic development. In particular, little is understood about the function of microRNAs expressed prior to the maternal to zygotic transition, when post-transcriptional control of gene expression is widespread. Seeking to elucidate the function of microRNAs in early development, we have focused on the mir-35-41 microRNA cluster in C. elegans, which is expressed maternally and in embryos, and is essential for embryonic development and fecundity. Here I show that the mir-35-41 microRNA cluster regulates sex determination, preventing aberrant activation of male-specific gene expression in hermaphrodite embryos. Two predicted mir-35-41 target genes are required for the sex determination phenotypes of mir-35-41 mutant embryos, suggesting that they act downstream of mir-35-41. These target genes, sup-26 and nhl-2, both encode RNA binding proteins, thus delineating multiple new layers of post-transcriptional regulation of the sex determination pathway.

Most players in the sex determination pathway are regulated zygotically, after the inheritance of both sex chromosomes. In contrast, the maternal load of mir-35-41 is largely responsible for regulating sex determination. Because of this maternal contribution to an inherently zygotic process, I propose that mirs-35-41 act as a developmental timer, ensuring a period of naiveté in early embryos, and preventing premature decision-making in sex determination and possibly other developmental processes.

Using CRISPR/Cas-9 to manipulate the endogenous mir-35-41 seed match in the nhl-2 3’ UTR, I observe that
repression of nhl-2 by mir-35-41 is not only required for proper sex determination but also for viability, showing that a single microRNA target site can be essential. Our work thus also makes progress towards understanding the essentiality of this maternally-contributed microRNA family.

13: THE ROLE OF GASOTRANSMITTERS IN HYPOXIC AND CHALLENGING ENvironments

13.1

Introduction to gasotransmitters and the role of carbon monoxide (CO) in hypoxia-tolerant species

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Certain gases [carbon monoxide (CO), nitric oxide (NO) and hydrogen sulfide (H2S)] that were previously thought to be strictly pollutants, are now known to be endogenously generated and act as critical signaling molecules in many physiological processes. Further, these gasotransmitters have recently shown to elicit protective or therapeutic effects, making the study of these gases an emerging field in medicine, biochemistry and physiology. Carbon monoxide (CO) is naturally produced in the body from the regular turnover of heme (a major component of many heme-proteins) by heme oxygenase enzymes. Ironically, CO also binds tightly to the same site on hemoglobin as oxygen (creating carboxyhemoglobin), leading to an allosteric increase in hemoglobin-oxygen affinity. Too much carboxyhemoglobin can therefore limit oxygen delivery to tissues. However, a moderate increase in hemoglobin-oxygen affinity is thought to confer tissue protection during severe hypoxia. Increased hemoglobin oxygen affinity is consistently seen in species adapted to tolerate chronic hypoxia, yet this phenotype is not thought to be present in diving mammals. Our research highlights that some deep-diving mammals exhibit elevated levels of carboxyhemoglobin that correlate well with their increased heme-protein stores. This results in an increase in hemoglobin-oxygen affinity, which can improve tissue oxygen delivery during the severe hypoxia these animals face while diving. This is the first direct evidence of a hypoxia-tolerant animal utilizing CO as a mechanism to achieve increased hemoglobin-oxygen affinity.

13.2

Roles of NO and H2S signaling in hibernators

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Surviving winter at nearly subzero temperatures without food is an extreme physiological challenge common to several vertebrates, including the brown bear, crucian carp and freshwater turtles. Common to these hibernating species is the ability to undergo metabolic suppression and to tolerate end products of energy metabolism and oxidative stress at reoxygenation. These responses are potentially controlled at least in part via nitric oxide (NO) and hydrogen sulfide (H2S) signaling pathways. Analyses of type and distribution of NO and H2S metabolites in the blood of brown bears in the wild reveal significant changes in the sulfide distribution during hibernation, suggesting recycling of sulfide oxidative products into H2S, but no apparent major changes in NO metabolites [1]. These results suggest that H2S may contribute to controlling aerobic metabolism suppression in hibernating bears. Conversely, in anoxic and cold-acclimated turtle and crucian carp, NO metabolites of most tissues increase markedly during anoxia, particularly in the heart, suggesting a key role of NO in the protection against oxygen deprivation [2]. Surprisingly, anoxic turtle hearts retain similar levels S-nitrosation of mitochondrial complex I, a major NO-dependent modification preventing reactive oxygen species (ROS) generation at reoxygenation [3]. In addition, changes in H2S metabolites measured in cold-acclimated turtles during anoxia are less pronounced than for NO and limited to selected organs. In conclusion, these results suggest distinct biological roles of NO and H2S signaling in the aerobic and anaerobic suppression of hibernating endotherms and ectotherms, respectively.

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13.3 Carbon monoxide signaling in the control of breathing and impacts for high-altitude adaptation

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Animals living at high altitude are subject to strong selective pressure to improve oxygen delivery and utilization efficiency. Tibetan and Andean human populations developed distinct physiological adaptations to high altitude, including different ventilatory sensitivity to oxygen. This may be explained by variation in heme oxygenase 2 (HO-2; HMOX2), which is implicated in carotid body oxygen sensing. HO-2 produces carbon monoxide in the presence of oxygen which inhibits hydrogen sulfide production by inhibiting cystathionine γ-lyase activity. Furthermore, heme oxygenase activity may contribute to differences in hemoglobin concentration across these groups since it is a key player in heme catabolism. We conducted whole genome sequencing and genotyped HMOX2 variants in Tibetan and Andean populations to determine if HMOX2, previous demonstrating signals of evolutionary selection in Tibetans, is also under selection in Andeans and if the putatively adaptive HMOX2 variants differ across these groups. We also collected hypoxic ventilatory response measurements in Andean highlanders to determine if HMOX2 variants are associated with ventilatory sensitivity to oxygen. We found that while HMOX2 demonstrates signals of selection in Tibetans, the gene encoding the inducible heme oxygenase isoform, HMOX1, is under selection in Andeans. Whether the adaptive significance of HMOX1 is related to oxygen sensing in the carotid body, hemoglobin concentration, alterations in inflammatory profiles, and/or cytoprotective effects remains to be determined.

13.4 Hydrogen Sulfide and Oxygen Sensing: From Evolution to Function

Kenneth Olson1
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Life appeared nearly 4 billion years ago (BYA) in anoxic and sulfidic environments and nearly seven-eights of evolution occurred in these conditions. When oxygen appeared 0.6 BYA most organisms readily became dependent on this efficient O2 acceptor[1]. This necessitated development of elaborate O2 sensing mechanisms to monitor availability of environmental O2, ensure adequate O2 delivery to tissues and to regulate cellular metabolism. Although low Po2 is directly coupled to physiological effectors in these cells, the identity of this O2 “sensor” has remained enigmatic despite over 50 years of intensive research. In 2006 we proposed a novel O2 sensing mechanism based on the balance between constitutive cellular production of biologically active hydrogen sulfide (H2S) and its inactivation through oxidation, the latter directly coupled to O2 availability[2]. Since then numerous studies on a variety of animals and tissues have shown that 1) H2S production is O2 independent, whereas there are numerous mechanisms of O2-dependent H2S metabolism, 2) tissue H2S concentration is intimately coupled to O2 concentration and this is regulated at physiologically relevant Po2, 3) physiological responses to exogenous H2S are identical to hypoxic responses, most notably as the paradoxical hypoxic pulmonary vasoconstriction or dilation in terrestrial diving mammals, 4) compounds that inhibit or augment H2S production inhibit or augment hypoxic responses, respectively, and 5) H2S acts upon effector mechanisms known to mediate hypoxic responses[3]. Recent studies have shown that H2S mediated O2 sensing has both a rapid on/off component but can maintain its effectiveness for extended periods. These studies have employed “Kroghian” physiology and Occam’s razor to demonstrate that H2S mediated O2 sensing is a simple, yet elegant remnant of an anoxic past where sulfide-based metabolism became adapted to detect the molecule that eventually replaced it as the energetic center of redox metabolism. Support, NSF IBN 0235223, IOS 0641436, IOS 1051627, IOS 1443610.

14: THE ROLE OF THERMAL PERFORMANCE CURVES IN PHYSIOLOGY, ECOLOGY AND CONSERVATION
SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

14.1
The thermal performance curve: mechanisms, applications, and pitfalls for a concept that just turned 40 (happy birthday!)
Michael Angilletta

School of Life Sciences, Arizona State Univ.
In the autumn of 1978, Huey and Stevenson penned an influential paper to introduce the concept of the thermal performance curve (1979, American Zoologist 19: 357-366). This concept catalyzed a theory of thermal adaptation and helped biologists to understand why animals thermoregulate. In recent years, thermal performance curves have been used to explain everything from global patterns of biodiversity to extinction during global warming. Given how widely biologists have applied thermal performance curves, we should periodically question the implicit assumptions and consider ways to improve the concept. In this talk, I will focus on three assumptions taken for granted in most applications of thermal performance curves: 1) a curve reflects the thermal sensitivity of performance; 2) the thermal sensitivity of performance reflects the thermal niche of a genotype; and 3) thermal sensitivity does not depend on factors other than temperature. Because these assumptions are usually false, I will demonstrate ways to relax these assumptions when modeling thermal adaptation or thermal ecology in a changing environment.

14.2
Translating physiology to fitness using thermal performance curves
Timothy Clark

School of Life and Environmental Sciences, Deakin Univ.
Lab-derived thermal performance curves (TPCs) provide a tangible means for predicting the responses and fitness of wild ectothermic animals in the face of spatial and temporal thermal variability. The use of TPCs has become even more important in the current era of human-induced climate warming, whereby forecasts of the performance of wild animals can be made using characteristics of TPCs including their slope, magnitude and breadth. Given that a TPC for fitness generally follows a bell-shaped pattern across the naturally occurring temperature range of a species, many lab studies attempt to isolate physiological processes that follow the same pattern and position along the (horizontal) temperature axis, thus opening the door to a cause-and-effect understanding of the physiological mechanisms underlying temperature-dependent fitness. In this regard, the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis is based on the TPC for aerobic scope (maximum minus minimum aerobic metabolism) and has been touted (including in an IPCC report) as a universal mechanism underlying temperature-dependent patterns in fitness of ectothermic animals. Controversy surrounds the OCLTT hypothesis, with mounting evidence demonstrating that the hypothesis lacks relevance and predictive power for the majority of ectothermic vertebrates. In this talk, I will outline some of the controversy related to the OCLTT hypothesis, including the negative consequences of marketing a hypothesis as universal while ignoring studies that contradict the foundational basis and predictions of the hypothesis. Moving beyond OCLTT, I will highlight promising avenues that are deserving of our scientific attention. I hope to promote healthy skepticism to inspire broader, multi-disciplinary research programs investigating the use of TPCs in predicting the lifetime fitness of wild animals.

14.3
Assessing the role of acclimation and adaptation in thermal performance curves
Johannes Overgaard

Torsten N. Kristensen, Jesper G. Sørensen, Vanessa Kellermann, Kristian Beedholm, Volker Loechschke, Heidi J. Maclean

Zoophysiology, Aarhus Univ., Department of Chemistry and Bioscience, Aalborg Univ., Genetics and Ecology, Aarhus Univ., Sch of Biological Sciences, Monash Univ.
Environmental temperature is arguably one of the most important factors dictating the distribution of animals. All animals, including insects, must experience periods with temperatures that allow for growth and reproduction. It is, therefore assumed that thermal performance curves (TPC) for growth and reproduction have evolved to match the species thermal environment. Likewise, species can only exist in climates where they avoid lethal thermal stress. It is, therefore also assumed that tolerance to thermal extremes has evolved to match the occurrence in their environment. Nevertheless, there are few studies that have systematically investigated how capacity traits (growth and reproduction) and thermal tolerance traits (CT_{min} and CT_{max}) vary among and within species and how such variations relate to their natural distribution.

Capacity traits of ectotherms are often depicted using TPCs and textbook examples often highlight that temperate species have broader TPCs and lower optimal temperatures compared to tropical species. Furthermore, theory predicts that acclimation to low or high temperatures could shift the TPC and again it is
assumed that species originating from variable temperate environments are more plastic. Here we measure TPCs of three fitness components (fecundity, egg to adult viability and developmental rate) of flies developed at 20°C and tested at seven test temperatures using 22 species of Drosophila originating from a wide range of temperate and tropical environments. For 10 of these species, we also measured how thermal capacity changed following developmental acclimation to three additional temperatures (15, 23 and 27°C). In parallel with these studies we also measured thermal tolerance traits (CT_{min} and CT_{max}) and investigated how these traits varied among and within species. Using these data, we test if Drosophila TPC’s conform to the fundamental assumptions about the evolution and plasticity of TPCs. We found that cold tolerance varied strongly between the species with higher tolerance in temperate species whereas heat tolerance was similar among species. Similarly, we found a positive and predictable response to both heat and cold acclimation. These results suggest that adaptation to temperate environments have involved the evolution of increased cold tolerance while the level of plasticity was similar among temperate and tropical species. Contrary to our expectation for fitness traits we find that the breadth and optimum of TPCs is similar in temperate and tropical species and we also find that the plasticity of TPCs is very limited. This suggests that TPC’s in Drosophila species are stable within and among species irrespective of origin or acclimation treatments. Together our data suggest that thermal tolerance limits have evolved to overcome extreme environmental conditions whereas evolution of the temperature range for optimal population growth is more likely to be governed by temperatures that prevail during the thermally benign growing season. These results therefore support previous findings suggesting that species distribution of Drosophila can be modeled reliably using the tolerance traits CT_{min} and CT_{max} while models using thermal capacity traits are unable to make reliable predictions of current distributions.

14.4
Comparing thermal performance curves across traits: how consistent are they?
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Thermal performance curves (TPCs), describing the relationship between temperature and fitness, are commonly integrated into species distributional models for understanding species responses to climate change. However, the accuracy of these predictions rests on a number of underlying assumptions, primarily that measured TPCs are static in time and space. TPCs however are unlikely to be static, which will have implications for the use of TPC’s in climate change models. A few reasons TPCs may vary are: because different traits have different thermal sensitivities, traits vary with the timing and duration of the temperature exposure (short exposures: mins – hrs, long exposures: days – months), or because trait variation is sensitive to other factors rarely measured such as biotic interactions. Here I explore the extent to which TPCs in D. melanogaster vary across traits (fecundity, viability, activity and metabolic rate) and how the timing of the temperature exposure may influence the shape and descriptors of the TPC. Moving beyond intra-specific variation I also examine the role of species interactions in shaping TPC’s for metabolic rate.
Mechanisms underlying forelimb vs. hindlimb function during terrestrial locomotion in juvenile alligators
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Most biomechanical models of quadrupedal locomotion rely on the assumption that the animal has a center-of-mass (CoM) located equidistant from the pectoral and pelvic girdles. Previous work based on the whole-body mechanics of crocodilians suggest their caudally-displaced CoM imparts a braking role on their forelimbs (FLs) and a propulsive role on their hindlimbs (HLs). However, it remains unclear how crocodilians distribute these roles across the various limb joints involved in terrestrial locomotion. Here we use high-speed videography and force-plate ergometry to develop an inverse dynamics model for the limb joints in the American alligator (\textit{Alligator mississippiensis}). We test how mechanical energy production (propulsion) and dissipation (braking) are distributed amongst FL and HL joints during steady-state walking. Our preliminary results show the wrist and elbow spend larger percentages of stance phase flexing, whereas the ankle and knee spend more time extending. We also find a greater braking impulse in the FLs and greater propulsive impulse in the HLs, and no significant difference in mediolateral or vertical impulses between limb pairs. Furthermore, the transition from braking to propulsion occurs later in the FLs when compared to the HLs. These results highlight the disparities in limb joint mechanics between corresponding distal joints in the fore- and hindlimb of alligators and will serve as a first step in quantifying joint moments during walking. Joint moments will help determine which limb joints, and therefore which limb muscles, contribute most significantly to the FL’s net braking impulse and the HL’s net propulsive impulse in crocodilians. This work will improve our overall understanding of skeletal muscle function in the context of the whole-organism and allow us to effectively characterize functional muscle specializations during terrestrial locomotion.

Effect of PIT tagging on aerobic metabolism and growth of the Gulf killifish, \textit{Fundulus grandis}
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Passive integrated transponder (PIT) tags allow unambiguous identification of individual animal subjects in field and laboratory studies. Typically, PIT tags are implanted into the animal, raising the question of whether the tagging procedure could alter subsequently collected physiological data. Here, we evaluated the effects of PIT tagging on the standard metabolic rate (SMR) and specific growth rate (SGR) of the Gulf killifish, \textit{Fundulus grandis}, a small estuarine fish native to the Gulf of Mexico. SMR, determined by intermittent-flow respirometry, and SGR, determined as percent change in body mass per day, were measured prior to PIT tagging and once per week after tagging for one month. Our results demonstrate that PIT tagging has no effects on SMR or SGR for the duration of the study. SMR was positively related to body mass, as expected, with the slope of a log-log relationship of 0.81. In addition, the SMR of male fish significantly decreased over the course of the experiment, independent of PIT tagging. We also found that male fish had higher SGR than female fish, again independent of tagging. We conclude that body mass, sex of fish, and duration of the experiment may affect SMR or SGR, but that neither variable was influenced by PIT tagging between 1 and 4 weeks after tagging. We suggest that PIT tagging can be used in \textit{F. grandis}, and probably in other small fishes, with minimal or no impact on subsequent physiological measurements. Funding for this work was provided by the Greater New Orleans Foundation and the Audubon Nature Institute.

The effects of body mass on immune cell concentrations of terrestrial mammals
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Body mass is likely to affect the way organisms evolve, develop, and use immune defenses. Risk of exposure to parasites, physical constraints affecting transport and metabolism of defensive cells and their secreted molecules, and tradeoff options among alternative life histories all impinge on how hosts of different body sizes cope with infections. Here, we investigated whether and how body mass and blood neutrophil and lymphocyte concentrations were related among 300+ terrestrial mammalian species. First, we tested whether predictions derived from existing theories (Protecton Theory, basal metabolic rate constraints, or risk of parasite exposure) best-predicted slope coefficients. We then evaluated the predictive power of body mass for these leukocyte concentrations compared to sociality, diet, life history, and phylogenetic relatedness. Phylogeny was the most important predictor of
leukocyte concentrations; taxon explained 61% of variation in lymphocytes and 67% of variation in neutrophils. Body mass explained only a small portion of the variation (3% in lymphocyte and 9% in neutrophils), and other factors combined show similar importance. Our data for lymphocytes revealed a scaling coefficient close to, but not overlapping the slope predicted by the Protecton theory, whereas no hypothesis was supported for neutrophils. Indeed, the strong positive effect of body mass on neutrophils was unexpected: we found that, extrapolated to total cell numbers, a 3800 kg elephant circulates 13,300,000 times the neutrophils of a 15 g mouse, whereas their masses differ by only 250k-fold. We hypothesize that such high neutrophil numbers might offset i) higher parasite exposure that large animals face as they traverse more risk space per unit movement and/or ii) provide broad protection against the relatively higher cellular replication capacities of pathogens relative to that of large mammals.

15.5
Sequence analysis, expression, and preliminary functional characterization of Aedes aegypti sodium-dependent cation-chloride cotransporters
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The genome of the yellow fever mosquito Aedes aegypti contains three genes with sequence similarity to vertebrate Na-K-Cl cotransporters (NKCCs). One gene, aeNKCC1, codes for an ortholog to Drosophila melanogaster ncc69, which is a bumetanide-sensitive NKCC (1,2). Two genes, aeCC2C and aeCC3C, group within an insect-specific clade of transporters that have not been functionally characterized. These paralogs are consecutive in the genome, have similar exon structures, and have orthologs in all mosquito genomes that we have evaluated. Thus, they probably arose from a tandem gene duplication at the base of the mosquito lineage. In prior qPCR experiments (3), we found aeCC3 to be 100-fold more abundant in larvae than in adults. In larval tissues, aeCC2C was 2-fold more abundant in Malpighian tubules compared to anal papillae. In contrast, aeCC3C was nearly 100-fold more highly expressed in larval anal papillae compared to Malpighian tubules, suggesting a role in absorption. Consensus phosphorylation sites for Ste20-related proline alanine-rich kinase (SPAK) are present on aeCC3C and the aeCC2C-X1 splice variant but not aeCC2C-X2 splice variant. Additionally, aeCC2C-X1 has several consensus protein kinase A sites that are not found on aeCC3C or aeCC2C-X2. Both aeCC2C and aeCC3C lack a dileucine motif that targets human NKCC1 to the basolateral membrane (4) and is present in the aeNKCC1 sequence. Quantitative PCR with splice variant specific primers indicated that when both variants are measured, aeCC2C was approximately 200 fold more highly expressed in adult female hindgut tissue than in Malpighian tubule, whereas the aeCC2C-X1 variant was equally expressed in the two tissues. An antibody produced against an aeCC2-specific peptide detected bands at and above 120 kDa in adult and larval tissues and stains the basolateral membrane of larval Malpighian tubules. To evaluate aeCC2 function, we measured uptake of lithium, a tracer for sodium, into oocytes injected with cRNA encoding aeCC2. Following exposure to buffers containing lithium, oocytes were lysed in distilled water and lithium concentration was assessed by cation chromatography. Oocytes expressing aeCC2 transported lithium at greater rates than water-injected controls. These findings suggest potential roles for aeCC2 and aeCC3 in mosquito transepithelial Na+ secretion and absorption. Funding: NSF-IOS-1557230, Kenyon College, and State and Federal funds appropriated to the OARDC of the Ohio State Univ.

15.6
Hypoxia Avoidance Behavior in two Air-breathing Fishes
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Aquatic animals are known to be sensitive to low O2 environments. Aquatic hypoxia can limit an animal’s capacity for aerobic metabolism, reducing its ability for locomotion, growth, reproduction, and survival. It is thus important for a fish to be able to detect and avoid hypoxic environments or develop an auxiliary method for obtaining O2, such as breathing air. Air breathing allows a fish to access environments and maintain aerobic metabolism independent of environmental O2 levels. In this study, we test two air-breathing fishes, Polypterus and Pangasianodon, for the presence of a hypoxia avoidance behavior both when allowed to freely breathe air and when air access is denied. Fish were placed in a shuttle box consisting of two circular arenas attached to allow free movement between the two. The position of the fish was monitored and O2 was reduced on one arena and then returned to normal while the other side was reduced. Separate trials were run with a subsurface net to deny air breathing. Polypterus did not show a hypoxia avoidance behavior when allowed air access, however it
did when denied air access, similar to a solely water-breathing fish. *Pangasianodon* did not show a hypoxia avoidance behavior with or without air access even at O₂ levels below its air-breathing threshold and Pcrit. The lack of hypoxia avoidance behavior may correspond with the physiologically ecology of these two fishes. Hypoxia avoidance behavior may be important to *Polypterus*, being a demersal predatory fish, in the wild when surfacing to air breath maybe costly, (e.g., when predation risk is high, while stalking prey, or in deep water). In contrast, a blunted response to external hypoxia may be advantageous for *Pangasianodon*, usually found in surface water with convenient air-access. Internal PO₂ would be the more important to detect than external PO₂ to maintain O₂ delivery if frequently inhabiting hypoxic water but air-breathing is not naturally inhibited.

15.7
Parasitic infection-associated resemblance between locomotor muscles of dragonflies and obese vertebrates
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Metabolic disease with high similarity to vertebrate obesity and type 2 diabetes exists in natural populations of *Libellula pulchella* male dragonflies, and is caused by a protozoan gut parasite. Previous work examined how these parasites affect male *L. pulchella* flight behavior and flight muscle metabolism, and here I have extended those studies by examining infection effects on *in situ* muscle performance- and sarcomere traits relevant to *L. pulchella* flight performance, but that are also known to be negatively affected by vertebrate metabolic disease. I will show that infection impairs normal relationships between body mass, and flight muscle power output, and molecular composition. Moreover, infection affects *in situ* flight muscle endurance and causes a left-shift of the optima of flight muscle power-cycle frequency curves. Interestingly, power-frequency curves of infected *L. pulchella* resemble those of non-infected, teneral (i.e., physiologically immature) *L. pulchella* males. These findings show that effects of metabolic disease on skeletal muscle physiology and function in natural insect systems can be very similar to those observed in vertebrates maintained in laboratory settings.

15.8
Beneficial effects of fluctuating thermal regimes in the alfalfa leafcutting bee, *Megachile rotundata*
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Exposure to low temperature during metamorphosis often results in high mortality or sublethal effects. A growing number of studies have shown that exposing insects to recurrent brief warm pulses during low temperature stress (fluctuating thermal regimes, FTR) improves survival and ameliorates sublethal effects observed during exposure to constant low temperatures. However, FTR can fluctuate over many parameters. To determine which parameters are the most important, we exposed groups of developing *Megachile rotundata* pupae and emergence-ready adults to one of eight thermal profiles. All thermal profiles had base temperatures of 6°C, upper temperatures of 12°C or 18°C (peak temperature), different durations of exposure to the upper temperature (pulse length), and either a square or wave-based thermoprofile (shape). An additional group of insects were exposed to a constant low temperature of 6°C. Generalized linear models that treated components of the thermal profiles separately were better at explaining the variation in survival than average degree day models or other models that accounted for total temperature exposure. Within each life stage, bees exposed to the higher peak temperature (18°C) as a group had improved survival compared to the 6°C control and 12°C pulse thermal profiles. Within the 18°C peak temperature, bees exposed to the square profile had greater survival than bees under a wave-based profile, but that survival benefit did not hold for profiles with a peak temperature of 12°C. Survival of the eye-pigmented pupae and of emergence-ready adults appears to be not directly related to the total degrees accumulated, but to specific aspects of a thermal profile.

15.9
Intraspecific Variation in Thermal, Hypoxia and Acute High pH Tolerance in Rainbow Trout
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Increased temperatures due to climate change are predicted to reduce available habitat for coldwater fishes such as Rainbow Trout (*Oncorhynchus mykiss*) by up to 50% in North America. In addition, the indirect consequences of rising temperatures, such as decreased dissolved oxygen and altered pH can also have negative effects. The resilience of a species to altered environmental conditions is, at least in part, determined by a combination of intrinsic tolerance, the degree of intraspecific variation in tolerance, and the extent of phenotypic plasticity. In addition, when multiple environmental factors are changing simultaneously, phenotypic or genetic correlations among tolerance traits may also be important. Here, we examined intraspecific variation and plasticity in tolerance to elevated temperature, decreased oxygen, and elevated pH, environmental challenges which are facing Rainbow Trout populations in British Columbia. We determined Critical thermal maxima (*CT*ₘₐₓ), incipient lethal oxygen saturation (*ILOS*), and acute high (9.5) pH tolerance for
multiple strains of Rainbow Trout that are used in stocking programs that support the recreational fishery for this species. We detected significant differences in upper thermal tolerance, measured as CT\text{max}, among strains at the fry life stage (Blackwater River strain, mean = 28.2 ± 0.05 °C vs Carp Lake strain fry, mean = 28.4 ± 0.04 °C, p < 0.05) and ILOS (Blackwater River strain, mean = 12.1 ± 12 % sat. vs Carp Lake strain fry, mean = 11.3 ± 0.11 % sat. p < 0.05) but there was little difference in acute high (9.5) pH tolerance for the same strains. At the yearling stage, these trials were completed on individuals as repeated measures to determine if there was a correlation within individuals from particular strains with respect to tolerance of these traits. CT\text{max} and ILOS were significantly correlated (F (1,896) = 1369, p < 2.2e-16; R^2 of .604), indicating that more thermally tolerant fish tend to also be more hypoxia tolerant. We also detected significant plasticity in these traits with differences in CT\text{max} and ILOS across multiple acclimation temperatures (12, 18 and 24 °C, p < 0.05) within individual strains. We are currently examining the physiological and biochemical mechanisms that underlie these effects. Taken together, our findings will aid in the preservation of recreational fisheries and Rainbow Trout as a species by informing stocking programs as to which strains appear most resilient in the face of rapid climate change and hence most appropriate for stocking.

**15.10**
Adenosine A_1 receptor agonist-induced hibernation: effects of agonist and seasons on neuronal pathways

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Hibernation is an adaptive strategy characterized by metabolic suppression and a decrease in body temperature (T_b). Previous study in the Arctic Ground Squirrel (AGS) reported the role of A_1 adenosine receptor (A_1AR) in inducing hibernation. Treatments with N\textsuperscript{-}cyclohexyladenosine (CHA), an A_1AR agonist, promotes the onset of hibernation, but showed a seasonal difference of A_1AR sensitivity to its agonist. However, what regulates the seasonal control of the agonist response is still unknown. We test the hypothesis that thermoregulatory pathways are differentially activated by CHA depending on season.

CHA or vehicle (0.5mg/kg) was administered intraperitoneally and after 3 hours AGS were perfused with 4% paraformaldehyde and brains removed for immunohistochemical analysis. Free-floating immunohistochemistry was used to localize active nuclei as indicated by cFos-immunoreactivity (mouse anti-cFos 1:20,000, Millipore). Data analysis was performed in R.

In our results, CHA-induced hibernation correlates with higher activation in the Nucleus Tractus Solitarius (NTS). The NTS has been previously identified as a site of action of CHA in the rat. The Median Preoptic Nucleus (MnP0) and the Raphe Pallidus (rPA) are thermoregulatory nuclei; MnPO and rPA show a lower cFos activation in winter compared to summer AGS following CHA treatment (p<0.05 treatment by season ANOVA); thus CHA response in winter may be mediated by these nuclei, decreasing thermogenesis leading to hibernation onset.

Other brain regions show difference in neuronal activation as the Tuberalomillary Nucleus (TMN), a region regulating wakefulness. The TMN shows a decrease in cFos activation after CHA treatment. This suggests that CHA inhibits wakefulness to promote hibernation. The rPA shows a lower activation in winter compared to summer in non-treated AGS. This result correlates with the lower euthermic body temperature of AGS in winter compared to summer.

In conclusion, hibernation is characterized by a seasonal decreased in thermogenesis; and CHA-induced hibernation correlates with a further suppression of thermogenesis and decrease in wakefulness.

**15.11**
Characterization of the HIF-1 pathway in response to an acute heat stress in Antarctic notothenioid fishes

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The long evolution of Antarctic fishes in the cold, oxygen-rich waters of the Southern Ocean may have reduced their capacity to respond to hypoxia, thereby contributing to their low thermal tolerance. The transcription factor, hypoxia-inducible factor-1 (HIF-1), a heterodimer of HIF-1\textalpha and HIF-1\textbeta subunits, is the master regulator of oxygen homeostasis in all metazoans. In Antarctic notothenioid fishes, HIF-1\textalpha has a polyglutamine (Q) and glutamic acid (E) repeat that is longer in the more oxygen sensitive and hemoglobinless icefishes (16-34 amino acids) than in red-blooded species (4-16 amino acids). The functional effect of the polyQ/E repeat in HIF-1\textalpha of notothenioids is unknown, but in humans, the expansion of polyQ repeats causes protein aggregation and is associated with several diseases, while shorter polyQ repeats (~ <35-40 residues) are prevalent in transcription factors and enhance transcriptional activity and facilitate protein-protein interactions. We sought to characterize the activity of the HIF-1 pathway in Antarctic notothenioids during an acute heat stress induced by exposure to their critical thermal maximum (CT\text{max}).

Studies were focused on the heart ventricles of the red-blooded notothenioid, Notothenia coriceps (polyQ/E repeat = 9 amino acids), and the icefish, Chaenocephalus aceratus (polyQ/E repeat = 34 amino acids), held at ambient temperature or exposed to their CT\text{max}. HIF-1\textalpha was quantified in nuclear extracts using western blotting and HIF-1 DNA binding was measured using an
electrophoretic mobility shift assay. Transcript levels of lactate dehydrogenase A (LDH-A), a glycolytic enzyme regulated by HIF-1, were quantified using quantitative real-time PCR. Levels of HIF-1α and HIF-1 DNA binding were significantly lower in heart ventricles of C. aceratus than N. coriceps and did not increase in response to exposure to CT_{MAX}, nor did transcript levels of LDH-A change in response to exposure to CT_{MAX}. The significantly lower level of nuclear HIF-1α and HIF-1 DNA binding activity in hearts of C. aceratus may be due to the polyQ/E repeat preventing gene transcription and/or nuclear localization of the protein. Alternatively, the polyQ/E repeat may enhance HIF-1 transcriptional activity, requiring less protein to drive gene expression. Future studies will determine if the lack of a HIF-1-mediated response following exposure to CT_{MAX} is caused by the short duration of the CT_{MAX} experiment (3-4 hours), the loss of the ability of these fishes to respond to hypoxia, or CT_{MAX} not leading to hypoxic conditions in the heart. Funding was provided by grants from the National Science Foundation (ANT 1341663 to KOB) and Alaska INBRE through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103395.

15.11
Variation in thermoregulation and linking whole organism behavior to thermosensory neurophysiology in the porcelain crab, Petrolisthes cinctipes

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Small-scale shifts in species distributions are expected to occur under future climate scenarios for many species. These shifts can have consequences for population dynamics, and therefore it is important to understand when and why they occur. The intertidal crab Petrolisthes cinctipes currently experiences temperatures near lethal levels. However, the extent to which crabs move in response to temperature and the thermal thresholds that trigger migration to cooler microhabitats remain unknown. We tested for effects of body size and reproductive state on escape temperature (Tesc). In addition, we tested for the relationship between Tesc and the temperature of peak action potential firing frequency in sensory afferent neurons. We found that both size and reproductive state influence behavioral sensitivity to temperature. Small crabs tolerate significantly higher temperatures before they move to cool refuges (a higher Tesc) compared to large crabs. In addition, non-gravid crabs have significantly higher Tesc than gravid females. We also found that Tesc is positively correlated with peak neural performance of spontaneous action potentials (R^2=0.26). We find that behavioral sensitivity to temperature varies consistently with size and reproductive state. These findings have implications for species persistence, rates of dispersal and community dynamics. The vulnerability of marine organisms to global change is predicated on their ability to utilize and integrate these physiological and behavioral strategies to promote survival and reproductive fitness; understanding these strategies will allow predictions of species distributions under warming and the potential for extirpation.

15.12
Bone composition of an elite mammalian diver, the Weddell seal: Implications for the use of bone as a buffer.

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The Western painted turtle, Chrysemys picta bellii, can survive exceptionally long periods of anoxia, up to 177 days at 3°C, in part, because they utilize their skeletal system, including their shells, to buffer lactic acidosis that results from reliance on anaerobic respiration. Buffering is achieved, in part, using carbonates from their bones to maintain plasma pH at survivable levels. Studies of reptilian and amphibian bone have demonstrated this ability to be a generalized property of vertebrate mineralized tissues that correlates with its carbonate content. To determine whether this property might extend to mammalian deep-divers, we analyzed the composition of dermal bone from the Weddell seal (Leptonychotes weddellii), a species which voluntarily dives beyond its calculated aerobic dive limit, resulting in the accumulation of lactate in plasma. Bone from 2 adult and 5 pup seals, 3 adult painted turtles, and 3 adult dogs was dried, powdered, and total carbonates were measured using the nitric acid/CO2 evolution technique. Levels of CO2 measured in dermal bone from Weddell seal adults and pups (mean ± SEM, 774.3 ± 17.4 mmol/kg dry bone) were lower than those present in painted turtles (mean=1343.2 ± 159.1 mmol/kg dry bone), and similar to those in canine bone (mean=863.7 ± 9.5 mmol/kg dry bone). There were no differences in bone CO2 between adult and seal pups. ICP-OES analysis of bone ash indicated that calcium/phosphorous ratios in Weddell seal (mean=1.58 ± 0.01) are similar to those in painted turtles (mean=1.63 ± 0.02), but less than in dogs (mean=1.86 ± 0.03). There were no qualitative differences between the pup and the adult seals for any of these measurements. Based on these compositional analyses, we conclude that Weddell seal dermal bone does not possess exceptional levels of carbonate, unlike the acidosis-tolerant painted turtle bone, suggesting Weddell seals do not likely utilize bone as a buffer in the...
same way. Further studies are needed to determine the relative importance of mineralized tissue in buffering lactic acidosis in elite mammalian divers.

15.14

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Although coral skeleton biomineralization is responsible for creating the massive reef ecosystems found throughout the world, the cellular mechanisms are mostly unknown. Biomineralization requires the transport of Ca2+ and dissolved inorganic carbon to the subcalcicoblastic medium (SCM), the microenvironment located underneath coral tissues where the skeleton is built. Additionally, H+ are constantly produced as a byproduct of calcium carbonate precipitation and must be removed from the SCM to prevent acidification that would impair biomineralization. We investigated the potential role of Na+ / H+ Exchangers (NHEs) in this process. Although NHEs are essential for H+ secretion and pH regulation across the animal kingdom, they have not been characterized in corals. BLAST searches identified several genes encoding for NHE-like proteins in Acropora, Stylophora, and Orbicella corals. We focused on an Acropora gene that shares the strongest homology with mammalian NHE2. Bioinformatics analyses predicted AcroporaNHE2 is ~93 kDa in size, has 10 transmembrane domains typical of NHEs, a long extracellular C-terminus tail, and abundant potential glycosylation sites. To further characterize AcroporaNHE2, we generated specific antibodies and studied its protein abundance and cellular localization in A. yongei. Western blotting on membrane-enriched fractions detected a specific protein of ~114 kDa. The discrepancy with the smaller predicted size is most likely due to glycosylation, a possibility we are currently investigating. Immunohistochemical analysis revealed AcroporaNHE2 was highly abundant in the calcifying cells. AcroporaNHE2 was also present in desmocytes that anchor coral tissue to the skeleton, in intracellular structures resembling the Golgi apparatus in symbiocytes, and in the apical membrane of oral ectodermal cells. The high abundance of AcroporaNHE2 in calcifying cells suggests it is important for H+ removal from the SCM, therefore promoting biomineralization, as well as in intracellular pH regulation. However, those roles must be confirmed by functional experiments. This type of basic information about the cellular mechanisms behind coral biomineralization is essential to be able to predict responses to environmental change, as well as to identify potential species-specific mechanisms that determine differential vulnerability and resilience. This work was funded by the National Science Foundation (NSF) Ocean Sciences #1538495 and Emerging Frontiers #1220641.

15.15
Cortisol mediates claudin-28b abundance and its contribution to model gill epithelium barrier properties via the mineralocorticoid receptor

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Cortisol-induced tightening of a primary cultured gill epithelium model derived from the gill of freshwater (FW) rainbow trout (Oncorhynchus mykiss) occurs in conjunction with a significant reduction in the permeability of the paracellular pathway. With this, the molecular physiology of the epithelium tight junction (TJ) complex reorganizes so that select barrier-forming TJ proteins become more abundant. Using pharmacological blockers of corticosteroid receptors (CRs), it has been suggested that the molecular composition of the TJ complex is rearranged by cortisol through binding to the epithelium mineralocorticoid receptor (MR) as well as glucocorticoid receptors (GR). However, because pharmacological blockers of CRs in fishes can sometimes produce ambiguous results, this study considered how cortisol influenced gill epithelium permeability and TJ properties by transcriptional knockdown (KD) of the gene encoding MR. Following mr-KD a significant reduction in MR protein abundance was observed in the gill epithelium. In the absence of cortisol, mr-KD reduced epithelium transepithelial resistance (TER) and increased the paracellular flux of [3H]polyethylene glycol (MW 400 kDa, PEG 400). Cortisol treatment significantly increased TER and reduced PEG 400 permeability, and this was further enhanced in mr-KD preparations, indicating that a reduction in MR abundance allowed the epithelium to become tighter in the presence of cortisol. Cortisol treatment significantly increased the transcript and protein abundance of TJ proteins such as claudin (cldn/Cldn) -8d, and -28b. However, in mr-KD preparations, Cldn-28b protein abundance did not significantly alter in response to cortisol treatment, while Cldn-8d was significantly elevated as observed in control preparations. These data suggest that while the barrier protein Cldn-8d is responsive to cortisol through both the MR and GR, Cldn-28b protein abundance may be modulated by cortisol via the MR only. In this regard, there appears to be a distinction between MR and GR mediated pathways in the regulation of gill epithelium paracellular permeability.
15.16
Osmotic activation of motility and expression of aquaporin proteins in sperm from the gray treefrog *Dryophytes chrysocelis*

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Gametes of gray treefrogs, *Dryophytes chrysocelis*, are deposited into freshwater ponds. We tested the hypothesis that exposure to a hypotonic environment activates sperm motility. We also hypothesized that osmotic water uptake is facilitated by expression of water channel proteins from the aquaporin family. To test these hypotheses, we collected sperm from captive treefrogs maintained with food and water at 22°C and assessed motility of sperm immersed in solutions of varying osmolarity. We also assessed expression of mRNA and protein for two aquaporins, HC-1, a homolog of the water channel AQP1, and HC-7, a homolog of the glyceroporin (glycerol/water channel) AQP7, in sperm and testes from those warm-acclimated animals and from animals that were cold-acclimated during the autumn and winter. Sperm from gray treefrogs could be rendered immotile by immersion in isotonic (280 mosmol/l) PBS. Motility was increasingly activated at more hypotonic concentrations, to maximum motility at 50 mosmol/l, with diminished motility in more dilute solutions. The specific type of motility (e.g., forward moving sperm vs. “wobblers”) also varied with osmolarity. We detected mRNA for both the aquaporin HC-1 and the glyceroporin HC-7 in testes from warm-acclimated, cold-acclimated, and post-freezing thawed animals, but not in emitted sperm. Western blotting indicated both HC-1 and HC-7 were expressed in testes from animals in all three thermal conditions. Immunolocalization using confocal microscopy of cross sections of testes from warm-acclimated treefrogs indicated HC-1 protein expression in the mesentery surrounding each testis, in the epididymis, and in interstitial cells. No expression was evident in the immature spermatogonia or in mature spermatooza within the tubular lumen. HC-7 in warm testes was expressed in the interstitial tissues of the testes and, at low levels, in primary spermatocytes within the seminiferous tubules. No HC-7 expression was detected in mature sperm. We conclude that treefrog sperm require osmotic activation to acquire motility. That water uptake likely is achieved via water channel proteins, but the specific aquaporins expressed in mature spermatooza remains to be confirmed.

Climate change is impacting biodiversity worldwide, and the survival of some species may depend on their capacity for phenotypic plasticity to buffer the effects of environmental change on organismal function. Specifically, seasonal plasticity (via thermal acclimatization) compensates for the effects of temperature on performance, allowing some ectotherms that live in temperate climates to maintain physiological function despite seasonal variation in body temperature. Studies comparing different populations of the same species suggest that ectotherms from diverse thermal environments have evolved different degrees of plasticity. However, few studies have examined how animals that alternate between aquatic and terrestrial habitats at various life-history stages differ in their capacity for seasonal plasticity. Therefore, the goal of this study was to determine if differences in the stability of the thermal environment has resulted in differences in the seasonal plasticity of metabolism between terrestrial and aquatic life-history stages in the eastern newt (*Notophthalmus viridescens*). The life-history of eastern newts is unique among amphibians—aquatic larvae metamorphose into terrestrial efts, and after 2 to 7 years the efts metamorphose into aquatic adults. As adults, eastern newts maintain active in winter by upregulating the activities of oxidative enzymes in skeletal muscle to compensate for the negative effects of temperature on metabolism, but little is known about their capacity to acclimatize during the terrestrial eft stage. Because seasonal plasticity requires a predictable thermal cue in order to benefit the organism, we predict that the plasticity of metabolism will differ between aquatic adults and terrestrial efts. To test this hypothesis, we measured whole-animal rates of oxygen consumption and the activities of metabolic enzymes in skeletal muscle of both adult and juvenile eastern newts that were acclimated to either winter (8°C, 10L:14D) or summer (26°C, 14L:10D) conditions for eight weeks. Preliminary results indicate that rates of oxygen consumption are higher in winter compared to summer-acclimated adults, but do not differ between winter and summer-acclimated efts.

15.17
Does the capacity for seasonal plasticity differ between aquatic and terrestrial life-history stages in the eastern newt (*Notophthalmus viridescens*)?

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Plastic plasticity: phenotypic plasticity at one time scale changes plasticity at another time scale in *Tigriopus californicus*

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Phenotypic plasticity, or the expression environmentally mediated alternative phenotypes, is a key process through which organisms respond to changes in their environment. For instance, plasticity in response to changes in temperature may play a major role in mitigating the effects of climate change on organisms.
and ecosystems. Even considering only within generation plasticity in response temperature variation, plasticity occurs over a range of time scales. Differences in temperature experienced during development can result in irreversible phenotypic differences that persist throughout an organism’s life, whereas changes in temperature experienced later in life often result in reversible modifications of phenotypes. Despite the fact that both developmental and irreversible plasticity often affect the same important physiological traits, such as thermal tolerance, little is known about the capacity for developmental plasticity to influence the expression of irreversible plasticity later in life. In the current study, we examine the combined effects of developmental and irreversible plasticity on the thermal tolerance of the intertidal copepod Tigriopus californicus. Populations of T. californicus are found along the west coast of North America from Baja, Mexico to Alaska, USA, and are known to demonstrate local adaptation of upper thermal tolerance across this latitudinal range. Additionally, temperatures experienced during development result in plasticity of upper thermal tolerance in T. californicus, as in essentially all populations 25 °C-developed copepods tolerate higher temperatures than 20 °C-developed copepods. Our data reveal that variation in developmental temperature not only results in plasticity of adult thermal tolerance, but also changes the reversible plasticity of thermal tolerance in adults. Copepods developed at 25 °C demonstrated an increase in upper thermal tolerance when adult acclimation temperature was increased from 20 to 25 °C, whereas copepods developed at 20 °C had no change in tolerance in response to the same change in adult acclimation temperature. These results suggest that the extent to which phenotypic plasticity is able to mitigate the organismal consequences of climate change will depend on the interactive effects of plasticity across a range of time scales.

15.19 Diversification of Characteristics Related to Endothermy in Thunnus Tunas
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Birds, mammals, and certain fishes, including tunas, opahs and lamnid sharks, are endothermic, conserving internally generated, metabolic heat to maintain body or tissue temperatures above that of the environment. Bluefin tunas, among the most threatened, but commercially important, fishes worldwide are renowned for their endothermic physiology, maintaining elevated temperatures of the oxidative locomotor muscle, viscera, brain and eyes, and occupying cold, productive high-latitude waters. Less cold-tolerant tuna, such as yellowfin tuna, by contrast, remain in warm-tropical to tropical waters year-round, reproducing more rapidly than temperate bluefin tuna. Little is known of the genetic and environmental processes underlying the diversification of tuna. In collecting and analyzing sequence data across 29,556 genes, we found that parallel selection on standing genetic variation has driven the evolution of endothermy in bluefin tunas. This includes two shared substitutions in genes encoding glyceral-3 phosphate dehydrogenase, an enzyme which underlies thermogenesis in bumblebees and mammals, as well as four genes involved in the Krebs cycle, oxidative phosphorylation, β-oxidation and superoxide removal. Using phylogenetic techniques, we further illustrate that the eight Thunnus species are genetically distinct, but found evidence of mitochondrial genome introgression across two species. Phylogeny-based metrics highlight conservation needs for some of these species.

15.20 Hypoxia-Induced Oxidative Stress in Fundulid Killifish
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Aquatic hypoxia is a naturally occurring environmental stressor that has the potential to promote adaptation in fish populations. Additionally, aquatic hypoxia and fluctuations in environmental oxygen availability have been linked to an increase in the production of reactive oxygen species and oxidative stress. We investigated the relationship between hypoxia tolerance and resistance to hypoxia-induced oxidative stress in species of Fundulid killifish that differ in their tolerance of oxygen deprivation based on their time to loss of equilibrium at 3% O2 saturation. These fish species were exposed to acute hypoxic challenges normalized to the species’ critical pressure of oxygen (PcO2) for 12 hours with and without subsequent re-oxygenation. Lipid peroxidation, which is a marker of oxidative stress, was measured in the brain, muscle, liver, and gill using the xylenol orange assay. These data will be used to assess the relationship between hypoxia tolerance and oxidative stress during and following hypoxia exposure in closely-related species of Fundulid killifish.

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Integration of endocrinology, behavior and body temperature of the South-American tegu lizard *Salvator merianae*

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The tegu lizard *Salvator merianae* exhibits annual cycles of high activity during the spring and summer, and hibernation during winter, a seasonal rhythm that is independent of ambient temperature. *S. merianae* exhibits a unique combination of traits among lizards — thermogenic capacity and seasonal metabolic adjustments, with a regularly occurring hibernation phase — that makes this species an interesting subject for study of comparative aspects of endocrine regulation of seasonal changes in physiology and behavior. We investigated seasonal changes in body temperature (Tb), activity (overall dynamic body acceleration; ODBA) and plasma concentrations of hormones involved in regulation of energy metabolism (thyroid hormones T₄ and T₃; corticosterone) and reproduction (testosterone in males and estrogen/progesterone in females) across the annual cycle of a captive population of *S. merianae*. Following emergence from hibernation in August (late winter in southeastern Brazil), males maintained a higher average Tb than females (25.4±0.3 vs 24.3±0.1°C; *P*<0.001, respectively). The transition from low (June—hibernation) to peak (September—reproduction) levels of plasma testosterone in males (0.65±0.13 vs 4.82±0.83 ng.ml⁻¹; *P*<0.001, respectively) was positively related to ODBA (August: *r*=0.72; *P=*0.017) and Tb (August: *r*=0.93; *P*<0.001), suggesting that testosterone may trigger the end of dormancy like seen in some mammalian hibernators. Estradiol in females also peaked in spring coincident with reproductive behaviors. Progesterone in females gradually increased from low (June) to high levels in October (0.28±0.04 vs 0.85±0.08 ng.ml⁻¹; *P*<0.001) when putative ovulation occurs and gravid females build nests. Nest building behavior contributes to a higher ODBA in females relative to males (0.025±0.002 vs 0.020±0.001g; *P=*0.011; respectively). The thyroid hormones, known for influencing energy metabolism, varied seasonally with some sex-dependent differences. T₄ gradually increased from an annual nadir during prehibernation and hibernation to high concentrations during spring in both sexes. In contrast, T₃ was not seasonally modulated in males, but females showed a two-fold increase in T₃ during the spring reproductive season. This sex-dependent seasonal change in T₃ secretion may underlie reproductive physiology and behavior of female *S. merianae* by supporting increased metabolism and modulating energy allocation during annual cycles of folliculogenesis, nest building and oviposition. Corticosterone was significantly elevated during the active season in both sexes, suggesting its involvement in mobilization of energy stores and modulation of behavior and physiology. This is the first study to characterize the integrated seasonal profile of reproductive, adrenal and thyroid hormones, as well as Tb and activity in this endemic and physiologically unique South American lizard. Our findings provide greater understanding of tegu physiology and behavior, insights needed for management of free-living tegus in South America, as well as management of invasive populations in other parts of the world.

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**15.22**

Hot and Dry: Effects of heat waves and water limitation on metabolic and evaporative water loss rates

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Environmental temperature is important because it influences a range of animal processes, including behavior, energy use, locomotion, and reproduction. In addition to warming (increasing mean temperature), environments are expected to continue to exhibit an increased frequency of extreme temperature events, such as heat waves. Some animals can respond to heat waves by reducing their metabolic rates (after controlling for test temperature) to conserve energy. Heat waves often coincide with reduced water availability (e.g., drought), and water is critical to homeostasis. Therefore, a combined heat wave and drought may reduce rates of both energy and water use. To investigate, we employed a 2 x 2 factorial manipulation of temperature (field-parameterized heat wave vs. control diel regime) and water availability (ad libitum vs. absent) in fasted variable field crickets (*Gryllus lineaticeps*). After 4 days of treatment, we used flow-through respirometry to estimate metabolic rate (VCO₂) and evaporative water loss rates at 28°C. We will discuss whether temperature regime and water availability exhibit additive, synergistic, or antagonistic effects on rates of metabolism and water loss. Together, our results will provide new insight into the effects of shifts in co-varying environmental factors (e.g., combined heat wave and drought) on animals’ water and energy budgets.
15.23
Skeletal Muscle Thermoregulation and Metabolic Control in Hibernating Arctic Ground Squirrels
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Skeletal muscle shivering, brown adipose tissue (BAT), and metabolic rate are the identified major sources of heat production in mammals to maintain homeostasis. Recently, skeletal muscle non-shivering thermogenesis has been highlighted as a significant thermogenic source, which occurs via sarcoplasmic reticulum (SR) calcium ATPase (SERCA)-mediated ATP hydrolysis and ryanodine (RyR) mediated calcium leakage from the SR (1,2,3). The SLN/SERCA pathway may play a significant role in thermoregulation of hibernating animals due to the wide temperature range experienced and periodic rapid metabolic increase every 3-4 weeks, which briefly return squirrels to euthermic temperatures (inter-bout arousals). We hypothesized that skeletal muscle uncoupling is a significant source of thermogenesis and inhibiting this pathway can alter metabolic rates during hibernation in Arctic Ground Squirrels (AGS). This work aims to elucidate skeletal muscle SLN uncoupling and its connection with BAT heat production in thermoregulation. Initial studies sampled tissues from AGS housed at 4 °C across the hibernation season, and compared to AGS exposed to extreme cold (-10 °C) for 2 weeks during the hibernation cycle, to determine if increased metabolic stress would cause modulation of thermogenic pathways. The following season, AGS were treated with inhibitors of BAT-uncoupling or skeletal muscle-uncoupling during hibernation (4 °C). Rate of temperature increase and change in VO2 were compared to internal controls during inter-bout arousals to quantify the effect of suppressing BAT or skeletal muscle uncoupling throughout hibernation. Preliminary results show that SERCA/SLN expression is increased during periods of cold temperature stress and fluctuate throughout the hibernation cycle. Protein expression of SERCA, RYR, and SLN are seen to increase during the later portions of hibernation bouts and post hibernation season. Proteins were also seen to increase in squirrels exposed to extreme cold. This indicates that increased metabolic stressors and increasing body temperature during periods of rewarming could be achieved by recruitment of the SLN/ SERCA pathway. Additional results show that suppressing skeletal muscle uncoupling by SLN, using dantrolene (RyR inhibitor that decreases cytosolic calcium) caused a dose dependent reduction in metabolic rate during AGS rewarming. Inhibition of BAT thermogenesis did not reduce metabolic rate highlighting the importance of SLN in thermoregulation and metabolism. The SLN/ SERCA pathway appears to act in conjunction and independently of the BAT uncoupling pathway to increase and control thermogenesis and metabolism during periods of inter-bout and complete arousal at the end of the hibernation season. Thermogenesis control is directly linked to metabolic rate and energy loss regulation, as seen in hibernating AGS, and thus has the potential to innovate treatments for obesity and metabolic syndromes.

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References

15.24
Fat to the High-Altitude Fire: Thermoregulation in Deer Mice
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It has been shown that the ability to effectively thermoregulate is under a strong directional selection in deer mice (Peromyscus maniculatus) native to high altitude. These small endotherms are constantly exposed to a cold and hypoxic (CH) stress all-year round. Therefore, thermogenesis is a demanding metabolic process and is fuelled mainly by lipids. Recently, our lab has determined that the maximal rates of lipid oxidation during maximal bouts of thermogenesis are three-four times higher compared to the maximal lipid oxidation rates observed during exercise. We have also shown that highland native deer mice have a greater capacity to oxidize lipids in muscle compared to their lowland conspecifics. How these mice are able to oxidize lipids at higher rates for heat production than exercise, and how highlanders maintain elevated lipid oxidation rates compared to lowlanders, remains unclear. To address this issue, I used lowland and highland native deer mice born and raised in common laboratory conditions to examine potential population differences in lipid storage, mobilization, circulatory transport, muscle uptake and mitochondrial oxidation. I also examined the role of phenotypic plasticity on lipid oxidation pathways at
high altitude by acclimating mice to combined CH. Results suggest that unacclimated highlanders maintain increased capacity to uptake fatty acids from the blood into the gastrocnemius, increased capacity for mitochondrial oxidation, and increased intramuscular triglyceride stores compared to lowlanders. Both populations increased thermogenic capacity with CH exposure, but showed distinct phenotypic plasticity changes along the lipid oxidation pathway. Whether the differences and/or changes along the lipid oxidation pathway are responsible for the higher rates of whole animal lipid oxidation in highlanders is unclear. My project and future research will be the first step in understanding the mechanistic underpinnings responsible for the highest observed lipid oxidation rates seen in any mammal.

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### 15.25

Modeling energy use of overwintering hatchling turtles using over a decade of nest temperatures

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Many animals endure extended bouts of dormancy during which they are aphagic and thus, must rely on endogenous energy stores to survive and to fuel post-arousal activities. Because temperature directly influences metabolic rate in ectothermic animals, the thermal environment during dormancy profoundly affects energy use and conservation by dormant ectotherms. Moreover, temperature varies among microhabitats both spatially and temporally such that dormant animals inhabiting different microhabitats may incur very different energy costs. We investigated the potential variation in energy costs among hatchling painted turtles (*Chrysemys picta*) that overwinter in their natal nests. First, we measured rates of oxygen consumption (VO$_2$) for individual turtles at varying temperatures (-1.5 – 25°C) during a multi-month acclimation to winter. Hatchling turtle VO$_2$ was very sensitive to temperature (range of Q$_{10}$: 3.3 – 7.4), and was characteristic of reverse metabolic compensation as the turtles were acclimated to lower temperatures. We then used those data to generate a predictive model of VO$_2$ as a function of temperature and fit that model to the thermal profiles (recorded hourly from 15 September to 21 March) of 138 successful natural nests from 2001 to 2013. Mean nest temperature varied significantly (P = 0.004) across years with 2004-05 having the highest nest temperatures. However, predicted rates of energy consumption did not vary significantly among years (P = 0.116). The marked variation among all nests, regardless of year, and the ~4-fold difference between the lowest (7.7 ml O$_2$ g$^{-1}$) and highest (33.4 ml O$_2$ g$^{-1}$) predicted energy consumptions suggest that the microhabitat of the nest site has profound energetic implications for hatching turtles. Indeed, the literature suggests that hatching *C. picta* are in poor condition after having consumed 24.9 – 56.7 ml O$_2$ g$^{-1}$ during dormancy which means that turtles in some of the natural nests may have exhausted their energy reserves. A multiple regression analysis of all nests showed that predicted energy consumption was separately and positively correlated to both mean nest temperature (coefficient = 0.43, P < 0.001) and the thermal variability of a nest (coefficient = 0.80, P < 0.001). Not only does this suggest that hatching turtles in warm and thermally variable nests incur greater energy costs than those in cool and thermally stable nests, but the coefficients suggest that thermal variability has an even greater impact on energy use than does mean nest temperature. By using this simple energetic model, we were able to take advantage of more than a decade of thermal profiles from natural *C. picta* nests to better understand the energy budget of hatching turtles. We suggest that a similar approach could be used on any dormant ectotherm.

### 15.26

Regulation of muscle pyruvate dehydrogenase in high altitude deer mice

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Low O$_2$ availability at high altitude (HA) can impair the ability for animals to engage in aerobic submaximal exercise. However, deer mice (*Peromyscus maniculatus*) are surprisingly abundant in the high alpine. HA deer mice have higher aerobic capacities than low altitude (LA) mice and a greater reliance on carbohydrate oxidation (CHO) at equivalent submaximal intensities as an O$_2$-saving strategy (1). It is unclear if differences in muscle metabolic phenotypes are responsible for population differences in fuel use. However, activities of most glycolytic enzymes of gastrocnemius muscles are equivalent in HA and LA deer mice and resistant to hypoxia acclimation (1). We hypothesized that altered metabolic regulation of the CHO pathway in muscle allows higher rates of CHO oxidation during submaximal exercise at HA. To test this we used first generation lab born and raised LA and HA deer mice to determine activation of pyruvate dehydrogenase (PDHa) in muscle using electrical stimulation of varying intensity relative to maximal force production *in situ*. Results show an induction of PDH activity with contraction and a positive PDHα to intensity relationship in both populations. While maximal PDH activity values in normoxic HA mice were slightly higher than LA mice, the degree of PDHα activation was ~20% higher than LA mice. PDHα activation in hypoxia-acclimated HA mice were ~50% higher than normoxic HA mice at similar muscle
workloads. However, kinetics of the PDH reaction might be different under acute hypoxic condition, where muscle $O_2$ availability is decreased. By identifying trends in PDH activation with exercise, we can provide a mechanistic explanation for whole-animal fuel selection strategies during exercise in vivo. Funding provided by Natural Sciences and Engineering Research Council of Canada.

References:

**15.27**

d-Amphetamine Exposure to Early Embryonic Zebrafish
Reveal Neural and Developmental Consequences
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By 2016, more than 9.4% of US children aged 2-17 had been diagnosed with attention deficit disorder, and of those 6.1 million adolescents, 62% were medicated with prescribed amphetamines. Not only are rates of diagnosis of behavioral attention disorders and amphetamine prescription to treat symptoms growing, but also illicit use is more prevalent than ever among college aged and working adults who seek easy ways to improve productivity and concentration, and even decreasing appetite. With both prescriptive and illicit amphetamine consistently increasing, risks for exposure to nervous systems are more and more likely, especially during critical embryonic, childhood, or adolescent neurodevelopmental stages. To understand specifically how amphetamines affect somatic and neural growth, we exposed early stage zebrafish embryos (0-1 hour post fertilization, hpf, through 72 hpf) to four amphetamine treatment concentrations (0, 10, 20, 30 mg/ml). We assayed growth and survival parameters (average length, developmental stage, natural chorion emergence, mortality, and morphological anomalies), as well as locomotor behaviors to measure the effects of amphetamines on the development and function of escape circuits. At 24hpf, amphetamine-exposed zebrafish embryos yielded mortality rates ranging from 19-20% of total, while mortality among controls was only 12%. Likewise, general development parameters were significantly delayed in amphetamine-exposed embryos compared to controls. Average natural chorion emergence for controls was 49.2 hpf, while hatching rates were slowed in amphetamine-exposed embryos (61 hpf for 10 μg/ml, 57.8 hpf for 20 μg/ml, and 58.8 hpf for 30 μg/ml). Body lengths in amphetamine-exposed embryos were significantly shorter than controls: 10 μg/ml ($p = 0.00039$), 20 μg/ml ($p = 0.00007$), and 30 μg/ml ($p = 0.000321$), and showed more common morphological anomalies, including blunted tails, spinal curvature, and pericardial and yolk sac edema. Amphetamine-exposed fish also showed more prevalent spastic episodes during elicited escape behaviors. Common spastic phenotypes included a hyper-wound, “corkscrew” C-bend coil, and stuttered, choppy, or hyper-paralytic coils and bends, resulting in circular movement or active paralysis, significantly lengthening the time to complete the C-bend portion of escape behavior when compared to controls ($p < 0.0001$). Finally, in an attempt to understand the underlying causes of spastic behaviors in amphetamine-treated embryos, we measured differences in neurotransmitter receptor populations in the spinal cord that underlie the excitatory (NMDA)/inhibitory (gla1) signaling balance necessary for patterned locomotor behaviors like the escape response. Significant differences in inhibitory glycine receptor protein (glycine receptor alpha-1 protein, glra1) and excitatory glutamate receptor proteins (NMDAR) were measured in Floresence Arbitrary Units (FAU). Only in the 30ug AMP group did we see a significant increase in glra1 expression compared to controls ($p = 0.0074$) and to 20 ug AMP ($p = 0.0085$). There were no significant differences in FAU of NMDAR among treatment groups ($p > 0.2921$).

**15.28**

Developmental oxygen preconditions cardiovascular response to acute hypoxic exposure and maximal b-adrenergic stimulation of anesthetized Juvenile American alligators (Alligator mississippiensis).

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During embryonic development, environmental factors, such as oxygen have been shown to induce characteristic phenotypic changes thereby shaping organismal traits and potentially impacting post hatching animal performance. The embryonic American Alligator (Alligator mississippiensis) cardiovascular (CV) system has been extensively investigated in this regard. In response to incubation in 10% oxygen (hypoxia), alligator embryos possess larger hearts, lower heart rates, and maintain metabolic rate during hypoxic exposure. If these cardiovascular traits persist in the hatching and subsequent adults, they may convey a greater capacity to adjust cardiovascular function during periods of elevated oxygen demand or extended dives. We hypothesized that developmental hypoxia would result in a dampened response to acute hypoxia and an increased response to beta adrenergic stimulation. 4-year-old alligators that were previously incubated in control (21% oxygen) and hypoxic conditions were studied under anesthesia.
Animals were instrumented for measurements of intraventricular pressures, systemic and pulmonary blood flow during exposure to acute hypoxia and beta-adrenergic stimulation. At baseline, left ventricle stroke volume was greater while heart rate and dP/dt max were lower in the hypoxic incubated juveniles compared to control animals. In both groups, 5% oxygen exposure decreased heart rate and peak ventricular pressure however the response was dampened in the hypoxic incubated juveniles. Further dP/dt max was unchanged during exposure to 5% oxygen. Beta adrenergic stimulation increased stroke volume, blood flow and heart rate in both groups. Beta adrenergic stimulation during 5% oxygen exposure produced greater increases in total systemic blood flow and left ventricular stroke volume in the hypoxic compared to the control juveniles. Collectively our findings suggest that exposure to 10% oxygen during embryonic development has lasting effects on cardiovascular function in American alligators and these changes may impact animal performance during periods of elevated oxygen demand.

15.29
Chronic crude oil exposure affects physiology and sexual differentiation to zebrafish (Danio rerio).
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Crude oil is composed of environmental toxicants, such as Polycyclic Aromatic Hydrocarbons (PAHs), that affect physiological processes (e.g. cardiorespiratory performance), sexual differentiation, as well as morphological traits in aquatic organisms. Nevertheless, there is a little information about the factors controlling zebrafish development during chronic crude oil exposure, especially sexual determination. We hypothesized that chronic exposure to crude oil would disrupt sexual differentiation, as well as affect cardiorespiratory physiology and morphological traits in zebrafish (Danio rerio). To corroborate these hypotheses, zebrafish larvae and juveniles were chronically exposed through dietary exposure to standardized oil mixtures call “High-energy water accommodated fraction (HEWAF)” consisting of 2 g of oil blended into 1000 ml water. Four different conditions were used: three treatments using aquarium water and crude oil mixture added to food at different concentrations (10% HEWAF, 50% HEWAF, 100% HEWAF), and a control group fed only with food spiked with aquarium water. HEWAF exposure lasted 16 days, beginning from 20 and finished at day 35 post fertilization (dpf). Oxygen consumption, loss of equilibrium in hypoxic water, heart rate, body mass and length were measured at 25, 30 and 36 days post fertilization (dpf). Preliminary results showed that there were no significant differences in the time to loss of equilibrium, body mass and length. In addition, heart rate during 25 and 30 dpf was not different among groups. These data suggest that the early larvae were not unduly stressed by the crude oil exposure. However, with further development to 36 dpf, significant differences in the heart rate began to develop. In the 100% HEWAF treatment, heart rate was significantly decreased to 208 ±7 beats/minute from 236 ±4 beats/minute in the control group (P= 0.005). The difference between 100% HEWAF and 50% HEWAF treatment (230 ±7 beats/minute) was also significant (P= 0.034) but more modest. Those results suggest that, even in the absence of gross morphological changes in zebrafish, more subtle physiological responses occur due to lengthy periods of exposure to PAHs.

15.30
Development and Characterization of a Primary Cultured Model of the Larval Sea Lamprey (Petromyzon marinus) Gill
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The parasitic sea lamprey (Petromyzon marinus) is an extant agnathan. As a model that provides insight into chordate evolution, these aquatic vertebrates have generated significant research interest in comparative physiology/endocrinology and functional biology. With regard to the regulation of salt and water balance, the sea lamprey presents an opportunity to study an organism with distinct osmoregulatory strategies in different lifecycle stages. However, to the best of our knowledge there is no simplified surrogate model to study the physiology of gill epithelium function in lamprey. This study reports a primary culture method using larval sea lamprey cells derived from the gill tissue. Cultured gill cells were examined using molecular biology and biochemistry and found to express tight junction-associated proteins. In this regard, the preparation and maintenance of the primary cultured gill cells is discussed along with application of endocrine-mediated physiology studies of the sea lamprey gill cells.

15.31
Withdrawn
Participation of orexin receptor-1 in the modulation of respiratory motor activity in the bullfrog (Lithobates catesbeianus)

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Orexin releasing neurons are known to participate in many important physiological processes, such as sleep-wakefulness, feeding behavior, energy homeostasis, metabolism, hormonal secretion, and additionally, it has been shown that the orexinergic system also modulates respiration in rodents. Orexin’s effects on respiration likely involve participation in central chemoreflexes. Orexinergic neurons are profoundly affected by changes in CO₂ and pH as demonstrated by in vitro and in vivo experiments, which suggests that they are intrinsically chemosensitive. Furthermore, focal dialysis of SB-334867 (OX₁R antagonist) in the retrotrapezoid nucleus (RTN) or medullary raphe of conscious rats decreased the hypercapnic ventilatory response. However, there are currently no in vitro data demonstrating the role of this important neuropeptide in the modulation of respiratory control in amphibians.

Based on this background and the scarcity of studies on respiratory control in non-mammalian vertebrates, the main objective of this study was to investigate the contributions of orexin receptor-1 (OX₁R) to central respiratory motor activity and its participation in the CO₂ chemoreflex in adult bullfrogs. We hypothesized that orexin, acting through the OX₁R, potentiates central CO₂ chemoreflexes in adult frogs to stimulate ventilation during hypercapnia. Consequently, we predicted that inactivation of OX₁R’s by a selective antagonist would attenuate fictive lung ventilation in response to hypercapnia.

To this end, we used in vitro brainstem preparations transected rostral to the optic chiasma to keep hypothalamic orexinergic neurons intact. Brainstems were perfused with artificial cerebrospinal fluid (aCSF) containing SB-334867 – 10µM (OX₁R antagonist) during control (98% O₂, 2% CO₂) and high CO₂ conditions (95% O₂, 5% CO₂). All experiments complied with the guidelines of the Canadian Council on Animal Care.

Contrary to our hypothesis, our preliminary results show that OX₁R antagonism potentiated the normal response of fictive lung ventilation to hypercapnia, suggesting that orexin (via OX₁R) attenuates the hypercapnic chemoreflex in adult bullfrogs. Further experiments are underway to explain these results, however, it is possible that the orexinergic system of amphibians is controlled by autoregulatory mechanisms (thus imparting dose-dependence), as shown for mammals. This work will bring new insights to our understanding of the role of the orexinergic system in vertebrate physiology and its evolution.

Financial support: This research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada and by a scholarship for the Emerging Leaders in the Americas Program (ELAP).

Does the spotted gar, Lepisosteus oculatus, express a functional endothelial nitric oxide synthase?

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The evolution of endothelium-derived nitric oxide (NO) in blood vessels, and its associated signalling pathway, remains contentious in comparative cardiovascular physiology. The advancement of comparative genomics has provided insight into the evolution of the NO synthase (NOS) enzymes (NOS1, NOS2 and NOS3), in particular, that of NOS3 that is expressed in the endothelium of mammalian blood vessels. Until recently, the parsimonious view was that NOS3 evolved early in the tetrapods, as all three isoforms are found in amphibians, but only two isoforms (NOS1 and NOS2) are found in the genomes of teleost and chondrichthyan fishes. Recently, all three NOS proteins have been predicted in the genome of the non-teleost actinopterygian, Lepisosteus oculatus, which potentially reshapes our understanding of NOS3 evolution. This study aimed to characterise the nos3 gene and determine if NOS3 is a functional protein within the vasculature of L. oculatus. The predicted nos3 gene did not demonstrate a conserved synteny with the nos3 genes of mouse and Xenopus, respectively, compared to the conserved synteny that is found for the nos1 and nos2 genes. Phylogenetic analysis showed that the predicted NOS3 protein grouped with other vertebrate NOS3 proteins; L. oculatus NOS1 and NOS2 grouped with their respective isoforms. To confirm the genomic sequence, we cloned the L. oculatus nos3 mRNA, which was found to be 99% similar to the predicted sequence. We then designed a specific L. oculatus NOS3 antibody, and demonstrated NOS3-immunoreactivity in the adventitia of both small and large blood vessels, as well as in chloride cells in the gill filaments. To determine if the presence of NOS3 within the vasculature contributed to vasodilation, myography was performed on the dorsal aorta. Interestingly, addition of the NO
donor, sodium nitroprusside, had no effect on vascular tone indicating that NO generated by endogenous NOS3 is not involved in vasodilation. Taken together, these data suggest that *L. oculatus* does express a NOS3 protein within the vascular wall, but the function of NO does not appear to involve regulation of vascular tone.

**15.34**

Toxicity of crude oil extracts in chicken embryos

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Crude oil from oil spills can be a toxic contaminant to bird embryos, affected directly by contaminated water or by feathers and feet of contaminated birds during parental care. This study is part of a series of studies examining embryotoxicity of crude oil (CO) water accommodated fraction (WAF). In the current report we studied the embryotoxicity and blood physiology in fertile eggs injected with water accommodated fraction (WAF) and crude oil. We first investigated body mass (BM) of hatchlings, time of hatch and hatchability of eggs injected with 10, 30, 50, 70 or 90 µL of 100% WAF (2g-crude oil stirred in 1L-tap water) or vegetable oil (V-oil, sham) into the air cell on embryonic day 4 (D4) or D10 beside control (intact) eggs. Hatchability and hatching BM, ratio of BM to egg mass and time of hatch were not different in any volume or day injected, indicating the lack of major toxicity at the employed doses. Thus, we investigated the survival rate of embryos in eggs injected with higher doses: 1, 3 and 5 µL of CO or V-oil into the air cell along with control eggs on D4 and D10 since 1 µL of CO correspond to ~six-fold volume of crude oil contained in 90 µL of WAF in embryotoxicity. No effect on the injection itself was found since sham and control embryos showed the same survival rate. However, the survival rate of embryos injected with CO on D4 was lower than the control 88%, 94% and 100% for 1, 3 and 5 µL injected respectively (p<0.001). D10 embryos injected with 3 µL of CO had a 58% decreased survival from control (p<0.001) and a further decrease to just 5% of control was observed in embryos injected with 5 µL of CO (p<0.001). However, no effect on survival occurred at 1µL of CO injection (p=0.260). Based on these survival test results, we hypothesized that physiological variables would be negatively affected in D10 embryos suffering severe toxicity of CO (5 µL-injection), but that in lower doses, embryos would be able to show physiologically plasticity to mitigate toxicity of CO (1 µL-injection). To examine the hypothesis, 1, 3 or 5 µL of CO was injected into the air cell of d10 embryoated eggs and the arterialized blood was analyzed on d15 of incubation. Blood O2 transport variables as Hct, [RBC], [Hb] were actually increased (4.5%, 3.7% and 3.9% respectively) in response to injection of 1 µL of CO. However, counterintuitively, they were significantly decreased (8.9%, 9.1% and 7.8% respectively) by injection of 5 µL of CO. Blood gas variables (pH, [HCO3 ], PCO2, PO2) remained unchanged in 1 and 3 µL of CO injections, but 5 µL injection caused metabolic acidosis (pH 7.58 to 7.55) and decrease of 3.8% in arterial PO2. Lactate ([La]) was increased progressively at all doses injected (1% at 1 µL, 181% at 3 µL and 204% at 5 µL. Water concentration in blood increased 0.9% just in the 5 µL injected. The harmful CO effects in higher CO dose could be generated by a disturbance of chorioallantoic membrane, decreasing the O2 diffusing capacity, resulting in selective embryo hydration and acidosis because of the large increase in [La]. Our results suggest that lower doses of CO are mitigated by embryo plasticity while higher doses of CO are harmful to embryo.

**15.35**

Cardiac proteome changes in the western painted turtle in response to cold acclimation and anoxia

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The western painted turtle *Chrysemys picta bellii* is the most anoxia-tolerant tetrapod known, with survival lasting more than 170 days a 3°C. This ability allows the turtles to overwinter in frozen lakes in the northern reaches of the US, which can become anoxic during the winter. Successful entry into and exit out of metabolic depression is critical to surviving anoxia, and during anoxia the cardiac function of the painted turtle is suppressed, beating only once per minute at 3°C. Shutting down energy consuming processes, such as protein synthesis, is critical to entering metabolic depression; yet changes in abundance levels of particular proteins may be key to maintaining cardiac function under energy limiting conditions. Therefore, we conducted a study on the effects of cold acclimation, anoxia, and recovery on the cardiac proteome of adult western painted turtle. Turtles were held at 20°C, acclimated to 3°C, exposed to anoxia for 20 days, and allowed 6 days of aerobic recovery following anoxia. Ventricles were sampled and 2D fluorescent Difference Gel Electrophoresis (DIGE) was used to compare the proteome of the turtle ventricle at 20°C, following cold acclimation, exposure to anoxia, and recovery from anoxia. Proteins of interest were identified by MALDI-QIT-ToF mass spectrometry. While work has been conducted on changes in the cardiac transcriptome during anoxia, this is the first study of its kind on the proteome. As such, these data will be particularly instrumental in furthering our understanding of anoxia tolerance in the painted turtle.
Blood O₂ transport variables as Hct, [RBC], [Hb] were examine the hypothesis, 1, 3 or 5 µL of CO was injected doses, embryos would be able to show physiologically would be negatively affected in D10 embryos suffering 1µL of CO injection (p=0.260). Based on these survival control (p<0.001) and a further decrease to just 5% of embryos showed the same survival rate. However, the injection itself was found since sham and control cell along with control eggs on D4 and D10 since 1 µL of lack of major toxicity at the employed doses. Thus, we sham) into the air cell on embryonic day 4 (D4) or D10 Toxicity of crude oil extracts in chicken embryos 15.34 does not appear to involve regulation of vascular tone. tone indicating that NO generated by endogenous NOS3 donor, sodium nitroprusside, had no effect on vascular and crude oil. We first investigated body mass (BM) of studied the embryotoxicity and blood physiology in fertile Morphology and Physiology, Sao Paulo State Univ. Chrysemys picta bellii 2018 APS INTERSOCIETY 75 L. oculatus 15.36 our results suggest that lower doses of crude oil from oil spills can be toxic contaminant to Crude oil and seals are the only animal in nature known to have concentrations of CO in their blood at levels similar to those that have shown cytoprotective properties in other models. The source of high CO production in elephant seals is currently unknown; however, in other mammals, including humans, endogenous CO is produced from the natural turnover of heme stores (primarily from erythrocyte and hemoglobin turnover). Understanding the source of CO production and the potential cytoprotective effects in the elephant seal model will help inform about the potential use of CO as a therapeutic drug. Our hypothesis is that the elephant seal will exhibit a reduced red blood cell lifespan, which will increase heme turnover and CO production via heme oxygenase activity. Our novel method uses biotinylated red blood cells (RBCs) to measure total systemic volume of RBCs and lifespan in elephant seals directly, as opposed to the current Evans Blue dye method which estimates the volume of RBCs from plasma volume. Specifically, our method labels the membrane of approximately 1% of RBCs in the animal with biotin. Biotinylated RBCs can then be tracked via serial collection of small blood samples (~50ul) and analyzed with flow cytometry. We have successfully biotinylated 1% of the RBCs in a mouse analyzed these on a flow cytometer. Our next steps will be to 1) biotinylate 1% of RBCs from a vial of elephant seal blood (knowing that the RBCs will be larger), 2) biotinylate 1% of RBCs directly from an elephant seal, and inject the labeled cells back into the animal to measure RBC lifespan and volume in the living organism, and 3) to use a novel method of co-oximetry to check the effects of endogenous CO production on an elephant seal’s hemoglobin oxygen affinity curve.
signaling and expression programs responsible for the dichotomy in digestive responses. This work was supported by funding from the National Science Foundation (IOS 0466139 to Secor and IOS 1656138 to Secor and Castoe).

15.38
Rapid Evolution of Starvation Resistance in *Drosophila*: Physiological and Molecular Mechanisms
**Austin J. McKenna**, **Alaric Smith**, **Allen G. Gibbs**

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We subjected five replicate populations of *Drosophila melanogaster* to selection for starvation resistance and compared them to a founding control population that had been maintained at large population sizes to reduce linkage disequilibrium. After only one generation of selection, all five replicate selected populations survived longer without food than the control population, and starvation survival continued to increase by ~5 hr/generation over four subsequent generations. Previous studies have shown that long-term starvation-selected *Drosophila* contain more lipid, have lower metabolic rates and develop more slowly than controls. Lipid contents in our selected populations increased within three generations, and development tended to be slower within five. However, starvation-selected flies did not not have lower metabolic rates than controls. Samples were collected each generation for a genome-wide association study to link changes in SNP allele frequency with evolved phenotypic changes. Preliminary findings of the GWAS will be presented. Supported by NSF award IOS-1355210.

15.39
Testing the Functional Consequences of Genetic Variation in Insulin-like Growth Factor 1 (IGF1) in Lizards via Primary Culture Experiments
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Insulin-like growth factors (IGFs) are key hormone peptides regulating the Insulin and Insulin-like signaling (IIS) pathway, a pathway required for growth, metabolism, and reproduction. IGFs and other key proteins in the IIS pathway are highly conserved across vertebrate lineages including reptiles, but there are still gaps in our knowledge about the function of the IIS pathway and its members in reptiles [1,2]. Previous work has shown that although IGF2 is highly conserved across reptiles, IGF1 has experienced diversifying selection across the reptile clade. This is in contrast to mammals where IGF1 is under purifying selection [2]. Substantial amino acid diversity in IGF1 between green anole (*Anolis carolinensis*) and brown anole (*A. sagrei*) lizards is concentrated in a domain associated with IGF1 Receptor binding affinity [2,3]. In mammalian *in vitro* cell cultures, IGFs are known to be fibrogenic/mitogenic and involved in cellular proliferation [4,5]. Cell culture provides a model for studying physiological and biochemical function and preliminary insight on cellular and possibly organismal response to drugs, growth factors, and/or stressors, but optimization of this method and associated culture assays in non-model organisms is not well documented [6,7]. We describe the establishment of three fibroblast lines from *A. sagrei* (brown anole lizard) tail tips and their use in characterizing the function of reptilian IIS pathway with species-specific recombinant IGFs expressed in and purified from *E. coli*. To optimize and assess prolific response to IGF treatments, cells were seeded and synchronized before serial time- and dose-dependent exposure to recombinant brown anole IGF1 and IGF2 treatments. Cellularity proliferation in response to IGF dose and exposure time was assessed via growth curve analysis and BrdU assay. We then test the bioinformatic prediction that the amino acid sequence variation in IGF1 between green and brown anoles has a functional effect on cell proliferation via binding to the IGF1 receptor through the application of brown or green anole IGF1 or IGF2 to culture wells. Cellular proliferation in response to IGFs was directly assessed via growth curve analysis and indirectly via cell metabolism assays, after time- and dose-optimized exposure to peptide treatments. Expression of transforming growth factor-β1 (TGF-β1), cytokine expressed in response to IGF stimulation in human dermal fibroblasts, was quantified via qPCR [5]. Results to be discussed include insights and challenges of primary culture with non-model ectothermic organisms, functional verification and use of lab purified recombinant proteins from non-model organisms, and cellular response to IIS activation by IGF proteins in the context of known genetic sequence variation and conservation patterns.

IGFs are known to be fibrogenic/mitogenic and involved concentrated in a domain associated with IGF1 Receptor carolinensis across the reptile clade. This is in contrast to mammals gaps in our knowledge about the function of the IIS vertebrate lineages including reptiles, but there are still proteins in the IIS pathway are highly conserved across metabolism, and reproduction. IGFs and other key (IIS) pathway, a pathway required for growth, 15.39

Physiological and Molecular Mechanisms
Rapid Evolution of Starvation Resistance in
Foundation (IOS 0466139 to Secor and IOS 1656138 to

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Amitogen also involved in differentiation processes in

life history traits (Gen Comp Endocrinol).

Mechanisms of Life History Evolution: The Genetics and

Physiology of Life History Traits and Trade -Offs (eds Flatt

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Mechanisms governing reproduction vary widely across

and within taxa, yet numerous challenges often prohibit

the study of intrinsic physiological factors that influence

reproductive outcomes in non-model or wild organisms.

Weddell seals (Leptonychotes weddellii) in Erebus Bay, Antarctica offer a unique opportunity to elucidate the physiological processes regulating the establishment of successful pregnancy in marine mammals, due to a long-term demographic study and high site-fidelity of females. We determined pregnancy status of 77 adult female Weddell seals during the peri-implantation period (January/February) from 2014-2017, using transrectal ultrasound (embryos detectable ≥3mm diameter). Pregnancy rates were high (78%), and 16% of females that were detected pregnant returned the following year but did not produce a pup (i.e., lost the pregnancy). Hormone quantification and metabolomics approaches were used to determine biochemical pathways that were up- or down-regulated during early gestation for female Weddell seals that had a successful pregnancy (n=14) as compared to females that were not detected pregnant (n=10) or experienced pregnancy loss (n=12).

Hormones used to diagnose pregnancy in domestic animals, such as progesterone and estrogen, did not

References:


Modelling Human APOL 1 Variant Related Kidney Dysfunction In Guinea Pigs
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Humans of early West African ancestry are more prone to kidney dysfunction. Earlier studies demonstrate that the reason for this phenomenon is because of the mutation of APOL 1 gene in most humans of early West African ancestry. Although several APOL genes are expressed in mammals, APOL 1 is only naturally expressed in primates. The restricted expression of APOL 1 to only primates has limited the exploration of the functional role of APOL1 variants in kidney disease development. The aim of this preliminary study was to develop a guinea pig model of APOL1 variants and demonstrate possible biological basis of APOL 1-mediated kidney injury as is observed in humans given that both humans and guinea pigs are HDL mammals.

Expression of APOL1 gene variants in Guinea pigs was done by hydrodynamic gene delivery (HGD). 5 sets of male and female guinea pigs were injected with plasmids containing various APOL1 gene variants. APOL1 protein presence in Guinea pig plasma and kidney tissues was determined by WESTERN blotting and immunohistochemistry (IHC) respectively.

Induction with APOL1 gene variant in Guinea pigs resulted in a derangement of renal function as evidenced by creatinine accumulation and distorted renal histoarchitecture. There was a scattered inconsistent IHC staining for APOL1 in the experimental group although the WESTERN blot assay showed a consistently elevated protein presence in the same group compared to control. Subsequent studies will seek to improve on the APOL1 induction method, gather more genetic information in addition to determining the full extent of renal structure and function compromise in the Guinea pig specie.

Keywords: APOL1, Kidney dysfunction, FSGS, End stage renal failure, HDL

References:


differ between non-pregnant and pregnant Weddell seals during the peri-implantation period. However, pregnant individuals and seals that successfully produced a pup had significantly higher serum thyroxine levels than seals that failed to give birth. As thyroid hormones are potent regulators of metabolism, this suggested that there may be differences in the catabolism of tissues and substrate use to meet the demands of a growing fetus. An untargeted metabolomics approach identified 600 biochemicals in Weddell seal serum. Of these, 25 were significantly (p<0.05) and 50 slightly (p<0.10) different between non-pregnant and pregnant Weddell seals. Most of the metabolic shifts associated with pregnancy suggest a decrease in lipolysis and increased protein degradation and amino acid transport. This was evidenced by a decrease in the relative abundance of free fatty acids (long chain; polyunsaturated) and an increase in the metabolic end-products in a diverse set of metabolic pathways (alanine and aspartate; lysine; tryptophan; leucine, isoleucine, and valine; arginine and proline, creatine, and gamma-glutamyl amino acid metabolism pathways) in pregnant animals. Further, females that successfully carried their pregnancies to term had significantly higher abundance of nicotinamide, 1-methylnicotinamide, and N1-methyl-2-pyridone-carboxamide than females that experienced pregnancy loss, suggesting greater flux through the nicotinamide metabolic pathways. Increased splanchnic uptake of amino acids is critical for proper embryonic development, and disruption of nicotinamide synthesis has been implicated in miscarriages in humans and other mammals. This study is the first to pair ultrasonography with the metabolomic-fingerprint of pregnancy in a wild mammal, and can aid in the development of minimally-invasive markers of successful reproduction while monitoring wild populations.

15.42
Evolution of thermal tolerance in pumpkinseed sunfish (Lepomis gibbosus)
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Historical transplantations of non-native species to novel warm environments are natural experiments for studying the potential mechanisms of climate change adaptation. We compared the thermal tolerance of warm-adapted pumpkinseed sunfish that were transplanted ~140 years ago from North America to Europe with native pumpkinseed populations from southern Canada. We found that Spanish populations (Ter River and Susqueda Reservoir) have evolved greater heat tolerance than Canadian populations (Rice Lake and Otonabee River), as reflected by a higher critical thermal maximum (CT\textsubscript{max}). This greater heat tolerance of Spanish populations was not associated with differences in resting metabolism, hypoxia tolerance, haematology, or the maximal activities of anaerobic and aerobic enzymes in muscle or liver, suggesting that other physiological mechanisms may have contributed to the evolution of thermal tolerance. Evolved differences in thermal plasticity were also observed, with fish from the Canadian Rice Lake population showing a much greater increase in CT\textsubscript{max} with acclimation to warmer temperature. This was associated with greater plasticity in the mitochondrial respiratory capacity of the heart in Rice Lake fish (as reflected by measurements of oxidative phosphorylation in permeabilized cardiomyocytes), suggesting that adjustments in cardiac energy metabolism are important for thermal acclimation. Therefore, pumpkinseed appear capable of increasing heat tolerance via evolutionary adaptation and thermal acclimation, possibly underpinned by changes in heart function, which may help them cope with climate change in their native range. Supported by NSERC of Canada.

15.43
Calcium transport across the placenta in a placentotrophic lizard: New insights about gestation
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Lizards species of the genus Mabuya distributed in the Neotropics are characterized by some exclusive and very interesting features in their reproductive and developmental biology. These traits include viviparity with long gestation time (from 9 to 10 months), microlethal eggs, and associated with this type of oocytes, the development of a placenta that is both morphologically and physiologically the most complex and specialized within Squamata reptiles. These traits make them particularly important as they contribute with a system that allows to perform comparative physiological, metabolic, and developmental biology investigations.

Previous studies on Colombian Mabuya sp IV have provided clues about the morphology and physiology of their gestation, for example, the placenta fulfills the fundamental functions for gaseous exchange and the absorption, transfer or transport of nutrients, such as water, glucose, some lipids, proteins and ions. Here we report preliminary data on gene expression of calcium transporters in placental tissues of Mabuya sp IV by quantifying the relative expression of mRNA and characterizing its temporal dynamics through its gestation by real-time reverse transcription PCR. To do this we analyzed gene expression of Calcium-binding proteins as calbindin-D9K and D28K, the Plasma
Membrane Ca\(^{2+}\) ATPase (PMCA) and the Transient Receptor Potential cation channel subfamily V member 6 (TRPV6) in non-pregnant female’s oviducts and oviductal eggs, early, mid and advanced extraembryonic membranes from pregnant females. Individuals were capture by hand, killed by intrathoracic injection of lidocaine 2\% v/v and dissected. All work conducted with the animals was consistent with government guidelines on the ethical treatment of animals and all applicable regulations and follows the considerations of The Herpetological Animal Care and Use Committee (HACC). We found that all of the calcium transporters were present in the placenta and they were highly expressed at mid and late gestation. This gene expression prove calcium transfer to the embryo-fetus must be completely mediated by the placenta, it also highlights that calcium is one of the most important ions required during pregnancy, not only for the development of the skeleton from mid pregnancy, but also for the establishment of gestation, growth and fetal homeostasis. Our results emphasize *Mabuya* sp as the only reptile species where the presence of calbindin-D9K, a protein previously restricted to mammalian species, has been observed nowadays. Finally, due to the homology of tissues we can compare and associate it with eutherian mammals, demonstrating a conserved and similar biological function in this placentotrophic species.


15.44

A role for kisspeptin receptor in the pituitary gonadotroph in male mice

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The anterior pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) regulate gonadal development, gametogenesis and the secretion of the gonadal steroid hormones. The gonadotroph is primarily regulated by hypothalamic secretion of gonadotropin-releasing hormone (GnRH) from neurons of the rostral hypothalamus and is mediated by GnRH receptor signaling. Recently, kisspeptin (KISS1)/kisspeptin receptor (KISS1R) signaling in GnRH neurons has been shown by our group and others to play an essential role in HPG axis function. However, whether kisspeptin signaling via the Kiss1r could also regulate reproductive function at the level of pituitary is not yet established. Using Cre/Lox technology, we knocked out the Kiss1r gene in pituitary gonadotropes (PKiRKO). Our results revealed that PKiRKO males have normal external genital development, have normal ages of puberty as assessed by preputial separation and also have comparable body and testes weight to WT male mice. While there were no differences in basal serum LH and FSH levels, we observed a significant attenuation (P<0.05) in GnRH stimulated luteinizing hormone (LH) levels in PKiRKO male mice compared with WT male mice and this was associated with attenuated expression of *Gnhr*. To directly assess cellular response, calcium (Ca\(^{2+}\)) assays were performed on primary pituitary cell cultured *ex vivo*, and demonstrated that cells from WT male pituitaries were more responsive to GnRH (100nm) and kisspeptin (10nm) than were pituitaries from PKiRKO males. Overall, these findings indicate that the pituitary KISS1R may play an important modulatory role in augmenting pituitary responsiveness.

16: HIBERNATION AND DAILY TORPOR: ABSTRACT DRIVEN SESSION - 2

16.1

The Effect of Lipids on Hibernation and Cardiac Function

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Many small mammals and birds have developed specific mechanisms of energy saving, via active and controlled
calcium ATPase (SERCA) in the hibernators' heart. This of PUFA may be mediated via effects of the membrane homeostasis. As previously hypothesized, the influence of torpor, plays a pivotal role in maintaining body activity is tightly regulated during the heart, which activity is tightly regulated during torpor. Conversely, there is evidence for adverse effects of n-3 PUFA on hibernation performance. The heart, which activity is tightly regulated during torpor, plays a pivotal role in maintaining body homeostasis. As previously hypothesized, the influence of PUFA may be mediated via effects of the membrane lipid composition on the sarcoplasmic reticulum (SR) calcium ATPase (SERCA) in the hibernators’ heart. This trans-membrane pump is responsible for removing calcium into the SR and hence for continued cardiac function at low Tb in torpor. We tested the hypotheses that high proportions of n-6 PUFA in general, or specifically of LA, in PL are associated with increased cardiac SERCA activity, and allow animals to reach lower minimum Tb in torpor. SERCA activity and SR-PL fatty acid composition were assessed from hearts of hibernating and non-hibernating Syrian hamsters (Mesocricetus auratus), a granivorous, food-storing hibernator, and of hibernating garden dormice (Elomys quercinus), an insectivorous, fat-storing hibernator. In both species, we found that SERCA activity was strongly up-regulated by increased proportion of LA in SR-PL, but was negatively affected by the content of docosahexaenoic acid (DHA; C22:6 n-3). In hibernating hamsters, high levels of LA and low proportions of DHA were found in SR-PL. As a result, SERCA activity was significantly higher during entrance into torpor and in torpor compared to inter-bout arousal, i.e. phase of high MR and euthermic Tb between torpor bouts. A subgroup of hamsters, which remained euthermic throughout winter, displayed a phenotype similar to animals in summer, i.e. lower LA levels and increased DHA proportions in SR membranes. Similarly, a group of dormice, which delayed their mean onset of hibernation by almost 4 days (range 0-12 days), showed extremely high DHA levels prior to hibernation. Both hamsters and dormice with increased SERCA activities reached lower Tb during torpor. Interestingly, SERCA activity in torpor was three-times higher in garden dormice than in Syrian hamsters at similar DHA proportions in SR-PL. We conclude that (1) fatty acid composition of SR membranes modulates cardiac SERCA activity, hence determining the minimum Tb tolerated by hibernators, and (2) high DHA levels prevent hibernators from entering into torpor, but the critical levels differ substantially between species. These specific roles of PUFA in regulating cardiac activity, hence body homeostasis during torpor and hibernation might shed light on the benefit for humans to enter a torpor-like state during long-term spaceflight. Funding sources: Austrian Science Fund (FWF) [P27267-B25], Univ. of Veterinary Medicine Vienna (Austria), French Space Agency, IdEx H2E Projex of the Univ. of Strasbourg (France).

A systems level approach reveals incomplete caspase cascade function during mammalian hibernation

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Hibernating golden-mantled ground squirrels, Spermophilus lateralis, experience numerous conditions during the winter that are known to be pro-apoptotic in other mammal systems (e.g. extreme hypothermia, ischemia and reperfusion, acidosis, increased reactive oxygen species, bone and muscle disuse). Studies suggest that hibernators may invoke a protective phenotype to limit widespread apoptosis during the hibernation season. Could regulating apoptosis provide protection against the harmful conditions experienced during the hibernation season? To address this, we systematically examined the caspase cascade (caspases 1-12) for evidence of apoptotic signaling during hibernation. Caspases comprise a family of cysteine-aspartate proteases that are central to apoptosis and inflammation where upon proteolytic activation, they participate in a complex signaling cascade. Using ground squirrel liver, we determined the availability and activation status of caspases with western blots, performed caspase-specific proteolysis activity assays, and analyzed multiple caspase-mediated cellular events for indications of caspase signaling during hibernation. Surprisingly, we found the canonical apoptotic caspases 3, 6, and 9, as well as inflammatory caspases 11 and 12, appeared activated during hibernation. Caspase activation typically has dramatic effects on proteolytic activity. For instance, in other systems, when caspase 3, the “key executioner of apoptosis,” is processed into the active 17 kDa (p17) fragment, caspase 3 proteolytic activity increases up to ~10,000X vs. the procaspase form (p32). Caspase 3 activation, therefore, is thought to commit a cell to apoptosis. We found caspase 3 p17 increased ~2X during...
hibernation which may indicate significant apoptotic commitment. Did these winter-activated caspases display increased activity? We found no indications of dramatically increased caspase activity expected with a seeming caspase activation. To better understand the implications of caspase “activation” during hibernation, we used a systems-level approach to analyze numerous events downstream of caspase activation. We looked for indications of caspase 6 activity (nuclear lamin A cleavage), caspase 3 activity (degradation of the inhibitor of caspase-activated DNase (ICAD), inactivation of DNA repair enzyme poly (ADP-ribose) polymerase (PARP), and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) activity), and inflammatory caspase activity (IL-1β and IL-18 cytokine activation and serum transaminase levels). We found no evidence of downstream caspase activity. Despite the pro-apoptotic conditions of hibernation and the seeming caspase “activations”, there was no evidence suggesting widespread apoptosis and inflammation during ground squirrel hibernation. These data demonstrate the importance and utility of the systems-level approach in studying complex cellular signaling pathways like apoptosis in non-steady state physiological contexts like mammalian hibernation.

16.3 Changes in protein phosphorylation and acetylation correspond with suppression of mitochondrial metabolism during mammalian hibernation

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Small hibernators such as the 13-lined ground squirrel (Ictidomys tridecemlineatus) cycle between distinct metabolic states from late autumn through early spring. Periods of torpor last approximately 12 days during which body temperature (Tb) is maintained near 5°C and metabolic rate (MR) is very low. Torpor is spontaneously interrupted by arousals when MR increases approximately 100-fold and Tb rises to near 37°C within a few hours. During interbout euthermia (IBE) these high levels of Tb and MR are maintained for ~10 hours before animals enter another torpor bout. The whole-animal suppression of MR during torpor is mirrored at the mitochondrial level; state 3 respiration rates of liver mitochondria isolated from torpid ground squirrels can be 70% lower than those from IBE animals. This suppression of organelle function corresponds with decreases in maximal activities of electron transport system (ETS) complexes I and II during torpor, compared with IBE. We hypothesized that changes in post-translational modifications to mitochondrial enzymes were responsible for the rapid and reversible changes in mitochondrial metabolism between torpor and IBE. Differential 2D gel electrophoresis (DiGE) and Blue-native PAGE of proteins from purified liver mitochondria revealed that the isoelectric point or molecular weight of several proteins changed between torpor and IBE. MALDI mass spectrometry revealed that these proteins are involved in β-oxidation, the Krebs cycle, ROS detoxification and the ETS. Immunoblots for total acetylated protein of 2D gels showed that subunit 1 of ETS complex IV had 2.4-fold higher acetylation in torpor compared with IBE. Phosphoprotein staining revealed that the phosphorylation of the 75 kDa subunit of ETS complex I increased 1.5-fold during torpor, whereas phosphorylation of flavoprotein subunit of complex II decreased 4.6-fold. Given that differences in phosphorylation state of complexes I and II corresponds with differences in maximal enzymatic activity of these proteins between torpor and IBE, we attempted to manipulate enzyme activity by inducing dephosphorylation in liver mitochondria from torpid and IBE animals. In vitro treatment with alkaline phosphatase increased maximal activities of complex I in liver mitochondria isolated from animals in torpor, but had no effect on complex I from IBE animals. By contrast, exogenous phosphatase treatment decreased complex II activity in IBE liver mitochondria, but not torpor. These findings suggest that the rapid changes in mitochondrial and, perhaps, whole animal metabolism between torpor and IBE are mediated by post-translational modifications of key metabolic enzymes. Our future work will focus on understanding how changes in the cellular environment between torpor and IBE affect the activities of intramitochondrial kinases, phosphatases and deacetylases, which ultimately catalyze the changes that we observe.

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16.4 Perineuronal nets cover parvalbumin-positive neurons in ground squirrel cerebral cortex

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Thirteen-lined ground squirrels go through seasonal hibernation, which is characterized by extreme changes in physiology. These animals enter periods of torpor, where they exhibit near freezing body temperature, reduced blood flow, and low metabolism. Bouts of torpor are regularly interspersed with short returns to the physiological parameters typical of a summer, non-hibernating ground squirrel, called interbout arousals (IBAs). The brain is known to go through extreme changes during the transitions between torpor and IBA, specifically related to connectivity and synapse structure. The extracellular matrix could play a key role in this dynamic nature of the brain, particularly...
perineuronal nets (PNNs), which are protective and stabilizing structures known to regulate plasticity. Previous work investigating PNNs in the thirteen-lined ground squirrel brain showed that expression of PNNs was not global throughout the brain, but in specific regions, including areas of the hypothalamus, cerebral cortex, amygdala, and septum. Additionally, the overall presence of PNNs did not differ seasonally, as PNNs were found in all brain regions of both hibernating (Torpor and IBA) and non-hibernating (summer) animals. Here, we further characterize PNNs in the cerebral cortex using fluorescent *Wisteria floribunda* lectin histochemistry, focusing analysis on three areas: the anterior cingulate cortex, and two areas of the dorsal and lateral cerebral cortex just cranial to the hippocampus. Fluorescent Nissl staining paired with the lectin histochemistry revealed that PNNs surround neuron cell bodies and extend out along the projections, some extending more than 25 μm from the cell body. The PNNs were found specifically in cortical layers III and V. Within these layers, the nets do not cover all neurons, but are restricted to specific, multipolar neurons. Double labeling with the *Wisteria* lectin and a parvalbumin antibody revealed that the PNNs specifically surround parvalbumin-positive interneurons. The average neuron area covered by the PNN was 207.29 ± 8.49 μm² in layer III and 221.54 ± 6.69 μm² in layer V (n=11). PNN wrapped neurons were found at a frequency of 5.69 ± 0.62 cells/100 μm² in layer III and 6.99 ± 0.35 cells/100 μm² in layer V (n=11). There was no significant difference in count or average size among three time points analyzed (torpor, IBA, or summer/non-hibernating) in any of the cerebral cortex areas. Importantly, the three cerebral cortex areas quantified and analyzed here serve as representative areas. The entire cerebral cortex exhibited the same general PNN expression. Overall, this work supports previous PNN expression analyzed in other species, such as rats, during hypoxia and subsequently elevated mitochondrial II activity may reduce both the rate of succinate consumption was comparable to the rat using substrates (succinate/rotenone + ADP + CytC), whereas oxygen consumption was lower than 2015, and survival of beetles exposed to low elevation during differing winter conditions. Low elevation sites had higher expected overwintering energy costs, and were more sensitive to changes in precipitation than high elevation sites. Beetles at low elevation sites also spent more time below a freezing threshold than higher elevations. Together this suggests that variation in snow cover timing and duration will impact overwintering energetics of dormant insects, but the extent is dependent on the overall severity of winter, and not all elevations will be impacted similarly.

16.5

The impacts of snow cover variation across elevation on overwintering montane insects

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During winter, organisms are faced with multiple physiological challenges, including cold exposure and limited access to nutritional resources. To cope with these difficult environmental conditions organisms often enter dormancy. During dormancy individuals rely on energy reserves accumulated during resource-abundant summer months. Surviving a lengthy dormancy requires large energy stores and physiological modification. Metabolic rate dictates rate of consumption of energy stores, and is strongly influenced by environmental temperature. For organisms that overwinter beneath snow, temperature is modulated by snow cover, with snowy years being relatively warm and stable, while dry years are cold and variable. Impact of winter snow cover varies across elevation, winter snow fall increases with elevation while mean winter temperature decreases. We expect more reliable buffering at high elevations, but higher risk of cold damage when exposed to air temperature. The willow beetle *Chrysomela aeneicollis* lives in high elevation habitats in the Sierra Nevada Mountains, where it overwinters in leaf litter below its host plant for eight months each year. In these habitats there is considerable variation in snow fall across elevation and between winters, exposing populations to variable winter cold stress. To test the hypothesis that snow cover modulates winter energy use and protects from cold stress, in October 2015 we placed field-caught individuals under soil in plots exposed to natural snow cover or sheltered from snow. After seven months, survival was assessed and whole body lipid profile was measured using thin layer chromatography (TLC-FID). The experiment was repeated in October 2017, which was a dry winter with no significant snowfall until February and a minimum air temperature that was 5°C higher than 2015. During winter 2015 beetle survival did not differ between snow and no snow conditions, lipid stores decreased over the course of winter in both conditions, and beetles under snow had significantly lower lipids at the end of winter compared to beetles exposed to environmental temperature. During winter 2017, overall survival was higher than 2015, and survival of beetles that were not under snow was significantly higher than beetles that were under snow. This field experiment was coupled with metabolic rate measurements that were used to calculate expected winter energy use across elevation during differing winter conditions. Low elevation sites had higher expected overwintering energy costs, and were more sensitive to changes in precipitation than high elevation sites. Beetles at low elevation sites also spent more time below a freezing threshold than higher elevations. Together this suggests that variation in snow cover timing and duration will impact overwintering energetics of dormant insects, but the extent is dependent on the overall severity of winter, and not all elevations will be impacted similarly.
A comparative analysis of mitochondrial supercomplexes in vertebrates

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Mitochondrial electron transport chain enzyme complexes have recently been found to be arranged in supercomplexes (SCs), and it has been suggested that SCs may either improve electron transfer between individual complexes or stabilise individual complexes. In mammals, SCs are only observed when using very gentle detergents like digitonin and consist of CI and CIII with or without CIV in varying stoichiometries. Using selected species for vertebrate classes, we show here that heart mitochondrial SCs are much more stable in ectotherms, where they can be observed even in the presence of the much harsher detergent dodecyl maltoside (DDM). Reptilian SCs are the most stable, with fish and amphibians having a SC stability in between that of mammals and reptiles. Using mass spectrometry complexomics of isolated mitochondria with DDM, we found that turtle heart SCs consists of CI and CIII, and that all CI is found in the SC state. We observed no SCs containing CIV. We found that the content of SCs correlates with the content of cardiolipin in the mitochondrial membrane, but found no correlation between respiration rate, ROS production and SC content of isolated mitochondria. This suggests that SC formation depends on the phospholipid composition of the inner mitochondrial membrane and may thus depend on the body temperature of the species. Whether the mitochondrial membrane phospholipid composition has evolved to maintain SC integrity or if SC stability is a consequence of a given phospholipid composition is an interesting question to address next. Our present data does not support the view that SCs enhance electron transfer efficiency.

The Influence of Thyroid Hormone Manipulation on Cardiac Muscle Mitochondrial Function in Developing Chickens

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As chickens hatch, their thermoregulatory ability changes from an ectothermic to an endothermic phenotype. Associated with attaining endothermy at hatching is an increase in mitochondria function within different tissues correlated with endothermic capacity. Thyroid hormones (TH), key regulators of avian metabolism, are thought to influence development of endothermy. In birds, TH regulates skeletal muscle
growth, which directly impacts a chick’s ability to thermoregulate via shivering thermogenesis. To better understand the role of TH in the timing of hatching, development of thermogenic capacity, and metabolic rate, we manipulated plasma TH levels in chicken embryos beginning at 85% development (day 17 of a 21 day incubation) with either the thyroperoxidase inhibitor methimazole (MMI) or supplemental triiodothyronine (T3). After TH manipulation, we characterized mitochondrial function and reactive oxygen species (ROS) production in cardiac muscle from embryos and neonates using high-resolution respirometry coupled with fluorometry (Oxygraph-O2k). Additionally, we measured citrate synthase activity of cardiac and skeletal muscle. Thyroid hormone manipulation had a significant effect on cardiac mitochondria respiration with no influence on ROS production in externally pipped embryos. These results allow for a comprehensive view of the role of TH on the development of metabolic capacity of skeletal and cardiac muscle in the developing chicken. The development of increased metabolic capacity is essential for maturation of endothermic capacity at hatching in these animals.

17.4
Metabolic underpinnings of life history allocations: Mitochondrial function is fine-tuned to meet divergent energetic demands in two species of wing-polymorphic crickets

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Organismal fitness depends on having sufficient energy available to invest in life history traits such as activity, growth, maintenance, and reproduction. Through development, energetic demands fluctuate as organisms differentially allocate acquired resources amongst life history traits. Energetic constraints limit life history investments, and in turn can shape evolutionary patterns of biodiversity. To avoid constraints, variable life history demands are met via differential flux of ingested nutrients through catabolic and anabolic metabolic pathways. These pathways are linked by mitochondrial ATP production and consumption. Thus, changing energetic requirements may elicit concurrent shifts in mitochondrial function. We hypothesized that alternative life-history allocations are underpinned by differences in mitochondrial function, and predicted that ontogenetic shifts in energy demand are met by changes in mitochondrial bioenergetics. Many species of Gryllus field crickets have two morphs within populations, a flight-capable dispersal morph and a flightless reproductive morph. The polymorphism is maintained by a resource-based flight-oogenesis trade-off. Metabolic demands are highly divergent between morphs and change during ontogeny, making this an ideal system to test our hypothesis. Energy demands of the reproductive morph are high during early adulthood while investing in ovary synthesis but decline within a week, once reproductive maturity is reached. In contrast, high metabolic requirements of flight in dispersal morphs extend past the first week of adulthood. Here, we determined the extent to which mitochondrial function differs between adult wing morphs of two cricket species (Gryllus firmus and Gryllus lineaticeps) across ontogeny (1, 3 or 5 day-old). Mitochondrial function was assessed using high-resolution respirometry to measure respiration rates of isolated mitochondria from fat body when fueled by NADH (malate, glutamate, pyruvate) or FADH₂-generating (succinate) substrates. In early adulthood, when energy demands are elevated for both morphs, mitochondrial respiratory function is similar, but diverged gradually through ontogeny in both species. By day five, mitochondria from dispersal morphs exhibited a higher oxidative phosphorylation capacity compared to reproductive morphs. Cytochrome c oxidase (Complex IV) expression levels and specific activity were also elevated in dispersal morphs. Thus, increases in mitochondrial content and electron transport chain activity may serve to enhance ATP production capacity to meet the high energetic demands of flight. Overall, our results demonstrate a divergence of mitochondrial activity through development, which suggests that adjustments to the bioenergetic machinery of this organelle play an integral role in maintaining energetic homeostasis and supporting alternative life-history allocations. Additionally, these findings indicate that life history evolution has played a prominent role in shaping mitochondrial function.

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17.5
Cellular metabolism and oxidative stress as a possible determinant for longevity in small breed and large breed dogs.

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Among species, larger animals tend to live longer than smaller ones, however, the opposite seems to be true for dogs - smaller dogs tend to live significantly longer than larger dogs across all breeds. We were interested in the mechanism that may allow for small breeds to age more slowly compared with large breeds in the context of cellular metabolism and oxidative stress. Primary dermal fibroblasts from small and large breed dogs were grown in culture. We measured basal oxygen consumption (OCR), proton leak, and glycolysis using a Seahorse XF96 oxygen flux analyzer. Additionally, we measured rates of
reactive species (RS) production, reduced glutathione (GSH) content, mitochondrial content, lipid peroxidation (LPO) damage and DNA (8-OHdG) damage. Our data suggests that as dogs of both size classes age, proton leak is significantly higher in older dogs, regardless of size class. We found that all aspects of glycolysis were significantly higher in larger breeds compared with smaller breeds. We found significant differences between age classes in GSH concentration, and a negative correlation between DNA damage in puppies and mean breed lifespan. Interestingly, RS production showed no differences across size and age class. Thus, large breed dogs may have higher glycolytic rates, and DNA damage, suggesting a potential mechanism for their decreased lifespan compared with small breed dogs.

18: OMIC RESPONSES TO STRESS: ABSTRACT DRIVEN SESSION

18.1
Physiological mean-variance relationships among intertidal mussels depend on environmental context

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The ability of environmental variation to mask or, alternatively, to reveal physiological variation among individuals will almost certainly play a role in how populations respond to changing environmental conditions. Yet, the contributions of temporal and spatial environmental variation to inter-individual physiological differences remain poorly resolved. Each individual’s physiological profile depends on how its unique microhabitat conditions and past experience interact with the biochemical/physiological and behavioral mechanisms at its disposal. Stressful conditions promoted the expression of inter-individual variation in antioxidant capacities within a population of rocky intertidal zone mussels (Mytilus californianus). In a separate study, hotter individuals expressed higher levels of some antioxidants in the field. However, preliminary measures of inter-individual variation in oxidative damage and global protein expression patterns in the same manipulations appear to tell a surprisingly different story, with greater magnitudes of inter-individual variation observed under relatively benign conditions. There are several possible explanations for this contradiction between measures of variation in "defense" and "damage". Using transcriptomics and proteomics approaches, we are exploring which underlying biochemical networks contribute the most to these patterns of inter-individual variation. Highly variable networks, or key regulators thereof, represent possible avenues of selection under novel environmental conditions.

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18.2
Transcriptomic responses to low salinity among locally adapted populations of Olympia oyster, an estuarine foundation species

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The Olympia oyster (Ostrea lurida) is a foundation species inhabiting estuaries along the North American west coast. In California estuaries, O. lurida is adapted to local salinity regimes and populations differ in low salinity tolerance. In this study, oysters from three California populations were reared for two generations in a laboratory common garden and subsequently exposed to low salinity seawater. Comparative transcriptomics was then used to understand species-level responses to hyposmotic stress and population-level mechanisms underlying divergent salinity tolerances. Gene expression patterns indicate Olympia oysters are sensitive to hyposmotic stress: all populations respond to low salinity by up-regulating transcripts indicative of protein unfolding, DNA damage, and cell cycle arrest several days before mortality. Among O. lurida populations, transcriptomic profiles differed constitutively and in response to low salinity. Despite two generations in a common garden, transcripts encoding apoptosis modulators were constitutively expressed at significantly different levels in the most tolerant population. Expression of cell death regulators may facilitate cell fate decisions when salinity declines. Following low salinity exposure, oysters from the more tolerant population expressed a small number of mRNAs at significantly higher levels than less tolerant populations. Proteins encoded by these transcripts regulate ciliary activity within the mantle cavity and may function to prolong valve closure and reduce mortality in low salinity seawater. Collectively, gene expression patterns suggest sub-lethal impacts of hyposmotic stress in Olympia oysters are considerable and that even oysters having garnered greater low salinity tolerance via natural selection will be vulnerable to future freshwater flooding events.
18.3 Unexpected natural modification of mt-DNA alters centenarian bivalve physiology and ecology

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Mitochondrial DNA (mtDNA) is normally maternally inherited and encodes for subunits of respiratory chain complexes and ATP synthase, among many other processes. The integrity of mtDNA is crucial for all eukaryote species function, from sub cellular processes to species ecology and evolution. Mitochondrial DNA mutations have been associated with cellular energetic and redox homeostasis dysfunction, and can affect individual well-being and longevity. Bivalves are the only zoological group in which Doubly Uniparental Inheritance (DUI), characterized by the presence of 2 divergent mtDNAs within different tissues of male individuals, is frequently observed. The F-mtDNA, maternally inherited, is found in somatic tissues of both sexes and female gonads whereas the M-mtDNA is inherited exclusively in male gonads (Dégletagne et al 2015). Our recent investigations highlighted the existence of this particular mtDNA inheritance system in long-lived populations of the ocean quahog, Arctica islandica. This clam is distributed throughout the North Atlantic shelf regions. Due to different environmental regimes (salinity, temperature, oxygen), the maximum lifespan of its populations varies between >500 years around Iceland and 35 years in the Baltic Sea.

Our new analyses of mitochondrial marker gene sequences in somatic tissues indicate North Atlantic populations to be genetically homogenous, but reveal the existence of a “divergent” mtDNA haplotype with a genetic difference ~6% in all somatic tissues (gill, mantle, foot) of 10% of Iceland animals, male and female. This “divergent” mtDNA is similar to the M-mtDNA which is present in 100% of A. islandica male gonads. We sequenced the “divergent” mtDNA and showed that the sequence of all genes is modified but that gene order is conserved. Using transcriptomic and biochemical approaches, we highlighted that this “divergent” mtDNA is exclusively expressed and reduces the maximum capacities of respiratory chain complexes (complex I, III and cytochrome-c oxidase) by approximately 30% compared to the normal F-mtDNA type. These results combined with our analyses of mitochondrial respiratory capacities, ROS production, and data on individual growth and longevity suggest that these animals carrying this “divergent” mtDNA in their somatic tissues, and among those especially the female specimens, could be the ones with an extremely long lifespan prognosis. Altogether, these new findings will allow us to enlarge the discussion about the link between natural mitochondrial genome modifications and animals’ phenotype and ecology.


18.4 Integrating the effects of food availability and sirtuins on stress tolerance to multiple levels of biological organization

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Sirtuins remove acyl groups, which represent several metabolites, from proteins and thereby regulate enzyme activity through modifying post-translational modifications (PTMs). Levels of sirtuins are generally upregulated in response to caloric restriction and affect a number of cellular processes in mammals. We investigated the effect of sirtuin (SIRT) and food on the stress tolerance of several closely related mussel species. We discovered that overall levels of the mitochondrial sirtuin SIRT5 are three times higher in gill of the heat-sensitive mussel Mytilus trossulus than the more heat-tolerant Mediterranean M. galloprovincialis, which replaced the former from the warmest part of its range in California over the last century. Despite the higher constitutive levels of SIRT5, the heat-sensitive species only showed an increase with heat stress. Further, inhibiting SIRT activity during heat stress increased SIRT5 levels in the heat-sensitive but not the -tolerant species, suggesting an active signaling feedback pathway. Using proteomics, inhibitor studies showed that sirtuins affect molecular chaperones, oxidative stress proteins, metabolic enzymes and signaling proteins during heat stress, suggesting a general influence on the cellular stress response (CSR). We extended the studies to test for the effect of food availability and frequent heat stress during acclimation on levels of sirtuins and the effect of sirtuin inhibition on the CSR, several measures of gill performance (particle velocity, ciliary beat frequency and clearance rate) and respiration in M. californianus. Our initial results suggest that the changes initiated by food availability and SIRT inhibition integrate up to affect gill respiration and interact to affect clearance rate at the level of the whole organism. Finally, these results are dependent on the circadian rhythm of mussels, in part because SIRT5 levels undergo circadian changes in
abundance. Our studies suggest that integration across levels of organization requires a high temporal resolution and several types of performance measures at the level of the organ and the organism.

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18.5
Evolution of higher rate of living leads to enhanced inducibility of gene expression: evidence from heat shock genes in Drosophila

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For most organisms, the ability to respond to stressful and changing environments is essential for survival and Darwinian fitness. In response to stress, individuals undergo shifts in both behaviour and physiology. Species/populations with early reproduction and short lifespan may be under pressure to respond to changing conditions rapidly because reproductive success is determined in a narrow window of time. Additionally, a higher metabolic rate may predispose faster activation of underlying molecular responses to a stressor. Either way, one might expect species with a high “rate of living” – typically smaller and faster growing species – to also be quicker in up-regulating stress-mitigating pathways. In this study, we address whether there is such a relationship between rate of living (ROL) and rate of cellular response to stress using populations of Drosophila melanogaster which have been selected for highly divergent ages of reproduction for over 1000 generations. One selection treatment, here designated “Fast” for simplicity, goes through a complete generation every 9 days, while it’s “Slow” counterpart reproduces every four weeks; each treatment has five-fold replication at the population level and each population is kept in large cohorts. In response to selection, Fast and Slow flies now differ markedly in many traits. Fast flies develop in seven days, compared to 9 – 10 days for Slow. Fast flies are also smaller-bodied at reproductive maturity. These lines also differ in sperm production, food requirements, desiccation and starvation tolerance and lifetime fecundity. Most important for this study, Fast and Slow flies have dramatic differences in longevity, with Slow flies living 2-3X longer. To investigate the relationship between stress response and ROL, flies from both selection treatments were exposed to temperatures known to up-regulate heat shock proteins (Hsps). We predicted that, due to their higher ROL, Fast flies would mount a stress response by up-regulating Hsps more rapidly in response to heat shock than Slow flies would. To measure the rate of heat shock response, we set up a time-course of heat exposure for flies from both selection treatments. We then measured gene expression of Hsps using qRT-PCR. We found that, in line with our predictions, Fast flies increase expression of Hsps quicker than Slow flies, consistent with a link between ROL and kinetics of the stress response. Unexpectedly, Fast flies not only ramped up HSP expression faster but also displayed increased overall expression than Slow flies. We are following up this work by looking at other stressors to determine if there is an innate difference in sensitivity to stress between the two selection treatments. Supported through NSERC Discovery Grants to AC and CDM.

19: ENERGETICS:
ABSTRACT DRIVEN SESSION

19.1
Polygenic mapping reveals genetic associations with variation in routine metabolic rate in Fundulus heteroclitus

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Variation in metabolic rate is thought to be a key factor influencing the ecology of individuals, populations and species. Consequently, identifying the genetic mechanisms that underlie variation in this trait is critical for understanding not only the potential for evolution of metabolic rate, but also the processes through which evolution of metabolic rate is likely to occur. Despite this clear need to link genetic variation with phenotypic variation in metabolism, there are few empirical datasets available that address this important issue. In the current study, we assess variation in routine metabolic rate in three populations of Atlantic killifish, Fundulus heteroclitus. There are two subspecies of this species along the east coast of North America: F. h. heteroclitus from Florida, USA to New Jersey, USA, and F. h. macrolepidotus from New Jersey to Nova Scotia, Canada. The northern subspecies has previously been shown to have a higher routine metabolic rate than the southern subspecies. However, this pattern could be to the result of either genetic differences between the subspecies or the result of developmental plasticity caused by the different environmental conditions at the northern and southern end of the species range. Here, we take advantage of a New Jersey population located in a region of genetic admixture between the subspecies to assess the relationship between genetic variation and variation in metabolic rate in a relatively large sample of fish (286) collected from a single location. Through the use of random forest models, we identified 62 single-nucleotide polymorphisms (SNPs) that explained up to 43% of the variation in routine metabolic rate among individuals. This suggests that metabolic rate is polygenic to a large
extent, and that a substantial amount of variation in this key physiological and ecological trait is genetically determined. Additionally, several of our identified SNPs demonstrated allele frequency differences among populations that were consistent with population-level variation in metabolic rate. Thus, it is possible that variation at a subset of the 62 SNPs we identified may contribute to variation in metabolism among populations as well as among individuals. Many of these SNPs were located within or nearby genes that have plausible functional connections to differences in metabolism, including several upstream regulatory proteins of central cellular kinases such as phosphatidylinositol 3-kinases, mitogen-activated protein kinase and the mechanistic target of rapamycin, as well as other genes involved in metabolic pathways such as monocarboxylate transporter 7.

19.2 Evolutionary variation in hypoxia tolerance in Fundulidae killifishes
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Hypoxia is a pervasive stressor in the aquatic environment, and fish exhibit a variety of mechanisms for coping with hypoxia that arise from phenotypic flexibility and/or evolutionary adaptation. We investigated the evolved variation in hypoxia tolerance and the hypoxia acclimation response in killifish from the family Fundulidae, a widely distributed group that naturally experiences daily and seasonal cycles of hypoxia. We compared resting O2 consumption rate (MO2) and three indices of hypoxia tolerance – critical O2 tension (Pcrit), O2 tension (PO2) at loss of equilibrium (LOE), and time to LOE in severe hypoxia – across 8 species (F. heteroclitus, F. confluentus, F. rathbuni, F. grandis, F. diaphanus, F. similis, Lucania goodei, and L. parva). We also examined the effects of acclimation to constant hypoxia (2 kPa O2) or to intermittent cycles of nocturnal hypoxia (12h normoxia during the day, and 12h of 2 kPa O2 at night) in a subset of 5 species. Larger fish generally had lower mass-specific MO2 and were more hypoxia tolerant (Pcrit and PO2 at LOE) after accounting for evolutionary relatedness using phylogenetically independent contrasts. Acclimation to constant hypoxia increased hypoxia tolerance in all species, but there was interspecific variation in the magnitude of the response. Acclimation responses to intermittent hypoxia were idiosyncratic, with changes occurring in only some species. This variation in hypoxia acclimation responses was not generally associated with variation in MO2 or with the relative severity of the O2 stress to each species (as reflected by acclimation PO2 relative to Pcrit). Our results suggest that there is appreciable evolutionary variation in hypoxia tolerance and the hypoxia acclimation response across the Fundulidae. Supported by the Natural Sciences and Engineering Research Council of Canada, the National Science Foundation, the National Institute of Environmental Health Sciences, the American Physiological Society, the Society for Experimental Biology, and the Louisiana Sea Grant program.

19.3 Protein turnover: A biochemical basis for endogenous variation in growth and energy metabolism
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Protein turnover is regulated by complex, counteracting dynamics of synthesis and degradation. When the balance of these processes favors synthesis over degradation, protein growth occurs. Protein turnover is energy intensive, with protein synthesis often being the major consumer of metabolic energy in animals, accounting for ~50% of total ATP consumption. Variation in protein turnover will therefore have important implications for metabolic energy expenditure and growth. While the regulation of protein turnover by exogenous (environmental) factors has been of long-standing interest in the field of comparative biology, less effort has been directed towards understanding endogenous (genetic) regulation of protein turnover and its role in genetically determined growth variation. We used factorial crosses of inbred lines of the Pacific oyster (Crassostrea gigas) to produce larval families that showed classical hybrid vigor (i.e., the superior growth of hybrids over both parental lines). We then tested for variation in protein turnover among families of larvae with contrasting growth phenotypes. Despite a lower growth rate than hybrid larvae, inbred larvae had higher fractional rates of protein synthesis (synthesis rate as a percent of whole-body protein content). Consequently, protein depositional efficiency (the ratio of protein accretion to total protein synthesized) was much lower for inbred families – reflecting higher protein degradation rates in these slow-growing larvae. We next assessed energy use and costs for protein turnover. During growth, respiration was dependent on larval size and did not differ among families (i.e., was not genetically determined). Differences in protein turnover between inbred and hybrid families did, however, have major effects on energy expenditure. Inbred larvae used ~2-fold more ATP to support protein synthesis, relative to hybrid larvae. Our analysis provides a mechanistic explanation for hybrid vigor and suggests that genotypes with increased energy demand for protein synthesis, as well as high degradation rates, may be limited in their ability to allocate ATP to other essential processes. This
work was supported by funding from the U.S. National Science Foundation (Emerging Frontiers No. 121220587).

19.4
Bioenergetics of protein metabolism under experimental environmental change
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The identification of the integrative processes that regulate physiological traits remains a key goal in biology. Furthermore, in multicellular organisms it remains challenging to define how processes at the cellular level can be used to predict responses at the whole-organism level under changing environmental conditions. In general terms, such responses are regulated by complex genotype-environment-phenotype interactions; for most organisms, however, the details of these interactions are not understood. In this study, we present findings based on a combination of physiological and genetic analyses focused on the study of biological variation in growth and metabolic energy (ATP) allocation. Crosses of pedigreed lines were used to produce contrasting phenotypes in developmental stages of a highly fecund marine invertebrate mollusc (Crassostrea gigas). On average, during growth 82% of the allocation of cellular ATP was accounted for by only three processes: protein synthesis and degradation (48%), sodium-potassium regulation (20%), and calcification (14%). Significant variation on this average hierarchy of ATP allocation was observed across different larval families, with noteworthy physiological consequences. For instance, variation in ATP allocation to protein synthesis predicted 72% of growth variation. Analysis of genetic, environmental, and physiological components of variation revealed a tradeoff between ATP allocation for fast growth and susceptibility to environmental stress. This bioenergetic framework suggests that a biochemical ‘tipping point’ can be identified as a predictive index of sublethal-to-lethal stress responses.

19.5
Dietary antioxidants and flight exercise affect the extent to which antioxidants are delivered to the mitochondria and how female birds allocate nutrients to eggs.
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Physiological challenges during one part of the annual cycle can carry over and affect performance at a subsequent phase, and antioxidants could be one mediator of trade-offs between phases. We performed a controlled experiment with zebra finches to examine how songbirds use nutrition to manage trade-offs in antioxidant allocation between endurance flight and subsequent reproduction. Our four treatment groups included a factorial combination of exercise/sedentary groups and diet supplemented/non-supplemented groups with the supplement including water- and/or lipid-soluble antioxidants. Dietary Vitamin E was delivered to mitochondria within 22 hrs of ingestion but only in exercised and not sedentary birds. After flight training, birds were paired within treatment groups for breeding. We analyzed eggs for lutein and vitamin E concentrations and the plasma of parents throughout the experiment for non-enzymatic antioxidant capacity and oxidative damage. Exercised birds had higher oxidative damage levels than non-exercised birds after flight training, despite supplementation with dietary antioxidants. Supplementation with water-soluble antioxidants decreased the deposition of lipid-soluble antioxidants into eggs and decreased yolk size. Flight exercise also lowered deposition of lutein, but not vitamin E, to eggs. These findings have important implications for future studies of wild birds during migration and other oxidative challenges. Supported by NSF (IOS-0748349 & IOS-135417 to S.R.M.), USDA (RIAES-538748 to S.R.M.), and AOU and Sigma Xi student research awards to M.M.S.
plasticity in the context of explicit regulatory mechanisms, which can replace hypothetical “genes for plasticity” in evolutionary models.

20.2
Evolution of the Acclimation Responses to Hypoxia and Cold in Deer Mice Native to High Altitudes
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High-altitude environments provide fertile ground for investigating the evolution of phenotypic plasticity in the physiological systems underlying animal performance. The cold and oxygen-depleted (‘hypoxic’) environment at high altitudes requires that endothermic animals sustain high rates of O2 consumption for thermogenesis and locomotion while facing a diminished O2 supply. This appears to result in strong directional selection for high aerobic capacity (VO2max) in hypoxia in deer mice (Peromyscus maniculatus) native to high altitude. I will present our work on the evolution of phenotypic plasticity of VO2max in high-altitude deer mice. Hypoxia acclimation augments hypoxic VO2max much more in highlanders than in low-altitude populations of deer mice and white-footed mice (P. leucopus). This is associated with higher pulmonary O2 extraction, arterial O2 saturation, cardiac output, and tissue O2 extraction. The evolution of hypoxia-induced plasticity in systems-level function involves coordinated changes at tissue, cellular, and transcriptomic levels of organization. In contrast, cold acclimation has a similar effect on VO2max in highlanders and lowlanders, and the effects of cold acclimation arise primarily from increases in cardiac output and tissue O2 extraction. Therefore, high-altitude adaptation has augmented plasticity in response to chronic hypoxia, but not chronic cold, and involves a series of integrated changes across the O2 pathway.

20.3
Cold adaptation drives evolution of metabolic plasticity in Drosophila melanogaster
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Metabolic pathways are strongly affected by temperature, and the thermal sensitivity of metabolism frequently evolves in response to variable thermal environments leading to differences in metabolic plasticity. Metabolic plasticity can occur across the levels of the biological hierarchy, from transcript or metabolite networks, pathway flux, and organismal performance. In some cases, plasticity at one level of the hierarchy can canalize important responses at another level. We use data from an integrative study of metabolic plasticity in response to cold adaptation in Drosophila melanogaster to illustrate this principle, and show that evolution of metabolic plasticity can preserve organismal function in response to thermal stress.

We show that adaptation of Drosophila melanogaster to acute low temperature exposure increases rates of growth, activity, respiration, catabolism and anabolism in flies that have not received a cold exposure. During cold exposure, cold-hardy flies shut down catabolism to a greater degree than do cold-susceptible flies, leading to greater plasticity in nutrient catabolism and greater metabolic flexibility. At the metabolite level, this flexibility in catabolism allows them to maintain metabolic and energetic homeostasis more effectively. Thus, plasticity in metabolic flux can respond to selection imposed by acute low temperature exposure, and can be key to the maintenance of homeostasis in variable environments.

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20.4
Evolution of osmoregulatory flexibility during transitions between marine and freshwater habitats in fishes
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Adaptive divergence between marine and freshwater environments is important in generating phyletic diversity within fishes, but the mechanistic basis of adaptation to fresh water remains poorly understood. We examine divergence in osmoregulatory abilities between populations of Fundulus heteroclitus that are native to brackish (BW-native) or freshwater (FW-native) environments along a salinity gradient. We find that BW-native fish showed a reduced ability to regulate plasma Cl– in fresh water, while FW-native fish showed a reduced ability to regulate plasma Na+ in brackish water, suggesting that cell–cell junctions are involved in the divergence of physiological abilities. We used population genomics to identify genomic regions that affect fitness between BW and FW environments, and genome wide association to reveal the genetic variation that is associated with variation in salinity tolerance. Our analyses implicate candidate genes likely involved in evolved physiological capabilities, some of which support hypotheses about the importance of cell-cell junctions.

21: CARDIOVASCULAR:
ABSTRACT DRIVEN SESSION - 2

21.1
Assessing the full significance of the Bohr/Haldane effect for gas exchange in the tissues
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Despite the fact that the Bohr and Haldane effects are of equal size at the molecular level due to their thermodynamic linkage, the influence of the Bohr effect...
on the utilization of blood borne oxygen has been deemed secondary to the influence of the Haldane effect on the uptake of carbon dioxide by the blood. Here we show that the opposite is the case. Using a simple two-ligand, two-state formulation we modelled the simultaneous oxygen and proton binding to hemoglobin as well as the resulting acid-base changes of the surrounding solution. When the Bohr effect is blocked in this model system, we see a dramatic increase in the oxygen affinity, with a fall in oxygen half saturation pressure ($P_{S0}$) from 27 to 6 mmHg. We also show that the $P_{S0}$ and the Bohr factor are not independent but directly related. Thus, everything else being equal, varying the number of Bohr groups from 0 to 8 per tetramer results in an increase in the Bohr factor from 0 to -0.9 and an increase in $P_{S0}$ from 6 to 46 mmHg at a constant $P_{CO_2}$ of 40 mmHg. Therefore, changes in hemoglobin structure that lead to changes in the Bohr factor will inevitably also change hemoglobin oxygen affinity. The full extent of the impact of the Bohr effect on oxygen unloading cannot be assessed by comparing oxygen equilibrium curves measured in the lab at different constant $P_{CO_2}$ or pH because each of these curves are already shaped by the Bohr/Haldane effect.

### 21.2

Weddell seals selectively limit guanylyl cyclase-mediated vasodilation: Implications for perfusion of the brain during diving

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In terrestrial animals, the nitric oxide-cGMP (NO–cGMP) signal transduction pathway plays a critical role in inducing vasodilation, including the systemic response to hypoxia. Diving mammals experience profound hypoxia during submergence, while they maintain selective vasoconstriction in peripheral tissues, suggesting that hypoxic vasodilation may modulate cardiovascular responses to diving. The objective of this study was to investigate NO–cGMP signaling in Weddell seals, using tissues including arteries collected at necropsy. We focused our investigation on soluble guanylyl cyclase (GC), which produces the vasodilator cGMP upon stimulation by NO. GC protein abundance, baseline activity, and NO-responsiveness were consistently lower in Weddell seals compared to terrestrial mammals. In seal lung homogenate, GC produced less cGMP (pmol·mg protein$^{-1}$·min$^{-1}$) than the lungs of dogs (-80±144), sheep (-472±96), rats (-664±104) or mice (-1160±104; $P<0.004$). GC activity was also lower in seal brain, heart, skeletal muscle, kidney, and artery homogenates compared to the same tissues in sheep, indicating that seals produce less vasodilatory cGMP at baseline and in the presence of NO. Amino acid sequences of the GC enzyme alpha subunits differed between seals and terrestrial mammals, impacting hydrophobicity and polarity of the seal proteins and potentially affecting their structure and function. GC abundance, activity, and NO-responsiveness declined with maturation in some seal tissues: NO-stimulated GC activity declined 15-fold in lung, 25-fold in muscle and 39-fold in heart of adults versus pups. Reduced cGMP production via low GC activity in selected adult seal tissues could support the dive response by potentiating sympathetically-mediated vasoconstriction. Yet, peripheral vasoconstriction in diving Weddell seals is not homogenous, with arterial blood perfusion distributed to favor the brain and heart. To better understand tissue-specific vasoregulation during diving, we compared NO-cGMP signal transduction in the brain versus the kidney (which experiences vasoconstriction, even during short dives). Seal GC was more responsive to stimulation by a NO-donor in the brain (58-fold activity increase) than in the kidney (24-fold increase), consistent with the prioritization of cerebral perfusion during diving. Nos3 expression was also high in the seal brain relative to the carotid artery and the renal circulation (renal artery and kidney tissue; $P=0.01$), which could improve NO production and vasodilatory potential in the hypoxic brain. Conversely, Pde5a expression was highest in the seal renal artery ($P=0.04$), identifying this as a site of cGMP scavenging and supporting vasoconstriction in the kidney. Taken together, these results suggest that Weddell seal-specific tissue alterations in the expression and function of proteins in the NO-cGMP signal transduction pathway may be critical features of the diving response. Funded by NSF Office of Polar Programs 1443554 and NIH U54 HG003067-08.

### 21.3

Developmental differences in anoxia-induced gene expression in the heart of the painted turtle

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The Western painted turtle, Chrysemys picta bellii, is the most anoxia-tolerant tetrapod known, capable of surviving 170 days of anoxia at 3°C. During anoxia, the turtle suppresses its cardiac metabolic rate in order to decrease energy consumption to levels that can be met by glycolysis. Although many of the functional changes that occur during anoxia have been described, the
transcriptomic changes have received less attention. Turtles also exhibit developmental differences in anoxia tolerance; hatching turtles survive for only 40 days at 3°C. Our study compared the transcriptomic changes that occur in the turtle ventricle during 20 days of anoxia followed by 6 days of recovery at 3°C in both adult and hatching painted turtles. The anoxia-tolerant adult turtles exhibited several unique changes in gene expression after anoxia and recovery, including a significant decrease in mRNA expression levels of 58 ribosomal proteins after anoxia, \((p = 0.001)\), all of which increased in hatchlings \((p = 0.001)\). After reoxygenation, ribosomal protein expression levels returned to control levels for both development stages. These results suggest that downregulation of genes encoding ribosomal proteins, and of the process they regulate, may be essential for cardiac survival during anoxia in adult painted turtles.

**21.4**
The influence of cellular stretch on extracellular connective tissue deposition in cultured trout cardiac fibroblasts

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Rainbow trout undergo cardiac hypertrophy and extracellular collagen deposition in response to cold (4°C) acclimation. During cold acclimation, whole blood viscosity initially increases, which in turn leads to increased vascular resistance and ultimately places more workload on the heart. At the cellular level, myocytes, fibroblasts, and endothelial cells within the myocardium are thought to undergo increased deformation as a result. In mammals, cellular deformation stimulates mechanosensory cellular components and initiates a signaling cascade through mitogen activated protein kinase (MAPK) pathways, influencing the gene and protein expression of collagen, angiotensin and transforming growth factor-beta1 (TGF-ß1). Previously, we have demonstrated that exogenous human TGF-ß1 is able to increase collagen synthesis in trout cardiac fibroblasts, as well as differentiate these cells into myofibroblasts. Therefore, it is expected that many of the major remodeling pathways are conserved throughout vertebrates, such as mechanotransduction through extracellular physical cues. We hypothesized that trout cardiac fibroblasts are deformed in response to vascular resistance, and predicted that stretching these cells would result in the activation of MAPK pathways and ultimately collagen remodeling. Fibroblast cultures from rainbow trout ventricles were subjected to 10% stretch on a rubber membrane for 10 min, 20min, 24h and 3d and then their MAPK pathways were compared to that of control cells that were cultured on the same type of membrane, but not stretched. p38 MAPK and extracellular-regulated kinase (ERK) phosphorylation was quantified using Western blotting. No changes were detected after 10min of stretch, however, after 20min, p38 MAPK phosphorylation increased by 4.2-fold compared to cells that were not stretched \((P<0.05)\). While ERK was not significantly phosphorylated, an interesting finding was noted. The total amount of unphosphorylated ERK was increased by 1.7-fold in stretched cells after 20min, without a change in total protein. After 24h of stretch at 10% deformation, p38 continued to be phosphorylated by 4.7-fold more than control cells \((P<0.05)\). At this time, collagen type I was reduced by 70% in stretched cells. Collagen types I and III are currently being quantified after 3d of stretch. The results of this study provide support for the hypothesis that cardiac remodeling in trout is initially triggered by vascular resistance, leading to cellular deformation and mechanotransduction.

**21.5**
Re-Assessment of the Biochemistry of Metabolic Acidosis Using Metabolite and Reaction H\(^+\) Coefficients Computed From Multiple Competitive Cation Binding

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**Background:** The purpose of this research was to complete pH dependent calculations of H\(^+\) balance during intense exercise corrected for pH and competitive cation binding. **Methods:** Metabolite accumulation data were acquired from prior published research of intense exercise conditions causing muscle anoxia, and where volitional exhaustion occurred within 3-4 min (1-3,5,9-11). Such data, adjusted for muscle water, pathway flux and carbon numbers where appropriate, were used to quantify the capacities of each reaction of non-mitochondrial ATP turnover in contracting skeletal muscle as well as profile each metabolite production curve over time. Muscle ATP turnover during intense exercise was modeled based on the data obtained from Spriet et al. (9,10). Data for H\(^+\) dissociation constants for metabolites were obtained from the NIST database (6), Robergs (7), Kushnerick (4), and Vinnakota et al. (12). Custom software was completed (LaBVIEW, Austin, Texas, USA) to allow computations of pH dependent H\(^+\) and competitive cation binding. **Results and Conclusions:** Computed data results revealed that the total H\(^+\) load of this intense exercise condition, where negative numbers indicate H\(^+\) release, was -174.6 mmol/L. The net H\(^+\) balance for the sum of the CK, AK, AMPD, myosin ATPase and amino acid deamination reactions was 32.87 mmol/L. The combined glycogenolytic and glycolytic H\(^+\) balance, including lactate production, was -33.54 mmol/L. The overall H\(^+\) balance of ATP hydrolysis equated to -44.57 mmol/L. Lactate production had a H\(^+\) balance of 44.14 mmol/L (assuming
a total lactate accumulation of 45 mmol/L). Total
metabolic H^+ buffering was 129.33 mmol/L. Structural
buffering was 49.53 mmol/L, with an estimated total
muscle buffer capacity for this pH change (7.0 to 6.1) of
55.03 Slykes. The H^+ release to lactate ratio was close to
4.0 (174.6/45=3.88). When correcting past errors of a
lactic acid origin of metabolic acidosis, muscle H^+ release
during intense exercise is up to 4-fold larger than previously
assumed based on the now disproven lactic acid construct.
Lactate production removes approximately 25% of the 
intracellular metabolic H^+ release of intense muscle contractions. The data have
important implications to the teaching of metabolic
biochemistry and interpretations of research data
concerning systemic and cellular acid-base balance.

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22: MAMMALIAN MOLECULAR PHYSIOLOGY:
ABSTRACT DRIVEN SESSION

22.1
Obesity-related gene expression during fasting in a
naturally obese marine mammal
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Adipose tissue is a complex energy storage and
endocrine organ that regulates metabolic homeostasis
via adipocyte-derived hormones (adipokines). Excess
adiposity in humans is correlated with increased
adipokine levels, insulin resistance, and inflammation,
and is a major risk factor for metabolic pathologies. With
some of the largest subcutaneous adipose stores in the
animal kingdom, marine mammals may provide valuable
insights into the role of adipose tissue in health and
disease. Capital breeding marine mammals rely on lipid
stores in blubber to sustain fasting periods associated
with terrestrial breeding and migration. Northern
elephant seals (Mirounga angustirostris) undergo two
annual fasts during breeding and molting haul-outs,
losing up to half of their fat mass over a period of 1-3
months while maintaining high circulating fatty acid and
glucose levels and insulin resistance, similarly to obese
humans. We examined changes in expression of genes
associated with obesity in humans in blubber of fasting
adult female elephant seals using quantitative PCR.
Target genes included the adipokines leptin, adiponectin,
and retinol-binding protein 4 (Rbp4), fat mass and
obesity-associated gene (Fto), and angiopoietin-like 4
(Angptl4), among others. Normalized gene expression
values (dCt) were compared between early breeding, late
breeding, early molting, and late molting groups using
linear mixed models. Expression values were significantly
different between groups and the majority of adipokine
genes were upregulated in late fasting compared with
early fasting seals, despite a concomitant decline in fat
mass. This suggests a mechanism by which elephant seals
maintain insulin resistance and lipid oxidation during
fasting periods characterized by high energy expenditure
and provides insights into rapid weight loss in mammals.

22.2
The Weddell seal skin transcriptome reflects local
mechanisms in endocrine regulation of molt
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Michelle Shero2,4, Donald Thompson, Jr.5, Jennifer
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Center
Pinnipeds replace their fur each year in an annual pelage
molt, the timing of which is tightly regulated to conserve
energy. While various hormones are known to influence
hair growth, the role of local gene expression in
endocrine control of molt is not well understood. To
characterize how serum hormones and skin gene
expression dynamics impact molt, we examined hair
follicle cycling in prime-age female Weddell seals
(Leptonychotes weddellii) during the Antarctic summer
(November-February) (n=121, each seal handled once or
twice). Hair cycle phases of seals were determined
histologically from skin biopsies, and associated hormone
profiles were assessed by radioimmunoassay. Skin
transcriptomes were profiled with Illumina RNA-Seq
during three of the observed hair cycle phases: early
anagen (initiation of hair growth), late anagen (rapid hair
growth), and telogen (quiescence) (n=3 per phase).
Weddell seal hair follicles entered early anagen in
November/December, at least a month prior to the onset
of visible shedding, and did not enter telogen until late
January. Animals with higher serum concentrations of
prolactin, cortisol, and estrogens and lower concentrations of thyroxine completed molt later, suggesting these hormone levels slow hair growth. The Weddell seal skin transcriptome changed drastically across the molt, with >4000 genes differentially expressed between hair cycle phases. The late anagen transcriptome was most distinct, and featured enrichment of genes in a number of signaling pathways likely involved in regulating the molt. For example, during late anagen, expression levels of receptors for prolactin (PRLR) and thyroid hormone (THRB) were upregulated, while those for estrogen (GPER1) and cortisol (NR3C1) were downregulated. Thyroid hormone deiodinases were also differentially expressed across molt stages, with upregulation of DIO3 expression during late anagen suggesting that thyroxine is more rapidly inactivated in skin at this phase. Local changes in both hormone receptors and modifiers thus may alter the biological signal of circulating hormones during molt. Our study is the first to examine how skin transcriptomics reflect the mechanisms of molt in pinnipeds, and our findings offer key context for interpreting the roles of hormones in regulating this life history event.

22.3
Development of a biomarker panel of chronic stress in free-ranging marine mammals
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Increasing anthropogenic disturbances in marine ecosystems such as fishing, oil-drilling, and noise pollution associated with human activity can have detrimental effects on the reproduction and survival of apex predators such as marine mammals. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased circulating glucocorticoid hormones, which alter expression of target genes encoding metabolic enzymes and other mediators of stress. Prolonged or repeated HPA axis stimulation may increase catabolism of nutrient stores and suppress the immune and reproductive functions, impacting the fitness of marine mammals. Our objective was to develop and validate a biomarker panel of stress that can discriminate between acute and chronic stress states in marine mammals. We previously characterized endocrine and metabolic profiles and identified genes that were differentially expressed in response to single and repeated adrenocorticotropic hormone (ACTH) administration in juvenile northern elephant seals (Mirounga angustirostris). We found that bladder genes upregulated during the response to repeated ACTH administration include those encoding lipid particle proteins (perilipins PLIN1 and PLIN4, cell death activator CIDe-A), oxidative stress enzymes (GPX3 and MGST1), adipokines (ADIPOQ and LEP), lipid transporters and lipid metabolism enzymes (ABCA6, ABCA10, AZGP1, and ACSL1), and an adipogenesis promoting transcription factor (DKK1). Downregulated genes include an adipogenesis inhibitor (TGFB1) and an adipokine that inhibits gluconeogenesis and inflammation (C1TQNF3). To validate our biomarker panel, we collected blood and blubber samples from 30 juvenile northern elephant seals of varying body condition and baseline stress states. Endocrine (cortisol, aldosterone, total triiodothyronine (T3) and reverse T3) and metabolic (triglyceride) markers were measured in blood using immunoassays and colorimetric assays. Expression of candidate genes in blubber was measured using real-time polymerase chain reaction. Gene marker expression levels were significantly correlated with elevated stress hormones, decreased triglycerides, and body condition (standard length/auxiliary girth) in elephant seals. These markers provide insights into molecular mediators of the stress response and its physiological consequences, such as depletion of lipid stores in blubber, and can be used as a potential diagnostic panel for differentiating stress states in free-ranging marine mammals.

22.4
Insights into the structure-function relationships of I-band titin and its evolution across mammals
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The giant elastic protein, titin plays a key role in vertebrate striated muscle, where it acts as a molecular spring responsible for passive and active muscle elasticity. Titin spans an entire half sarcomere from Z-disk to M-line and its elastic I-band region is composed of three domains: the proximal tandem Ig segment; the unique N2A (skeletal muscle), N2B (cardiac muscle), or N2BA (cardiac muscle) sequence; and the PEVK region. Through alternative splicing, titin can be expressed as isoforms of varying lengths which correlate with the passive properties of different muscle types. Muscles with long titin isoforms have low passive stiffness whereas muscles with shorter isoforms have higher passive stiffness. However, little is known about how the variability of titin contributes to differences in muscle performance across a wide range of muscle types and species. A more extensive description of I-band titin could reveal evolutionary trends across vertebrates. In this study, we developed an annotation tool to characterize the PEVK region of the titin gene across 41 mammalian species with a range of muscle physiologies. Our results reveal contrasting patterns of constraint and divergence across the PEVK region, suggesting two distinct sub-regions with distinct evolutionary dynamics. The PEVK-N region shows a relatively
conserved length and exon structure over evolutionary time, but evidence of diversifying selection and more variable amino acid content. In contrast, the PEVK-C region varies dramatically in length and exon number across mammals, but these exons tend to be more similar. These data suggest that “essential” PEVK-N exons play key roles in vertebrate muscle function but the total length of the PEVK-C region, rather than selection on any particular exon, dominates the evolution of the PEVK-C. It is possible PEVK-N exons are necessary for increasing titin stiffness in active muscle whereas alternative splicing of the PEVK-C region determines the stiffness of the titin “spring”. Future work can focus on disentangling the effects of natural selection acting on specific codons, and how both regions contribute to evolutionary adaptation of titin.

23: THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION - 2

23.1 Social cues can push amphibious fish to their thermal limits
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Social context can impact how animals respond to changes in their physical environment. We used an aggressive, amphibious fish, the mangrove rivulus (Kryptolebias marmoratus) with environmentally-determined sociality to test the hypothesis that social interactions would push fish to their thermal limits. We capitalized on the propensity of rivulus to emerge from warming water and demonstrated that social stimuli, produced by their reflection, increased emersion threshold without changing critical thermal maximum, effectively diminishing thermal safety margins. When rivulus were denied air access, surface behaviours dramatically increased, supplanting social interactions. This suggests that assessing the terrestrial environment is crucially important. We conclude that social stimulation narrows the scope for survival in naturally stressful conditions.

23.2 Developmental changes in oxygen consumption and hypoxia tolerance in the heat- and hypoxia-adapted Tabasco line of the Nile tilapia (Oreochromis niloticus)
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The genus Oreochromis is among the most popular of the tilapiine cichlid tribe for aquaculture. However, their temperature and hypoxia tolerance, if tested at all, is usually tested at temperatures of 20-25°C, rather than at the considerably higher temperatures of 30-35°C typical of tropical aquaculture. We hypothesized that both larvae and adults of the heat- and hypoxia-adapted Tabasco line of the Nile tilapia, Oreochromis niloticus, would be relatively hypoxia-tolerant. Oxygen consumption rate (MO2), Q10, and aquatic surface respiration (ASR) was measured using closed respirometry at 2 (~0.2g), 30 (~2.5g), 105 (~10-15 g) and 240 (~250 g) days of development, at 25°C, 30°C and 35°C. MO2at 30°C was inversely related to body mass: ~90 µM O2/g/h in larvae down to ~1 µM O2/g/h in young adults. Q10 for MO2 was typical for fish over the range 25-35 °C of 1.5-2.0. ASR was exhibited by 50% of the fish at PO2s of 15-50 mmHg in a temperature-dependent fashion. However, the largest adults showed notable ASR only when PO2 fell to below 10 mmHg. Remarkably, Pcrit for MO2 was 12-17 mmHg at 25°C-30°C and still only 20-25 mmHg across development 35°C. These values are among the lowest measured for teleost fishes. Noteworthy is that all fish maintain equilibrium, ventilated their gills, and showed routine locomotor action for 10-20 minutes after MO2 ceased at near anoxia, and when then returned to oxygenated waters, all fish survived, further indicating a remarkable hypoxic tolerance. Remarkably, data assembled for MO2 from >30 studies showed a >2000X difference, which we attribute to calculation/conversion errors. Nonetheless, Pcrit was very low for all Oreochromis sp., and lowest in the heat- and hypoxia-adapted Tabasco line.

23.4 Acute thermal tolerance, not hypoxia tolerance, affects the temperature sensitivity of hypoxia tolerance in marine fishes
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There is considerable interest in understanding the combined effects of ocean warming and deoxygenation on species distribution and abundance. Recent studies have used the temperature sensitivity of the critical oxygen tension for standard oxygen uptake rate (Pcrit) as an index of hypoxia tolerance to model changes in species-specific habitat suitability and availability with projected changes in ocean temperature and oxygen content. Though this approach is theoretically well-founded, data to validate the models are limited to a handful of disparate taxa. Many questions remain regarding the effects of variability in hypoxia tolerance and thermal tolerance on species’ responses to changes in environmental temperature and oxygen availability. In this study we asked whether species that are more tolerant of hypoxia and high temperature show reduced temperature sensitivity of hypoxia tolerance. We measured the effect of acute temperature increase on standard oxygen uptake rate (MO2,standard) and Pcrit in 9
species of marine cottid fishes with native habitats spanning the inter-to-subtilidal environment. We then compared the temperature sensitivity of $P_{\text{crit}}$ ($\beta_{\text{Pcrit}}$) in these species to $P_{\text{crit}}$ at 12°C, a common environmental temperature at which previous investigations have characterized the mechanisms underlying variation in hypoxia tolerance of these species. To determine whether $\beta_{\text{Pcrit}}$ varied with acute thermal tolerance we also measured critical thermal maximum ($CT_{\text{max}}$) in 8 of these species and compared $CT_{\text{max}}$ with $\beta_{\text{Pcrit}}$. The effects of acute temperature increase on $P_{\text{crit}}$ were directly correlated with temperature effects on $MO_{2,\text{Standard}}$. There was no relationship between $\beta_{P_{\text{crit}}}$ and $P_{\text{crit}}$ at 12°C and a marginally significant relationship between $\beta_{P_{\text{crit}}}$ and $CT_{\text{max}}$. Species exhibiting tidepool occupancy, a proxy for variability in habitat temperature and oxygen availability, generally had lower $\beta_{P_{\text{crit}}}$, though this was not unique to tidepool occupants. Together these data suggest: (1) variation in whole-animal hypoxia tolerance is not related to the temperature sensitivity of hypoxia tolerance and (2) acute thermal tolerance may be an important predictor of the temperature sensitivity of hypoxia tolerance, potentially by constraining thermal performance breadth of aerobic metabolism.

### 23.5

**Critical windows in rainbow trout embryos: Effects of thermal shifts on survival, growth and oxygen consumption**

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Rainbow trout (*Oncorhynchus mykiss*) are a native anadromous species that inhabit a variety of fresh water rivers and lakes throughout the western portion of the United States. Currently, populations of rainbow trout in California, Oregon, Washington, and Idaho have been designated as threatened or endangered. Environmental temperature is a crucial abiotic factor that influences many physiological functions in fish, and global climate change is contributing to species declines. Understanding temperature effects during development is likely to provide insight into the overall thermal biology of a species and its persistence in a changing climate. To assess the effect of temperature on development of rainbow trout, we examined phenotypes of hatchery embryos reared in various incubation temperatures (5°C, 10°C, 15°C and 17.5°C). To identify the presence of embryonic developmental plasticity within critical developmental time points embryos incubated in 5°C were exposed to either 10°C, 15°C or 17.5°C during gastrulation, organogenesis, system integration or growth windows. A 3-dimensional critical window model was then applied. An increase in constant incubation temperature increased oxygen consumption rate ($VO_2$), and decreased hatching survival, mass, and time to 50% hatch. Thermally shifting embryos into increased temperature during distinct windows of development also reduced survival at hatch, and this was most evident following exposure during organogenesis. Likewise, increased temperature reduced hatchling mass, specifically when exposure occurred during system integration. Thermally shifted embryos exhibited a decrease in $VO_2$ when measured at hatch at 5°C compared to 5°C constant embryos. This decrease in $VO_2$ was most evident during later developmental windows. Collectively, these results suggest rainbow trout embryos are most sensitive to increased temperature during organogenesis and system integration. Critical window studies such as this one performed across species will eventually allow for the identification of commonalities in plasticity between different species, and allow us to understand when during development particular phenotypic traits are most influenced by temperature.

### 24: COMPARATIVE ASPECTS OF ACID-BASE REGULATION

#### 24.1

Evolutionary patterns of acid-base regulation in vertebrates.

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Acid-base regulation is a tightly regulated process and pH disturbances in vertebrates are rapidly corrected to restore homeostasis. In most vertebrates investigated to date, acute (<96 h) exposure to elevated environmental $CO_2$ (hypercarbia) results in a rapid reduction in both blood pH ($pH_e$) and intracellular pH ($pHi$); recovery of $pHi$ occurs in conjunction with $pHe$ compensation (coupled pH regulation). However, coupled pH regulation may be limited at $PCO_2$ tensions far below levels that some fishes naturally encounter. Previously, three hypercarbia tolerant fishes had been shown to not compensate $pHe$ but completely and rapidly regulate $pHe$ during acute exposure to >3 kPa $PCO_2$ (preferential $pHi$ regulation). Here we test the hypothesis that preferential $pHi$ regulation confers improved hypercarbia tolerance, by measuring $CO_2$ tolerance as well as intra- and extracellular pH regulation in a broad range of phylogenetically separated fishes. Contrary to previous views, we show that preferential $pHi$ regulation is the most common strategy for acid/base regulation within adult fishes and that this strategy is associated with improved hypercarbia tolerance. While preferential $pHi$ regulation has not previously been observed in adult amniotes, we have recently found that embryonic snapping turtles and alligators exhibit preferential $pHi$ regulation but that this trait is lost later in development. We hypothesize that preferential $pHi$ regulation is an embryonic pattern of acid-base regulation in vertebrates.
that is either retained or lost during development, depending on the severity of acid-base challenges experienced during adaptation to their environment. By conferring hypercarbia tolerance, preferential pHi regulation may have been a key expectation that facilitated evolutionary transitions in vertebrate evolution, such as the evolution of air breathing.

24.2 Evolutionarily conserved mechanisms for acid-base sensing

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The levels of carbon dioxide, pH, and bicarbonate collectively determine the acid-base status of an organism. Because acid-base disturbances can compromise cellular function, maintaining acid-base homeostasis is essential for all organisms. To be able to modulate compensatory responses, organisms must be able to sense the acid-base disturbance in the first place. The bicarbonate-stimulated enzyme soluble adenyl cyclase (sAC) is an evolutionarily conserved acid-base sensor that modulates diverse physiological processes via the cAMP signal transduction pathway. In coral cells, sAC senses acid-base disturbances from both internal and external origin and regulates intracellular pH, a function likely to apply to many (all?) cells from other organisms. In acid-base regulatory organs such as gill and kidney, sAC senses blood acid-base disturbances and modulates compensatory acid and base secretion and absorption. In response to elevated bicarbonate, sAC stimulates intestinal NaCl absorption across the intestine of marine bony fish. sAC is abundantly present in coral calcifying cells, mollusk mantle, fish otolith organ, and shark rectal gland, suggesting universal acid-base related regulatory control of epithelial ion transport. In hagfish heart, sAC regulates heart beat rate. The presence of sAC in the nucleus of some cells and in hemocytes, erythrocytes, and myocytes suggest regulatory control over diverse other physiological processes. However, sAC is by no means the only acid-base sensor. For example, research in mammals has identified a subset of G-protein coupled receptors (GPCRs) that stimulate transmembrane adenyl cyclases (tmACs) in response to extracellular acidosis. Because sAC is stimulated by bicarbonate and the GPCR-tmAC complex is stimulated by protons, coordination of both sensing mechanisms might allow discriminating between metabolic and respiratory acid-base stress. Additionally, tmAC and sAC activities can have opposite effects, implying exquisite regulatory mechanisms that allow differential physiological regulation within cells by the same messenger molecule, cAMP. Funded by NSF grants IOS # 1354181 and 1754994, and UCSD CRES.

24.3 Bicarbonate-sensing soluble adenyl cyclase in fishes

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The evolutionarily conserved acid-base sensor soluble adenyl cyclase (sAC, adcy10) is important for regulation of acid-base status (i.e. [CO2], pH, and [HCO3]) in cells, organs, and whole-animals. sAC is highly expressed in the base-secreting V-H+-ATPase (VHA)-rich gill cells of both leopard shark (Triakis semifasciata) and round ray (Urolophus hallieri), where it locally senses alkalosis and triggers VHA translocation to the cell membrane and subsequent base secretion. sAC is also highly expressed in acid-secreting Na+/K+-ATPase (NKA)-rich gill cells, where it likely regulates acid secretion. Both acid- and base-secreting cells are mitochondrion-rich and have large glycogen stores that serve as an energy source for each ATPase. Glycogen abundance diminished in both NKA-rich cells from sharks experiencing acidosis, and in VHA-rich cells from sharks experiencing alkalosis, demonstrating differential energy utilization during acid and base secretion, respectively. Furthermore, glycogen intracellular localization in isolated gill cells was consistent with that of NKA and VHA: in NKA-rich cells, glycogen was always present near the cell membrane; in VHA-rich cells, glycogen was normally present throughout the cytoplasm but was at the cell membrane during alkalosis. Intriguingly, the co-localization of VHA and glycogen at the cell membrane during alkalosis was blocked by the sAC-selective inhibitor KH7, implicating sAC as a regulator of energy metabolism in base-secreting cells. sAC is also present in other fish species; rainbow trout (Oncorhynchus mykiss) have >20 sAC splice variants with potential differential intracellular and tissue localization and functions. We are currently investigating the role of sAC in sensing and regulating acid-base stress in fish using rainbow trout (very CO2 intolerant) and white sturgeon (Acipenser transmontanus; very CO2 tolerant) as model species because they preferentially regulate extracellular and intracellular pH, respectively. Thus far we have found sAC is present in the liver and gills of both species, and that exposure to elevated CO2 induced significant changes in carbonic anhydrase, VHA, and cAMP abundance in a tissue- and species-specific manner. Overall, we propose that sAC may be an essential acid-base sensor within fishes (elasmobranchs, teleosts, and basal actinopterygians), and that sAC activity has differential regulatory effects depending on acid-base status, species, tissue type, and even cell subtype; all of which are currently being investigated. Funded by NSF Postdoctoral Fellowship in Biology and APS Porter Physiology Development Fellowship to JNR, NSERC Discovery grant to CJB, and NSF IOS#1354181 to MT.
The alkaline tide: acid-base regulation during digestion
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Gastric acid secretion during digestion leads to a rise in the bicarbonate concentration in the bodily fluids, whilst pancreatic base secretion will tend to decrease bicarbonate levels. Because gastric functions precede the intestinal secretions, many vertebrates experience an initial rise in plasma bicarbonate upon ingestion of food, and this “alkaline tide” may last for hours or even days in some animals that ingest very large meals. The alkaline tide probably represents the most common acid-base disturbance in the life of any vertebrate. Yet, surprisingly little is known about the attending compensatory mechanisms. In air-breathers (amphibians, reptiles and mammals), arterial pH remain rather unaffected due to a concomitant reduction in air-convection requirement vertebrates that provides for a rise in PCO₂. Air-breathers therefore provide for a respiratory compensation of the metabolic alkalosis, which is consistent with ventilation being primarily regulated to govern acid-base balance. In fish where ventilation is more committed to oxygen, the ventilatory compensation is more benign. I will discuss the alkaline tide in connection with the large metabolic and phenotypic changes that occur during digestion, and will emphasize studies based on animals that ingest large meals upon extended periods of fasting. I will review how the use of specific proton pump inhibitors demonstrate that the ventilatory responses are indeed indicative of pH regulation and I will discuss unpublished data demonstrating the pancreatic secretions are of paramount importance in dampening the rise in plasma bicarbonate concentration that results from gastric acid secretion.

25: EVOLUTION OF METABOLIC PROTEINS

25.1
Molecular evolution of cytochrome c oxidase in hypoxia tolerant fish
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Cytochrome c oxidase (COX) is an ancient, multi-subunit enzyme that is considered to be older than the rise in atmospheric oxygen (O₂) that occurred 2.4–2.1 billion years ago that led to the expansion of eukaryotic biodiversity [1] Though its original function is unclear, the modern COX is the final step of the O₂ transport cascade in mitochondria and essential for aerobic metabolism. It catalyzes the final transfer of electrons from the electron transport system (ETS) to O₂, reducing it to water while simultaneously pumping protons to generate a proton electrochemical gradient for ATP synthesis via the F₁F₀ ATP-synthase. Given its functional importance, COX was thought to be highly conserved. In fact, variation in COX function has been observed in organisms that encounter naturally occurring hypoxia in which adaptive modifications of the O₂ transport cascade have been well described. For example, in species of intertidal sculpins (Family Cottidae) that inhabit the nearshore environment and vary in whole animal hypoxia tolerance, there was a relationship between COX apparent O₂ binding affinity and the critical oxygen tension (Pcrit), where more hypoxia tolerant species showed lower COX apparent O₂ binding affinity compared to less tolerant species. In silico analysis revealed interspecific sequence differences on the catalytic COX3 subunit that could affect interaction with membrane phospholipid cardiolipin and subsequently the path of O₂ travel [2]. In mammalian models, hypoxia induces an isofrom switch of COX4-1 to COX4-2 which renders the COX protein insensitive to ATP inhibition [3]. Although it was previously shown that the teleost COX4-2 gene was not responsive to hypoxic exposure [4], recent data suggests that the COX4-2 transcript is upregulated and that the isofrom switch may occur in the anoxia-exposed crucian carp Carassius carassius [5]. In this talk, I will present what is currently known of modifications to COX function and regulation in teleosts adapted to environmental O₂ limitation, and what is still unknown about this complex enzyme.


25.2
Function and evolution of cellulase and hemicellulase enzymes within invertebrates that do not consume significant amounts of plant cellulose.
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Classic cellulase, endo-β-1,4-glucanase and the hemicellulases, β-1,3-glucanase or laminarinase and endo-β-1,4-mannase are expressed widely amongst the invertebrate animal groups. It is now widely recognized that these enzymes are endogenously produced and are not solely synthesized by symbiotic microorganisms such as bacteria and protozoans. Their presence is commonly used to indicate the ability of an animal to digest cellulose and hemicellulose. The implication is that animals consume and digest plant cellulose and hemicellulose. This may be true for primarily herbivorous or wood feeding invertebrates. However, in animals such as algal and deposit feeding invertebrates, such as crustaceans, molluscs and echinoderms, that do not consume substantial amounts of plant cellulose and hemicellulose, the function is not clear. Thus, the function of the enzyme in these species needs a
reappraisal. This will be attempted here by 1. describing the reaction catalyzed and structure of the enzyme to infer the preferred substrates, and 2. correlating if the substrates are present in the diet and hence ascribe a function. The endo-β-1,4-glucanase and β-1,3-glucanase may also be involved in non-digestive functions such as innate immunity given these enzymes are expressed in non-digestive tissues. They may act as β-glucan binding proteins to stimulate the phenol oxidase system. The cellulase and hemicellulase enzymes are distributed in a gradient of aquatic to terrestrial species and within different feeding specializations ranging from algal feeders, mixed deposit and leaf litter feeding species, those which are primarily herbivorous and omnivores. Hence there is the potential to examine the evolution of the enzymes and this will be attempted in this presentation. This will be done with respect to the colonization of land and the adoption of an herbivorous leaf litter diet. The function of the cellulase and hemicellulase enzymes within omnivorous species that do not consume significant amounts of low grade plant material will also be reviewed.

25.3 Evolutionary phylogenomics of UCP1 and sarcolipin: key players underlying adaptive thermogenesis across Eutheria?
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Two proteins—uncoupling protein 1 (UCP1) and sarcolipin (SLN)—have been implicated to underlie non-shivering thermogenesis (NST) in brown adipose tissue (BAT) and skeletal muscle, respectively, of eutherian mammals. BAT has long been recognized to play a key thermoregulatory role in small-bodied and hibernating species, and is considered to be especially important for neonates of cold-tolerant forms. However, given that body size and energetic considerations are expected to alter the selective advantage of BAT-mediated NST, we posited that evolution of large body mass or reduced thermogenic capacity would be accompanied by relaxed selection and/or inactivation of the ucp1 locus. More recently, SLN—a 31 residue helical peptide bound to SERCA pumps in the sarcoplasmic reticulum—has been shown to increase heat production in vitro by partially uncoupling Ca\(^{2+}\) re-sequestration from ATP hydrolysis, leading to the hypothesis that SLN may contribute to muscle NST in vivo. To date, however, little is known on the regulation of SLN or its potential role in facultative muscle NST outside of a few small-bodied model systems (rodents and rabbits) that reside within the same mammalian clade. To address these questions, we first mapped ucp1 sequences from 133 mammals onto a robust species tree and show that inactivating mutations occurred in at least 8 of the 18 traditional placental orders. Selection and timetree analyses further reveal that ucp1 inactivations temporally correspond with strong secondary reductions in metabolic intensity (xenarthrans and pangolins), or coincided with a ~30 million year episode of global cooling that promoted sharp increases in body mass evident in the fossil record. These findings shed light on the potential energetic costs of BAT and challenge the thermogenic importance of UCP1 in nearly half of all eutherian lineages. Furthermore, while a number of studies conducted on lab mice contend that loss of SLN hinders muscle-based NST or that cold exposure increases SLN expression, our calculations suggest that this mechanism should only contribute ~2% of the heat produced by a single contraction-relaxation cycle. Additionally, as SLN is nearly undetectable in fast-twitch glycolytic fibers that form the bulk of the limb musculature, any NST linked to SLN may be expected to be imperceptible in these small mammals. Intriguingly, SLN mRNA and protein levels are several magnitudes higher in skeletal muscles of pigs, dogs, and humans than lab rodents, raising the possibility that SLN-NST may be of greater thermogenic importance in larger mammals. However, like ucp1, this locus may also be expected to have evolved under relaxed selection or even be pseudogenized in very large or tropically distributed species. In contrast to this expectation, not only is SLN functionally intact in all mammals (except possibly sloths), but its primary structure has remained exceptionally well conserved despite highly divergent body sizes and thermoregulatory capacities. This remarkable degree of sequence conservation—in species for which NST should have no apparent adaptive benefit—is not readily compatible with a thermogenic function, though in line with a role in regulating intracellular Ca\(^{2+}\) homeostasis by modulating the (apparent) Ca\(^{2+}\) affinity of SERCA. Thus, unequivocal support for an adaptive thermogenic role by SLN in vivo is lacking. Suggestions that SLN plays a meaningful thermogenic role in larger bodied mammals (or birds) is also without empirical evidence and remains purely speculative. This work was supported by Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grants to KLC (RGPIN/238838-2011 and RGPIN/6562-2016).

25.4 Evolution of Metabolic Proteins: Pyruvate dehydrogenase in anaerobiosis
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Without oxygen, most vertebrates die within minutes as they cannot meet cellular energy demands with anaerobic metabolism. However, fish of the genus Carassius ( crucian carp and goldfish) have evolved a
specialized metabolic system that allows them to survive prolonged periods without oxygen by producing ethanol as their metabolic end-product. Here we show that this has been made possible by the evolution of a pyruvate decarboxylase, analogous to that in brewer’s yeast and the first described in vertebrates, in addition to a specialized alcohol dehydrogenase. Whole-genome duplication events have provided additional gene copies of the pyruvate dehydrogenase multienzyme complex that have evolved into a pyruvate decarboxylase, while other copies retained the essential function of the parent enzymes. We reveal the key molecular substitution in duplicated pyruvate dehydrogenase genes that underpins one of the most extreme hypoxic survival strategies among vertebrates and that is highly deleterious in humans.

26: HARNESSING NATURALLY EVOLVED TORPOR TO BENEFIT HUMAN SPACEFLIGHT

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26.1

Enhancing Metabolic Flexibility in Humans: Insights from Hibernation to Benefit Spaceflight

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Many mammals from diverse phylogenetic lineages can safely and reversibly lower their metabolic rate and enter torpor, a depressed metabolic state that enables substantial energy savings. Some species undergo daily torpor and continue to forage during active periods. Others hibernate, characterized by multi-day torpor bouts interspersed with periodic arousals to euthermia. Most hibernators cease feeding completely and obtain energy from oxidation of endogenous fuel (primarily lipid). Multiple lines of evidence suggest that the ability to induce natural torpor or hibernation is accompanied by resistance to a variety of pathologic stressors including ischemia-reperfusion, hemorrhagic shock and radiation injury. Thus, engineering the ability to induce a state of “synthetic torpor” in species that are unable to do so naturally would be advantageous for the treatment of multiple pathologic states, routine surgery, and long duration spaceflight missions - when food resources are limited and cosmic radiation impairs survival. What constrains humans from adopting this shifting phenotype for energy savings and cellular protection? This talk will discuss (1) why mimicking mechanisms of natural hibernation/torpor holds more promise for engineering synthetic torpor in humans than do current medical practices that rely on direct body cooling to reduce energy demands; (2) why size matters in translating natural hibernation to humans; and (3) what key discoveries in the field are needed to engineer synthetic torpor for beneficial use in non-hibernating species.

26.2

Central mechanisms of torpor induction

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In mammals, torpor is a state that is characterized by an active reduction in metabolic rate with a progressive decrease in body temperature (1). At the moment, the neural pathways responsible for the metabolic suppression that characterizes torpor are not known. Mice are facultative heterotherms, and torpor in these rodents can be reliably triggered by changing environmental conditions (2). This characteristic consents therefore to trigger torpor almost on command and the marker of neuronal activation cFos can be used to identify groups of neurons activated at torpor onset. The metabolic suppression that characterizes torpor requires a reduction in the activity of metabolically active organs. Most of these organs, such as the brown adipose tissue, are controlled by the putative sympathetic premotor neurons located within the Raphe Pallidus (RPa) (3). To enter torpor, these neurons have to be necessary inhibited. The inhibition of these neurons in rats induces indeed a state the resembles torpor under many aspects (4). To pinpoint the neural circuits actively inducing the metabolic suppression, a retrograde tracer was injected within the Rpa region. The main aim was to identify populations of neurons projecting to the RPa neurons with torpor-related activity.

Here preliminary results showing the neural regions with torpor-related activity and projections to the neurons within the RPa will be presented.

The experiment was funded by the Univ. of Bologna


26.3
The relationship between sleep and torpor
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Torpor is a strictly regulated process, associated with pronounced changes in activity, sensory functions, physiology and metabolism. However, many of the mechanisms that regulate this dramatic physiological state remain poorly understood. The nature of the relationship between torpor and sleep has been a controversial topic for several decades. In mammals, sleep and energy metabolism are intimately linked, as evidenced by the numerous bidirectional connections between the neural centres/circuits that govern these processes (1, 2). The maintenance of waking and sleep is regulated by several subcortical structures, which provide neuromodulatory action on the forebrain (3). In addition, wakefulness and sleep are shaped by the interaction of two processes: the homeostatic process, and the circadian process, which provides a temporal framework for specific waking behaviours, sleep and metabolism (4). Several studies implicated the role of hypothalamic centres of thermoregulation and energy homeostasis in the occurrence of torpor both in hibernators and non-spontaneously hibernating species (5, 6). Mice are facultative heterothermic species, and readily display torpor bouts in response to food deprivation (7, 8). It has been proposed that fasting can elicit a torpor response by decreasing energy expenditure and body temperature, while blocking normal cold-induced thermogenesis responses. However, less is known about the specific fasting-related signals which initiate entry into torpor. Behaviourally, torpor in mice resembles sleep, but our study suggests that brain activity measured by EEG is significantly depressed in torpid animals. However, early studies in ground squirrels and our observations in mice suggest that animals during torpor are able to respond to sensory stimuli and may exhibit spontaneous or provoked behaviours. While it appears that torpor is a state neurophysiologically distinct from both waking and sleep, evidence suggests that torpor and sleep are closely related. Specifically, torpor bouts induced by fasting are initiated from deep sleep, while daily torpor in Djungarian hamsters and pharmacologically induced torpor in mice are followed by sleep with high EEG slow-wave activity. The functional role of deep sleep after torpor is debated, but it has been suggested that sleep is important for recovery processes, which could be related to energy homeostasis, synaptic structure and function, and for a renormalisation of learning and waking performance after torpor. We conclude that further studies are necessary to investigate the effects of torpor on brain function and the relationship between torpor and sleep.
systems) that can reasonably be launched and carried on-board. As mission lengths increase, load limits will quickly be reached. Although developing regenerative life support systems will be critical for future exploration, an added strategy could be to induce some level of metabolic depression or induced torpor in astronaut crewmembers to prolong the supply of such consumables. Astronauts in states of induced torpor may also be able to remain better shielded from the harmful effects of radiation during the journey, a medical risk factor that remains to be resolved before such missions can be realistically undertaken. In addition to the safety and practicality of the astronauts on-board, logistical considerations must also be taken into account according to the given mission architecture, for example the activities involved on the space journey and level of autonomy of the spacecraft itself. The practical application of such states will be discussed from the perspective of the astronaut crewmember.

27: PHYSIOLOGY FROM THE NEOTROPICS: RHYTHMS, TEMPERATURE AND SEASON

27.1
Seasonal physiology of a hibernating and facultative endothermic lizard
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In southeastern Brazil, seasons are characterized by warm and wet spring/summers and mild and dry winters, with small changes in photoperiod. In this scenario, the tegu lizard *Salvador merianae* shows a robust annual cycle of intense daily activity/foraging during spring/summer and hibernation during winter, which starts as early as in the first year of post-hatch life. During autumn, lizards gradually decrease feed intake until eventually hibernate, maintaining a reduced metabolic rate without food ingestion for about 3 months. Important to highlight is that this seasonal metabolic cycle is a temperature-independent phenomenon. In addition, when adults, they show a capacity for increasing the temperature difference between core body and ambient during the reproductive season, in spring, just after hibernation. This presentation will be focused on seasonal variation of metabolism, behavior, cardiorespiratory parameters and hormones involved in energy metabolism and reproduction in this unique species of lizard inhabiting tropical and subtropical biomes in South America. Financial support: FAPESP and CNPq (Brazil) and Northern Arizona Univ. (NAU-USA).

27.2
Orexin in ectotherms: modulatory role on ventilation
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Orexins (or hypocretins) are neuropeptides that are involved in regulating many physiological functions such as sleep, feeding, temperature, energy balance, and breathing. Interestingly, the amino acid sequences of orexins A and B are highly conserved across different groups of vertebrates and the distribution of these neuropeptides has been described in all classes of vertebrates. In most groups, orexinergic neurons are located in the hypothalamus, but instead of being found exclusively within a single nucleus, they are located in various hypothalamic nuclei. Despite being located in a restricted spot, these neurons project widely to the brain including areas involved in respiratory regulation and this modulation is dependent on the phase (light or dark) and, in mammals, is also dependent on the state of activity of the animal (sleep and wakefulness). Additionally, these neurons are considered to be intrinsically CO2/pH sensitive. This presentation will discuss the orexinergic modulation on breathing in vertebrates, giving emphasis in our recent findings on neotropic amphibian and reptilian species. Financial support: FAPESP and CNPq.
We introduce a non-invasive methodology that can contribute to long-term studies of cardiorespiratory performance, and its respective temperature influences, in in vivo larval stages of aquatic anuran amphibians. These studies are essential to our understanding of the effects of temperature on cardiorespiratory physiology during a particularly vulnerable life stage.

27.4 Plasticity of 24h body temperature rhythms in a South American subterranean rodent

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Plasticity in the expression of 24h rhythms has been a subject of increasing interest. This plasticity can involve a change in the time of day a certain behavioral or physiological process occurs (such as a change from nocturnal to diurnal) or a change in the amplitude of rhythms, such as in the daily body temperature rhythm. The subterranean rodent tuco-tuco (Ctenomys aff. knighti) is among the species which display rhythmic plasticity. This animal is nocturnal under standard lab conditions but mostly diurnal in the field. It also shows drastic changes in amplitude of the body temperature rhythm when in the field, with changes in maximum, minimum and mean body temperature values. This plasticity was revealed by several years of data collection on body temperature rhythms of tuco-tucos in the laboratory and in outdoor enclosures. The differences between the rhythms observed in the laboratory and in the field suggest an interaction of the circadian system with a wide set of biotic and abiotic factors that differ between these two conditions. A lot of these variables are related to daily energetic challenges, which are much greater in the field than in the lab. This herbivorous rodent forage by intense digging, in a semi-arid habitat where vegetation is sparse and there are great daily changes in environmental temperature. Noteworthy, food availability and temperature have been changing in the last years, in their natural habitat in Argentina, especially with the increase in rainfall. Increase in the plasticity of rhythmic patterns have been registered along these years and investigation of this phenomenon may provide insights into the role of environmental changes on timing of activity and physiological functions.

28.1 On the Dynamics of Actomyosin Binding

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Animal skeletal muscle exhibits very interesting behavior at near-stall forces (when the muscle is loaded so strongly that it can barely contract). Near this physical limit, the actin-myosin cross bridges do more work than their energy releasing molecules, Adenosine TriPhosphate (ATP) suggest they can. It has been shown that the advantageous utilization of thermal agitation is a likely source for this increased capacity. Here, we propose a spatial-temporal mechanical model to illustrate how this may occur. The physical interpretation of the system energy also informs how Brownian motion and the probability of actomyosin binding are related, granting a greater physical understanding for binding rate functions that are prevalent in muscle contractile theories.

28.2 Effect of Substrate Compliance on Coordinated Landing in Rhinella marina.

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The musculoskeletal system must dissipate mechanical energy to decelerate the body during common modes of terrestrial locomotion like running downhill, decelerating or landing from a jump. The ability to safely dissipate energy upon impact is influenced by the mechanical properties of the environment which can vary greatly in nature. Substrate compliance is one property that may affect the performance of animals as they attempt to safely decelerate their body upon impact. Rhinella marina offers an ideal model to understand how substrate stiffness may modulate controlled deceleration behavior as hopping is their main form of locomotion and well-coordinated landings have been well documented. In this study we use high-speed videography and force-plate ergometry to compare landing performance of R. marina (N = 5) across four compliance treatments relative to body weight (BW); 0, 2.5, 5 and 10 mm BW⁻¹. Landing performance was normalized and assessed by determining the ratio of energy dissipated by the forelimbs relative to the total energy of the system – animal’s center of mass and substrate. We also use inverse dynamics analysis to determine how the energy dissipated by the forelimb is distributed across different joints and how such patterns change with substrate dynamics. Our findings suggest the increased compliance decreases the relative amount of
energy that has to dissipated by the forelimbs during landing. This work will provide fundamental insight into the relationship between the properties of the substrate and the energetics and control rapid deceleration during terrestrial locomotion.

28.3
The interaction of incompressible fluid and extracellular connective tissues in lobster muscle
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Vertebrate skeletal muscle is reinforced by a complex network of fibrous collagen, comprising the endomysial, perimysial, and epimysial layers of intramuscular connective tissue. The physical interaction of these connective tissues with the incompressible muscle fibers they surround has been shown to influence passive tension generated by stretched muscle (1,2), and may also influence active force generated during contraction. Though this interaction is mechanically important in the muscles of vertebrates, its relevance in the muscles of non-vertebrate animals has received relatively little study.

Using a combination of scanning electron microscopy (SEM) and mechanical testing of muscle, we investigated the interaction of connective tissues and incompressible muscle fibers in the claw closer muscle of the American lobster (Homarus americanus). Cross sections of claw closer muscle were visualized using SEM and compared to skeletal muscles of three vertebrates: bullfrogs, mice, and alligators. In separate experiments, passive tension was measured from lobster claw closer muscles before and after a perturbation of intramuscular fluid volume. In vertebrates, perturbations of fluid volume have been shown to influence passive tension by altering the interaction of muscle fibers and connective tissues (2). Micrographs of lobster claw closer muscle differed from those of vertebrates in that fibrous connections between adjacent muscle fibers were relatively scarce. Distinctions between endomysial, perimysial, and epimysial layers of connective tissue were not apparent. Contrary to its effect on vertebrate muscle, altering the fluid volume of claw closer muscle did not alter passive tension generated in response to stretch.

Together, these results suggest that interactions between incompressible muscle fibers and intramuscular connective tissues may not be mechanically relevant in the claw closer muscles of lobsters, and that connective tissue may play a lesser mechanical role in the claw closer muscles than in typical vertebrate skeletal muscles. These results suggest that the mechanics of the extracellular matrix are not uniform across all skeletal muscle, and raises questions about the function and evolutionary history of connective tissue in vertebrate muscle.

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References

28.4
Effects of passive integrated transponder tagging on cortisol release by the Gulf killfish Fundulus grandis
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Passive integrated transponder (PIT) tagging has many advantages as a method for individual identification of animals in field and laboratory studies. One potential drawback, however, is the stress that results from implantation of PIT tags, especially in smaller animals, which could have adverse physiological effects. This study assessed the effects of PIT tagging on levels of the stress hormone cortisol in the Gulf Killfish, Fundulus grandis, an estuarine fish of the Gulf of Mexico. First, we optimized a non-invasive procedure for measuring cortisol excreted by individual fish into their aquarium water. Water samples were acidified to release bound cortisol, which was then concentrated by solid phase extraction prior to quantification by enzyme-linked immunoassay. The optimized procedure allowed high rates of recovery of known amounts of cortisol added to aquarium water. Then, using this procedure, cortisol was measured in water samples from fish one week before, immediately after and weekly for four weeks following PIT tagging. Within the first 2 h of tagging, cortisol release rates were dramatically elevated compared to values measured prior to tagging. This immediate cortisol release was significantly higher than that of fish handled identically except not implanted with PIT tags. By one week after PIT tagging, however, cortisol release returned to control levels. The results suggest that PIT tagging causes an immediate stress response, which subsides within one week. Therefore, individuals should be allowed to recover one week after PIT tagging prior to other experimental manipulations. Funding for this work was provided by the Greater New Orleans Foundation and the Audubon Nature Institute.
28.5
On the role of the visual and vestibular systems in stabilising perching in zebra finches
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Birds have outstanding abilities to balance on two legs and maintain upright posture on both rigid and highly flexible moving perches. However, to our knowledge, there has been no formal study investigating which sensory modalities are used to control upright posture in birds and, more importantly, how these are combined. From observation, we hypothesized that birds use a combination of visual, vestibular and proprioceptive information to balance on a perch. We used a perch torque sensor to measure the torques and forces exerted on the perch under different circumstances to explore this question.

To test the degree to which vision contributes to balancing ability, we compared perching ability in the light and dark. Zebra finches (Taeniopygia guttata) were assessed for their ability to perch using high speed infrared videography and perch torque measurements in light and dark conditions over several weeks. In the dark perch torque root-mean-squared (RMS) deviation from 0 was higher in light conditions (p=0.003), suggesting that perching ability was impaired in the dark. We then assessed the role of the vestibular system in maintaining upright posture while perched by damaging vestibular hair cells with amino glycoside antibiotics, and measuring perching ability in the light and dark over a period of 8 weeks, over which loss of vestibular function and consequent recovery as inner ear hair cells regenerated was recorded. Again, we found root-mean-squared deviation from 0 was higher in light conditions than in the dark, but not significantly so (p=0.06). This was attributed to birds being overall less active after treatment, as reflected both in the videography and RMS data (p=0.01), which was especially evident in light conditions.

28.6
Research of development of adaptive processes to psycho-emotional stress in medical students
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Psycho-emotional stress that develops in dynamics of studying in Univ. is a common factor of development of the vast group of neurogenic diseases.

The objective of the research was to study the intersystem relations (integration) which are the base of development of organism’s resistance to the effects of stress factors, i.e. the development of adaptive syndrome.

The object of the research was a group of medical students of 1-3 years which were examined in conditions of educational process. The peculiarities of intersystem integration of cardiorespiratory system based on coupling index (CI), and integralational brain activity based on intellectual workability in conditions of correction test (2 minutes of test, total percentage of completed work, number of mistakes) were studied.

The research of complex of psychophysiological indicators in the dynamics of studying revealed the formation of chronic emotional stress in medical students, the severity of which depends on the individual characteristics of the psychophysiological status of students.

Formation of adaptation to the educational stress has the stage character (3stages) – the initial period is accompanied by activation of non-specific adaptive mechanisms in almost all students (1st stage). From the 2nd year, only in 40% of the students an adequate adaptation to the training load was formed – intellectual performance increases, with the background of rationalization of its vegetative support. Sustainable intersystem relationship that reveals increasing the body’s integrity (2nd stage) is formed.

In second group of students (43%) non-specific adaptive mechanisms (1st stage) on the 2nd year of study goes into a stage of specific adaptation (2nd stage), but the severity of changes in psychophysiological indexes is strongly pronounced. On the 3rd year of study it leads to the depletion of adaptive capabilities (3rd stage).

In 17% of students it was seen that the stage of activation of non-specific adaptive mechanisms rapidly changed by their depletion, that is accompanied by decrease of intersystem integration and intellectual workability. This is the evidence of prevalence of disintegrational processes in the organism.

Based on results of the research, a conclusion can be made that the formation of adaptive optimum to psycho-emotional stress occurs only in 40% of students. The majority (60%) of students show either the initially insufficient adaptive capabilities, or their excessive intensity, that naturally leads to psychological and vegetative disorders in the organism.

28.7
A comparison of thermal performance among latitudinally separated populations of the intertidal barnacle Balanus glandula
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Intertidal species must adapt to life in a biphasic environment, which alternates daily between marine and terrestrial conditions. These two environments present
very different thermal regimes, with water temperatures generally being more stable and less variable than what animals experience in air. Few studies have measured the energetic cost of exposure to thermal extremes during emersion. Moreover, it is not known how the water temperatures experienced during immersion influence thermal tolerance in air. We measured the energetic costs of low tide exposure in the intertidal barnacle Balanus glandula, collected from three populations along the west coast of the United States with distinct thermal environments. Barnacles were collected from southern California (a site with both warm air and water temperatures), northern California (cold air and water temperatures), and northern Washington (warm air and cold water temperatures). All animals were acclimated to a laboratory tidal cycle with native water temperatures and a common low tide temperature of 18 °C. To measure performance, we exposed barnacles from each population to a 5-hour low tide at 10, 15, 20, 25, 30, 35, or 38 °C, followed by a 6-hr high tide. We monitored oxygen consumption throughout the full tidal cycle using a fluorometric O2 sensor system. Overall, aerial respiration was greater than full tidal cycle using a fluorometric O2 sensor exchange. Cardiovascular function and active or passive heat exchange. We monitored Midland-painted turtles (Chrysemys picta) as our partial thermoregulator in 2017 and Eastern box turtles (Terrapene carolina) as our strict thermoconformer in 2014 and 2015 using similar equipment and protocols in Southwest Ohio. Metabolic rates were calibrated under laboratory conditions using body temperature, heart rate, and body mass and proxies for oxygen consumption. We found that thermoconformers used approximately 25% of the total daily energy than partial thermoregulators. Although we found no significant difference within species throughout their active season, we found a significant difference in the daily energy expenditure between box turtles and painted turtles and time of day within each species. Oxygen pulse was similar to previously reported turtle species, and in the field the average oxygen pulse decreased from the beginning to the middle of the active season for painted turtles but increased for box turtles. Our study shows the energetics of thermoconformity and partial thermoregulation in two turtle species and quantifies a significant difference in the daily energy expenditure using heart rate and body temperature as more accurate proxies for oxygen consumption, and begins to disentangle the relationship between cardiovascular function and active or passive heat exchange.

28.9
Clocks and meals keep mice from being cool
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Daily torpor is used by small mammals to reduce daily energy expenditure in response to energetic challenges. Optimizing the timing of daily torpor allows mammals to maximize its energetic benefits and, accordingly, torpor typically occurs in the late night and early morning in most species. The regulatory mechanisms underlying such temporal regulation have however not been elucidated. Direct control by the circadian clock and indirect control through the timing of food intake have both been suggested as possible mechanisms. Here,
feeding cycles outside of the circadian range and brain-specific mutations of circadian clock genes (Vgat-Cre \textsuperscript{fl/+}, Vgat-Cre \textsuperscript{fl/fl}; Vgat-Cre \textsuperscript{Cre+ Bmal1\textsuperscript{fl/+}}) were used to separate the roles of the circadian clock and food timing in controlling the timing of daily torpor in mice. These experiments revealed that the timing of daily torpor is transiently inhibited by feeding, while the circadian clock is the major determinant of the timing of torpor. Torpor never occurred during the early part of the circadian active phase, but is preferentially initiated late in the subjective night. Food intake disrupted torpor in the first 4-6 h after feeding by preventing or interrupting torpor bouts. Following interruption, re-initiation of torpor was unlikely until after the next circadian active phase. Overall, these results demonstrate that feeding transiently inhibits torpor while the central circadian clock gates the timing of daily torpor in response to energetic challenges by restricting the initiation of torpor to a specific circadian phase.

28.10
Expression of TRP channels in notothenioid fish
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The Southern Ocean connects all the world’s oceans and yet is highly isolated due to the Antarctic Circumpolar Current, the benthic topography, and the thermal environment. This isolation, combined with the strong selective drive of the cold stenothermic environment, has enabled the broad niche diversification of a single fish clade: the nototheniids. While notothenioids are well adapted to typical Antarctic temperature regimes between -2 and +1 degrees Celsius, their metabolic scope is highly limited above 2 degrees C and temperatures above 5 degrees C can be lethal. Given this high thermal sensitivity we wondered about the presence and abundance of molecular thermosensors in these fish. The candidate molecular thermosensors in both vertebrates and invertebrates are a class of cation channels called transient receptor potential potential (TRP) channels. We investigated the expression of TRP channels in four species of notothenioids: Harpagifer antarcticus, Neopagetopis ionah, Parachaenichthys charcoti, and Parachaenichthys georgianus, and compared them with Notothenia coriiceps, a nototheniid for which the complete genome is available. We found the genome contained approximately 15 TRP channels, similar to other teleost fish and approximately half that of mammals. Of those, the notothenioids expressed between five and ten of the channels. All four species with transcriptome data expressed TRPV1, TRPM5, TRPM2, and TRPC1. Channels in the TRPC subclass are not thought to be thermosensitive. This leaves three channels as candidates for determining thermal sensitivity in these fish, and the range of temperatures that might activate these channels remains to be determined. Further study of these channels may help us gain insight into how these fish might cope with or avoid warming waters of the Southern Ocean, which in some places are expected to warm 0.5 degrees C per decade.

28.11
Investigating Changes in Thermal Physiology in Response to a Gut Infection in the Dragonfly, Libellula pulchella
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A key goal in thermal physiology is to understand the factors that induce physiological thermal plasticity and their underlying mechanisms. Recently, insulin signaling and disruptions to metabolic homeostasis have been demonstrated to impact thermoregulatory strategies and thermal performance regimes in a number of species. Here we use an endothermic dragonfly, the 12-spotted skimmer, Libellula pulchella, as a model to test how a disruption of insulin signaling affects thermal physiology at organismal and molecular levels. \textit{L. pulchella} often harbor a protozoan gut parasite which disrupts insulin signaling, changes flight behavior, and induces a pathology similar to mammalian type 2 diabetes and obesity. One unresolved question regarding this host-parasite interaction is whether this infection produces a thermally plastic phenotype. Here we test if infected \textit{L. pulchella} differentially regulate their thoracic temperature in the field, and whether thermal reaction norms of flight muscle force production differ between infected and healthy individuals. Lastly, we examine the effects of infection on the thermal performance of key carbohydrate and fatty acid enzymes in an attempt to test potential metabolic correlates of thermal plasticity.

28.12
Stage-specific oxygen limitation of thermal tolerance in Schistocerca cancellata
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Performance decreases drastically as body temperature increases above a thermal optimum, yet the proximate cause of this heat stress is unknown. One controversial hypothesis, known as oxygen- and capacity- limited thermal tolerance, suggests that metabolic demand during warming outstrips the energy supplied by aerobic respiration, decreasing performance until death. Therefore, reducing oxygen availability should make animals more susceptible to heat stress. This idea has mixed support in adult insects, but younger life-stages have less developed tracheal systems that may make them more susceptible to oxygen limitation. We tested this hypothesis by rearing South American locusts
The effect of salinity on expression of aquaporins 1 and 5 in the gastric caeca of Aedes aegypti mosquito larvae

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The Aedes aegypti mosquito is a vector that can transmit viral diseases such as Zika, dengue, chikungunya and yellow fever. The larvae of this mosquito can inhabit freshwater (FW), brackish water (BW) and sewage contaminated water. Each of these habitats impose different challenges to osmoregulation and survival. The gastric caeca (GC) are digestive and putative osmoregulatory organs located at the anterior portion of the midgut that have been shown to secrete ions from hemolymph to the GC lumen. External salinity is known to alter the structure of the GC ion-transporting and resorbing/secreting cells; however, the movement of water across the GC has not been studied. Aquaporins (AQPs) are water and/or solute channels which mediate water flux across cell membranes. Here we compare the expression of A. aegypti AQP1 and AQP5 in the GC of larvae reared in FW and BW. Water-specific AQP1 was found on the apical membrane of the GC of both FW and BW-reared larvae and did not show changes in protein abundance in response to external salinity. However, localization of AQP1 along the apical membrane became more dispersed in BW-reared larvae compared to FW-reared larvae and coincided with the effect of salinity on ion-motive ATPases in the GC ion-transporting cells. AQP5 transports water and small solutes and was localized to the basal membrane of the GC. Whole mount and cross-sectional immunohistochemistry of the GC showed greater AQP5 immunoreactivity in BW-reared larvae compared to FW-reared larvae, however quantification of AQP5 protein abundance in the GC remained unchanged between the groups. Knockdown of AQP5 resulted in decreased survival of larvae reared in BW. This is the first study to report on the protein expression and localization of aquaporins in the GC of larval mosquitoes and results suggest that aquaporins in the GC play a role in the osmoregulatory strategies mosquitoes employ to cope with alterations in habitat salinity. Funding was provided by NSERC.

Comparative and functional analysis of Na+/Ca2+ exchangers across Nematodes

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The super-family of Na+/Ca2+ exchangers include transporters that exchange sodium for calcium (NCX), Na+/Ca2+/K+ exchangers (NCKX) which exchange sodium for potassium and calcium, and Ca2+/Cation exchangers (also called NCLX) which exchange sodium or lithium for calcium. In mammals there are three genes encoding various isoforms of NCX transporters, five NCKX genes and a single NCLX gene. Here, I will give an overview of my lab’s data on the comparative genomics of Na+/Ca2+ exchangers across nematode species. We have uncovered a surprising level of diversity within this super-family of exchangers across diverse nematode species. We observed several examples of gene gain and loss, but perhaps most surprisingly was the apparent absence of NCLX-type exchangers from a subset of nematode species that we sampled, these were: Brugia malayi, Loa loa, Ascaris suum, Trichinella spiralis, Trichuris muris, Trichuris suis, and Trichuris trichiura. This was most unusual considering that this cohort includes some of the most basal nematode species. This diversity suggests that mechanisms regulating calcium homeostasis vary in accordance with physiological demands of individual species. In addition to our comparative physiology data, my lab has also functionally characterized an NCLX-type exchanger in Caenorhabditis elegans called NCX-9. During neural circuit formation, migrating axons must interpret secreted guidance cues to facilitate proper path finding and navigation. Defects in the detection or expression of these secreted cues have been shown to perturb guidance, and this system of axon guidance via secreted cues is evolutionarily conserved across invertebrate and vertebrate species. Yet, despite the central and conserved role of secreted guidance cues in the control of neural development, the mechanism of guidance cue secretion is very poorly understood. The mammalian NCLX has been shown to regulate secretion of insulin in pancreatic β-cells, and we have found that the C. elegans orthologue of NCLX (called NCX-9) functions with the guidance cue UNC-129/BMP to regulate proper patterning within a motor neuron circuit during development. We also show that NCX-9 functions at the mitochondrion and is expressed in non-neuronal hypodermal cells in vivo in which UNC-129/BMP is also expressed. Our findings on NCX-9 are
the first description of a role for NCLX in patterning neural circuits.

28.15
Identification of the first member of the gap junction protein family in the protozoa Trypanosoma cruzi, the etiological agent of Chagas disease.
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Introduction: The presence of member of the gap junction protein family has been described in vertebrates (connexin and pannexin) and invertebrates (innexin), but has not been reported in unicellular organisms. The aim of this study was to identify an innexin protein and perform its characterization in Trypanosoma cruzi (T. cruzi), etiologic agent of Chagas disease.
Methodology: The search of genomic sequences, homologues of gap junction proteins were performed using TritrypBD genomic database. The topology was analyzed with PROTTER software. The innexin structural stability was performed in Modeller 9.10 and dynamic simulation carried out in Amber16 software. The dye uptake assay in epimastigote form of T. cruzi was used for innexin functional characterization. Probenecid (400 µM), flufenamic acid (50 µM) and heptanol (2 mM) were used for pharmacological characterization. A FITC-Dextran (~70,000 Da) was used to control for cell membrane damage.
Results: T. cruzi presents a homologue of innexin proteins with a length of 257 amino acids and present a highly conserved innexin motif YYQWV. The innexin three-dimensional modeling, showed that the channel model with 8 subunits is more stable than with 6 subunits modeled at 10 ns. While the channel with 6 subunits is closed, the model with 8 subunits presents a pore diameter of 10-12 Å. Moreover, it presents a gradient of electrostatic potential, being electropositive in extracellular regions and electronegative in intracellular regions. Epimastigotes showed an innexon-like activity with permeability to YOPRO-1, DAPI, ethidium bromide and Evans blue, induced by extracellular Ca²⁺/Mg²⁺ free solution and blocked by 400 µM probenecid, 50 µM flufenamic acid or 2 mM heptanol.
Conclusions: These findings suggest the presence of homologues of connexin proteins in T. cruzi. This could be a new molecular target for future studies on the parasite biology.
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28.16
Trade-offs in reproduction and regeneration in Anolis lizards.
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Life-history theory has been used as an analytical framework to explain the variation across species in their life history strategy through traits such as birth size, growth, age of maturity, reproductive patterns, and longevity. The energetic investment in regeneration—particularly of large amounts of tissues such as limbs or tails—is predicted to be negatively correlated with investment in other life history traits, such as reproduction. However, the physiological mechanisms of trade-offs between reproduction and tail regeneration are unclear. Across animals, the Insulin and Insulin-like Signaling (IIS) network regulates cellular processes including pre- and post-natal growth and development, reproduction, longevity and wound healing following tail autotomy. Anolis lizards that regenerate tail tissue and produce a single egg every 1-3 weeks provide an opportunity to study the life history patterns associated with tissue regeneration, and the role of the IIS network in regulating this trade-off. Female brown anoles (N=40) were split into two treatment groups: Control vs Tail Autotomy. Lizards were kept on a limited diet of 5 crickets weekly. Reproduction was tracked for a period of one month prior to tail autotomy and 3 months post autotomy through measures of egg number, mass, and frequency of oviposition, along with tissue generation post-autotomy. Hatchling size was measured through mass, snout vent length and tail length. Maternal tissues will be assessed for differential expression of IIS hormones and regulators between the treatments. We predict that if tissue regeneration and reproduction are negatively correlated, on a limited resource diet, reproductive females will decrease investment into eggs, slow, or halt reproduction when regenerating tail tissue. Results will be discussed along with future plans for recovery of tradeoffs through manipulation of the IIS network.
The effect of food availability, temperature and sirtuin inhibition on the metabolic rate of California mussel gill tissue

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Recent work has demonstrated the importance of food availability in modulating the tolerance of marine organisms to several environmental stressors. Preliminary studies provide evidence that sirtuins (deacetylases that respond to caloric intake and maintain cellular homeostasis) mediate the response to differences in food availability and heat stress in intertidal mussels. To investigate the role of sirtuins in mediating physiological performance under conditions of varying food availability and thermal regimes California mussel (Mytilus californianus) were acclimated to one of two algae food rations (high or low) and to daytime low tide periods where air temperatures were ramped to 20 or 30°C for three weeks. Post acclimation, half of the mussels in each treatment were exposed to a sirtuin inhibitor during the evening high tide period. During the subsequent low tide, all mussels were exposed to an acute aerial heat shock of 33°C during the daytime low tide period, after which mussels were returned to acclimation conditions. Prior to (baseline) and following sirtuin inhibition (experimental), metabolic rate of gill tissue was assessed by closed respirometry every 3 hours during high tide periods (i.e. 1am, 4am, 1pm, 1pm) for two consecutive tidal cycles (i.e. 4 days total). For the baseline measurements, the availability of food had a significant effect on the metabolic rate, with the high food groups having a higher metabolic rate than low food groups, and metabolic rate was highest at 4pm. Day time temperature acclimations at low tide (20 or 30°C) did not have an effect on metabolic rate. For the experimental measures, food availability significantly increased metabolic rate, similar to what was seen in baseline measurements, and exposure to a sirtuin inhibitor significantly decreased metabolic rate. Time of day and low tide temperature did not have an effect on metabolic rate. Metabolic rate is a measure of aerobic performance, specifically metabolic demand. The baseline data indicate that the degree of temperature change experienced at low tide does not affect metabolic demand during the subsequent high tide period, but that metabolic demands shift on a circatidal rhythm, with highest energy demand in the daytime high tide period 3 hours after low tide. Increased feed availability (i.e. energy supply) is met by increases in metabolic demand in both the baseline and experimental measures, supporting recent findings that food availability plays a vital role in the physiology of the intertidal mussel. Additionally, sirtuin inhibition decreased metabolic demand of mussels, supporting previous preliminary work that sirtuins are an important mediator that links cellular energy state and physiological stress in the California mussel. Taken together, the results provide evidence that sirtuins likely play an important role in modulating the metabolic demand in intertidal mussels but do not influence how mussels are affected by low tide temperature or food availability at the tissue level. This research is funded by the National Science Foundation (IOS-1557496 & IOS-1557500).

The importance of tidal acclimation in assessing the physiological responses of the intertidal crab Carcinus maenas to emersion

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Animals inhabiting the intertidal zone are exposed to abrupt changes in environmental conditions associated with the rise and fall of the tide. The most predictable of these changes is the transition between aquatic and aerial media. For convenience, the majority of laboratory studies on intertidal organisms have acclimated individuals to permanently submerged conditions. However, this is not representative of the daily fluctuations intertidal organisms experience in their natural habitat. We used the green crab Carcinus maenas to identify whether individuals acclimated to a simulated tidal regime of continuous emersion-immersion (tidal) exhibited different physiological responses compared with individuals that were held permanently submerged (non-tidal). Oxygen consumption, PaO₂, venous pH, L-lactate and hemocyanin concentration were measured in
individuals from both acclimation groups subjected to 6h-6h-6h cycle of immersion, emersion, and re-immersion. Both tidal and non-tidal crabs exhibited a 50% decline in oxygen consumption during the 6h emersion period but experienced no change in PaO₂. This was associated with a concomitant decline in venous pH and increase in L-lactate in non-tidal crabs, whereas these remained unchanged in tidal crabs. Pre-emersion oxygen consumption rates were rapidly regained in both groups when the crabs were re-immersed, as were pH and L-lactate levels in non-tidal crabs. Tidal crabs maintained higher hemocyanin concentrations than non-tidal crabs throughout the experimental regime. These results suggest acclimation of C. maenas to submerged conditions results in a loss of important physiological mechanisms to tolerate emersion. Hemocyanin plays a crucial role in oxygen transport and acid base homeostasis during emersion in C. maenas and the elevated levels in tidal crabs are the likely driver behind the physiological differences between the two groups. The results of this study show that caution must be taken when acclimating intertidal organisms to laboratory conditions, as it risks abolishing important physiological responses that play a critical role in the physiological performance of an organism in situ. These results further underscore the importance of acclimating organisms to realistic ecological scenarios that incorporate a multifaceted design within the laboratory environment. This study was funded by a NSERC Discovery grant awarded to Iain J. McGaw.

28.19
Hypoxia and the metabolic phenotype in Daphnia.
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Low oxygen induces many animals to mount compensatory responses to improve oxygen utilization and/or anaerobic metabolism. In vertebrates, the coordination of these compensatory responses falls to the HIF pathway, which simultaneously induces expression of genes for glycolytic enzymes, glucose transporters, angiogenic factors and erythropoietin. The net result is improved vascular delivery of oxygen and glycolytic capacity. The hypoxic response in invertebrates is less studied, and expected to be different because of the role of the vasculature and the use of extracellular Hb. Daphnia are an intriguing model because they induce Hb genes in response to environmental hypoxia, causing the animal to turn red. This phenotypic plasticity is seen in lakes that undergo summer thermal stratification, with corresponding gradients in oxygen levels. Although the nature of the Hb response is well studied, it remains unknown whether this is sufficient to sustain normoxic metabolism or whether systemic hypoxia results, necessitating induction of glycolytic metabolism and/or glycolytic gene expression. The objective of this study is to explore the extent to which the Hb response is coupled to glycolytic gene induction in relation to the depth and duration of hypoxia, and whether there is any evidence of local (microevolutionary) adaptation in glycolytic gene patterns. Daphnia were collected from a series of lakes in the Canadian Shield, differing in the extent to which late summer stratification occurs. Animals were compared before stratification (June) and after stratification (September/October). In several lakes, animals that differed in Hb level (pale vs red) showed no difference in glycolytic enzymes (lactate dehydrogenase, pyruvate kinase, enolase). Other lakes showed pronounced increases in levels of both Hb and the suite of glycolytic enzymes, which for the most part increased in parallel with each other. These results suggest that in at least some conditions, Hb induction is sufficient to sustain normoxic metabolism and that there is a flexible coupling of control of Hb and glycolytic genes in response to hypoxia. Current studies integrate metabolic studies with activity patterns of the animal, assessing the potential of glycolytic metabolism and glycogen stores to sustain the animal during diurnal migrations in the water column of stratified lakes. Supported through NSERC Discovery Grants to WN and CDM.

28.20
Establishing an index of habitat quality and reproductive success for the Northern Fur Seal
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Northern fur seals (NFS; Callorhinus ursinus) support lactation using a central place foraging strategy, alternating foraging trips to sea with shore visits to nurse pups. Prey abundance and availability influence trip duration. The frequency and length of shore-stays influences the rate and amount of milk pups receive. Pup mass, influenced by these factors, is positively correlated with post-weaning survival. Overall, the NFS population on the Pribilof Islands, AK, has declined ~3.5% annually for the past two decades. However, despite continued decline on St Paul Island (SNP), pup production increased ~27% on St George Island (STG) between 2012 – 2016. Maternal females forage in colony-specific areas at sea, each characterized by a distinct oceanographic environment that may influence prey availability and explain the inter-island variation in pup production. We hypothesized that the variability in maternal foraging trip durations (MFTD) could provide an index of offshore habitat quality and explain known differences in pup mass and post-weaning survival; the objective was therefore to identify correlative relationships between colony-averaged MFTD and pup mass between seasons.
Between 2010-2017, the attendance patterns of 239 maternal females were monitored throughout the lactation period using VHF radio transmitters at six Pribilof Island colonies. Additionally, inter-annual variability in the relationship between MFTD and pup mass was assessed for females at Polovina Cliffs, a SNP colony. Likelihood ratio tests identified factors influencing the variability observed in the colony-averaged MFTD (Colony, Year, & Julian Day; \( P << 0.01 \)). Colony average MFTD were longer for SNP females than STG (\( P << 0.01 \)). For Polovina Cliffs, there was a negative correlation between average MFTD and average female pup mass (\( P = 0.03, r^2 = 0.59 \)), whereby pups lost 0.51kg mass per day mom spent foraging. Regional and temporal variability in colony average MFTD was consistent with trends in oceanographic environment, pup mass, and pup production. Record-breaking warm ocean temperatures, as seen in 2016, corresponded to decreased biomass of important NFS prey species. Subsequently, MFTD increased and pup mass decreased. Longer average SNP MFTD corresponded to the observed decrease in pup production. The interannual and regional correlations between colony-averaged MFTD and pup mass suggests that population-level metrics can be used as a sensitive indicator of habitat quality and reproductive success.

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### 28.21

**Hypoxia induces differential changes in thermoregulation and metabolic rate base on body size in the bumblebee *Bombus impatiens***

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Facultative endothermy is utilized in bumblebees to warm up flight muscles in preparation of flight and also to maintain hive warmth. *Bombus impatiens* is a species of bumblebees that divides labor based on size of the bee. The larger bees are more likely to forage and when in the hive are found at the edges of the colony, while the smaller bees are found in the center of the hive and are more likely to incubate and feed larvae (Jandt & Dornhaus, 2009). In another colonial bee (*Apis mellifera*), oxygen concentrations vary within the hive and have been measure during winter hibernation as low as 10% (Nerum & Buelens, 1997). We investigated whether bees of differing sizes react differently to several levels of hypoxia using two different regimes of oxygen change. One starting with low oxygen concentration of 2.5% then increasing from 7.5, 10, 15, to 21% and one that started high at 21% and gradually decreased down to 2.5%. We measured thorax temperature using an infrared camera and simultaneously measured metabolic rate using flow-through respirometry. Our results suggest that larger bees when challenged with hypoxia are more likely to decrease thorax temperature and therefore metabolic rate than their smaller counterparts. In addition, larger bees required a higher metabolic rate than smaller bees to maintain the same thorax temperature and overall smaller bees maintained higher thorax temperatures despite having a higher surface area to volume ratio. This indicates that larger bees, those that are more likely to forage, have higher basal metabolic rates and do not thermoregulate as well at low oxygen levels. While the smaller bees increase temperature in lower oxygen environments.

Citations:


### 28.22

**Does individual variation in heat loss influence thyroid and metabolic responses to cold?**

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Triiodothyronin hormone (T3) is known to play a role in thermoregulation and heat production in birds and is typically higher in cold environments. Additionally, T3 has been shown to correlate positively with metabolic rate; however, these relationships are typically noisy, with a large amount of variation around the mean. One potential cause for this variation is individual-level differences in insulation and consequently heat loss (conductance). For a given temperature, birds losing more heat would perceive their environment as colder and might therefore show a stronger physiological response; leading to the prediction that thermal conductance should correlate positively with T3 levels and metabolic rate. Here, we exposed indoor captive red knots (*Calidris canutus islandica*) to sequential decreases in temperature (15°C to -10°C; by -5°C) while measuring their oxygen consumption to calculate minimal thermal conductance. We also measured T3 blood levels before and after metabolic rate measurements with the expectation that birds with the highest heat loss would show higher T3 levels and higher metabolic responses. Although we found that T3 increased with
the decline in temperature, as expected, our results only provide weak support for the effect of individual heat exchange on T3 and metabolic response. Potential explanations for the correlation between thyroid hormone, metabolic responses and heat conductance will be discussed.

28.23
Does the risk of overheating limit maximum rates of energy expenditure in breeding birds?

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During breeding, adult birds may spend many weeks feeding their chicks, resulting in a sustained energy expenditure of ~4x the parent’s resting levels. But why don’t parents work even harder, especially if there are presumed fitness benefits to raising more nestlings? The heat dissipation limit theory of Speakman and Król (2010), proposed that maximum sustained rates of energy expenditure are constrained by an individual’s capacity to dissipate metabolic heat. To test this, we studied breeding tree swallows (Tachycineta bicolor), which forage for up to 15 hours per day gathering insects to feed nestlings. We predicted that if an individual’s capacity for energy expenditure is limited by its ability to dissipate body heat, then individuals with an increased capacity to dissipate heat will feed their nestlings at higher rates. To increase the capacity for heat dissipation we experimentally increased the size of the brood patch in females, by trimming the overlying feathers. We also implanted small temperature-sensitive tags, which allowed us to monitor body temperature and feeding rates remotely. Our preliminary analyses suggest that risk of overheating does in fact limit parental performance. Because the risk of overheating may increase with climate warming, such risks may exacerbate the ongoing population declines of many small birds. Funding: Trent Univ.; Natural Sciences and Engineering Research Council (Canada).

28.24
Evidence for the influence of triiodothyronin on maximal heat production in birds

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Triiodothyronin (T3) is considered to be a regulatory hormone of thermoregulation in mammals and birds. Indeed, low ambient temperatures elicit both heat production and higher T3; which has been linked to non-shivering thermogenesis. T3 has also been shown to be correlated with basal heat production (BMR) in birds. However, whether T3 plays a significant role in avian maximal heat production and cold endurance remains unknown (i.e. positively correlates with). Here, we studied indoor captive red knots (Calidris canutus islandica) maintained under three thermal treatments over a complete annual cycle. Each month we measured variation in body mass, basal metabolic rate (BMR), maximal thermogenic capacity (Msum), and thyroid hormones: triiodothyrinin (T3) and thyroxine (T4); and subsequently investigated correlated variation. T3 levels were positively correlated with BMR across seasons and treatments, confirming previous observations. T3 also correlated positively with Msum, thus suggesting a potential influence of tissue heat production independent from shivering. Our results support recent findings showing improvement of maximal thermogenic capacity independent from muscle size variation in birds.

28.25
Withdrawn

28.26
Depressing mitochondrial function during paradoxical anaerobism leads to an alcoholic fish

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Skeletal muscle mitochondria were isolated from desert pupfish (Cyprinodon spp) that were acclimated to ecologically relevant temperatures of 28 °C (M28) and 33 °C (M33). The respiratory control ratio (RCR) of M33 pupfish was ~2.6X lower than that of M28 pupfish, initially suggesting mitochondria were damaged by the warmer temperature. However, state 4 respiration was below expected values in both M28 and M33 pupfish suggesting little proton leak. Further, membrane potential was lower in the M33 pupfish than M28 pupfish and did not change with assay temperature. These data suggested a reduced proton motive force in M33 pupfish. Analyses of respiration as a function of membrane potential suggest a block in substrate utilization in M33 pupfish. M33 pupfish also experience reduced production of reactive oxygen species (ROS) from the Q site of complex I. Taken all together, the data suggest pupfish may be limiting mitochondrial use to avoid ROS production by restricting access of substrates to the mitochondrion. These results are consistent with our measurements of oxygen consumption in intact fish. Specifically, fish acclimated to 33 °C demonstrate extended periods of paradoxical anaerobism in which
oxygen consumption is absent despite the presence of oxygen. These fish produce ethanol as an alternative end product of metabolism and key indicators such as alpha smooth muscle actin in the liver and cytochrome p4502E1 analogue are consistent with an alcoholic lifestyle.

28.27
The impact of developmental hypoxia on the cardiovascular chemoreflex in embryonic snapping turtles (Chelydra Serpentina).

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Developmental hypoxia is a common challenge in developing reptiles which can have lasting effects on the functional phenotype of the embryo and hatching. Prior studies have shown several aspects of cardiovascular function are altered in reptile embryos by chronic hypoxic incubation. In this study we expanded on our prior studies to investigate the impact of chronic developmental hypoxia on the cardiovascular limb of pharmacologically induced activation of a chemoreflex in developing snapping turtle (Chelydra serpentina) embryos. We measured arterial blood pressure and heart rate in embryos incubated in 21%O2 and 10%O2 at 70 and 90% of incubation. We hypothesized that hypoxic incubation would blunt the reflexive response to phenylbiguanide (PBG) and sodium cyanide (NaCN) late in incubation due to withdrawal of vaga1 input on the heart. Chronic hypoxic incubation produced smaller embryos that were hypotensive at 90% of incubation, similar to previous studies. NaCN (0.1, 1, 10mg kg\(^{-1}\)) and PBG (800ug kg\(^{-1}\)) produced a hypotensive bradycardia, similar to the cardiovascular response produced during acute hypoxia in both incubation conditions and time points previously observed. The cardiovascular response was attenuated with hexamethonium in response to PBG, while atropine completely abolished the response to PBG and reduced the response to NaCN, suggesting that the cardiovascular reflex is mediated through the vagus nerve. This study has confirmed the presence of the cardiovascular limb of a chemoreflex in embryonic snapping turtles and that it is mediated through the vagus nerve acting on cardiac tissue.

28.28
Scaling of Major Organs in Hatchling Female American Alligators (Alligator mississippiensis)

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Large non-avian and avian reptile species grow most rapidly during their early life history, with ‘clutch’ effects for different parentage. The effect of body mass on the relative growth, metabolism, organ size and morphological/anatomical features of animals is an active and long-studied area of biology, ecology and comparative animal physiology. Allometric equations represent relationships between a morphological size or physiological rate and body mass as \(Y = aM^b\), where \(Y\) is a size or rate, \(a\) is elevation, \(b\) is the exponent describing the shape of the line, and \(M\) is animal body mass. We measured visceral organ size in hatching female alligators up to 6 months-old from 5 clutches at 8-10 timepoints during their first order of magnitude of growth (~45 g to ~500 g wet body mass). We tracked each individual alligator (N=86) from original egg/clutch identity. Clutches differed in initial egg mass, initial hatching wet mass with yolk, snout-vent length (SVL) and head length (HL). The interaction between initial egg mass and clutch identity was significant for initial hatching wet mass, but only egg mass, not clutch, had a significant effect on initial SVL and HL. Kidney and liver mass showed biphatic scaling with body mass for both wet and dry values, a ‘breakpoint’ of ~100 g wet body mass. Kidney and liver wet mass showed slopes \(b>1.0\) as animals increased in wet body mass from ~45 g to ~100 g; kidney and liver wet mass slopes were significantly lower \(b<0.8-0.9\) for larger animals >100g. Lung and heart wet mass did not show biphatic scaling with body mass and \(b<0.8-0.9\). Within kidney and liver mass, below (‘small’ alligators) and above (‘larger’ alligators) the breakpoint (~100 g body mass), wet or dry organ mass slopes tended to be similar between clutches. Within lung and heart wet or dry mass between clutches, all clutches had statistically identical slopes. Combined clutch data for wet mass showed distinct regressions with slopes >1.4 for small alligators’ kidney and liver mass, compared to larger alligators’ kidney and liver mass slopes as well as all alligators’ lung and heart mass slopes. The slope for wet heart mass was larger than other slopes, except wet liver mass for larger alligators. Overall, there was variation in size of hatching alligators due to egg size and clutch effects (genetic effects); however, for a given whole animal size, the relative organ mass of alligators was reasonably similar across clutches through the first order of magnitude of rapid growth. Hatchling alligators appear to undergo a very early period of rapid kidney and liver growth following hatching, with higher rates than lung or heart tissue. Clutch, egg mass, and hatching size influence organ size, and each factor should be accounted for in future studies exploring reptile morphology and physiology in order to assess environmental versus clutch/genetic contributions. This study strongly indicates that given common environmental conditions and diet, alligators dedicate similar energetic resources to visceral organ
growth, regardless of clutch, and that variability in crocodilian organ size may be driven primarily by environmental variation or ecological conditions.

28.29
Effects of Δ9-tetrahydrocannabinol (THC) on Zebrafish Embryo Development
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Δ9-tetrahydrocannabinol is the psychoactive constituent derived from the angiosperm Cannabis sativa, and found in the commonly used recreational drug marijuana. Now being examined for its medicinal purposes, C. sativa has become a relevant topic for political and social debate due to its ability to trigger the endocannabinoid system, resulting in powerful therapeutic effects like anti-emesis, anti-anxiety and anti-spasticity, but also remaining controversial because of its accompanying THC-induced hallucinogenic effects. While research involving the physiological actions of THC have been conducted, very few studies have investigated the deleterious effects on early embryological development. As a preliminary component of my thesis research regarding the reestablishment of balanced excitatory and inhibitory signaling in a muscle spasticity mutant, I examine the developmental effects of THC on the developing zebrafish from 2 hours post fertilization to five days post fertilization. Somatic growth parameters (body length, maturation rates, natural chorion emergence rates and morphologic abnormalities), as well as the achievement and production of smooth, effective and fast-maturing escape behaviors indicate the phenotypic consequences of early embryonic exposure to THC.

28.30
Evolution of the Development of Respiratory Physiology in Deer Mice Native to High Altitude
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High altitude is a challenging environment characterized by unavoidable and unremitting hypoxia (low oxygen), which can constrain O2 supply to support physiological processes. Based on studies in adults, control of breathing appears to be altered in many highland taxa to optimize O2 uptake, but few studies have investigated the control of breathing at early developmental stages. We examined the development of the hypoxic ventilatory response (HVR) at post-natal age (P) 7, 14, 21, and 30 in animals that were born and raised in captivity, comparing populations of deer mice (Peromyscus maniculatus) native to high altitude to populations of deer mice and white-footed mice (P. leucopus) native to low altitude. Breathing, arterial O2 saturation (SaO2), and heart rate were measured during step-wise decreases in inspired PO2. The HVR appeared to develop between P7 and P14, as reflected by robust increases in breathing in response to hypoxia in P14 mice but not P7 mice. This was associated with significant development of the carotid bodies – the peripheral chemoreceptor that initiates the hypoxic chemoreflex – between P7 and P14. Differences in breathing pattern arose in highlanders at P30, who then breathed deeper but less frequently than lowland mice, consistent with differences we have previously observed in adults. Blood-O2 binding affinity decreased with age, consistent with decreases in SaO2 during hypoxia, but highlanders had consistently higher binding affinity and SaO2 than lowlanders. Therefore, high-altitude mice express a high blood-O2 binding affinity very early after birth, which should help safeguard arterial oxygenation, but evolved changes in the control of breathing do not become apparent until later in post-natal development.

28.31
A legged limitation on insect size? Scaling of tracheal systems in scarab beetles
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Scaling limitations on oxygen supply are often invoked to explain the small size of insects and sometimes other invertebrates. However, body size does not affect the safety margins for oxygen delivery at rest, during flight and during growth of insects, suggesting that oxygen supply matches demand across insect size. In vertebrates, oxygen transporting structures scale isometrically or hypometrically, in the latter case, matching the hypometric scaling of metabolic rate. In insects, there is some evidence for hypermetric scaling of tracheal structures, suggesting that larger insects must invest relatively more in tracheal structures in order to be large, and potentially providing upper limits on insect size. We tested this idea using 15 scarab beetle species ranging in mass from 0.05 to 31 g by using microCT to assess the scaling of the tracheal system, flight muscles, and the brain. Most of the air sacs in the body of scarab beetles form globular, grape-like clusters, appearing morphologically quite different from the air sacs of other insects. However, around the flight muscles, air sacs appear complex in shape similar to those seen in other insects. Total and thoracic air sac scaled isometrically, as did flight muscle volume, consistent with matching convection to tissue oxygen need during flight. The percent of volume occupied by air sacs was greater in the thorax than the head or abdomen, consistent with the high demands of flight. Head and brain volumes scaled hypometrically, and air sacs became an increasing proportion of the head as beetles increased in size,
consistent with the need for greater convection to supply the brain in larger beetles, or alternatively with the use of air sacs to expand head volume for other purposes. Air sac volumes scaled hypometrically in the abdomen. In contrast to all other body segments, the tracheal system of the femur (the segment of the leg containing most leg muscle) scaled strongly hypermetrically. The morphology of the femoral tracheae suggest that they are compressible and used for convective gas exchange in the leg, consistent with the hypothesis that gas exchange to larger legs requires increasingly larger relative investments in tracheal structure and function. These data support the hypothesis that insect maximal size may be partly constrained by gas exchange within the leg. Supported by NSF IOS 1122157.

28.32
The effects of a bacterial endotoxin LPS: neuromuscular junction and cardiac function in fruit fly (Drosophila melanogaster) and blowfly (Phaenia sericata) larvae.
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Gram negative bacterial septicemia inflicts humans as well as other animals. The immunological response to bacterial infection activates cascades of defense cytokines and antibody formation. Two common culprits in mammals are Pseudomonas aeruginosa (P.a.) and Serratia marcescens (S.m.). The induced cytokines and defense response to the surface antigens on bacteria accounts for some of the immune response but also the secretion of lipopolysaccharides (LPS) is responsible for a large degree of the immune response. The direct action of bacterial LPS endotoxin was shown to enhance synaptic transmission and hyperpolarize the membrane potential at low dosage but block glutamatergic receptors and decrease observable spontaneous events at a high dosage. The dosage effects are LPS type specific. The hyperpolarization is not due to a voltage gated potassium channels or due to activation of nitric oxide synthase (NOS). Comparative effects of LPS on heart rate (HR) were examined in larvae. Acute direct exposure of in situ heart tubes with saline containing at 500 µg/ml LPS from two common bacterial strains (P.a. and S.m.) showed a dose-dependent effect on HR but different responses for the two fly models. LPS is likely altering ionic balance of the pacemaker potential by inducing a hyperpolarization of the cardiac muscle. Currently, we are investigating lower doses of LPS. The significance of these findings is to gain a better understanding of the direct mechanism of action of LPS on synaptic function and its effects on HR, induced without the effect of an immune response as occurs in intact animals. Knowing the acute and direct actions of LPS exposure on larvae in these species may aid in understanding the underlying mechanisms in other animals during septicemia. We thank Ms. Kameron Roach, Ms. Amanda Paschal, Ms. Alexandra Stanback, Mr. Jaylen Scott, Mr. Mohsin Akhtar, Mr. Jate Bernard and Ms. Nicole Audia for helping in conducting these experiments. Funding was provided by personal funds (R.L.C.), student tuition, and a “Sustaining Excellence-2014” competition grant from the Howard Hughes Medical Institute (Grant #52008116) awarded to the Univ. KY (VM Cassone, PI). The authors confirm that the HHMI funder had no influence over the study design and content.

28.33
Examination of predicted cardiac parameters based on ventricle wall thickness in the Northern bobwhite quail, Colinus virginianus.
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Experimental evidence of avian cardiac function for a number of species has been understandably limited due to a number of factors including animal size constraints. However, a number of predictions related to cardiac function have been put forward based on available data for this vertebrate group. In this study we directly measure ventricular pressure parameters of Northern bobwhite quail, Colinus virginianus in an effort to test existing models of cardiac parameters in this species. We measured right and left intraventricular pressures under general anesthesia. Heart rate, intraventricular pressures, contractility, change in pressure over time and time of relaxation were measured in adult birds. In an effort to investigate maximal pressure parameters the beta adrenergic agonist dobutamine (100 ug/kg) was injected into the right ventricle. At the completion of the study cardiac mass as well as right and left ventricle wall thickness were determined. Birds maintained baseline mean intraventricular pressure of the left and right ventricles at 4.8±0.3 kPa and 1.9±0.4 kPa respectively. Peak systolic pressure was 12.3±0.4 kPa and 3.5±0.4 kPa in the left and right ventricle respectively. Diastolic pressure was 0.5±0.2 kPa and 0.9±0.3 kPa in the left and right ventricle respectively. Baseline heart rate was 300±16 beat min⁻¹ and increased to 360±17 beat min⁻¹ following dobutamine injections. All pressure parameters increased following dobutamine injection, with the right ventricle pressure parameters increasing to a greater degree (~40% vs ~20%) compared to the left ventricle. Our findings deviate from those values that would be predicted based on an existing predicted values suggesting revision of the model is necessary.
Heart rate and angiogenesis in chicken embryos exposed to the environmental contaminant TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin)

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TCDD is a persistent pollutant that can be delivered to the mammalian fetus from the mother via placenta and breast milk and via yolk in birds. TCDD is teratogenic and cardiotoxic for chicken embryos, as evident from an enlarged heart and dilated ventricle cavities with thin walls as well as cardiac septal defect. TCDD also causes angiogenic malformations and a reduced number and size of blood vessels in chicken embryos, as well as decrease in vascular endothelial growth factor-A (VEGF-A). However, whether and when TCDD affects early cardiovascular development in the embryo is unclear. Thus, we investigated the effect of TCDD on heart function and vasculature development at the early stages of development in the chicken embryo. We used shell-less cultured embryos that allow full access to embryo and chorioallantoic membrane (CAM) development until embryonic day 5 (E5). The eggs of Gallus gallus were incubated until day E2, after which the whole egg content was transferred to a sterilized petri dish covered in plastic wrap. The embryos in the petri dishes were then incubated at 37.5°C. Beside controls, the embryos were exposed to one topical application of 0, 0.002, 2 and 10 ng TCDD/egg on E2. Another population received repeated injections of 0 and 2 ng TCDD/egg daily (E2, E3, E4). Finally, a third population was dosed with 0, 2, 10 and 20 ng TCDD/egg before the incubation (EO) for heart rate analysis and exposed once to 0, 0.002, 2ng for angiogenesis analysis. Videos for heart rate analysis and photographs for angiogenesis analysis were made before the injection and daily at the same time until E5. Heart rate was analyzed by counting the number of heart beats in a 30 sec film and extrapolating to 1 min. Angiogenesis in the CAM was analyzed by a vascular index generated by counting the number of vessels that crossed each of several concentric circles placed 2mm distant for each other until cover all the CAM from the umbilical vessels. Heart rate in control embryos was 128.5±3.4 (E2), 154.6±2.8 (E3), 178.2±1.4 (E4) and 123.3±5.9 beats.min⁻¹ (E5). Vessel density index measured on the CAM of control embryos at E5 was 3.2±0.7, 11.2±0.8, 19.4±1.3, 32.8±2.5, 49.4±5.8, 39.2±6.8, 8.6±4.7 and 0.6±0.5 vessels/concentric circle for 2-16 mm in 2 mm distance increments, respectively, from the umbilical vessels. Neither heart rate nor vascular index were significantly changed in any of the described treatments in these early stages. Despite the toxicity and cardiovascular effects of TCDD in late chicken embryos, TCDD does not affect cardiovascular form or function in early stage chick embryos. Past literature shows differences in heart rate and angiogenesis between E5 and E10. Other current experiments from our lab show the sharpest increase of mortality in ovo occurs from E9 to E15 when embryos were exposed to dioxins (2-50ng) at E4. This leaves for future investigation the identification of the beginning of the critical window for appearance of TCDD-induced cardiovascular malformation, which now appears to be between E5 and E15.

Baroreflex changes with body size in the Green iguana

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The baroreflex is a mechanism that continuously regulates acute changes in arterial blood pressure (Psys) at every heartbeat. Mechanoreceptors (i.e. baroreceptors) localized at the arterial wall sense variations of Psys by the displacement of the arterial wall during the cardiac cycle. The typical baroreflex response includes rapid changes in heart rate (HR) that regulates blood volume to re-stabilize Psys. The magnitude of this response is denoted baroreflex gain (G50) and indicates the baroreflex sensibility. Increased body size may affect vascular compliance, which is often related to a decreased G50. In reptiles, which may exhibit massive increases in body mass (BM) that can reach 10,000 fold during their life span, bigger BM is often related to decreased HR and increased Psys possibly affecting G50 responses. To test if G50 is affected by increased BM, we measured Psys and HR of 7 Green iguanas (Iguana iguana), an arboreal lizard species, with body sizes ranging from 31 to 819g, representing a 27-fold increase in BM. Animals had their femoral artery occulsive catheterized and recordings were made 24h after surgery within a climatic chamber set to constant 35°C. Heart rate was derived from blood pressure pulsatile signals and G50 was calculated using the sequence method. Psys increased with BM (Psys=0.55BM⁰.¹¹, R²=0.72), whereas HR and G50 decreased with BM (HR=57.6BM⁻⁰.¹¹, R²=0.56; G50=125.4BM+188.5, R²=0.67). Increased Psys suggests perfusion pressure is greater in larger individuals which may facilitate tissue perfusion. The decreased HR suggests a lower specific rate of oxygen consumption of bigger animals, such as observed in mammals and other reptiles. The decreased G50 indicates that larger animals possess a decreased vascular compliance or distensibility. This higher sensitivity to regulate acute pressure changes in smaller iguanas suggests that they are better able to circumvent abrupt orthostatic challenges, such as during climbing, when compared to adult individuals, probably increasing their chances to avoid predation.

Withdrawn
Changes in the gut microbiota over the course of gestation in oviparous Eastern fence lizards (Sceloporus undulatus)
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In humans, pregnancy significantly alters the structure of the gut microbiome. Specifically, gut communities exhibit decreases in alpha diversity and increases in beta diversity (higher inter-individual variability) over the course of pregnancy. Here, we investigated whether similar trends occur in evolutionarily distant hosts. We collected repeated fecal samples from gestating Eastern fence lizards (Sceloporus undulatus), and recorded the date of egg laying to determine the time of gestation for each sample. Additionally, fecal samples were collected from non-gestating females. Bacterial inventories were conducted by sequencing the 16S rRNA gene and community profiles were determined using QIIME2. We found that over the course of gestation, lizard gut microbiol communities exhibited decreases in alpha diversity (Faith’s phylogenetic diversity and number of observed OTUs). Additionally, inter-individual variation was higher towards the end of gestation. The relative abundance of the candidate phylum Melainabacteria was lower in lizards towards late-gestation. The presence of Melainabacteria was detected in 60% of samples from non-gestating individuals, 70% of samples from early-gestation individuals, but less than 40% of samples from late-gestation individuals. Overall, our results are similar to previous results observed in humans, suggesting similar interactions between gestation and the gut microbiome in these disparate lineages. We hypothesize that hormonal, immunological, or metabolic changes associated with gestation may underlie these community shifts. Further studies should investigate the functional effects of these altered communities over gestation in varied animal groups, including humans.

Comparative analyses of gene expression in snakes yields insight into conserved mechanisms underlying intestinal regeneration
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Some species of snakes possess feeding ecologies that involve extremely long periods of fasting between meals. Rather than maintaining an energetically costly digestive system, these snakes have evolved the capacity to downregulate digestive form and function at the conclusion of digestion and maintain this dormant state throughout the fast. Following feeding, the digestive system is rapidly restored to a fully functional state within just 24 hours through major increases in cell growth and proliferation, metabolism, and overall digestive function. In contrast, frequently feeding snake species do not possess this extreme capacity for regulation, and instead maintain an active digestive system at all times. Here, we compared responses in the intestine of multiple snake species, including those that do and do not regenerate upon feeding, to identify transcriptional responses associated with the intestinal regenerative phenotype in snakes. By comparing and contrasting responses across phenotypes and species, we develop a set of explicit hypotheses about signaling mechanisms underlying regenerative growth in snakes, which includes a surprisingly major role of stress-response signaling pathways.

Cold-inducible RNA-binding protein as a potential regulator of embryonic gonadogenesis in the red-eared slider turtle
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Temperature-responsive genes, such as those coding for heat shock proteins, play a vital role in embryogenesis and their patterns of spatial expression are shared among vertebrate species. While the hierarchy of expression is not conserved, many of the genes regulating developmental processes, such as gonadal development, are shared between taxa. Vertebrates exhibit multiple forms of sex-determining pathways, including genetic and environmental sex determination. The red-eared slider turtle (Trachemys scripta elegans) exhibits temperature-dependent sex determination (TSD), where extrinsic thermal cues trigger gonadal differentiation during the thermosensitive period. During the thermosensitive period, embryos respond to relatively cooler or warmer temperatures to initiate male and female development, respectively, but we do not yet fully understand how temperature affects the molecular mechanisms of sex determination. In this study, we are targeting a candidate protein for driving sex determination in species with TSD, cold-inducible RNA-binding protein (Cirp). Cirp is a heat shock protein present in the gonadal tissues of many vertebrates and it has a potential regulatory role in the sex-determining pathway for T. s. elegans. Cirp has sexually dimorphic, temperature-dependent expression in T. s. elegans and other TSD species. In addition to Cirp, we are also investigating the potential for intron-retention (IR) to impact sex determination in T. s. elegans. IR has been proposed as a regulatory mechanism for sex-specific development in a variety of taxa. RNA-binding proteins can impact gene expression by: 1) stabilizing the bound transcript for transport into the cytoplasm, or 2) triggering degradation of the bound transcript by...
recognizing a retained intron that contains a premature stop codon. The objectives of the proposed research are to evaluate the role of Cirp in gonadogenesis in T. s. elegans by determining the transcript targets of Cirp and evaluating those targets for intron retention. This will allow us to determine if Cirp binds to ovarian-inducing or testis-inducing transcripts and if the bound transcripts contain retained introns with premature stop codons, thereby implicating Cirp as a regulator of gonadal development in T. s. elegans. Turtle eggs were purchased from Concordia Turtle Farm (Concordia, LA) and incubated under fluctuating temperatures within the range of naturally-occurring, sub-surface soil temperatures to mimic an incubating nest. At the start of the thermosensitive period, eggs experienced thermal shifts in the form of heat waves to initiate gonadal differentiation. The duration of the heatwaves were selected to initiate bipotential gonadal development, to allow for testis development, or to trigger ovarian development. Gonads have been harvested from embryos for use in immunoprecipitation and RNA-seq to capture mRNAs bound to Cirp. We will evaluate potential impacts of IR using a T. s. elegans de novo transcriptome assembly derived from genes expressed at male-and female-producing temperatures in developing T. s. elegans embryos. Transcripts will be aligned to the closely-related painted turtle (Chrysemys picta) proteome to identify alignment gaps that correspond to retained introns. We are presently validating the binding efficiency of the Cirp antibody, which has not been specifically developed for T. s. elegans. The proposed research represents the first year of a multi-year project investigating the functional role of Cirp in vertebrate sex determination and its impact on the nuclear expression of reproductive genes. This study was approved by ISU IACUC. Funding by: NIH (#1R15ES023995-01), APS Porter Physiology Development Fellowship, ISU Mockford-Thompson Fellowship, and Beta Lambda Phi Sigma Biological Honor Society.

28.40 Determinants of growth in hybrid sunfish: asymmetries in expression of maternal and paternal myogenin genes. Chris Moyes1, Rachel Soon-Shiong1, Zhilin Chen1, Shawn Garner1, Bryan Neff2

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Sunfish are an intriguing model to study the evolution of metabolism and growth because of inherent differences in their hypoxia tolerance and life history strategies. However, these species can also unidirectionally hybridize when precocious male bluegill (BG, Lepomis macrochirus) sneak into pumpkinseed (PS, L. gibbosus) nests to fertilize eggs. Interestingly, though the BG fathers are phenotypically small, the hybrids demonstrate larger body size, as is seen in other models of heterosis. Previous studies have shown that BG are much less hypoxia tolerant than PS, but their hybrids have very low tolerance for hypoxia, consistent with a metabolic dysfunction. In this study, we took advantage of the unidirectional hybridization to explore the maternal and paternal patterns of expression of genes associated with body size. We considered that the large body size of hybrids might result from elevated expression of one or more growth factor genes, a dysfunction that might arise if hybrids escaped epigenetic silencing of maternal or paternal alleles that is seen in other models of growth. We surveyed the sequences of a series of growth factors and, where possible, created primers that could discriminate between each parental species. We then examined PS/maternal and BG/paternal expression in hybrids. Though most of the genes examined (myoD1, IGF2, IGFBP5, MEF2, follistatin) showed similar expression of BG and PS genes, myogenin showed marked differences in maternal and paternal gene expression. Specifically, in about one third of the hybrids, BG/paternal myogenin expression was more than an order of magnitude lower than PS/maternal expression. Since myogenin stimulates muscle growth, clearly this depressed myogenin expression was not consistent with the larger body size of hybrids. However, we continued to explore whether this pattern might be a reflection of the genotypes of the small-bodied precocious BG males. We sequenced upstream promoter regions of BG myogenin to see if we could find genetic markers linked to low expression individuals. We identified a series of diagnostic sites for 4 alleles of BG myogenin (A1, A2, A3, A4). We found that 90% of the hybrids displaying very low BG myogenin expression possessed the A4 allele, and 10% the A3 allele. In contrast, the A4 allele was found at about 55% frequency in BG. This appeared to be consistent with a model where enrichment of the A4 allele in hybrids might reflect an overrepresentation of this allele in the genotypes of the small male sneakers and satellites. However, genotyping of males that adopted the sneaker/satellite life history strategy were not found to differ from the population at large in myogenin allele frequency. Though the unexpected allele-specific suppression of myogenin is intriguing, it appears not to be linked to a phenotypic outcome in either BG or hybrids. Supported through NSERC Discovery Grants to CDM and BDN.

28.41 Hydrogen sulfide metabolites in tissues of normoxic and anoxic freshwater turtles (Trachemys scripta) Birgitte Jensen1, Christopher Kevii2, Angela Fagoe1

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Since the discovery that at low levels hydrogen sulfide (H2S) acts as a neuromodulator in the brain, H2S has
gained acceptance as a vital signaling molecule controlling key physiological functions, such as metabolism, cytoprotection, oxygen sensing and vascular tone. Here, we investigate whether and how H$_2$S metabolism remodeling is involved in the adaptation to anoxia of freshwater turtles (Trachemys scripta), one of the very few vertebrates capable of overwintering up to several months in complete absence of oxygen without major tissue damage. As H$_2$S is inactivated by O$_2$, we speculate that H$_2$S signaling could have a prominent impact on tissue metabolic depression during anoxia and cytoprotection.

Turtles were acclimated to low temperature and to normoxia or anoxia. After 9 days of exposure, tissue samples were collected. Sulfide content was analyzed with a fluorescent monobromobimane assay coupled with reverse-phase high-performance liquid chromatography (RP-HPLC) to identify major H$_2$S storage pools, namely bound sulfane sulfur (mainly persulfides, polysulfides and thiosulfate) and acid-labile sulfide (mainly iron sulfur clusters).

The total pool of sulfide (including bound sulfane sulfur and acid-labile sulfide) in tissues was lowest in the lung, moderate in liver, kidney and brain, and highest in red blood cells, where we found a very high content of bound sulfane sulfur, which may reflect the high protein thiol content of these cells. Overall, there was no major difference in sulfide content between the normoxic and anoxic turtles, except for a decrease of the bound sulfane sulfur pool in the anoxic brain. Whether changes in the free H$_2$S are linked to anoxia-adaption in turtles remains to be investigated further.

**28.42**

Effects of hind limb immobilisation and castration on [$^3$H]ouabain binding site content and Na$^+$, K$^+$-ATPase isoform abundances in rat soleus muscle

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The Na$^+$, K$^+$-ATPase (NKA) protein plays a critical role in skeletal muscle function via maintenance of the [Na$^+$] and [K$^+$] gradients across sarcolemmal and t-tubular membranes, and is comprised three $\alpha$ (\$\alpha_1\$ - \$\alpha_3\$) and three $\beta$ (\$\beta_1\$ - \$\beta_3\$) isoforms. No study has yet investigated the possible effects of testosterone suppression on skeletal muscle NKA particularly after immobilisation. We therefore investigated the effects of hindlimb immobilisation and testosterone suppression via castration surgery on rat soleus muscle NKA as measured by [$^3$H]ouabain binding site content and NKA isoform abundances.

**Methods:** Eight week old male Fischer rats underwent sham or castration surgery, and then after 7 days were subjected to 10 days of immobilisation of one hindlimb. For both sham and castration groups, soleus muscles were obtained 7 d after surgery from non-immobilised controls, following 10 days immobilisation and after 14 days of recovery, from both the cast and non-cast leg.

**Results:** Within the sham group, after immobilisation, the [$^3$H]ouabain binding site content in the cast leg was 26% lower than in the non-cast leg (p = 0.023) and 34% lower (p = 0.001) than in the non-immobilised control group (P = 0.012), but did not differ at 14 d recovery compared to either the non-cast leg or non-immobilised control group. There were no differences in the NKA $\alpha_1$, $\alpha_2$, $\alpha_3$, $\beta_1$ or $\beta_2$ isoform abundances in the cast leg compared to either the non-cast leg, or the non-immobilised control group, after immobilisation, or at 14 d recovery.

Within the castration group, the [$^3$H]ouabain binding site content in the cast leg after immobilisation was 34% lower (p = 0.001) than in the non-immobilised control group and remained depressed by 34% (p = 0.001) at 14 d recovery after immobilisation. The $\alpha_2$ isoform in the cast leg was 60% lower than in both the non-cast leg (p = 0.004) and non-immobilised control group (p = 0.004) and remained 42% lower than the non-immobilised control group at 14 d recovery (p = 0.039). The $\beta_1$ isoform in the cast leg after immobilisation was 26 % lower than in the non-cast leg (p = 0.018), but did not differ at 14 d recovery compared to either the non-cast leg or non-immobilised control group. The $\beta_2$ isoform in the cast leg after immobilisation was 71% lower than the non-cast leg (p = 0.004) and 65% lower than non-immobilised control group (p = 0.012), but did not differ at 14 d recovery, compared to either the non-cast leg or non-immobilised control group. There were no differences in the abundances of the $\alpha_2$ and $\alpha_3$ isoforms between legs or groups. The $\beta_1$ isoform abundance could not be detected in either sham or castration groups.

**Conclusions:** Thus the [$^3$H]ouabain binding site content and $\alpha_2$ were decreased with immobilisation, and remained depressed at 14 d recovery in the castration group. With impaired restoration of immobilisation-induced lowered NKA $\alpha_2$ isoform and of the number of functional NKA in rat soleus muscle.

**28.43**

Preliminary survey of homeodomains in Lumbriculus variegatus

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Several animal clades contain members capable of body part regeneration. In particular, many annelids have remarkable regenerative abilities. A recent review of annelid regeneration noted the utility of examining a wide variety of annelid species to help uncover
conserved versus derived regenerative abilities (1). The annelid L. variegatus is a superb model organism for regeneration research (2), but is poorly characterized at the molecular level. Since Hox genes code for transcription factors that help establish positional identity during animal development and regeneration (3), we sought to study the expression of Hox genes during L. variegatus regeneration. As a first step we cloned Hox genes from L. variegatus. Genomic DNA was extracted from L. variegatus and conserved homedomain sequences were amplified via PCR with degenerate primers (4). PCR products of the expected size were inserted into the pGEM-T Easy vector and sequences from 18 plasmids were placed into six different groups based on sharing greater than 90% amino acid identity. A member of each group was compared to homedomain sequences from other organisms and named Lva (L. variegatus) followed by the paralog group it is part of (if one could be ascertained). We identified Lva-Scr (95% a.a. identity to earthworm P. excavatus (Pex) Scr), Lva-Lox2 (97% a.a. identity to Pex-Lox2), and Lva-Lox5 (95% a.a. identity to Pex-Lox5), all part of the central group Hox gene cluster, and an XloX paralox (92% identity to Pex-XloX), part of the ParalHox gene cluster. Two sequences do not show high similarity to any homedomains (less than 84% a.a. identity to any Pex homedomains screened). To our knowledge this is the first report of homedomain sequences from the Lumbriculidae family of annelids. Our study may pave the way for analyzing Hox gene expression along the anterior-posterior axis of adult L. variegatus and during regeneration following amputation.

References:

Whole Genome de novo Sequencing of the Atlantic and Pacific Bluefin Tuna Genomes
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Accurate genome assembly is a critical first step for in depth studies of physiology, population genetics, and evolution. Here, we report the first long-read draft assemblies of the Atlantic (ABFT) and Pacific (PBFT) bluefin tuna genomes. We extracted high quality genomic DNA from one individual from each species, which was sequenced with single-molecule, real-time (SMRT) sequencing technology. Long read sequence data (average read lengths >15kb) was generated to over 80X genomic coverage for each sample. Diploid assemblies were generated using the FALCON-Unzip assembly algorithm designed for long sequence reads (>10kb). Genome assembly completeness and the degree of heterozygosy of each genome assembly was assessed with the benchmarking universal single-copy orthologs dataset (BUSCO, version 3). The FALCON-Unzip genome assembly algorithm generated a 1.59 Gb assembly for ABFT and a 1.24 Gb assembly for the PBFT. The primary N50 was 4.58 Mb for ABFT and 4.96 Mb for PBFT. In both assemblies, the largest contigs were in the 20Mb range approaching theoretical sizes in the chromosomal arm scale. The assembly completeness analysis using the BUSCO gene model set found 95% completeness for ABFT and 93.2% completeness PBFT. The degree of heterozygosy in each assembly found 85.9% duplicated BUSCOs in ABFT and 52.2% duplicated BUSCOs in PBFT, suggesting a higher degree of haplotype merging in the PBFT assembly. Coverage estimates across each assembled base in the two assemblies found ~5% collapse in the ABFT assembly and ~30% collapse in the PBFT assembly. Based on these findings the genome sizes were re-estimated to be 1.67 Gb for the ABFT assembly and 1.61 Gb for the PBFT assembly. These estimated genome sizes are roughly twice the haploid genome sizes published to date for these species suggesting that the assemblies have resolved a majority of the polymorphisms inherent within these individual genomes. Additional data types will be required to finish the annotation of these genomes. The high-quality initial draft assemblies of the Atlantic and Pacific bluefin tuna genomes resulting from long-read technology provide a more complete picture of gene-content and structural variation to help guide future studies of physiology and evolution in tunas.
CLINICAL PHYSIOLOGY: ABSTRACT DRIVEN SESSION

29.1 Blood glucose levels and hyperbaric pressure in SOD2 enzyme knockdown mice.
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Introduction: Large numbers of studies have been conducted in the search for the mechanism underlying CNS-oxygen toxicity (CNS-OT), the outcome of which may be fatal when diving with closed-circuit apparatus. In our previous studies in rats, we found a linear correlation between the partial pressure of oxygen at 4–6 atmospheres absolute (ATA), blood glucose levels (BGL), and changes in the membrane potential of the mitochondria. In transgenic mice, knockdown of the antioxidant enzyme Mn-superoxide dismutase (SOD2), which is found only in the mitochondria, resulted in an increase in oxidative stress. In light of this information, it would appear that plasma glucose is influenced by oxidative stress, which in turn depends on the activity of the enzyme SOD2.

Hypothesis: We hypothesized that underexpression of SOD2 would result in greater elevation of BGL than may be seen in the WT as oxygen pressure increases. The purpose of the study was to verify whether BGL might serve as a marker for the development of CNS-OT.

Methods: The study was conducted on 2 groups of mice: 1. Knockdown SOD2; 2. Wild type (WT). Latency to CNS-OT was measured by preliminary exposure of animals to hyperbaric oxygen (HBO) at 5 ATA, and this was used to derive the time for subsequent exposure at the lower pressures. Mice were exposed to HBO from 2–5 ATA in increments of 1 ATA/wk for 60% of their latency to CNS-OT (no convulsions). BGL were measured before and immediately after each exposure. We evaluated the influence of hyperglycemia and hypoglycemia on latency to CNS-OT by injection of 25% glucose solution and 6 U/kg insulin, respectively, prior to HBO exposure.

Results: Glucose levels increased after HBO exposure at 3–5 ATA in the WT mice, whereas in the transgenic mice blood glucose levels increased after HBO exposure at 2–5 ATA. Latency to CNS-OT did not differ between the transgenic mice and the WT on exposure to 5 ATA. However, after the induction of hyperglycemia, latency in the WT mice was prolonged in comparison with the transgenic mice, and compared with the latency observed without hyperglycemia. No change was noted in the transgenic mice. Following the induction of hypoglycemia, latency in the transgenic mice was shorter than it had been without hypoglycemia. No change was noted in the WT mice.

Conclusion: The induction of hyperglycemic and hypoglycemic states showed that transgenic mice with knockdown of the antioxidant SOD2 are more sensitive to oxidative stress. This may be an indication that the mitochondria play a significant role in the development of CNS-OT. Further investigation of mitochondrial activity will be required to assess this theory.

29.2 A proposed role for the mammalian dive response in sudden unexpected death in epilepsy
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Epilepsy patients die suddenly nearly thirty times more frequently than the general population. Victims are often found dead, in bed. Seizures are occasionally witnessed prior to death, but often only a hint of one is discovered (e.g., a tongue bite). This enigmatic phenomenon is known as sudden unexpected death in epilepsy (SUDEP). Clinicopathological reports indicate that SUDEP is most commonly associated with prolonged apnea, severe bradycardia and pulmonary edema. This paper explores important parallels between the physiopathology of SUDEP and that induced by the human form of the mammalian dive response (MDR), as the latter is known to include apnea, hypertension, bradycardia, and the translocation of a large fraction of the total blood volume into the pulmonary vasculature. The author hypothesizes that the MDR is triggered during apneic-generalized-tonic-clonic seizures and, in the worst instances, the drastic cardiovascular adjustments induced by this response result in SUDEP.

29.3 A COPMARITIVE STUDY OF PULMONARY SLOWLY ADAPTING RECEPTORS BETWEEN RABBITS AND RATS
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Pulmonary mechano-sensory receptors provide important inputs to the respiratory center for control of breathing. However, our knowledge regarding the receptor structure-function relationship is still limited. In the current studies, we explored the relationship by comparing the morphology and function of pulmonary slowly adapting receptors (SARs) between rabbits and rats. Sensory units of SARs were recorded from anesthetized, open-chest and artificially ventilated animals and their electrical activities in response to lung inflation were compared at 3 different constant airway pressures (10, 20 and 30 cm H2O). We found that the discharge frequencies were higher in rabbits than in rats. Peak discharge frequencies at pressures of 10, 20 and 30 cmH2O were 83±6, 139±8 and 200±11 impulses/sec for rabbits and were 49±6, 78±7 and 94±9 impulses/sec for rats, respectively (P<0.001). For morphological studies,
tracheal or bronchial smooth muscles were histochemically labeled with anti-Na+/K+-ATPase (a3 sub-unit). We found that receptor size is larger in rabbits than rats. Averaged receptor sizes were 368.7±20.5 mm² (n=78) in rabbits and 222.9±12.9 mm² (n=73) in rats (P<0.0001). Our results demonstrate that SARs are larger in size and discharge more in rabbits than in rats. It is possible that action potentials are generated from generator potentials, which are, in turn, determined by the local potential on the sensing surface of the receptor. In summation, the larger the surface of a receptor, the greater the generator potential and the discharge frequency, and the lower the activating threshold. Our results support the theory that larger receptors in a sensory unit may result in a lower activating threshold and higher discharge frequency. (Supported by a grant from VA Merit Review Award PULM-024-17S)

29.4

Contribution of Group II Metabotropic Glutamate Receptors in the Dorsal Medullary Neuronal Groups during Hypertension Development

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[Background] It has been known that the nucleus tractus solitarius (NTS) and the area postrema (AP) in the dorsal side of medulla oblongata are crucial sites for regulating blood pressure and controlling baroreflex function via glutamatergic transmission. Although ionotropic glutamate receptors in the NTS and the AP are main receiving docks from baroreceptor sensory information, group II metabotropic glutamate receptors (mGluR2/3) are thought to be modulating baroreflex signal transmission to set the blood pressure properly. Inappropriate baroreflex signalings may cause different set point of blood pressure, e.g. resulting in hypertension, and thus we hypothesized that mGluR2/3 signals are essential to control blood pressure in normal range before developing hypertension.

[Methods] The mGluR2/3 agonist (LY379268; 4, 10, 40 µg/day) was continuously applied onto the dorsal side of the medulla oblongata for 6 weeks, using implantable mini-osmotic pump through a foramen magnum catheter in spontaneously hypertensive rats (SHR) starting at 6 weeks of age. Thereafter, blood pressure was measured twice a week using tail-cuff method more than 9 weeks.

[Results] The systolic blood pressure (SBP) of sham control group increased to 200 mmHg at age of 13 weeks, while SBP in experimental groups showed less than 175 mmHg at 10 µg/day and less than 165 mmHg at 4 µg/day. The 40 µg/day dose of LY379268 application caused low vitality and bradycardia, and thus we considered that this dose was not feasible even though lower SBP was observed. Even after finishing 6 weeks of LY379268 application, SBP in experimental groups was still remaining around 170 mmHg, indicating that blood pressure could be controlled in normal range without mGluR2/3 stimulation in the dorsal medullary neuronal groups.

[Conclusions] Our results suggest that continuous stimulation of mGluR2/3 in the dorsal medullary neuronal groups could prevent hypertension development in SHR and, therefore, the agonist may become a possible prophylaxis medication for hypertension-prone patients.

29.5

The evolving cholecystokinin 1 receptor as a unique G protein-coupled receptor permanently activated by singlet oxygen (GPCR-pABSO)

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The typical class A G protein-coupled receptor cholecystokinin 1 receptor (CCK1R) is unique in that it is permanently activated by singlet oxygen which is normally generated in a Type II photodynamic action. We have previously shown that CCK1R is activated permanently by photodynamic action with the chemical photosensitisers sulphonated aluminium phthalocyanine (SALPC) in freshly isolated rat pancreatic acinar cells. Such permanent photodynamic activation of CCK1R with SALPC could be reproduced in the rat pancreatic acinar tumor cell AR4-2J, and in CHO-K1 cells ectopically expressing the human CCK1R. Further, both CCK1R in AR4-2J cells and ectopically expressed CCK1R in other cell lines (CHO, HEK) could be permanently activated by photodynamic action with genetically encoded protein photosensitisers KillerRed and miniSOG. Ongoing work is examining the potential for CCK1R in Peking duck pancreatic acinar cells and CCK1R analogues from invertebrates to be permanently activated by photodynamic action with SALPC, KillerRed, miniSOG or enhanced variants of KillerRed and miniSOG. Since the CCK-CCK1R system is evolutionally conserved from ancient times the evolution of permanent CCK1R activation by singlet oxygen is actively pursued in our laboratory. The outcomes of such experiments will provide useful tools or toolkits for remote in vivo photon-driven manipulations of the CCK-CCK1R system in animal behaviors such as feeding and higher CNS functions from invertebrates to birds, mammals and non-human primates.

References

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30: OSMOREGULATION ION REGULATION: ABSTRACT DRIVEN SESSION - 1

30.1 Going against the gradient: Active NH₄⁺ excretion by the ammonia tolerant hagfish (*Eptatretus stoutii*).

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Hagfishes (*Eptatretus stoutii*) feed on decomposing carrion drops during which time they may be exposed to high concentrations of total ammonia ([T ammonium] = [NH₃ + NH₄⁺]). Unlike most fishes, hagfishes are incredibly resilient to high environmental ammonia (HEA) exposure, readily surviving at water [T ammonium] of 20 mM but limiting plasma [T ammonium] accumulation to ~5 mM despite large inwardly directed NH₃ + NH₄⁺ partial pressure (ΔPNH₃) and electrochemical gradients (ENH₄⁺) respectively. To test the hypothesis that hagfish used active transport to excrete ammonia under such conditions, hagfish were exposed to sequentially higher [T ammonium] (0-20 mM) for 48 h, and plasma [T ammonium] was measured after 6, 12, 24 and 48 h. In all cases, plasma [T ammonium] were consistently maintained at ~30-70% lower than environmental [T ammonium], despite sustained inwardly directed ΔPNH₃ (~3800 µTorr) and ENH₄⁺ (~40 mV) gradients, supporting our hypothesis. The possibility that NH₄⁺ excretion was facilitated by secondary active transport using Na⁺/NH₄⁺ (H⁺) exchange via apical NHE (Na⁺/H⁺ Exchanger) antiports was then examined at both the organismal and tissue (gill) level. When hagfish were acclimated to 10 mMHEA for 24h and then transferred to HEA-containing artificial seawater (ASW) or Na⁺-free artificial seawater (NFASW), plasma [T ammonium] increased by ~40%, suggesting that Na⁺ was a necessary substrate for ammonia stabilization. In a separate series, HEA-acclimated were infused with ¹⁵C-methylamine (¹⁴C-MA), a radiolabeled analogue of NH₄⁺ prior to transfer to HEA-ASW or HEA-NFASW for measurement of apparent JNH₄⁺ (inferred from J¹⁴C-MA). While minimal JNH₄⁺ was observed in control hagfish not acclimated to HEA following transfer to HEA-containing ASW, JNH₄⁺ increased by ~60-fold in the HEA-acclimated hagfish following transfer to HEA-ASW; yet only a 14-fold increase was observed in animals transferred to HEA-NFASW. These results further support the hypothesis that Na⁺ is a required counterion for active NH₄⁺ excretion. Next, we utilized a newly established in situ hagfish dual gill perfusion/perfusion technique to characterize the role of the NHE, using amiloride, and ¹⁴C-MA. After the intact hagfish were acclimated to 10 mM HEA for 24 h, the afferent gill pouch arterioles were surgically cannulated and perfused with ¹⁴C-MA/4 mM [T ammonium] hagfish saline while the water ducts draining the gills were cannulated and perfused with 10 mM HEA-ASW spiked with amiloride (500 µM), a putative NHE inhibitor. Amiloride application resulted in a 52% reduction of JNH₄⁺, compared to control fluxes suggesting that there was appreciable Na⁺/NH₄⁺ exchange. We conclude that an active Na⁺/NH₄⁺ (H⁺) exchange mechanism, likely mediated via NHE, is used by the hagfish to excrete ammonia against large inwardly directed ΔPNH₃ and ΔNH₄⁺/H⁺ gradients which may be encountered when feeding on decomposing animal carcasses on the ocean floor. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

30.2 Ammonia transporter expression and distribution in organs of Caribbean subpopulations of the mosquito, *Aedes aegypti*, collected from freshwater and high ammonia habitats.

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Surveys of the presence and abundance of mosquitoes in various urban habitats have demonstrated that *A. aegypti* may select septic tanks as a preferred breeding site, and emerging adults are larger and have significantly higher nutrient reserves. Sewage is typically rich in ammonia (NH₃/NH₄⁺) and whilst ammonia serves as an important nutrient for microorganisms and plants, it is toxic to animal cells at relatively low levels. Insects are generally more tolerant to high ammonia in comparison to other animal groups and this may explain the prevalence of *A. aegypti* larvae in high ammonia environments. Important ammonia-excreting organs of these larvae which are in contact with the surrounding environment, the anal papillae, express four ammonia transporters; RH proteins AeRh50-1 and AeRh50-2, and Amt/Mep proteins, AeAmt1 and AeAmt2. We have previously shown using RNAi that each RH andAmt protein functions in ammonia excretion at the anal papillae, as well as contributing to hemolymph ammonia homeostasis in mosquitoes from a laboratory colony. The objective of the present study was to investigate ammonia transporter gene expression within Caribbean subpopulations of *A. aegypti* mosquitoes developing and emerging from clean freshwater or high ammonia sewage breeding sites (i.e. septic tanks). We hypothesized that the populations of *A. aegypti* collected from septic tanks would be highly specialized in their ammonia transporting capabilities to tolerate living in these habitats through measurable differences in ammonia transporter expression. We examined the
expression and distribution of Rh and Amt mRNA and protein in the organs of larvae and adults using quantitative PCR, Western blotting, and immunohistochemistry. Results are presented as a comparison between freshwater and sewage collected mosquitoes. We conclude that these populations of A. aegypti show vast plasticity in ammonia regulatory and excretory pathways that may contribute to their successful inhabitancy of these high ammonia environments.

### 30.3 Potential Role of a Rh Channel in Delivery of Ammonium from Coral Host Cells to Their Endosymbiotic Algae

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**Intro:** Coral reefs are facing mounting threats in the Anthropocene Era, yet basic coral biology remains largely uncharacterized. The purpose of this study is to examine the unexplored physiological mechanisms allowing for the exchange of nutrientous molecules, such as NH$_3$/NH$_4^+$, between corals and their endosymbiotic algae. We identified a Rhesus protein (Rh) in the coral Acropora yongei and provide evidence it regulates nitrogen supply to their endosymbiotic algae. **Methods:** Molecular biology and immunohistochemistry techniques were employed to clone A. yongei Rh (ayRh) and investigate its physiological role in the coral-algal symbiosis. Custom rabbit-anti-ayRh antibodies were developed by GenScript USA and validated. **Results:** The cloned ayRh cDNA sequence coded for a 52 kDa protein with 12 predicted transmembrane domains typical of Rh proteins. Phylogenetic analysis determined ayRh to be part of the invertebrate Rhp1 subgroup, members of which enhance the diffusion of NH$_3$ gas. Immunocytochemistry on coral tissues and isolated cells revealed ayRh was colocalized with the vacuolar H$^+$-ATPase (VHA) in the host-derived symbiosome membrane which surrounds the algae and mediates molecule exchange between host and endosymbiotic cells. Localization of ayRh in the symbiosome membrane fluctuated on a diel trend as the percentage of cells exhibiting symbiosome ayRh-localization was significantly greater in the daytime compared to the nighttime (52 ± 5% v. 31 ± 2%; n=3). **Conclusions:** We propose ayRh and VHA in the symbiosome membrane constitute a novel nitrogen concentrating mechanism whereby ayRh facilitates the diffusion of NH$_3$ and VHA acidifies the symbiosome space resulting in NH$_4^+$ trapping. The diel variations in symbiosome ayRh localization suggest a mechanism that allows coral host cells to regulate algae metabolism and growth. Enhanced supply of NH$_3$/NH$_4^+$ during daytime may provide algae with nitrogen only when required for the maintenance of photosystems and the production of photosynthesis-derived products that are translocated to the coral host. During nighttime, diminished nitrogen supply may limit algal growth and maintain coral control over the symbiosis.

### 30.4 The dual-purpose saltwater mitochondria rich (MR) cell of sea lampreys (Petromyzon marinus): an organ of osmoregulation and ammonia homeostasis

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The life cycle of sea lampreys comprises a prolonged 3-7 year filter-feeding, larval (“ammocoete”) phase, followed by a multi-stage metamorphosis which prepares them for the blood-feeding juvenile “parasitic” stage in sea water. The goal of our study was to determine how gill structure and function changes following metamorphosis to enable sea lamprey to cope with the dual challenges of osmoregulation and high rates of ammonia production arising from the ingestion of vast quantities of protein-rich blood. Ammocoetes, captured from rivers draining into the Northumberland Strait, were held in freshwater (T=19°C) for 3-4 months, where approximately 40% of animals entered metamorphosis. Rates of ammonia excretion averaged 100 nmol/g.h, but decreased by 50-75% during metamorphosis, before increasing several-fold following metamorphosis. Immunohistochemistry and western blot analyses revealed that changes in J$_{Am}$ were accompanied by a marked redistribution of ammonia-transporting, Rhcg-like glycoproteins (Rhcg). In ammocoetes, Rhcg-like proteins and V-ATPase had a punctate distribution on the lamellae. Following metamorphosis, however, Rhcg-like protein abundance massively increased, co-localizing with Na$^+$/K$^+$-ATPase (NKA) on the basolateral membrane of saltwater mitochondria rich (MR) cells, within the intra-lamellar spaces. Saltwater acclimation and feeding lead to further increases in Rhcg-like protein abundance in SW MR cells, during which time most ammonia was excreted via the gills. V-ATPase abundance decreased markedly, however. We propose that ammonia excretion across the lamprey gills in saltwater is via Rhcg-like proteins, which transport NH$_4^+$ into the cytosol from which it is excreted to the water via an apical Na$^+/$(NH4$^+$)/H$^+$ antiporter. We conclude that in addition to osmoregulation, the SW MR cells augment ammonia excretion by juvenile lampreys when they are ingesting large quantities of protein-rich blood during their saltwater parasitic phase.
Impact of sugar beet de-icing liquid on salt and water balance in mayfly nymph, *Hexagenia limbata*

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With increasing awareness of the negative environmental impact of road salt (NaCl) as a de-icing agent, alternative road de-icers are being developed and marketed as environmentally friendly. This study investigated osmoregulatory changes in freshwater (FW) mayfly nymphs (*Hexagenia limbata*) following chronic exposure to beet juice de-icer (BJD), an alternative to conventional road salt. As benthic macroinvertebrates, mayflies can act as water pollution indicators as they are particularly sensitive to runoff contaminants such as road salts and metals. Knowing that beet juice contains high concentrations of K⁺ which is an important ion in maintaining electrochemical gradients; it was hypothesized that excess runoff into FW may disrupt ionoregulatory mechanisms of mayfly nymphs, in part since K⁺ settles in the sediment where some nymph species, such as *H. limbata*, burrow. A seven day chronic toxicity test was first carried out to establish LC₅₀, after which mayfly nymphs were exposed to a sub-lethal dose. Following exposure, measurements of hemolymph ions (K⁺, Na⁺, Cl⁻, NH₄⁺ and H⁺) as well as Na⁺-K⁺-ATPase and V-type H⁺-ATPase activity in tracheal gills, rectum and Malpighian tubules were used to evaluate any perturbations in systemic salt and water balance. The LC₅₀ value for BJD was measured at 40 ppt and the sub-lethal dose of 32 ppt was further used for the seven day exposure. Hemolymph Na⁺ and pH were significantly higher in BJD nymphs compared to FW nymphs. In contrast, no significant difference was recorded in K⁺, Cl⁻ and NH₄⁺ hemolymph concentration although the trend showed an increase in Cl⁻ and a decrease in K⁺ and NH₄⁺ in BJD nymphs compared to FW nymphs. To our knowledge, this is the first study to explore the physiological effects of BJD on FW organisms. This is an important initial step in identifying any potential risk to FW ecosystems that relate to the introduction of BJD products as an environmentally friendly alternative to conventional road salt.

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Exploring how a shifting gut microbiome may influence the hibernation phenotype

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Some animals hibernate during periods of food scarcity, surviving up to nine months without eating. This alters the abundance and composition of the hibernator’s gut microbiota, which, because of the microbiota’s role in supplying its host with metabolites, may influence the hibernator’s own metabolism. To explore this possibility, we orally gavaged 13-lined ground squirrels with ¹³C-labeled substrates that are degraded only by microbes, and then used stable isotope-based techniques to 1) measure the effects of hibernation on the microbiota’s function, and 2) track the movement of microbial-derived metabolites through the squirrel. To measure microbiota function, we used cavity ring-down spectrometry to monitor in real time the δ¹³C of the squirrel’s exhaled CO₂. Higher values indicate higher rates of substrate metabolism by the microbiota. We found that season significantly influenced these ratios, which peaked in summer and reached a nadir in winter, indicating a diminished microbial metabolic capacity during hibernation. Next, to track microbial-derived metabolites, we used NMR-based metabolomics to identify how ¹³C-labeled metabolites in gut contents and host tissues varied with season (results pending). This approach will reveal the potential for hibernation-induced microbiota changes to influence the squirrel’s metabolic plasticity, the hallmark of the hibernation phenotype.

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Unraveling the Complexity of Seasonal Phenotypic Flexibility in Small Birds via Omics Integration

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Non-migratory small birds in seasonal climates must contend with changes between summer and winter conditions, including alterations in temperature and food availability and type. These birds typically respond by upregulating the capacity for muscular shivering thermogenesis in winter, including changes to either or both muscle masses and cellular metabolic intensity. To better understand the mechanistic basis of seasonal phenotypic flexibility, we conducted a large-scale metabolic profiling of pectoralis muscle in two small North American songbird species, American goldfinches.
(Spinus tristis) and black-capped chickadees (Poecile atricapillus) during summer and winter seasons. These muscle samples were analyzed using non-biased, global metabolomics profiling technology based on UHLC/MS/MS² platforms. We integrate these non-targeted metabolomics data with previously published transcriptomics data for the same tissues to gain insight into how combined gene expression and metabolic profiles can provide a more detailed mechanistic understanding of the cellular responses to seasonal changes in organismal metabolic capacity of two small songbirds. This ‘omics integration has provided an unprecedented view into the complex regulatory and biochemical mechanisms orchestrating seasonally flexible phenotypes. The metabolomics work was supported by the Department of Biology, Univ. of South Dakota startup funds to BWMM, whereas the transcriptomics work was supported by the National Science Foundation IOS 1021218 to DLS and partially supported by the National Science Foundation IOS 1354934 and IOS 1634219 to ZAC.

31.3
Withdrawn

31.4

The Role of Microbial Symbionts in Bonnethead Shark Seagrass Digestion

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Sharks, uniformly accepted as carnivores, have guts specialized for a high-protein diet. However, the bonnethead shark (Sphyra tiburo) has been shown to consume copious amounts of seagrass (up to 62.1% of gut content mass)¹. Bonnetheads were found to digest 51.2% of the organic matter in seagrass², as well as possess cellulose-component-degrading enzymes (β-glucosidase)² in their hindguts. This indicates likely involvement from the gut microbiome as part of the seagrass digestion process. In order to understand the role that the microbiome plays in bonnethead shark seagrass digestion, gut contents and mucosal scrapings were collected along the digestive tract of wild-caught bonnetheads (n=4) and bonnetheads that were fed a 90% seagrass and 10% squid diet in the lab (n=5). Using 16S rDNA sequencing, we determined the taxa of microbes present along the bonnethead shark digestive tract. Using gas chromatography of gut content fluid, we determined the concentrations of short-chain fatty acids, the primary end-products of microbial fermentation, in the different gut regions. Data collection is in progress. Results of this work show that the bonnethead shark is digesting seagrass at higher efficiency than would be expected for a “carnivore,” and digestion may be aided by microbial symbionts. These findings have ecological implications because they show that bonnethead sharks are omnivorous and play a different role (including nutrient transport) than assumed within fragile seagrass ecosystems. Funding: UCI Newkirk Center for Science and Society, the UCI Microbiome Initiative, National Geographic, and an NSF GRFP. References: ¹Bethua DM, Hale L, Carlson JK, Cortés E, Manire CA, Gel- sleichter J (2007) Geographic and ontogenetic variation in the diet and daily ration of the bonnethead shark, Sphyra tiburo, from the eastern Gulf of Mexico. Mar Biol, 152: 1009–1020. ²Leigh SC, Papastamatiou YP, and German DP (under review) Seagrass digestion by a notorious ‘carnivore.’

31.5

Will abalone survive climate change? Comparative digestive physiology and the effect of temperature stress on abalone across the Pacific Ocean

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Abalone in the northeastern Pacific Ocean have experienced massive population declines over the last century due to historic overfishing, and more recently, disease and stress. Withering syndrome (WS), a bacterial disease, infects the digestive systems of North American abalone and results in starvation and eventual death in affected animals. WS has led to population declines in all species infected (Crosson et al. 2014), one of the largest threats to the Endangered black abalone, and affects animals more often and more severely during periods of thermal stress (Moore et al. 2009). Despite the severe impact of WS and the ongoing threats of thermal stress from climate change, the understanding of mechanistic impacts of WS and heat stress on abalone digestive function is unknown. This study aims to fill this critical knowledge gap by determining the digestive strategy of abalone and characterize their gut function. To do this, we examined gut function in wild red abalone (H. rufescens) and compared their gut function to distantly related New Zealand pāua (H. iris), because this species is thought to be basal with respect to the Haliotids and serves as a WS-free control, as WS does not exist in New Zealand. We tested two hypotheses: (1) Abalone use a rate-maximizing digestive strategy; and (2) Abalone digestive function is unable to keep pace with increased metabolic demand at the highest temperatures they experience in the wild, thereby making them more vulnerable to heat stress and WS in the face of climate change. To test hypothesis 1, we collected wild red abalone from northern California and wild pāua from Wellington, New Zealand, and measured enzyme activities, fermentation byproducts, and microbiome communities in distinct gut regions. To test hypothesis 2, we conducted 4-week thermal stress experiments with
both species. Abalone/pāua were divided into two temperature regimes, ambient seawater and 6°C above ambient. We fed animals known diets, and collected fecal material daily. After 4 weeks, we measured organic matter digestibility, as well as digestibility of individual macronutrients (protein, carbohydrate, and lipid) digestibility to determine how thermal stress impacts the animals’ ability to extract nutrients and energy from their diet. We also measured metabolic demand at both temperatures to determine whether the changes in digestive function enable animals to extract enough energy to meet their increased metabolic demand at elevated temperatures. Preliminary results show that metabolic demand is significantly elevated during the thermal stress treatment. We predict that ongoing analyses will show that digestive efficiency will not keep pace with increased metabolic demand and abalone will thus have a net energy deficit during thermal stress. This is the first detailed study on abalone digestion, and collaborations with international fishing, aquaculture, and management agencies is enabling this physiological data to be integrated into management.

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31.6

Quickly Becoming an Omnivorous Lizard: interactions of diet, physiology, and ecology lead to dynamic changes in a rapidly evolving system

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After an experimental relocation, a population of Italian Wall Lizards, Podarcis sicula, on the island Pod Mrčaru in Croatia became omnivorous and morphologically distinct from its source population on the nearby island of Pod Kopište in <36 years (~30 generations). Additionally, the new omnivores consumed >2x more food than their Pod Kopište counterparts. Observed changes in the Pod Mrčaru lizards, such as the presence of valves in the hindgut, suggest a shift in digestive tract function to accommodate this drastic dietary change.

Determining what an animal eats and how it digests its food requires a multi-faceted approach that considers not only diet, but also digestive tract structure and function. Using Chemical Reactor Theory, a gut can be modeled as a series of chemical and physical reactions, predicting chemical and physical adaptations in the gut to accommodate dietary input. This theoretical framework provides the “ultimate” reasons for why specialization of digestion should arise. Thus, we hypothesized this new population of lizards would have physiological and morphological adaptations for plant eating. We characterized the shifts in form and function of these lizards’ digestive tracts in the context of diet and ecology. By comparing the new and source populations, we can test the “proximate” mechanisms through which specialization arises, and the consequences of specialization on organismal performance.

We measured digestive performance of the newly omnivorous lizards of Pod Mrčaru and the Pod Kopište source population on three experimental diets: plant, insect, and mixed. When fed daily, the newly omnivorous Pod Mrčaru lizards had a higher digestibility of plant diets than their source population, attributable to better digestibility of plant-proteins in the Pod Mrčaru lizards. The two populations did not differ in digestibility of the insect diet. However, when fed half as often, a different pattern emerged. Male lizards fed every two days showed no population differences in digestibility of each diet. Surprisingly, female Pod Mrčaru lizards were more efficient at digesting the insect only diet—overall and with respect to carbohydrates and protein—than their Pod Kopište counterparts.

To understand the mechanisms of these performance differences, we investigated the digestive physiology of each of these populations of lizards in their ecological contexts, on a wild diet. We found no differences in gut morphology, apart from the already discovered cecal valves. Digestive enzymes corresponding to major nutrient classes and specific plant- or prey- based food sources did not differ in proximal regions of the gut, where the lizards would have produced these enzymes endogenously. However, in the distal intestine, where we primarily expect microbial digestion to occur, several enzyme activities were higher in the plant eating Pod Mrčaru lizards, although not just enzymes associated with plant digestion This implicates endosymbiotic microbes as the likely source of the functional shift in the gut over this short time span.

The Pod Mrčaru lizards proportionally more plant material in the summer, up to 70% of their diet compared to 30% in spring. The Pod Kopište population, on the other hand, consumed 4-7% plant material year round. While stomach contents revealed no sex differences in diet, stable isotope analyses of the lizards’ tissues indicated differences in C and N assimilation by population, sex, season, and even year. Analyses of gut morphology and digestive enzyme activities also reveal interactions of population, sex, and season. Our results suggest that males and females accommodate this dietary shift using different strategies.
Overall, the physiology and ecology of the lizards in this system are dynamic and context dependent, with the hindgut playing a central role in this rapid evolutionary shift. This research was funded by NSF, CNRS, the US Department of Education, the Company of Biologists, and Sigma Xi.

32: CONDUCTING MECHANISTIC INVESTIGATIONS IN COMPARATIVE PHYSIOLOGY USING IN VITRO AND EX VIVO SYSTEMS

32.1
Induced pluripotent stem cells from 13-lined ground squirrels: to learn “hibernation” in a dish?
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Hibernating mammals survive profound hypothermia (<10°C) without injury, a remarkable feat of cellular preservation that bears significance for potential medical applications. However, mechanisms imparting cold-resistance, such as cytoskeleton stability, remain elusive. Using the first iPSC line from ahibernating mammal (13-lined ground squirrel), we uncovered cellular pathways critical for cold-tolerance. Comparison between human and ground squirrel iPSC-derived neurons revealed differential mitochondrial and protein quality control responses to cold. In human iPSC-neurons cold triggered mitochondrial stress, resulting in reactive oxygen species overproduction and lysosomal membrane permeabilization, contributing to microtubule destruction. Manipulation of these pathways endowed microtubule stability upon human iPSC-neurons and rat (a non-hibernator) retina, preserving its light responsiveness after prolonged cold exposure. Furthermore, these treatments significantly improved microtubule integrity in cold-stored kidneys, demonstrating the potential for prolonging shelf-life of organ transplants. Thus, ground squirrel iPSCs offer a unique platform for bringing cold-adaptive strategies from hibernators to humans in clinical applications.

32.2
Molecular manipulations: the power of cell culture for defining mechanisms of anoxia tolerance
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The freshwater turtle Trachemys scripta has been studied as a model of anoxia tolerance for nearly 40 years due to its ability to survive prolonged bouts of anoxia and repeated reoxygenation without apparent functional damage. T. scripta can survive without oxygen as long as 24h at room temperature to weeks at 3°C during winter hibernation. Originally, research on this model was largely observational, whole animal physiology. More recent studies have focused largely on the brain, since this is the most sensitive organ to oxygen deprivation in vertebrates. However, manipulation of an intact animal to determine molecular mechanisms of anoxia tolerance is difficult. Microdialysis of intact brains or the use of brain slices have proven useful for some studies such as the mechanisms of neurotransmitter balance, but the thickness of the samples can limit utility. Since 2007, our lab has utilized primary cell cultures of brain tissue which allows for direct manipulation at the cellular and molecular level. Neuronal cell cultures are established by digestion of brain tissue in an enzyme cocktail for 4 hours followed by density gradient separation to isolate the neuronal layer. Cell cultures are incubated for 3-4 weeks in Minimum Essential Medium (MEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin at 30°C in a 5% CO2 incubator. Our lab has utilized these cell cultures to pharmacologically manipulate molecular pathways including inhibition of the Adenosine A1 Receptor (A1R) and protective kinase pathways as well as knocking down expression of neuroprotective genes such as Neuroglobin, Hsp72 and Bcl-2 utilizing turtle target specific small inhibitory RNAs (siRNAs). Our results indicate that during anoxia/reoxygenation blocking of A1R and decreased expression of Neuroglobin and Hsp72 increased ROS production suggesting that these play an important role in neuroprotection by decreasing oxidative stress damage, while decreasing the expression of Bcl-2 triggers the activation of the apoptotic pathway. Our current study focuses on the role and regulation of Methionine Sulfoxide Reductase (Msr), an antioxidant and repair mechanism that is highly conserved from prokaryotes to eukaryotes and has been shown to be neuroprotective during anoxia and oxidative stress. We are using both MsrA knock down with target specific siRNA and plasmid transfection to examine the expression, role, and regulation of MsrA in anoxia and under oxidative stress. The induction of FOXO3a protects against cell death when cells are exposed to oxidative stress. Preliminary studies suggest that the induction of FOXO3a also increases MsrA levels. As it may be possible pharmacologically to increase FOXO3a expression, this suggests a way to increase cell survival under conditions that induce oxidative stress. The ability to manipulate cells in culture at the molecular level thus provides an additional tool by which we can define critical protective pathways of survival without oxygen and suggest therapeutic targets for further study. All work is approved by the FAU IACUC.
32.3 Identifying anti-inflammatory properties of serum that could protect the lungs of deep-diving seals
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Seals are accomplished diving mammals, which rely on lung collapse to limit nitrogen absorption and prevent decompression injury. Repeated collapse and re-expansion exposes the lungs to multiple stressors, including ischemia/reperfusion, alveolar shear stress, and inflammation. There is no evidence, however, that diving damages pulmonary function in these species. To investigate potential protective strategies in deep-diving seals, we examined the inflammatory response of seal whole blood exposed to lipopolysaccharide (LPS), a potent endotoxin. IL6 cytokine production elicited by LPS exposure was 50-500× lower in blood of healthy northern elephant seals and Weddell seals compared to that of healthy human blood. In contrast to the ~6× increased production of IL6 protein from LPS-exposed Weddell seal whole blood, isolated Weddell seal peripheral blood mononuclear cells, under standard cell culture conditions using media supplemented with fetal bovine serum (FBS), produced a robust LPS response (~300×). Induction of IL6 mRNA expression as well as production of IL6, IL8, IL10, KC-like and TNFα were reduced by substituting FBS with an equivalent amount of autologous seal serum. Weddell seal serum, elephant seal serum, and harbor seal serum also attenuated the inflammatory response of RAW 267.4 mouse macrophage cells exposed to LPS. Cortisol level and the addition of serum lipids (free fatty acids) did not impact the cytokine response in cultured cells. These data suggest that seal serum possesses anti-inflammatory properties, which may protect deep divers from naturally occurring inflammatory challenges such as dive-induced hypoxia-reoxygenation and lung collapse. Funded by NSF Office of Polar Programs 1443554. [Reference: Bagchi, A., Batten, A. J., Levin, M., Allen, K. N., Fitzgerald, M. L., Hückstädt, L. A., Costa, D. P., Buys, E. S. and Hindle, A. G. (2018). J. Exp. Biol. 221, 10.1242/jeb.178491]
to study the cellular mechanisms modulating a natural tolerance to ischemia/reperfusion.

Research supported by National Science Foundation Office of Polar Programs (Award # 1443554) and Univ. of California Berkeley funds.

33: INTEGRATING PHENOTYPES AND FUNCTIONAL GENOMICS TO UNDERSTAND MECHANISMS OF REMODELING AND GROWTH

33.1

A new perspective from snakes on conserved vertebrate stress and growth pathways underlying intestinal regeneration

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Some snake species undergo remarkable feats of physiological remodeling and organ regenerative growth upon feeding. These species tend to substantially downregulate physiological function and atrophy organs while fasting, and must rapidly regenerate tissue and upregulate physiological function upon feeding. This begs the question of what molecular mechanisms do snakes use to direct such regenerative processes. In this study, we focus on regenerative growth in the snake intestine, and use a comparative experimental design that includes snake species that do, and those that do not, undergo regeneration upon feeding. Here we integrate phenotypic data, together with data on gene expression and protein expression, to characterize regenerative growth in snake intestinal tissues. Our results provide surprisingly clear inferences of shared conserved stress and growth pathways that underlie regenerative intestinal growth in snakes, and new evidence for the importance of stress response signaling in tissue regeneration.

33.2

Using natural genomic variation and experimental approaches to understand the function and evolution of the Insulin and Insulin-like signaling network in reptiles

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The Insulin and Insulin-like Signaling (IIS) network integrates signals from the environment and physiological stressors to regulate cellular and organismal growth, reproduction, and aging (i.e. life history traits). Evidence from model organisms indicates the IIS network may regulate trade-offs between these life history traits. Reptiles have considerable diversity in these life history traits, relative to mammals and classical model organisms; however, little is known about the function of the IIS network in this clade. Previous research found evidence of positive (diversifying) selection in key regulators and nodes within the IIS network in reptiles, particularly the interactions between the hormones (IGF1 and IGF2) and their receptors within squamates (snakes and lizards) [1]. Here I summarize what we know and don’t know about the interaction of molecular stress responses with the IIS network to regulate life history traits and trade-offs in snakes and lizards [2,3]. I present recent findings from my lab on (1) the genetic variation in the IIS pathway in Anolis and Scleropus lizard clades, and (2) the quantitative expression of regulators of the IIS pathway with ontogeny in the brown anole lizard, Anolis sagrei. I then describe the tools my lab has been developing to facilitate the study of IIS in reptiles. These tools include genomic sequence data; quantitative assays for IIS proteins and mRNA; the establishment of fibroblast cell culture from lizard tails and organs; production of species-specific recombinant proteins (regulators of the IIS pathway) for experimental manipulations; and organismal dosing of recombinant proteins using slow-release microspheres. Finally, I provide an overview of ongoing projects within my research program that are using these tools to understand the role of IIS in regulating cellular and organismal growth and aging, tissue regeneration, and life history trade-offs.

Funding provided by Auburn Univ.


33.3

Transcriptome Dynamics in Hibernation: Cause or Consequence of Physiology?

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Small-bodied hibernators including the 13-lined ground squirrel (Ictidomys tridecemlineatus) exploit winter’s
Cardiomyocyte polyploidization creates a barrier to heart regeneration in zebrafish

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The adult mammalian heart lacks any appreciable capacity to regenerate ischemia-damaged muscle. By comparison, injured hearts of neonatal mice and some non-mammalian vertebrates, including salamanders and zebrafish, mount an impressive regenerative response driven by myocardial proliferation. Despite these differences in regenerative capacity, the variables that promote or restrict cardiomyocyte proliferation following injury remain incompletely characterized. One often-noted difference between non-regenerative and highly regenerative hearts is the DNA content of their cardiomyocytes. Non-regenerative hearts contain a majority of polyploid cardiomyocytes that form early in life when their diploid predecessors complete one round of DNA replication without cytokinesis. By contrast, highly regenerative hearts contain a majority of cardiomyocytes that are diploid, or assumed to be diploid based on nucleation studies. Although these observations reveal an inverse correlation between myocardial ploidy and cardiac regeneration, a causal relationship has yet to be investigated. Here, we demonstrate that experimental induction of cardiomyocyte polyploidization is sufficient to suppress the high regenerative capacity of the zebrafish heart. We learned that zebrafish cardiomyocytes are diploid, both during homeostasis and regeneration, and become susceptible to polyploidization upon inhibition of the RhoGEF Ect2. To create adult animals with mosaic hearts composed of diploid and polyploid cardiomyocytes, we engineered a transgenic system for transient, cardiomyocyte-specific inhibition of Ect2 during hyperplastic growth of the zebrafish heart. Following cardiac injury, diploid cardiomyocytes outcompeted their polyploid neighbors in producing regenerated muscle, demonstrating that elevated ploidy reduces or abolishes cardiomyocyte proliferation in vivo. Moreover, mosaic hearts composed of roughly equivalent percentages of diploid and polyploid cardiomyocytes failed to regenerate, indicating that a critical percentage of diploid cardiomyocytes, one greater than 50%, is required for heart regeneration. Taken together, our data identify cardiomyocyte polyploidization as a significant barrier to heart regeneration and suggest that maximizing the percentage of diploid cardiomyocytes surrounding infarcted myocardium may improve the regenerative capacity of the human heart.
34: THERMAL BIOLOGY: ABSTRACT DRIVEN SESSION - 3

34.1
Geographic variation in bumblebee thermal tolerance: implications for past and future range shifts
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Recent shifts in the geographic ranges of bumblebees (genus Bombus) in North America and Europe appear to be tightly linked to changes in climate. Warming temperatures could explain range contraction at the southern edge, but the failure of many bees to move northward in response to warming climates remains enigmatic. As with many other species, we know little about the mechanistic links between changing temperatures and bumblebee physiology. To address this gap, we first measured critical thermal limits of B. vosnesenskii workers reared from queens collected across the geographic range of the species (from southern CA to northern OR). We found strong divergence in cold (but not heat) tolerance across latitude and altitude, with CTmin closely tracking winter minimum temperatures of the queen collection sites. We then asked whether thermal tolerance could predict past distribution of this species (hindcast) by filtering historic climate data with measured thermal limits. Finally, we used projected climates to predict future range shifts.

34.2
Defying the temperature size rule in flight: Bigger bees perform better at higher temperatures
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The temperature-size “rule” was coined because, both in the field and lab, ectotherms are often smaller in hotter environments. However, neither the evolutionary causes nor the physiological mechanisms for this pattern are clear. As for other insects, bees tend to be larger at higher altitudes and smaller when reared at warmer temperatures in the lab. Larger bees can be endothermic during flight, so it is possible that thermal effects on body size are partially mediated by temperature effects on flight performance. Plausibly, endothermic, larger bees may be more able to fly in cooler temperatures but heat-limited under hotter conditions. If so, such a pattern could provide a partial evolutionary explanation for the temperature-size rule in bees. In the field in Panama, we measured thorax and air temperature to assess the effect of size on thermoregulatory ability. We measured leaf and flower surface temperatures and air temperatures in sun and shade in the tropical forest canopy, to assess the thermal environment of foraging stingless bees. We then measured the flight performance (whether or not bees could fly, flight durations) and flight metabolic rates across a range of air and body temperatures in the lab that reflected thermal conditions in the field using ten species of stingless bees that varied in body size from 2-120mg in body mass. Smaller species flew with body temperatures much closer to air temperature than larger species, which fly at thorax temperatures up to 10°C in excess of air temperature. This is partially explained by the scaling of heat gain and loss as a function of body volume; we found that smaller bees had steeper cooling curves, indicating that they gain and lose heat more rapidly. In the lab, contrary to predictions, the critical thermal maximum temperature at which flight ceased was lower in smaller species. These data suggest that larger bees may have developed capacities to tolerate higher body temperatures because they are routinely exposed to higher temperatures during flight due to their endothermy. Also, the shape of thermal performance curves varied strongly by species, suggesting that other ecological or phylogenetic effects on thermal tolerance are important determinants of flight performance in stingless bees. This research was supported by USAID, the Smithsonian Tropical Research Institute, and NSF IOS 1558127.

34.3
One for all or all for one: Emergent thermal physiology of ant colonies along tropical mountain ranges
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How do individual physiologies relate to group thermal adaptation? Using 16 Neotropical ant species as social models, we compared both individual and group-level thermal adaptations among tropical habitats. Here, we report new patterns of how within-colony thermal tolerance variation and group thermoregulatory tradeoffs scale to biogeographic clines in temperature. Differences in thermal tolerance breadth across habitats were often due to changes in small-caste (weak-link) thermal performance, and these biogeographic patterns appeared to be more actively selected for by thermal variation than by mean environmental temperature. We go on to describe the role synchronous broods play in group thermoregulatory trade-offs across elevation clines of living, self-constructed nests (bivouacs) of the army ant Eciton burchellii. Together, these findings suggest that considering social scales of thermal adaptation is key to accurately predicting the effects of climate change on highly social species.
Simultaneous stress: effects of hypoxia-temperature interactions on mortality, thermal tolerance, and transcriptome of *Drosophila melanogaster*

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Insects live in diverse, multifaceted environments, where several abiotic factors occur concurrently. Despite the relevance, stress responses to multiple, interactive stressors are not well studied. When stressors are combined the results are often unpredictable because we do not know how animals sense and respond to interactive environmental stressors. To understand how oxygen and temperature stressors interact across several organizational hierarchical levels, we studied how combined oxygen partial pressure (PO2)-temperature stressors affect subsequent mortality, thermal tolerance and the transcriptome. We exposed male *Drosophila melanogaster* to a PO2-temperature treatment consisting of 21 (normoxia), 10 or 5 kPa O2, with control (23°C), cold (4°C) or hot (31°C) temperature. After 2h recovery, we measured mortality, chill coma recovery time (CCRT) and heat knock down time (HKDT) as measures of cold and heat tolerance, and ran microarrays to measure the global gene expression profile. Cold treatments resulted in low mortality, regardless of the PO2 of the treatment. Hot treatments had higher mortality, especially at 5 kPa O2, and longer CCRT and shorter HKDT in comparison to the cold treatments. We identified genes and pathways associated with the protective effects of combined hypoxia and cold, as well as transcripts correlated with longer CCRT and shorter HKDT. These genes, and their associated pathways, provide targets for future studies on oxygen-temperature stress, and how they may interact to affect insect thermal tolerance and differential survival.

Funded by: Sapere Aude (Danish Council of Independent Research, Natural Sciences) and National Research Foundation of South Africa.


**34.4**

**34.5**

Cold tolerance of insects is arguably among the most important traits defining the distribution of this species rich group (Addo-Bediako et al., 2000). Even so, very little is known regarding the causes of cold injury. For many insects it has been observed that cold injury coincides with a cellular depolarization caused by hyperthermia and extracellular hyperkalemia that develops during chronic cold exposure (Andersen et al., 2017). However, prior studies have been unable to determine if cold injury is caused by direct effects of hypothermia, toxic effects of hyperkalemia or by the depolarization that is associated with these perturbations. Here we use a florescent DNA staining method to estimate cell viability of muscle and hindgut tissue from *L. migratoria* and show that the cellular injury is independent of the direct effects of hypothermia or toxic effects of hyperkalemia. Instead, we show that chill injury develops as a consequence of the cellular depolarization caused by these perturbations: 1) Regardless of whether the cells were depolarized by hypothermia or hyperkalemia, they accumulated injury to a similar extent when at similar membrane potentials. 2) The combination of hypothermia and hyperkalemia additively induced injury in both muscle and ileum tissue. 3) Depolarization and cell injury also occurred in conjunction when the means of depolarization was TEA. We further hypothesized that the depolarization-induced injury was caused by opening of voltage sensitive Ca2+ channels causing a Ca2+ overload that triggers apoptotic/necrotic pathways. To test this we pharmacologically manipulated intra- and extracellular Ca2+ concentration as well as Ca2+ channel permeability and demonstrate that injury is prevented if transmembrane Ca2+ flux is prevented by removing extracellular Ca2+ or blocking Ca2+ influx. Together these findings provide the first demonstration of a causal relation between cold induced hyperkalemia, depolarization and the development of chill injury through Ca2+ mediated necrosis/apoptosis.


34.6
How to assess *Drosophila* heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits
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Temperature is a critical determinant of ectotherm distribution, and accordingly, climate change will likely affect future distributions of many species. Prediction of such distributional changes calls for simple and comparable measures of heat tolerance. These measures should ideally correlate closely with the characteristics of the species current thermal environment. The time to heat death is obviously dependent on the intensity of the heat stress and a recent model (thermal tolerance landscapes – TTLs) uses the negative exponential relation between temperature and heat knockdown time to parametrise the interaction between temperature (heat stress intensity) and duration across a range of stressful temperatures. In the present study we established TTLs for 11 *Drosophila* species representing different thermal ecotypes by measuring knockdown time at 9-17 stressful temperatures (at 0.5°C intervals). All species displayed the expected exponential relation between temperature and knockdown time (average $R^2=0.98$).

Previous analyses of TTLs have described an apparent trade-off between tolerance to acute and chronic heat stress in ectotherms. Here we show that this trade-off is an erroneous artefact that can arise from experimental noise and extrapolations of limited datasets used to feed the TTL model. In accordance with this conclusion we do not find any general trade off between chronic and acute heat tolerance between our 11 species. Our study also addresses another important debate related to the measurement of heat tolerance in ectotherms. Thus, it has been debated if dynamic heat tolerance assays are better or worse at describing ectothermic heat tolerance. In contrast to the static tolerance assays, dynamic assays gradually increase temperature until knockdown resulting in a knockdown temperature rather than a knockdown time. The comparability of the static and dynamic assays has been questioned, but here we show that static and dynamic assays give comparable information on heat tolerance. Thus, the constants derived from a TTL can be used mathematically model dynamic knockdown temperature and we confirm the validity of this model with empirical measurements of dynamic knockdown temperatures in all 11 species. Finally, we show that both dynamic and static assessment of heat tolerance correlate closely with the environmental gradients that characterise the 11 species studied. Heat tolerance assessed by static and dynamic assays correlations to precipitation of the driest month and maximum temperature of the warmest month combined ($R^2=0.68-0.71$). These simple laboratory measures of heat tolerance are therefore powerful predictors of the species fundamental thermal niche and both static and dynamic measures of heat tolerance could therefore also be useful to predict the consequences of future climate change.

34.7
Evolution of body size toward temperature-dependent oxygen conditions in 188 rotifer species
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Recent reports suggest the important mediating role of oxygen concentration in body size-to-temperature response, especially in aquatic systems. However, the knowledge of the causal connections is still in a phase of conceptions. We tested the relationship between body size evolution and temperature/oxygen preferences in a large group of rotifer species using a comparative phylogenetic approach. Each species was defined by eight parameters describing its environmental preferences, namely minimum/maximum/optimum/range of temperature/oxygen concentration in the habitat of living. These data were obtained from the published studies. The PCA analysis showed that both range of temperature and of oxygen concentration clustered together, inversely to minimum temperature and minimum oxygen (Factor 1; 38 % of variance explained), while optimal temperature clustered inversely to optimal oxygen concentration (Factor 2; 30 % of variance explained). Factor 3 explained 14 % of variance, but its eigenvalue was still above 1, so we included it in further analyses. This factor differentiated temperature from oxygen and was driven mainly by the inverse connection (a trade-off?) between temperature range and temperature minimum, and between oxygen range and oxygen minimum. We further tested the relationship between species body size and species-specific environmental characteristics using published data on 188 rotifer species and corrected for their phylogeny. A multiple regression analysis for post-PCA scores showed the significant positive relationship of body size with Factor 1 ($p < 0.001$), a tendency of the relationship with Factor 3 ($p = 0.05$), but no relationship with Factor 2 ($p = 0.12$). The interpretation of this pattern is that body size of rotifers increases with wider ranges of preferred temperature and oxygen concentration, but is not related to optimal values of these variables. Instead, body size tends to be affected by minima of temperature and oxygen. Our study points out some interesting patterns in temperature/oxygen preferences in a large group of aquatic organisms. The new findings are that (i) the evolution toward wide thermal preferences was accompanied by the evolution toward wide oxygen preferences, (ii) the evolution toward optimal temperature/oxygen conditions, thus guaranteeing
maximal performance, has evolved separately from temperature/oxygen range, (iii) the interspecific correlation between body size and joint thermal and oxygen conditions discloses that, in the evolutionary scale, body size of aquatic organisms is selected not only by temperature, but by a combination of thermo-oxygenic conditions.

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35: OSMOREGULATION ION REGULATION:
ABSTRACT DRIVEN SESSION - 2

35.1
The role of the pyloric ceca in ion balance in rainbow trout: Integrating across techniques to understand active calcium transport
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The gastrointestinal tract (GIT) and dietary ions contribute additional routes and sources to maintaining ion balance in fish. Within the GIT of rainbow trout are vermiform ceca projecting from the anterior intestine ampule. The role of these structures in ion balance has been shown on a broad scale, averaging across all ceca combined using more traditional techniques. Instead, we use the Scanning Ion-selective Electrode Technique (SIET), which offers a powerful tool for studying ion transport, providing rapid and precise measurement of ion transport across GIT tissues. Using SIET we explored active and passive transport in individual ceca isolated from the Anterior, Middle, and Posterior sections of the anterior intestine. The impact of feeding and environmental manipulation exposure to Ion Poor Water (IPW)) revealed a dynamic response in ion transport that was specific for location within the ceca. Furthermore, correlations with enzyme activities within those locations demonstrated an increase in Na⁺/K⁺-ATPase (NKA) and Citrate Synthase (CS) activity that paralleled changes in ion transport. Overall, feeding and IPW exposure each altered ionoregulation within the caeca in a zone-specific pattern, with the Anterior and proximal portions of the caeca being paramount. Increased carbohydrate and protein metabolism fueled the increased ATP demand of NKA through CS.

35.2
Cellular mechanism for teleost otolith calcification, and their responses to acid-base disturbances
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Otoliths are calcium carbonate structures found in the inner ear of teleost fish, specifically within small epithelial sacs (“otolith epithelium”) and surrounded by an alkaline fluid (“endolymph”). The otolith organ is essential for perceiving gravity, equilibrium, soundwaves, and acceleration. The otolith calcifies in a ring-like pattern similar to a tree that provides valuable information about age, diet, growth rate, and geographical location that is essential for fisheries and ecology research. In addition, recent studies reporting enlarged otolith size in fish exposed to hypercapnia have, raised concerns about potential impacts of future ocean acidification on the balance and hearing of fishes. To promote calcium carbonate precipitation for otolith growth, the surrounding otolith epithelium must transport calcium and bicarbonate into the endolymp, and remove protons from the endolymp. However, the cellular mechanisms behind otolith calcification are poorly understood, especially in marine fish. This information would help identify potential species-specific mechanisms in otolith calcification and regulation that could affect isotope incorporation rate or determine differential responses to ocean acidification. Using immunohistochemical techniques, we identified two ion-transporting cell types in the otolith epithelium of Pacific mackerel (Scomber japonicus). The first type of ionocyte expresses Na⁺/K⁺-ATPase and Na⁺/K⁺/Cl⁻-co-transporter, while the second type of ionocyte is enriched in vacuolar H⁺ pump and a cytosolic carbonic anhydrase. Both types of ionocytes also express the evolutionary conserved acid-base sensing enzyme soluble adenylyl cyclase. To further investigate how otolith calcification may be affected by acid-base disturbances, otolith epithelia were dissected and exposed to elevated [HCO₃⁻] levels simulating blood conditions typical of fish exposed to hypercapnia. Immunohistochemistry of whole-tissue, sections, and isolated ionocytes as well as scanning electron microscopy were employed to analyze potential changes in protein localization and ionocyte morphology. Funded by NSF GRFP and GRIP to GTK, and NSF IOS # 1754994 to MT.

35.3
No water, no problem: a metabolomics analysis of desiccated annual killifish embryos
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Water is essential for life and the threat of dehydration puts organisms at risk. However, some organisms have adapted mechanisms for survival when faced with water stress. The annual killifish (Austrofundulus limnaeus) survives in ephemeral ponds in the coastal deserts of Venezuela and their embryos have the remarkable ability to tolerate anoxia for months. In addition, A. limnaeus must also contend with the seasonal dehydration of their ponds, which they survive through mechanisms that likely highly limit gas exchange. Survival is attributed to the ability of the embryos to enter a state of drastic
metabolic dormancy (diapause) as a part of their normal development. Embryos of *A. limnaeus* exhibit unique resistance to desiccation unseen by aquatic vertebrates. However, dehydration tolerance of these embryos has received much less attention than other aspects of their biology, and many questions remain regarding how long embryos can survive without water and the molecular mechanisms that support survival. To address these unknowns, we assessed the dehydration tolerance of *A. limnaeus* embryos during diapause and across post-diapause embryonic development. We then performed a metabolomics analysis (Metabolon) on embryos exposed to short and long term dehydration stress at two developmentally unique stages (diapause and 4 days post diapause). We sought to identify the metabolic pathways that may provide this species with its remarkable abilities to survive under extreme dehydration stress. Early findings suggest different molecular mechanisms are at play that allow embryos to survive dehydration stress compared to those that support survival of anoxic stress. Further metabolite profiling of desiccated embryos will provide insight into the unique molecular physiology behind embryological survival with no water. (Funding: NSF Grant IOS 1354549 to JEP).

### 35.4

**Distinct ion transport properties in airways of the marsh rice rat (Oryzomys palustris)**

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The airway epithelium is a critical barrier to the external environment and is protected by a thin layer of fluid known as the airway surface liquid (ASL). ASL volume is driven by osmotic gradients that occur via active ion transport, evaporative water loss and contact with hyper- or hypo-osmotic aerosols. The marsh rice rat (*Oryzomys palustris*) is a medium-sized rodent from the *Cricetidae* family that inhabits salt marshes, which are areas of coastal wetlands marked by periods of flooding, high salinity, and extreme temperatures. In the current study, we examined the ion transport and fluid secretion mechanisms of the marsh rice rat airway. We found limited amiloride-sensitive Na⁺ absorption and forskolin and IBMX-mediated Cl⁻ secretion. In contrast, a large carbachol-mediated increase in Cl⁻ secretion that was sensitive to DIDS, the calcium-activated chloride channel (CaCC) inhibitor CaCCinh-A01, and pretreatment with barium was observed. Consistent with that, marsh rice rat airway cultures exhibited fluid secretion to carbachol. When challenged with 3.5% NaCl apically to mimic exposure to sea water and sea water aerosols, marsh rice rat cultures exhibited a prolonged expansion of the ASL volume. These data suggest that the marsh rice rat airway may have evolved unique ion transport mechanisms to facilitate survival in the dynamic salt marsh environment.

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### 36: **ANIMAL INTESTINAL MICROBIOMES: COMMUNITY DIVERSITY AND SERVICES PROVIDED TO THE HOST**

#### 36.1

It's not easy eating green: The importance of the gut microbiome in facilitating herbivory

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Herbivory, or feeding primarily on plant material, is a relatively common feeding strategy for animals. This feeding strategy is advantageous given the high abundance of plants in most ecosystems. However, herbivory is also physiologically challenging because plants often have low protein concentrations, high amounts of indigestible materials such as fiber, and defend themselves with toxic chemicals. In response, herbivorous animals have enlisted the help of microbial partners to cope with these various challenges. I will discuss experimental work in various animal systems examining the physiological contributions of the gut microbiome that allow animals to thrive on herbivorous diets. For example, using a series of experimental manipulations consisting of whole-organism feeding trials, microbiome reduction, and microbial transplants, as well as metagenomic and metatranscriptomic approaches, it was demonstrated that herbivorous woodrats (*Neotoma* spp.) rely on their gut microbiota to consume plant toxins. Additionally, captive feeding trials demonstrated that the gut microbiome of lizards respond to high fiber diets and help lizards cope with these challenging diets. These studies and others across various animal taxa will be important for uncovering universal and novel microbial adaptations to herbivory.

#### 36.2

The enteric microbial communities of sharks, fishes, island-dwelling lizards, and abalone: dietary and phylogenetic considerations

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All animals have microbial communities associated with them in one way or another, and usually, the most diverse community is in the digestive system. These enteric microbes can participate in a number of metabolic pathways that affect digestion, metabolism, and immune function in the host. As a field, we are just scratching the surface of what microbial diversity exists in animal guts, and how these communities provide
services for their hosts. In this talk, I will share the microbial diversity in the guts of four different taxonomic groups that show dietary variation: bonnethead sharks, prickleback fishes and zebrafish, Italian wall lizards, and abalone. Each of these systems has unique characteristics that make their intestinal microbial community of interest. The bonnethead shark (Sphyraena tiburo) is the only shark known to consume and digest seagrass. A spike in β-glucosidase activities in their hindguts, as well as elevated (~50%) digestibility of plant fiber—an unexpected result for a “carnivore”—suggest that the microbes matter in this shark’s ability to digest plants. Prickleback fishes (family Stichaeidae) feature dietary diversity, sister-taAx with different diets, and convergent evolution of herbivory, all within sympatric species, making this group an excellent model for investigating the role of diet and host evolutionary history in microbial community diversity. Moreover, I will share data on how diet switching experiments affect the microbiome diversity in prickleback fishes and zebrafish (Danio rerio), the latter of which is becoming a model for microbiome research. Italian wall lizards (Podarcis sicula) showed rapid evolution (~30 generations) of morphological characters relating to a shift to an omnivorous diet, but the shifts in digestive physiology occurred mostly in the hindgut, concomitant with targeted changes in its microbiome. Finally, the abalone (family Haliotidae) are iconic herbivorous molluscan taxa found globally, yet we know little about the digestive process in their guts. Hence, I will share the first efforts to integrate their digestive strategy and enteric microbial diversity with regards to how they make a living and potentially deal with the deadly “Withering Syndrome”, which has decimated abalone populations on the west coast of the USA. Overall, by better understanding how intestinal microorganisms correlate with different diets and phylogenetic histories, and coupling these data with functional data on digestive physiology and metabolism of the host, we can move towards understanding the range of services that microorganisms provide to their host organisms.

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36.3

Host genetic background contributes to resistance to microbiota disruption and host development in an evolution model organism

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Background: Communities of gut microbiota play an important role in stimulating development of organs and the immune system of the host, and in maintaining host homeostasis. The host genetic background can influence microbiota composition, in part via the immune response to microbes. In fact, several diseases that are marked by inflammation and gut microbiota disruption, such as Irritable Bowel Syndromes (IBS) and Inflammatory Bowel Diseases (IBD), are known to have high heritability (1,2). However, these are also marked by a strong environmental stressor or microbiota challenge early in development, indicating a gene by environment interaction that drives susceptibility to these diseases. To determine the extent that the host genetic background contributes to the developmental response to microbiota disruption, we adapted the evolution and biomedical model organism, threespine stickleback fish (Gasterosteus aculeatus), for microbiota manipulation experiments. Stickleback are ideal model organisms for these studies due to their large clutch sizes, genetic variation within and between populations that is similar to human genetic variation within and between populations, extensive knowledge about developmental and behavioral variation between and within populations, and the tools available to study host-microbe interactions. I previously found that a lab-adapted anadromous population, which originally spent part of its life in the ocean and part freshwater, had a larger immune response to microbes than a lab-adapted freshwater population (3). These two populations shared ancestors, but have been separated for thousands of years. Methods: We developed gnotobiotic protocols to manipulate the microbiota of both lab-raised and wild embryos of stickleback (3). We then raised fish from several different populations in germ free conditions with no detectable microbes (GF), with complex communities added back (conventional, CV), with single microbeAg (monoassociations, MA), or with mock communities of up to eight members (mock). We also added antibiotics or environmental contaminants to the water with the fish. We compared the somatic development, immune system transcripts, and behavior of the fish to determine how these microbiota manipulations affected fish development. Results: We found that the populations varied in their response to these manipulations, indicating that the genetic variation between the populations contributed greater to the relationship between microbes and the host than the variation within the populations. Interestingly, we observed differences in the development of some somatic markers of development but not others very early in development (14 days post fertilization). Future directions: We will use these results as a basis for future studies to identify the critical windows in development in which disruptions to gut microbiota result in short- and long-term consequences to host health, and determine the extent to which the host genetic background contributes to the ability of healthy gut microbial communities influence to fitness. We are also working
with collaborators to determine whether changes in the immune responses to microbes occur early in the switch from anadromous to freshwater phenotype.

### 36.4

**Gut microbial community dynamics in arctic ground squirrels: microbiobically-activated urea-nitrogen use across the annual cycle of hibernation and activity**  
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Arctic ground squirrels (AGS) are hibernation extremophiles, spending up to 9 months annually in torpor, subsisting on endogenous body reserves of lipid and protein. We have shown that gut microbial diversity and metabolic activity in AGS is influenced by the physiological state of the squirrel, and that microbial community composition reflects differences in availability of nutrient substrates across the annual cycle (Stevenson et al., 2014a,b). Urea-nitrogen salvage (UNS) -- the diffusion of urea into the gut, its degradation by ureolytic gut microbes to NH\(_4\) and CO\(_2\), and the subsequent incorporation of microbiobically-activated urea-N (MLUN) by the host -- is posited as an important N-conservation mechanism of hibernators; however, little is known about the extent to which MLUN contributes to host synthetic processes, the identity, number or activity of ureolytic microbes in the gut, or how these aspects change seasonally. Therefore, we injected squirrels with either unlabeled or \(^{15}\)N-labeled urea periodically across their annual cycle. The magnitude of gut ureolysis was assessed via quantification of \(^{13}\)CO\(_2\) in breath. To determine host use of MLUN, tissues were collected for analysis of \(^{15}\)N. Cecal samples were collected to enumerate ureolytic microbes and determine expression of urease genes, and fecal and cecal samples were collected to isolate and characterize ureolytic bacteria. The breath of squirrels was enriched in \(^{13}\)CO\(_2\), suggesting active ureolytic bacteria in the gut in both hibernation and summer euthermia, and several taxonomically diverse ureolytic bacteria were isolated and identified. Tissues were enriched in \(^{15}\)N to a greater extent in hibernating squirrels compared to summer active squirrels, evidence of the use of MLUN in host protein synthesis across the annual cycle. Enumeration of ureolytic bacteria and analysis of expression of urease genes in the gut is ongoing. Our results indicate that AGS contain a diversity of ureolytic gut bacteria that are active across the annual cycle and confirm the potential importance of UNS in N-conservation in hibernation. All work followed approved UAA-IACUC protocols. FUNDING was provided by an Institutional Development Award (IDEA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103395 and the National Institute of Diabetes and Digestive and Kidney diseases (IR15DK110784 to KD). The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH. Support was provided to KC from the Alaska Native Science and Engineering Program and Sloan Scholar, Alfred P. Sloan Foundation’s Indigenous Graduate Partnership (SIGP) Program, awarded in 2015-16.

### 37: COMPARATIVE PHYSIOLOGIC: SYSTEMS-LEVEL APPROACHES TO COMPARATIVE PHYSIOLOGY

**Species-Specific Responses of Juvenile Rockfish to Elevated pCO\(_2\) and Hypoxia**  
Cheryl Logan\(^1\), Holly Doerr\(^2,3\), Melissa Palmisciano\(^2,3\), Cori Hume-Flannery\(^4\), Andrew Cline\(^5\), Evan Mattlasi\(^6\), Neosha Kashef\(^5\), David Stafford\(^5\), Susan Sogard\(^6\), Eric Bjorkstedt\(^6\), Scott Hamilton\(^6\)

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Global climate change is predicted to trigger large-scale changes in ocean carbonate chemistry and hypoxia over the next several decades. Projected decreases in mean ocean pH and oxygen levels will be exacerbated in coastal upwelling systems if climate change increases the frequency and magnitude of upwelling events. We compared the effects of exposure to low pH (elevated pCO\(_2\)), hypoxia, and a combined stressor treatment on metabolic physiology and transcriptomic profiles of juvenile rockfish (genus *Sebastes*) over acute (<2 weeks) and chronic timescales (>4 months) under both fluctuating and static treatments. To compare high-pCO\(_2\) and hypoxia tolerance among species, experiments were conducted simultaneously on congeners that inhabit California kelp forests yet differ in early life history traits. Our findings indicate that congeners express different sensitivities to elevated pCO\(_2\) and hypoxia levels, and that hypoxia appears to have a stronger effect than elevated pCO\(_2\) at multiple levels of biological organization. The capacity of long-lived, late to mature, commercially important fish to acclimatize and adapt to a changing ocean over the next 50–100 years is likely dependent on species-specific physiological
37.2
The role of small noncoding RNAs in the regulation of metabolic dormancy and extreme stress tolerance
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1Department of Biology, Portland State Univ., 2Department of Biology, Saint Louis Univ., 3Department of Anatomy, Physiology & Cell Biology, Univ. of California at Davis School of Veterinary Medicine
Small noncoding RNAs (ncRNAs) are ubiquitous, abundant, and diverse. These short RNA sequences (15-30 nucleotides) can have a wide range of cellular functions most of which have to do with regulation of gene expression. However, there are a growing number of examples where these sequences may be playing roles in cell and organismal physiology independent of direct gene regulation. While some classes of small ncRNAs, such as miRNAs, are relatively well understood, there is an enormous diversity of sequences that have interesting expression patterns but unknown origins and functions. Exploring the small ncRNA transcriptome promises to shed new light on mechanisms of gene regulation, cellular and organismal physiology. The annual killifish, Austrofundulus limnaeus survives in ephemeral ponds by producing stress tolerant embryos that can enter into diapause, a state of developmental and metabolic arrest. Using RNAseq, we have explored the small ncRNA transcriptomes of A. limnaeus during transitions into and out of anoxia (1,2), and during entrance into metabolic dormancy (3). This work has identified small ncRNA sequences that may help to mediate survival of anoxia in embryos, including sequences that are derived from the mitochondrial genome. In addition, we have identified a number of interesting sequences that may mediate environmental cues into the developmental program and regulate phenotypic plasticity. Importantly, many of these sequences have unknown origins and functions and may represent new origins and mechanisms of action for small ncRNAs. Funded by NSF IOS-1354549 (JP), NSF DEB-1501414 (JP&CR), NSF GRFP DGE-1057604 (CR).

37.3
Using Proteomics to Investigate Regulation of Stress Tolerance by Sirtuins in Mytilus Mussel Congeners
M. Christina Vasquez1,2, Lars Tomanek2
1Biology, Loyola Marymount Univ., 2Biology, Cal Poly San Luis Obispo
Understanding physiological tolerances of marine organisms to environmental stressors is key to predicting species adaptability under climate change. Along the Pacific Coast of the U.S.A. intertidal mussel congeners of the genus Mytilus vary in their physiological tolerances to stress, with the invasive M. galloprovincialis being heat tolerant but vulnerable to hyposalinity while the native M. trossulus is vulnerable to heat stress and tolerant of hyposalinity. Our research findings suggest that sirtuins (SIRT), a group of NAD+-dependent deacetylases, influence the environmental stressor tolerances in these two mussel species. Our work uses proteomics to understand the role of sirtuins in regulating the cellular stress response (CSR) in mytilid mussels exposed to a variety of environmental stressors (i.e., submerged or aerial heat, hypoxia, food limitation, and hyposalinity). Mussels are acclimated to laboratory conditions in tidal simulators with a 12 h light:dark cycle and 6 h square wave tide to assess the effect of the circadian and circatidal rhythm. At the end of acclimation, mussels are exposed to chemical sirtuin inhibitors that reduce the activity of SIRT1, 2, 3, and 5. Following inhibition, mussels are then exposed to experimental conditions for 6 h during low or high tide to heat, oxygen and salinity stress. Our studies have found a clear effect on CSR proteins (antioxidants, superoxide dismutase; molecular chaperones, HSP70) key to maintaining cellular homeostasis under stress, proteins related to energy metabolism (isocitrate dehydrogenase), cell signaling (ERK2), and translational regulation (Musashi1). Moreover, through western blot analysis of SIRT5, our studies highlight the stark difference in stress tolerance between the two mussel congeners possibly due to sirtuin regulation. This comparative proteomics reveals sirtuins as regulators of the CSR and stress tolerance and may allow us to make predictions regarding climate change effects on these competing species.

37.4
Metabolic response to stress in marine mammals
Cory Champagne1,2, Jane Khudyakov3, Dorian Houser1, Daniel Crocker4
1Dept of Biological Research, National Marine Mammal Foundation, 2Dept of Biological Sciences, Univ. of Washington Bothell, 3Dept of Biological Sciences, Univ. of the Pacific, 4Dept of Biology, Sonoma State Univ.
Marine mammals are exposed to a range of stressors, including increased levels of anthropogenic disturbance in the marine environment. The generalized stress response is largely mediated by the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA-axis stimulates glucocorticoid release and results in metabolic alterations that facilitate the response to, and recovery from, proximate stressors. Frequent or chronic stress, however, can result in persistent activation of the stress response with maladaptive consequences on health, survival, and fitness. Far less is known about the metabolic effects of stress responses in wildlife species in comparison to model species commonly used in biomedical studies. We therefore investigated the hormone and metabolic response to stress in two marine mammal species: the bottlenose dolphin (Tursiops truncatus) and the northern elephant seal (Mirounga
**angustirostris**). We experimentally induced stress in dolphins under managed care (n = 5) with a single out-of-water stress test, and in free-ranging seals (n = 7) by repeatedly administering adrenocorticotropic hormone (ACTH). Blood samples were collected before, during, and after stress manipulations. To understand the influence of stress on whole-animal metabolism, we characterized the glucocorticoid response and used metabolomics to evaluate changes in the circulating metabolome. Stress had profound influences on metabolism—in both species, over half of the circulating metabolites were significantly altered during the stress response. As anticipated, stress stimulated carbohydrate metabolism, evidenced by increased circulating carbohydrates increased (e.g. glucose, lactate, and pyruvate) in both species, whereas amino acids showed a varied response to stress. The greatest influence of stress was on lipid metabolism, both in the magnitude of change and in the number of compounds affected. Multiple pathways of fat metabolism were altered, including lipolysis, carnitine-mediated transport, β-oxidation, and corresponding tricarboxylic acid cycle activity. The enhanced effect of stress on fat metabolism may be advantageous in marine mammals commonly characterized by high adiposity. Preliminary analysis suggests that there may be a combination of compounds that exhibit differing responses to acute and chronic stress that may help inform stress states in marine mammals.

**37.5**

From Genome to Phenome: Exploiting 13-Lined Ground Squirrel "Omnics" to Achieve a Deeper Understanding of Hibernation.

Katharine Grabek1,2, Thomas Cooke3,4, L. Elaine Epperson5, Kaitlyn Spees5, Gleyce Cabral5, Shirley Sutton1, Dana Merriman5, Sandra Martin9, Carlos Bustamante1

1Biomedical Data Science, Stanford Univ., 2R&D, Fauna Bio Incorporated, 3Department of Genetics, Stanford Univ., 4Biology, MIT, 5Center for Genes, Environment and Health, National Jewish Health, 6Brazil Scientific Mobility Program, CAPES Foundation, 7Cardiovascular Medicine, Stanford Univ., 8Biology, Univ. of Wisconsin Oshkosh, 9Cell and Developmental Biology, Univ. of Colorado, Anschutz Medical Campus

Hibernation is a highly dynamic phenotype whose timing, for many mammals, is controlled by a circannual clock and accompanied by rhythms in body mass and food intake [1]. When housed in an animal facility, 13-lined ground squirrels exhibit individual variation in the seasonal onset of hibernation, which is not explained by environmental or biological factors, such as body mass and sex [2]. We hypothesized that underlying genetic factors instead drive variation in this timing. In this project, we first increased the contiguity of the 13-lined ground squirrel draft genome assembly by using a long-range scaffolding technique [3]. We next employed a modified ddRAD sequencing protocol [4] to characterize genetic variation in 153 13-lined ground squirrels. Combining this with datalogger records, we estimated high heritability (61-100%) for the seasonal onset of hibernation. Applying a genome-wide scan with 46,996 variants, we identified 2 loci significantly, and 12 loci suggestively, associated with hibernation onset. These 14 markers alone accounted for 48% of the variance in the phenotype. The most significant marker (SNP 1, p=3.8x10⁻⁷) was located near prolactin-releasing hormone receptor (PRLHR), a gene that regulates food intake and energy homeostasis. Other loci were located near genes functionally related to hibernation physiology, including muscarinic acetylcholine receptor M2 (CHRM2), involved in the control of heart rate, exocyst complex component 4 (EXOC4) and prohormone convertase 2 (PCSK2), both of which are involved in insulin signaling and processing. Finally, to further refine genes affected by these loci, we applied an expression quantitative trait loci (eQTL) analysis using existing transcriptome datasets. We identified significant expression associations for 8/14 loci. Our results highlight the power of applying a genetic mapping strategy to hibernation and present new insight into the genetics driving its seasonal onset.

References:


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38.1

**Metabolic suppression mechanisms for fasting and hypoxia**

Jean-Michel Weber^1^ 

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Metabolic suppression is a key strategy to cope with prolonged fasting or environmental hypoxia. My talk will address two alternative physiological mechanisms that reduce overall energy expenditure when food or oxygen are in short supply: (1) the inhibition of substrate cycles during long-term fasting, and (2) the inhibition of ion pumps via remodelling of membrane lipids during chronic hypoxia. Rabbits fasting for 6 days decrease their metabolic rate by 28%. After 2 days, in vivo tracer kinetics show that the rates of appearance of glycerol (R_a glycerol or lipolysis; +59%) and nonesterified fatty acids (R_a NEFA; +73%) are strongly stimulated, but these responses are abolished after 6 days without food. Simultaneous lipolysis and re-esterification form the triacylglycerol/fatty acid cycle (TAG/FA cycle): a substrate cycle that consumes ATP without net synthesis of any product. By combining tracer kinetics and indirect calorimetry, flux through this cycle can be quantified from R_a glycerol, R_a NEFA, and NEFA oxidation. We have found that TAG/FA cycling is first activated (+47% after fasting 2 days), but that it falls well below baseline after 6 days (-31%). Therefore, when fasting is prolonged, the inhibition of the TAG/FA cycle reduces total energy expenditure. If food deprivation was to have the same effect on other major substrate cycles (glucose, fructose and others), this mechanism alone would play an important role in supporting metabolic suppression.

Fish and mammalian champions of hypoxia tolerance like goldfish and naked mole rats show an outstanding capacity for metabolic suppression. They are able to reduce their rate of oxygen use dramatically by downregulating ion pumps, anabolic pathways, and energy metabolism. Because ion pumping by membrane-bound ATPases is the most energy costly physiological process in resting tissues, we have looked for new mechanisms of ion pump inhibition that could help survival in hypoxic environments. We have recently discovered that goldfish restructure membrane lipids extensively to cope with chronic hypoxia (4 weeks at 10% air saturation). They modulate both the fatty acid composition and the cholesterol content of their membranes in ways that are fully consistent with well known mechanisms of ion pump inhibition. More specifically, they increase membrane cholesterol (in muscle and gills), and decrease membrane unsaturation (in gills and liver) by reducing the relative abundance of polyunsaturated fatty acids, but particularly DHA (docosahexaenoic acid or 22:6 n-3). These large changes in lipid composition are interesting because ion pumps like Na^+/-K^+/-ATPase and Ca^{2+}/-ATPase are inhibited by cholesterol and activated by DHA. Therefore, these observed membrane remodelling responses to low oxygen could be a novel mechanism used by hypoxia tolerant animals to achieve extreme levels of metabolic suppression. We are presently exploring this possibility through direct measurements of ion pump activity during acclimation to hypoxia and by characterizing how mammalian membranes are affected by long-term hypoxia, using the naked mole rat as a model.

38.2

**Now or Later: Differential fates for glucose and fructose in a nectarivore**

Morag Dick^1^,^2^, Antonio Alcantara-Tangoanan^1^, Yazan Shamli Oghli^1^, Kenneth Welch^1^,^2^,^3^ 

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Hummingbirds fuel their high energy active lifestyle with the fructose and glucose in their nectar diets. These sugars are used to fuel immediate energy needs and build the fat stores to fuel future fasting periods. Remarkably, hummingbirds can quickly shift from fuelling energy-demanding hovering flight from stored fat to either fructose or glucose alone within 20 mins of feeding. However, if and how hummingbirds partition dietary fructose and glucose towards immediate oxidation to fuel foraging behaviour or towards fat storage is unknown. The high fat turnover rates of hummingbirds (50% fat turnover rate in less than 24 h) means that nectar consumed during the day is also required to fuel that night’s energy requirements. As such, metabolic partitioning of glucose and fructose to balance the energy needs of now and later may be an important strategy. Theoretically, the distribution of sugar transporters and metabolic pathways suggests preferential oxidation of glucose and prioritization of fructose for lipogenesis. Using acute and chronic stable isotope tracer studies we are examining how dietary sugars are allocated towards immediate oxidation or fat synthesis in ruby-throated hummingbirds (*Archilochus colubris*). First, we tested for preferential use for *de novo* lipogenesis by feeding hummingbirds diets with either the glucose or fructose enriched with ^13^C for 5 days. The isotope incorporation into fat was measured via the breath fatty acid signature each morning after an overnight fast. We found higher incorporation of stable isotopes into the fat stores when glucose was enriched compared to fructose suggesting
preference for glucose as a substrate for fatty acid synthesis. Secondly, we are testing for immediate preferences to determine if glucose or fructose is partitioned towards fuelling foraging behaviour. These studies improve our understanding of the adaptations that support the energy needs of hummingbirds during both feeding and fasting periods and the role played by the partitioning of a seemingly simple diet. Funding for this work is provided by the Natural Sciences and Engineering Research Council and Human Frontier Science Program.

#### 38.3

**Feeding the Machine at the Top of the Food Chain: A carnivore conundrum**

**Terrie Williams¹, Anthony Pagano¹, Jason John¹, Nicole Thometz²**

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A defining feature of the 276 species of fish- and meat-eating mammals is a diversity of form and function that enables them to serve as apex predators from mountain tops to ocean depths and from tropical to polar regions. Critical to this success is the integration of physiology, environmental synergy and energetic efficiency that matches how animals are built with the environmental conditions in which they forage and live. Such integration is especially evident in the marked changes in organ morphology and physiology that occurred 30-50 MYA as terrestrial mammals reinvaded the oceans. In this study, we use extant carnivorous mammals to examine the role of metabolic demand and acquisition along the evolutionary pathways leading to modern marine mammals. Maintenance, locomotor and field metabolic rates were measured for fully terrestrial (puma, leopard, grizzly bear), transitional (sea otter, polar bear), and fully marine (bottlenose dolphin, monk seal, Weddell seal) species to characterize the energy demands of each lifestyle. To assess the capacity of the digestive system to process food to meet those demands, we also examined the length of the small intestine of 50 mammalian species specializing in terrestrial, transitional or marine diets. We found that the maintenance metabolism of carnivorous mammals depended on the degree of aquatic specialization, and ranged from 1.3 to >2.2 times predicted basal levels of non-carnivorous mammals of similar body mass. Locomotor costs were as predicted for similarly-sized swimming or running mammals regardless of diet preference. Field metabolic rates of marine mammals, except deep-diving seals, reflected the thermal energetic demands of immersion and averaged 1.8 times that predicted for terrestrial carnivores. An important pre-adaptation for marine living was remodeling of the omnivore and herbivore gut to support these elevated metabolic rates and to fuel seasonal adiposity. Consequently, maintenance energetic demand (kcal/day) was correlated to small intestinal length (m) according to, Energetic demand = 141.9 x small intestinal length^1.33 (n = 16 species).

Transitional species in this study retained both terrestrial and marine characteristics that included the comparatively high resting metabolic rates of marine-adapted species coupled with relatively short intestinal lengths of their terrestrial ancestors. Thus, the intestinal morphology of the polar bear is similar to that of terrestrial black bears and grizzly bears but must assimilate more calories to meet the higher metabolic demands of marine living. Two different solutions for fueling the metabolic overhead created by this reduced digestive capacity are displayed by these transitional species. Polar bears rely on a diet of calorically-dense, lipid rich prey, while sea otters graze throughout the day and night in areas of abundant protein prey resources. The latter fueling strategy of otters has the added benefit of prolonging the heat increment of food processing to offset thermal metabolic demands. Both fueling strategies enabled ancestral mammalian groups to take advantage of plentiful oceanic prey across evolutionary time. Today, access to critical food resources needed to preserve energy balance within the processing capacities of these transitional carnivorous mammals has been compromised by rapid changes in ecological and environmental conditions. The ensuing energy imbalance is manifested as a reduction in body condition in polar bears and sea otters during periods of high energetic demand with a concomitant increase in uncertainty for long-term survival. (Supported by ONR, NSF-DBI, NSF-Polar.)

#### 38.4

**Fuelling locomotion and thermogenesis in high altitude native deer mice**

**Grant McClelland¹**

¹Biology, McMaster Univ.

Alpine regions are challenging for high-altitude animals due to unavoidable low O₂ availability and low temperatures. Reduced aerobic scope in hypoxia may limit the ability to engage in sustained exercise or to avoid hypothermia. However, deer mice (*Peromyscus maniculatus*) have a wide altitudinal distribution, up to ~4300m, and have evolved elevated cold-induced (thermogenic capacity) and exercise-induced maximum oxygen consumption (VO₂max) in hypoxia. Thermogenesis is fuelled by very high rates of lipid oxidation but submaximal exercise relies to a greater extent on carbohydrates than seen in lowland natives. The metabolic phenotype of skeletal muscle and their responses to cold and hypoxia acclimation do not necessarily reflect variation in fuel use. These data suggest a role for metabolic regulation of muscle enzymes or modifications of other parts of substrate pathways in setting fuel use at altitude. Possibilities that
will be discussed include changes in substrate availability, muscle uptake capacities, and/or selective recruitment of fibre metabolic pathways.

39: MECHANISMS OF CHANGE, PHYSIOLOGICAL RESPONSE TO ENVIRONMENTAL STRESSORS SPONSORED BY THE SOCIETY OF EXPERIMENTAL BIOLOGY

39.1 Sublethal effects and biomarkers of crude oil exposure in anadromous fish
Sarah Alderman1
1Integrative Biology, Univ. of Guelph

Crude oils are a complex mixture of chemicals and represent a pervasive environmental stressor. Canada sits on the world’s third largest crude oil reserve, found as bitumen in the Athabasca oil sands. As plans to bolster the export capacity of this resource intensify, so too do concerns for the added risk of spills and environmental contamination. For example, existing and proposed oil pipeline routes carry bitumen across coastal watersheds, and release of bitumen here could threaten salmon populations that depend on these waters for their anadromous life cycle. My research investigates the physiological and performance impacts of environmentally-relevant bitumen exposure in salmon at different life stages, with an aim to understand the implications of a spill on the capacity of these fish to transition from freshwater to seawater. I have connected molecular and histological changes in the hearts of juvenile salmon with reduced swimming performance, supporting a causal link between crude oil induced cardiotoxicity and impaired aerobic capacity, and warning of reduced migration success in exposed fish. In addition, exposing fish during smoltification blunts the molecular response in the gill required for seawater acclimation, which could further impede the success of their seaward migration and transition to the oceanic life phase. I have shown that exposure during development carries a lasting impact, with high mortality continuing for months even when fish are removed to uncontaminated water, and changes in brain morphology of surviving fish are evident nearly one year later. While the organismal outcomes of these changes in the brain are not known, this finding supports an emerging interest in the neurotoxic effects of crude oil exposure. Finally, analysis of the plasma proteome of exposed fish revealed changes consistent with elevated tissue damage as well as several proteins that may be useful as biomarkers of crude oil exposure. This research is funded by the National Contaminants Advisory Group at Fisheries and Oceans Canada.

39.2 Physiological responses to social stressors
Kathleen Gilmour1
1Department of Biology, Univ. of Ottawa

Many species of fish establish social hierarchies as a consequence of competition for limited resources such as feeding territories or mates. Typically, dominant fish enjoy preferential access to the limited resource and are aggressive towards more subordinate fish. Subordinate fish, by contrast, may exhibit behavioural inhibition including reduced activity and feeding. These behavioural differences are accompanied by distinctive physiologies, particularly with respect to regulation of the stress axis, and metabolism and growth. Subordinate fish often exhibit chronic elevation of the glucocorticoid stress hormone cortisol, and typically do not feed or grow as well as dominant fish. Indeed, the combination of high cortisol levels and low food intake can remodel liver metabolism in subordinate fish to place greater reliance on on-board energy reserves. This presentation will focus on regulation of the stress axis and regulation of growth and metabolism as a result of social interactions in rainbow trout (Oncorhynchus mykiss) and the cichlid Neolamprologus pulcher. Whereas juvenile rainbow trout form pecking order social hierarchies, N. pulcher is a cooperative breeder in which a breeding pair is dominant over a series of subordinate helpers. These contrasting social systems provide a useful framework in which to explore physiological responses to social stressors.

Research supported by the Natural Sciences and Engineering Research Council of Canada.

39.3 Exploring thermal physiology: Effects of environmental temperature in embryonic to larval frogs and juvenile to adult copepods
Casey A. Mueller1
1Biological Sciences, California State Univ. San Marcos

The environment is a driving force that has immediate and long-term effects on animal phenotypes. Yet, animals are not passive entities, and phenotypic plasticity is an important avenue by which animals respond to the environment. Plasticity is an important component of thermal responses, particularly when temperature changes occur during development, or fluctuate over short time scales (minutes to hours). One of the main objectives of my lab’s research is to understand how temperature shapes phenotypes across different life stages, time scales, and populations. We are exploring the immediate and long-term effects of developmental temperature in the Baja California chorus frog (Pseudacris hypochondriaca) and examining how different populations of a supratidal marine copepod (Tigriopus californicus) respond to temperature changes across different time scales. Chorus frogs are the most
abundant and successful amphibian on the west coast of North America. Chorus frog embryos demonstrate immediate responses to incubation temperature (10°C-25°C). Warmer temperatures reduce time to hatch, and oxygen consumption rate (Vo₂) is higher and more variable at 20°C. However, there is a limited temperature effect on embryonic growth, with mass similar between temperatures throughout embryonic development and at hatch. The developmental environment may also have long-term effects in shaping phenotypes later in life. Chorus frog larval Vo₂ and critical thermal maximum (CTmax) is influenced by a combination of embryonic temperature and larval acclimation temperature. Timing of thermal changes is also an important consideration in juvenile and adult organisms, particularly those experiencing highly variable environments. *T. californicus* inhabit supratidal ‘splash pools’ (above the tidal zone) that are exposed to drastic changes in daily temperature and a latitudinal thermal cline from Baja California, Mexico, to British Columbia, Canada. When juvenile and adult copepods are shifted from 20 to 30°C at 2.5°C intervals every 48 h, we observe no temperature effect on Vo₂ measured at each temperature. These results suggest the copepods undertake rapid metabolic compensation. However, when Vo₂ is measured at 0 to 6 h after a temperature increase from 20°C to 25°C, an immediate increase in Vo₂ occurs before rapidly returning to 20°C levels by 6 h. The exact time course of this response varies between copepod populations from Oregon and southern California. Collectively, these studies indicate that a full understanding of a species thermal physiology must include both temporal (changes throughout development, environmental variability) and spatial considerations (geographic population).

### 39.4 Effects of Acute and Chronic Thermal Exposure on the Swimming Performance and Aerobic Scope of Sheepshead Minnows (*Cyprindon variegatus*).

**Amanda Reynolds Kirby¹, Edward Mager¹, Dane A. Crossley II¹**

¹Biological Sciences, Univ. of North Texas

Estuarine fish experience daily fluctuations in abiotic factors, which makes them an ideal model organism to study physiological acclimation responses to environmental stressors. One such species is the eurythermal Sheepshead minnow (*Cyprindon variegatus*), which has the capacity to tolerate environmental temperatures from below freezing (-1.9°C) to the point of protein degradation (43°C). It has been suggested that these fish have the largest thermal capacity recorded. However, questions remain about their physiological strategies at these temperatures. To investigate this adult mix-sexed minnows were placed in aquaria where water temperature was changed 1°C daily from 21°C until target temperatures (10 and 32°C) were reached and then held at target temperature for four weeks. For this study, individuals were transferred to a Blazka-style swim respirometer to assess swim performance (Ucrit and Uopt), metabolic rate (SMR, MMR, and aerobic scope) and cost of transport (COT). Ucrit and Uopt in 10°C minnows is decreased by 50% compared to 21 and 32°C minnows. Aerobic scope in 32°C minnows is 5.5 times higher than 10°C minnows but neither were different from 21°C minnows. COT and COTmin is not altered by thermal acclimation. To ascertain if observed results were temperature dependent or a product of acclimation, minnows (n=5) were acutely exposed (2°C change per hour) to target temperatures. Preliminary results show Ucrit was unchanged but exhibited a 2.5 fold increase in MMR and aerobic scope. This suggest that swim performance is a temperature dependent response but metabolic rate is not. This research was made possible by a grant from The Gulf of Mexico Research Initiative.

### 39.5 Hypoxia and ammonia exposures have differential, developmental-stage specific, and long-term consequences on the stress response in zebrafish

**Nicholas Bernier¹, Catie Ivy¹, Kristina Mikloska¹, Tegan Williams¹**

¹Department of Integrative Biology, Univ. of Guelph

Eutrophication and climate change are increasing the incidence of severe hypoxia and high environmental ammonia (HEA) in freshwater fish nursery habitats yet the short- and long-term consequences of these challenges on stress responsiveness in later life are largely unknown. To identify the potential programming effects of these environmental stressors in fish, we explored the consequences of HEA and hypoxia exposure during early life on the stress response of zebrafish. Larval exposure to HEA stimulated the endocrine stress axis, inhibited neuronal differentiation but also increased ammonia tolerance in later life. While early life HEA had little impact on the cortisol stress response to a repeat HEA exposure, it abolished a later life stress response to a novel vortex stressor in both larval and adult fish, suggesting that early-life HEA exposure can have persistent effects on the stress response. Larval hypoxia exposure also stimulated the endocrine stress axis, inhibited neuronal proliferation and differentiation, and increased hypoxia tolerance in later life. In contrast to the effects of HEA, larval hypoxia exposure inhibited the cortisol stress response to a repeat hypoxia exposure in larval fish, but had no effect on the cortisol response to a novel vortex stressor, and no sustained effects on the stress response to hypoxia in adults. Finally, although anoxia exposure in embryos had no effect on the stress response to hypoxia in later life, adults derived from anoxia-exposed embryos exhibited dominance during dyadic interactions and had lower whole body cortisol.
levels. Anoxia-exposed embryos raised to adults were also more aggressive and had higher whole body testosterone levels. These results suggest that acute embryonic anoxia can favor the development of a dominant and aggressive phenotype, and that a disruption in sex steroid production may contribute to the programming effects of environmental hypoxia. Overall, while early life environmental challenges can affect the larval stress response and stress phenotype of adult zebrafish, our results also show that the programming effects of early life environmental stressors are both life stage- and stressor-specific, and dependent on exposure history. Acknowledgements: Supported by an NSERC Discovery Grant to NJB.

41: PLENARY LECTURE

41.1
Ecophysiology: Physiology can Inform Ecology, and Ecology Can Inform Physiology
Raymond Huey
1Biology, Univ. of Washington, Seattle
My training (late 1960s) was initially in animal ecology and subsequently in evolutionary biology. Not surprisingly, my approach to ecophysiology naturally reflects that ecological and evolutionary perspective. For most of my career, ecologists and evolutionary biologists largely ignored physiological data and insights. That began shifting about 25 years ago when biologists and others started becoming aware of climate warming, but also began to appreciate that the biological impacts of climate warming (and of other environmental perturbations) are inevitably mediated by and transformed by basic physiological processes. I will begin my talk by briefly summarizing my view of the history of ecophysiology in the 20th Century as well as highlight a few papers that were key to my thinking, and then examine three case studies in ecophysiology. The first two demonstrate that a basic physiology perspective can sometimes be fundamental to understanding certain ecological issues: in fact, a failure to consider physiology can sometimes lead to incorrect answers. The third example demonstrates how an ecological perspective can lead to an awareness of physiological 'unknowns' and thus can motivate novel physiological studies. Overall, my talk develops the theme -- hardly original with me -- that ecophysiology provides synergies to multiple fields. Research supported by NSF grants (1978-2017).
B

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